AN IN VIVO INVESTIGATION OF SOME FACTORS INVOLVED IN SMOOTH SURFACE DEMINERALIZATION IN ADOLESCENTS UNDERGOING ORTHODONTIC TREATMENT WITH FIXED APPLIANCES

BY

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A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

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DEDICATION

Learning occurs as a result of interactions between the student and his/her environment. There were many in my environment who had a "hand" in completing this research project and from whom much was learned.

Dr. George Bowden was the force behind the project. Both he and Nora Nolette spent many hours plating, culturing and counting organisms. Without them, the project would never have gotten off the ground. Of course, now I owe my next-born to Nora!

The other members of my committee, Dr. Robert Baker and Dr. Alan Milnes, were only too willing to offer advice, particularly from a clinical perspective. "Coach" listened well, and Al knew the ins and outs of in vivo research.

I must also thank Anna for preparing the culture media. Ed, Martha and Dennis were there in the lab keeping all things in perspective and making sure that I stayed on course with the Mac. Without Darrell's programming "genius", I would still be loading data onto the mainframe. And the ladies in the dental library always took the time to help me locate another reference.

The children and their parents who participated in this study could not have been more cooperative. They donated their own time to this project and even arrived for appointments in spite of blizzard conditions.

My own children, Erik and Katie, and my wife, Kath, kept one another company all the while that I was at school. They sacrificed much so that I could pursue my goal, and their smiling faces and

words of encouragement helped me make it from day to day. I am anxious to resume my role as father and husband on a more full-time basis now that the goal has been achieved.

Finally, I want to acknowledge and thank God. He has been and will always be a part of everything that takes place in my life.

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ABSTRACT

White spot demineralization of the enamel surface is a potential adverse side effect of orthodontic treatment with fixed appliances. The presence of bands and brackets on the teeth may alter the oral environment to allow the numbers of certain bacteria to increase. Of particular interest are the so-called "cariogenic" organisms, Streptococcus mutans and Lactobacillus. An imbalance in the oral microflora in caries susceptible individuals could result in the formation of white spot lesions in the cervical area on the labial surface of certain teeth.

The intent of the present study was to evaluate longitudinally some of the "cariogenic factors" (caries history, diet, fluoride intake, microbiology) and attempt to correlate these factors with white spot formation in a group of orthodontic patients wearing fixed appliances. If a relationship between these factors and white spot demineralization can be shown, it may be possible to devise a protocol for predicting white spot formation in orthodontic patients with fixed appliances. In this way, individuals who are at risk for white spot demineralization may be identified and additional preventive measures implemented.

Forty-five subjects, all eighteen years or younger and undergoing orthodontic treatment with fixed appliances in the Graduate Clinic of the Faculty of Dentistry, University of Manitoba, were examined over a sixteen month period for white spot formation in the cervical area on the labial surface of an upper lateral incisor and a lower incisor before and after placement of an edgewise appliance. Plaque and white spot index scores were assigned to

these surfaces at each of the five examination sessions (times 0 to 4). A baseline DMFS and the fluoride concentration in each subject's home drinking water were determined. Sugar consumption was estimated from five-day diet surveys completed by the subjects before and after banding/bonding. Saliva samples were obtained at baseline (time 0) and at each of the clinical sessions (times 1 to 4) following placement of the appliance. Plaque samples were collected from the test surfaces one month (time 1), five months (time 2), nine months (time 3) and twelve months (time 4) after fixed appliance therapy had begun. The isolation frequency/persistence of S. mutans and Lactobacillus in all samples was measured. The concentration and percentage contribution to the total cultivable flora of these organisms were determined in the saliva and plaque samples, respectively. The saliva samples obtained before placement of the appliance and at the end of the study were incubated on commercially available culture media in addition to the laboratory media. Statistical analyses were used to correlate white spot formation with the clinical and microbiological measurements.

White spot lesions occurred in fourteen upper lateral incisors and five lower incisors. Lactobacillus levels remained stable over time whereas a significant increase ($p=0.03$) in the levels of S. mutans in plaque from the cervical area of upper lateral incisors which developed white spot lesions was observed at time 4. Of particular interest was a significant increase ($p=0.0002$) in the mean percentage contribution of S. mutans to the total cultivable flora in plaque from lower incisors with white spot lesions at time 1. This result suggests that low risk (lower incisors) rather than

high risk (upper lateral incisors) sites may provide more pertinent information regarding a person's susceptibility to white spot development. Comparison between the commercial and laboratory cultures indicated a positive correlation for the salivary concentration of S. mutans and Lactobacillus at the beginning and at the end of the study, respectively. However, the colony counts were less on the commercial culture at both times which indicates that laboratory cultures are more accurate in determining the salivary concentrations of S. mutans and Lactobacillus. Clinical measurements alone were not correlated with the development of white spot lesions which confirms the importance of both clinical parameters and microbiological tests in assessing risk of white spot lesion formation.

CHAPTER 1

A. INTRODUCTION

Enamel demineralization during orthodontic treatment with fixed appliances is a potential problem that persists in orthodontics today even though dental decay in the pediatric population is on the decline (Sheridan, 1988). The caries process results from the interplay of three principal factors: the host (teeth and saliva), the microflora, and the diet. Placement of bands and/or brackets on the teeth alters the oral environment to allow the numbers of certain bacteria to increase, particularly Streptococcus mutans and Lactobacillus. Both of these microorganisms have been implicated in dental caries, S. mutans with lesion initiation and Lactobacillus with the progression of a carious lesion (Bowden et al., 1983; Fitzgerald et al.,1966; Ikeda et al., 1973; Loesche et al., 1975a). Individuals who are predisposed to dental decay may develop white spot or incipient lesions during the course of orthodontic treatment that, at the very least, produce an unesthetic appearance.

B. EPIDEMIOLOGY OF WHITE SPOT LESIONS IN ORTHODONTIC PATIENTS

Noyes (1937) made a radiographic assessment of carious lesion formation in 100 adolescents undergoing orthodontic treatment before the placement of and after the removal of edgewise appliances. Only a slight increase in the caries rate was recognized. However, the incidence of enamel etching was considerably more frequent and was attributed to the presence of orthodontic bands which accumulated dental plaque.

Bach (1954) retrospectively analyzed his orthodontic patient population for demineralization. He pointed out that appliance wear has traditionally coincided with the adolescent period during which there is an increase in the caries rate independent of the presence of appliances.

Zachrisson and Zachrisson (1971a) compared a group of adolescents who had worn full banded appliances for 18 to 24 months with an untreated group to determine the prevalence, severity and distribution of caries. The treated group did not have more caries than the control group. However, the location of carious lesions shifted from interproximal to buccal and lingual surfaces. Their findings suggested that surfaces completely covered by the band were protected while partially covered surfaces became more susceptible to enamel demineralization. An earlier study by Ingervall (1962) similarly concluded that the gingival one-third of the enamel surface exhibited a higher incidence of decalcification.

Gorelick et al. (1982) completed a cross-sectional study to determine the frequency of white spot formation in orthodontic patients. A control group of 50 children scheduled to begin fixed appliance therapy was examined for enamel decalfication prior to banding and bonding. Twenty-four percent of these patients had one or more nondevelopmental white spot lesions but only 3.6% of the teeth displayed demineralization that was clinically detectable. The experimental group consisted of 121 patients all younger than 18 years of age who had been treated with fixed appliances for approximately two years. At debanding, 11% of the teeth had a white spot lesion. The highest incidence occurred in the maxillary lateral

incisors. Overall, the maxillary anterior teeth were one-and-a-half times more likely to develop a white spot lesion than the mandibular anteriors. Maxillary incisors that were banded and those that were bonded had a similar incidence of white spot formation. Both groups had more decalfication than non-treated maxillary incisors. This study failed to demonstrate a relationship between the length of active treatment and white spot incidence.

Mizrahi (1982, 1983) completed a cross-sectional study comparing enamel opacities in pre- and post-treatment orthodontic patients. He defined opacity as any discrete area of white opaque enamel. The treatment consisted of multi-banded Begg appliances. Both the prevalence and severity of enamel opacity was greater in the post- than in the pre-treatment group. The increase was statistically significant on the buccal surface in the first molars, maxillary and mandibular lateral incisors and mandibular canines at the cervical-third of the crown. Males had a greater opacity severity than females, however there was no sex difference in opacity prevalence. The prevalence of enamel opacity was the same for the post-treatment group and a non-orthodontic control group.

Artun and Thylstrup (1986) concluded from their scanning electron microscopy (SEM) study of incipient carious lesions that new sites with a predilection for caries occur around bonded brackets. At the time of debonding, the environment is once again altered and the process of enamel demineralization apparently ceases and may be replaced by remineralization.

Another study by Artun and Brobakken (1986) assessed enamel opacity and white spot extension in a group of patients with

edgewise brackets applied to all teeth anterior to the first molars. Carious white spots occurred most often in the gingival-third of the crown. Maxillary lateral incisors and mandibular cuspids and premolars were more frequently affected than the other teeth. They postulated that the concave anatomy of the mesiogingival portion of the crown of a maxillary lateral incisor may predispose to voids between the bracket base and tooth surface thus enhancing plaque retention.

O'Reilly and Featherstone (1987) conducted an in vivo study to quantify the amount of demineralization that occurs around a bonded bracket four weeks after placement. Following extraction and sectioning of the premolars, microhardness tests indicated that up to 15% of the mineral was lost at a depth of 50um from the enamel surface in the areas cervical and occlusal to the bracket. However the demineralization was not clinically detectable as a white spot lesion.

C. HOST FACTORS IN WHITE SPOT LESION FORMATION

1. The Tooth Surface

a. Enamel Structure

Dental enamel is composed of inorganic and organic matter plus water. In mature enamel, the mineral content makes up approximately 96% of the total weight and consists of crystalline calcium phosphate [$Ca_{10}(PO_4)_6(OH)_2$] known as hydroxyapatite. The unique spatial arrangement of calcium and phosphorus atoms around the centrally positioned hydroxyl groups imparts stability to the crystal. The organic material appears as a fine, lacy network of high molecular weight protein, referred to as enamelin, along with a small amount of carbohydrate. Amino acid composition, structural conformation and interaction properties distinguish this protein from other extracellular structural proteins such as collagen, elastin and glycosaminoglycans. During enamel calcification and maturation, most of the enamelins are lost due to peptide bond hydrolysis leaving an organic matrix with a different amino acid composition and a low molecular weight (Glimcher, 1971).

Weatherell et al. (1974), Brudevold et al. (1956) and Robinson et al. (1971) have shown that the density of enamel as well as the concentration of calcium, phosphate and fluoride decrease while the magnesium, carbonate and chloride concentration increase from the surface inward. Robinson et al. (1971) found that the enamel protein content was inversely correlated with the percentage by weight of calcium and phosphorus. They also noted that the calcium-to-phosphorus ratio was constant throughout the enamel although it tended to decrease somewhat near the surface.

There are many minor inorganic constituents and trace elements in enamel. Some, including molybdenum, vanadium, selenium, zinc, lead and tin, may have a role in reducing the solubility and caries susceptibility of enamel (Weatherell and Robinson, 1973).

Fluoride is highly concentrated at the surface, but its content decreases rapidly to a low level in the enamel interior. The highest concentration of fluoride in newly formed teeth is near their incisal edge with a much lower concentration at the cervical margin. However, as the tooth ages the gradient is reversed and fluoride becomes highly concentrated in the cervical region. In addition, the cervical enamel has a more porous nature due to its less homogeneous nature (Hardwick and Leach, 1963).

b. Enamel Formation

Enamel matrix is synthesized in the rough endoplasmic reticulum of the ameloblasts and secreted through the Tomes' process located at the apex of each enamel producing cell. The matrix consists of keyhole-shaped enamel rods which extend from the dentinoenamel junction to the surface of enamel. Each rod is composed of hydroxyapatite crystals. Those that are located around the central axis of the rod run parallel to the longitudinal axis of the rod, while the crystals that are away from the rod center have an oblique orientation and those located between rods have a different orientation still. The concentration of crystals is slightly lower at the rod periphery than within and between the rods. Also, spaces or micropores filled with water and organic matter exist between

crystals. Thus, enamel may be thought of as a microporous solid (Fejerskov and Thylstrup, 1986).

The surface of developing enamel consists of pits and walls and takes on a "honeycomb" appearance. Each pit is occupied by a Tomes' process which secretes the rod portion of the enamel matrix whereas the wall or interrod enamel is secreted by adjacent ameloblasts. Rodless enamel may be found in the outermost layer of primary and in the cervical region of secondary teeth. Osborne (1973) attributed the appearance of this surface layer to the orientation of particular rod sheaths which reflect light back through the microscope to give a diffuse white structureless image. The densely packed crystals within this layer are arranged with their long axes perpendicular to the enamel surface (Gwinnett, 1967; Ripa et al., 1966).

The orientation of apatite crystals relative to the surface determines the solubility rate of enamel. The center of a rod where crystals run perpendicular to the tooth surface tends to undergo greater dissolution than the periphery where crystal orientation is more oblique (Sharpe, 1967). Silverstone et al. (1975) found that human dental enamel exposed to acid solutions underwent three basic etch patterns. Preferential loss of core material, called type 1 etching pattern, was most common. Removal of the peripheral region of the rods and a relatively unaffected rod core was seen in the type 2 etching pattern. The type 3 pattern exhibited a random distribution of both type 1 and type 2 etching patterns. The central solubility of enamel crystals may explain the white spot lesion phenomenon in

which a substantial portion of mineral has been lost but the gross morphology remains (Scott et al., 1971).

Immature enamel protein is referred to as amelogen. The secretory ameloblast and the cells of the stratum intermedium function primarily to produce enamel matrix and facilitate the influx of calcium phosphate. Shortly after amelogen is deposited outside the cell, mineralization of the matrix occurs as hydroxyapatite crystals orient along the long axis of the enamel protein and increase in length and diameter. After the initial matrix deposition and mineralization along the dentinoenamel junction, matrix formation and mineralization moves peripherally to the cusp tip, then laterally to the crown and finally ends in the cervical region of each tooth. The degree of mineralization within the central groove and at the cervical margin is usually less than within the rest of the tooth crown and may account for the increased caries susceptibility associated with these areas (Avery, 1987).

With the completion of matrix deposition, the ameloblast and its counterpart within the stratum intermedium become active in the absorption of organic matrix and water from the enamel. Thus, the spaces between hydroxyapatite crystals decrease during maturation. The central portion of the rod contains the most mineral while the rod periphery and, in particular, the rod sheath have more organic matrix as a result of their crystal orientation. The final process of mineralization takes place just before tooth eruption and most likely occurs in the rod periphery to give enamel its 96%+ mineral content. The distribution and extracrystalline location of the organic matrix explain the diffusion pathways created in enamel

during the latter stages of mineralization (Eisenmann, 1989; Scott et al., 1971; Simmelink, 1987).

c. Orthodontics and the Enamel Surface

Clinical studies by Zachrisson (1974) and others (Artun and Thylstrup, 1986; Lundstrom et al., 1980; Mizrahi, 1982; Pender, 1986) indicate that the presence of orthodontic bands and brackets can alter the smooth surface morphology of the teeth by creating plaque retentive areas (Glatz and Featherstone, 1985; Weitman and Eames, 1975). In the case of bonded brackets, demineralization of a part or all of the enamel surface (depending upon etching technique) may occur with placement of the appliance. The resin, in addition to the bracket, may contribute to plaque accumulation by its surface characteristics (Weitman and Eames, 1975). Zachrisson and Zachrisson (1971) reported an almost linear correlation between plaque accumulation and gingival caries in their study of oral hygiene during orthodontic treatment. Hardness testing results of a study by Glatz and Featherstone (1985) indicate that significant demineralization in the buccal surface gingival to a band or bracket can occur by four weeks.

Caries frequency does not necessarily increase with fixed orthodontic appliances, but the location of lesions usually changes (Artun and Brobakken, 1986; Ingervall, 1962; Zachrisson and Zachrisson, 1971). Risk of white spot lesion formation is higher in the cervical area on the labial surface of maxillary lateral incisors (Artun and Brobakken, 1986; Gorelick et al., 1982; Hirschfield, 1978; Mizrahi, 1983). Gorelick et al. (1982) found that twenty-three percent of maxillary incisors developed white spot lesions whereas

only 2.5% of mandibular central incisors were similarly affected in adolescents wearing bonded orthodontic appliances. In Hirschfield's (1978) study, a maxillary lateral incisor comprised over fifty percent of teeth with increased demineralization following full banded orthodontic treatment.

Neither Zachrisson and Zachrisson (1971) nor Gorelick et al. (1982) were able to demonstrate a direct relationship between the duration of fixed appliance therapy and the incidence of white spot lesions.

No study has compared white spot lesion formation in patients with fixed versus removable orthodontic appliances. Pender (1986), however, found comparable plaque levels in patients with maxillary fixed and maxillary removable appliances.

2. Saliva

Saliva is produced primarily by the major salivary glands and secondarily by the minor salivary glands of the oral mucosa. The composition and quantity of saliva varies depending on how, when and from where it is derived. Numerous conditions with diminished salivary production suggest an inverse relationship between caries and salivary flow (Karmiol and Walsh, 1975). Brown et al. (1976) conducted a longitudinal clinical study involving forty-two patients with radiosensitive tumors of the head and neck. All of the patients developed pronounced xerostomia which resulted in a shift to a highly acidogenic oral microflora. Prior to the onset of caries, levels of S. mutans increased. Following lesion initiation, plaque levels of Lactobacillus increased while S. mutans declined.

The secretions from the separate glands are pooled to form whole or mixed saliva in the oral cavity. The uneven distribution of whole saliva, which is related to gland orifice location and tooth morphology among other factors, contributes to the differences in caries susceptibility found throughout the mouth (Sreebny, 1984). Fissures and interproximal areas are less accessible to saliva than buccal and lingual surfaces, and usually more susceptible to decay (Kleinberg and Jenkins, 1964, McNamara et al., 1979). Work by Englander et al. (1959) demonstrated how the distribution of saliva affects plaque pH with a greater drop in the pH when salivary flow is restricted.

Dawes' (1983) theoretical model of salivary sugar clearance demonstrated that an increased unstimulated salivary flow rate and decreased volumes of saliva before and after swallowing enhance sugar clearance from the mouth. The rate of unstimulated salivary flow is directly related to the degree of hydration of the subject with decreased flow rates present during dehydration (Dawes, 1987).

More recent investigations have revealed that plaque is normally covered by a thin film of saliva which moves at different rates in different areas of the mouth (Collins and Dawes, 1987). Slow movement of the salivary film, as was calculated for the labial surfaces of upper anterior teeth, allows diffusants from plaque to accumulate within the saliva. The concentration gradient between plaque and saliva is subsequently reduced which prolongs the clearance of acids as well as other metabolic products from the tooth surface (Dawes, 1989; Dawes et al., 1989).

a. Composition

Saliva contains inorganic and organic compounds which influence the dental caries process. The buffering capacity of saliva depends chiefly on its bicarbonate (HCO_3^-) and phosphate (HPO_4^{2-}) ion concentrations. As the salivary flow rate increases, the bicarbonate concentration rises and the overall buffering capacity of the saliva increases. Bicarbonate helps to moderate saliva pH. By diffusion, bicarbonate becomes incorporated into the plaque matrix where it counteracts the drop in plaque pH which occurs following carbohydrate consumption and acid production (Abelson and Mandel, 1981; Frostell, 1979).

Other inorganic constituents of saliva related to dental caries include calcium, fluoride and hydrogen ions. The concentration of these components along with the buffer ions collectively determine the degree of saturation of saliva with respect to fluorapatite and hydroxyapatite (Dawes, 1983). Maintenance of enamel mineral and the mechanism for recalcification depend on the supersaturation of saliva, particularly by calcium phosphate salts (Gron, 1973).

1) Salivary Proteins

Salivary proteins make up an important part of the organic component of the oral fluid. Hay (1983) classified the oral functions of salivary proteins under: (1) antibacterial activity; (2) enamel maintenance; (3) plaque pH control, and others.

a) Antibacterial activity

Nonspecific antibacterial activity occurs with lysozyme, an enzyme which cleaves glycosyl bonds and thus destroys the peptidoglycan cell wall of bacteria. Another non-immunological

factor with antibacterial activity in saliva is salivary peroxidase. Through an oxidation reaction various enzymes of the glycolytic pathway are inactivated and bacterial inhibition occurs (Tenovuo, 1987).

Secretory immunoglobulin A (IgA) is the major immunoglobulin in saliva. Michalek et al. (1976) and Michalek and McGhee (1977) demonstrated through in vitro studies that passive transfer of antibody of either the IgA or the immunoglobulin G (IgG) class renders protection against S. mutans-induced dental caries. However, the mechanism by which increased antibody levels enhance the elimination of cariogenic microorganisms from the oral cavity and interfere with their metabolic function is incompletely understood. Secretory antibodies may inhibit bacterial adherence by binding to antigens and blocking surface determinants necessary for adherence, promote agglutination of bacteria, and interfere with bacterial enzymes (Williams and Gibbons, 1975).

b) Maintenance of enamel

Recalcification of lesions below the enamel surface appears to be controlled by salivary proteins; statherin and proline-rich phosphoproteins. Both of these proteins inhibit calcium phosphate precipitation, but their size prevents them from reaching the subsurface layer with the calcium and phosphate ions. Therefore remineralization occurs beneath the outer layer of enamel (Schlesinger and Hay, 1977).

c) Control of plaque pH

A number of salivary factors act to neutralize acids produced by bacterial glycolysis in plaque. In addition to the buffer anions

already discussed, salivary urea is metabolized by plaque bacteria to give ammonia which increases the pH of plaque. Arginine- and lysine-containing peptides (sialine) reduce or nearly prevent the decrease in pH which occurs in plaque incubated with glucose (Kleinberg et al., 1976).

D. MICROBIOLOGY OF INCIPIENT CARIES

1. Oral Microflora

The oral cavity is comprised of a number of structures , each of which provides a slightly different environment for microbial habitation. The teeth, the tongue, and the mucous membranes certainly differ in their surface characteristics. The properties of plaque and saliva as they relate to oral structures are also important in determining the presence or absence of particular bacteria(Gibbons et al., 1964a; Gibbons et al., 1964b).

a. Distribution

Numerous studies have shown that the oral microflora is heterogeneously distributed throughout the various microenvironments existing within the mouth (Gibbons et al., 1964a; Krasse, 1954a; van Houte and Green, 1974). Although all indigenous organisms can be found in each of the sites at one time or another, many organisms apparently have a preferred site for colonization. Normally Streptococcus salivarius inhabits the tongue while S. mutans and Streptococcus sanguis reside primarily on the tooth surface (Carlsson, 1967; Gibbons et al., 1964a; Krasse, 1954a; Socransky and Manganiello, 1971). Lactobacillus acidophilus is most frequently isolated from saliva whereas Lactobacillus casei is usually found in dental plaque and carious dentin (Fitzgerald, 1968a).

b. Composition

A person's oral microflora changes over time (Hardie and Bowden, 1974). At birth, the oral cavity is normally sterile but during the first twenty-four hours the resident flora begins colonization. The streptococci usually predominate the cultivable

microflora, and by twelve months of age the complexity has increased as additional microenvironments are created with the eruption of the primary teeth (Carlsson et al., 1975; McCarthy et al., 1965). The results from a study involving adult subjects indicated that changes in the microbial composition of the mouth continue throughout life (Ritz, 1967).

There are quantitative differences as well between individuals in the types of microorganisms from plaque and in saliva (Gordon and Jong, 1968; Richardson and Jones, 1958; Socransky and Manganiello, 1971). Littleton et al. (1970) isolated specific "caries-inducing" streptococci with different frequency in plaque samples from two groups of adolescent males.

c. Colonization

In vitro and in vivo studies have demonstrated that colonization by an organism varies directly with its ability to adhere or attach to oral surfaces (Gibbons and van Houte, 1971; Liljemark and Gibbons, 1972; van Houte et al., 1970; van Houte et al., 1971; van Houte et al., 1972). Also, the bacterial cell concentration in saliva influences the degree of colonization (Gibbons et al., 1972; van Houte and Green, 1974). For example, S. mutans has a rather low affinity for the enamel surface and its salivary concentration is normally quite low. Accordingly, S. mutans colonization is usually highly localized on the tooth surface (van Houte et al., 1974). Recent work has indicated that enzymes present in the oral cavity and multiple binding sites located on pellicle-covered hydroxyapatite surfaces influence colonization of the tooth surface as well (Gibbons and Etherden, 1982; Gibbons et al., 1983).

2. Streptococcus mutans

Animal studies along with clinical investigations have implicated the streptococci in the process of caries initiation (Carlsson, 1970; Duchin and van Houte, 1978; Fitzgerald and Keyes, 1960; Fitzgerald et al., 1960; Krasse, 1966; Krasse and Edwardsson, 1966; Krasse and Krasse, 1954b; Zinner et al., 1965). In 1924 Clarke isolated an organism from an early carious lesion which he named S. mutans (Clarke, 1924). This gram-positive coccus naturally inhabits the human mouth and tends to occur in short or medium chains. It forms highly convex to pulvinate colonies which are opaque when grown on mitis salivarius agar. The colony morphology is commonly rough but smooth and mucoid variants have been isolated (Edwardsson, 1970).

a. Classification

Polysaccharides located in or on the cell wall form the serotype -specific antigens of S. mutans. They consist of a combination of glucose, galactose, or rhamnose (Linzer, 1976). By using immunological specificity rather than phenotypic characteristics, seven serotypes (a-g) of S. mutans have been isolated and identified (Bratthall, 1970; Perch et al., 1974). In 1977, Coykendall reclassified the subspecies of S. mutans as species based on their DNA base compositions and observed four genetic types (Coykendall, 1977). More recently, Coykendall and Gustafson (1986) indicated that six distinct organisms comprise the "mutans" group of streptococci: (1) S. mutans (serotype c,e,f); (2) S. rattus (serotype b); (3) S. cricetus (serotype a); (4) S. sobrinus (serotype d,g,h); (5) S. ferus (serotype c); and (6) S. macacae (serotype c). Only S. mutans

and S. sobrinus were prevalent in humans, and S. sobrinus was not clearly associated with human dental caries (Gibbons, 1986).

b. Metabolism

S. mutans produces lactic acid from mannitol and usually sorbitol and can utilize ammonia as the sole source of nitrogen. This property allows S. mutans to grow in an anaerobic environment without exogenous amino acids (Carlsson, 1970b; Edwardsson, 1968). S. mutans is more aciduric than the other oral streptococci and becomes dominant in dental plaque associated with a carious lesion (Donoghue and Tyler, 1975; Minah and Loesche, 1977a; van Houte et al., 1982). Results from other studies indicate that there is little difference in the microbial composition of plaque covering carious and non-carious tooth surfaces in caries-active individuals (Mikkelsen et al., 1981; Milnes and Bowden, 1985)

c. Polysaccharide Production

1) Extracellular polysaccharides (EPS)

In the presence of sucrose S. mutans synthesizes extracellular as well as intracellular polysaccharides. The extracellular polysaccharides are water insoluble glucans and fructans which are produced by the action of glucosyltransferase and fructosyltransferase, respectively (Guggenheim, 1970a; Hamada et al., 1976).

a) Glucans

The glucans, in particular, are involved in plaque formation. De novo synthesis of glucans on various solid surfaces has confirmed their ability to promote adherence (Guggenheim, 1970b; Newbrun, 1976). Inoue and Yakushiji (1986) analyzed the glucans produced by S.

mutans for their chemical and morphological properties. They found that the insoluble glucans of all serotypes of S. mutans consisted of two components; a double-stranded fibril consisting of a long sequence of alpha (1-3)-linked glucose residues with alpha (1-6)-linked branches, and a single-stranded fibril composed of a sequence of alpha (1-6)-linked residues with alpha (1-3)-linked branches. The insoluble nature of the glucans was attributed to the alpha (1-3) linkage whereas the glucans' adhesive properties apparently stem from the alpha (1-6)-linked sequences.

2) Intracellular polysaccharides (IPS)

When high concentrations of exogenous sugars are available, most serotypes of S. mutans produce and store intracellular iodine-staining polysaccharides(IPS) (Gibbons and Socransky, 1962). IPS is a glycogen-like glucan with alpha (1-4)- and alpha (1-6)-linkages susceptible to the enzyme alpha-amylase (van Houte et al., 1970). The metabolism of IPS by S. mutans results in the production of ethanol and acetic acid in addition to lactic acid under conditions of limited exogenous glucose, whereas only lactic acid is formed from IPS in the presence of excess glucose (Huis In't Veld and Backer Dirks, 1978; Minah and Loesche, 1977b). Mutants of S. mutans with a reduced ability to synthesize IPS demonstrate less cariogenic activity (DiPersio et al., 1974;Tanzer et al., 1974).

3. Lactobacillus

a. Characteristics

Lactobacilli are gram-positive rods that grow best under microaerophilic conditions. They normally comprise about 1% of the oral flora and are either absent or present in insignificant amounts

in dental plaque (London, 1976). van Houte et al. (1972) found much lower proportions of lactobacilli in plaque than in saliva or on epithelial surfaces. Colonies grown on blood agar are characteristically dome-shaped with a smooth texture. At least nine species of Lactobacillus have been isolated from the human oral cavity.

b. Metabolism

Lactobacilli are acidogenic and aciduric. They can grow at pH < 5.0 and produce either lactic acid (homofermentative) or a combination of acetic acid, ethanol, carbon dioxide and lactic acid (heterofermentative). Lactobacilli isolated from active subsurface carious dentin in human teeth were three times more likely to be homofermentative than heterofermentative, and Lactobacillus casei was the most frequent isolate (Camilleri and Bowen, 1963; Shovlin and Gillis, 1969).

c. Polysaccharide Production

Fitzgerald et al. (1980) inoculated hamsters with lactobacilli from human teeth which were non-carious. Their findings indicate that lactobacilli, in contrast to S. mutans, do not synthesize intracellular polysaccharides nor detectable levels of extracellular polyglucans. However, Newbrun (1989) classified several species of lactobacilli as either glucan or heteropolysaccharide producers Another study isolated lactobacilli from carious dentin in human teeth. All lactobacilli were negative for intracellular polysaccharide storage (Shovlin and Gillis, 1969).

4. Smooth Surface Plaque Formation

The mechanism by which S. mutans attaches to the enamel surface is incompletely understood. Usually bacterial attachment and plaque development are preceded by the formation of an acquired pellicle from the saliva.

a. Acquired Pellicle

The acquired pellicle is a thin membrane or film that rapidly forms by selective adsorption of salivary proteins or glycoproteins to tooth surfaces (Mayhall, 1975; Sonju and Rolla, 1972). The hydroxyapatite surface has a net negative charge based on the arrangement of its phosphate groups and calcium ions. When immersed in saliva, ions of the opposite charge are attracted to the enamel surface and form a hydration layer. Normally this "neutralizing" layer is composed of 90% calcium and 10% phosphate (Arends and Jongebloed, 1977). Salivary glycoproteins attach to the apatite surface by displacing ions in the hydration layer and are adsorbed by hydrogen bonding and hydrophobic interactions (Sonju, 1986). The pellicle imparts selective permeability and influences the selective attachment of specific organisms to the enamel surface (Kraus and Mestecky, 1976; Meckel, 1965).

b. Bacterial Attachment

Shortly after the pellicle has formed, rapid and selective adsorption of oral microorganisms occurs. Bacteria tend to accumulate in areas where oral hygiene procedures are ineffective such as the gingival margin. Two types of adhesive interactions are required for plaque to form. First, bacteria must adhere to the pellicle surface to initiate plaque formation. Second, they must grow

and adhere to one another to allow plaque accumulation (Gibbons and van Houte, 1973).

In vitro and in vivo techniques indicate that different species of streptococci and lactobacilli attach to various oral surfaces with different efficiencies. Therefore the bacterial cell surface must contain a highly developed recognition system for identifying and interacting with different surface components. These specific ligands are called "adhesins" which bind to complementary components on the host tissues (Gibbons, 1984).

Numerous studies have shown that S. mutans can adhere independently of sucrose and glucan synthesis (Clark et al., 1978; van Houte and Duchin, 1975; van Houte et al., 1976). Staat et al. (1980) proposed a two reaction model for S. mutans adherence. Initial attachment involves a reaction between proteins associated with the bacterial cell surface and host receptors in the acquired pellicle. The nature of the bacterial surface protein is unknown but work by Gibbons and Qureshi (1978) supports the idea that blood group-reactive salivary mucins form the salivay receptor protein.

Following the initial weak attachment of S. mutans to the enamel pellicle, a stronger, more specific adherence occurs as a result of cohesion between S. mutans cells, particularly in the presence of sucrose. S. mutans "lectins" are cell surface proteins which recognize glucans and allow the cells to aggregate (Staat et al., 1980). According to Rolla et al. (1985), glucosyltransferase , which synthesizes glucans from sucrose, mediates the cellular accumulation. This enzyme can adsorb to acquired pellicle and is produced by S. mutans. Glucan chains synthesized by

glucosyltransferase, especially the alpha (1-3)-linked glucans, interact to form packed, fibrous aggregates which are insoluble in water.

c. Plaque Development

Several bacteria are involved in the initial colonization of the tooth surface, and their development on the enamel surface generally follows a pattern (Bowden et al., 1979; Hardie and Bowden, 1976). They adhere initially as separated cells or cell aggregates which eventually coalesce with bacterial proliferation (Duchin and van Houte, 1978). The plaque composition early on depends on the relative adherence ability of each organism while later changes in the bacterial make up are attributed to altered growth conditions (van Houte, 1982). Qualitative and quantitative changes are apparent between dental plaques from different individuals, from different sites within the same mouth and at different times (Hardie and Bowden, 1976).

Socransky et al. (1977) described three detectable phases in the development of plaque. The first phase coincided with initial colonization and was followed by a phase of rapid growth and separate localized concentrations of organisms some time after eight hours. Maximal total counts were achieved during the third or remodeling phase of plaque development. Continual internal and external remodeling takes place, and microorganisms that are able to multiply in the presence of metabolites from neighboring bacteria may eventually predominate the localized microflora.

1) Microbial Succession

Alexander (1971) defined succession as "the replacement of one type of community by another in response to modifications in the habitat. Commonly there are a sequence of continuous replacements of the microbial communities located in a particular place". Autogenic succession refers to the situation where the resident microbial population alters the environment such that it is replaced by a species more suited to the modified habitat. Conditions that favor this type of succession include: (1) depletion of essential nutrient supplies; (2) accumulation of toxic metabolic products; (3) increases in temperature; and (4) alteration in the pH or Eh

A study which evaluated plaque collected from the smooth surface of human incisors at increasing times following prophylaxis revealed a shift from coccal, anaerobic organisms to filamentous, anaerobic types following a drop in the oxidation-reduction potential (Eh) with growth of the resident populations (Ritz, 1967). This proliferation by the early colonizers alters the environment and leads to a progressive shift in the microflora throughout the course of plaque development (Kenney and Ash, 1969; Newbrun, 1989). Hurst and Collins-Thompson (1979) concluded that the lactic acid-producing bacteria become dominant in pure culture by lowering the pH to a point where other microorganisms can neither initiate nor sustain growth. Other interbacterial interactions that may influence colonization include competition for binding sites on the tooth surface, cell-to-cell binding via specific receptors, and IgA on the surface of indigenous oral bacteria which interferes with their growth or attachment (van Houte, 1976).

Allogenic succession, on the other hand, implies successional changes in response to alterations in the environmental conditions which are not of environmental origin such as: (1) growth and maturation of the host; (2) eruption or loss of teeth; (3) insertion of dental appliances; (4) change in dietary patterns and oral hygiene habits; and many other factors (Alexander, 1971).

Normally S. mutans and the lactobacilli are present in relatively low numbers in dental plaque. However with fluctuations in a number of factors such as salivary flow, carbohydrate consumption, fluoride exposure, etc., a shift can occur in the plaque flora. For example, cariogenic plaque is often dominated by S. mutans for an extended period of time in response to changes in the environment (Bowden et al., 1979).

Milnes and Bowden's (1985) nursing caries study reported significant differences between the levels of S. mutans, Lactobacillus and Veillonella at the sites which developed caries and the control sites in the same mouth. Another clinical investigation showed that the plaque microflora of progressing incipient carious lesions in a group of children included significant numbers of S. mutans, Lactobacillus and A. odontolyticus compared to control sites. Progressing lesions displayed an increased colonization by S. mutans, S. salivarius, A. odontolyticus, Lactobacillus and Veillonella, and a decreased colonization by S. mitior, A. naeslundii and A. viscosus relative to the caries free control sites (Boyar and Bowden, 1985)

5. Organisms and Fixed Orthodontic Appliances

A number of different studies have assessed the extent to which fixed orthodontic appliances alter the oral environment and

cause quantitative changes in the oral microflora. Dikeman (1962) found that the number of lactobacilli in saliva increased while the streptococci did not change following the placement of fixed appliances A study by Bloom and Brown (1964) found a significant increase in salivary lactobacilli after band placement which was directly related to the number of bands. Sakamaki and Bahn (1968) showed that band placement produced a five-fold increase in the count of lactobacilli, particularly at the gingival edge of the band, which decreased to pre-treartment levels after band removal.

Gwinnett and Ceen (1978) looked at plaque accumulation on bonded upper central incisors. They found that an individual's plaque pattern was maintained regardless of the presence or absence of bonded brackets. In another study using scanning microscopy, they identified the junction between enamel and resin gingival to the bracket base as the most common site for plaque retention and subsequent demineralization (Gwinnett and Ceen, 1979).

An investigation by Corbett et al (1981) compared plaque concentrations of S. mutans between banded and non-banded (pre-treatment) patients. The presence of bands produced a significant increase in S. mutans in all areas of the mouth. Mattingly et al. (1983) obtained consecutive plaque samples from ten orthodontic patients with direct bonds and found that the relative number of S. mutans increased for each succeeding sample. In another study, the levels of S. mutans in saliva and plaque from banded teeth initially declined but by three months exceeded pre-treatment levels. It was felt that the presence of bands increased the number of retentive

areas and resulted in an increase in S. mutans infectivity (Scheie et al., 1984).

E. DIET AND CARIES

1. Sugars

Diet is a necessary parameter in the caries process. The fermentable sugars, in particular sucrose, provide a substrate that suits the metabolic requirements of the cariogenic microflora (Fitzgerald, 1968; Jordan and Keyes, 1966). A study by De Stoppelaar et al. (1970) showed that plaque levels of S. mutans decreased significantly following carbohydrate restriction. Jay (1947) periodically restricted carbohydrate intake and saw a drop in the salivary Lactobacillus acidophilus counts. Epidemiological surveys in various parts of the world along with clinical observations in select groups supply additional evidence that caries prevalence is related to sucrose consumption (Harris, 1963; Hoover et al, 1980; Marthaler, 1979; Sreebny, 1982).

The form and frequency, rather than the total amount, of sugar that is consumed by an individual have a significant affect on caries activity (Gustafsson et al., 1954). Results from other studies contend that the frequency and length of sugar exposure are critical factors in cariogenicity (Geddes et al., 1978; von der Fehr et al., 1970).

Hefti and Schmid (1979) demonstrated a direct relationship between dietary sucrose concentration and the incidence of smooth surface caries in an animal model. Sucrose invariably induces more smooth surface lesions than other carbohydrates, especially in the presence of S. mutans (Edwardsson and Krasse, 1967; Guggenheim et al., 1966; Newbrun, 1967; Stephan, 1966; Tanzer, 1979).

The role of sucrose in plaque formation has been directly related to the development of smooth surface caries. Glucosyltransferases utilize sucrose specifically to form insoluble glucans (mutans) which serve as structural components of the plaque matrix. Dextrans (soluble glucans) and levans (soluble fructans) are also synthesized extracellularly and probably function as reservoirs of fermentable carbohydrate (Carlsson, 1970a; Guggenheim, 1970a; Newbrun, 1967; Newbrun, 1976). The soluble glucans reportedly also mediate interbacterial aggregation (Bourgeau and McBride, 1976).

Sucrose and other carbohydrates within plaque are utilized directly by the oral flora in the production of organic acids and energy. Sugars are transported into the cytoplasm of the oral streptococci by a phosphoenolpyruvate - dependent phosphotransferase system located in the cell membrane (Calmes, 1978; Hamilton, 1977; Schachtele and Mayo, 1973). The intracellular product, sucrose 6-phosphate, yields glucose 6-phosphate and fructose which are subsequently integrated into the Embden-Meyerhof pathway (St. Martin and Wittenberger, 1979).

Ellwood et al (1979) and Hamilton and Ellwood (1978) varied the glucose concentration and pH values under which Streptococcus mutans Ingbritt was grown. Glucose transport via the phosphoenolpyruvate phosphotransferase system (PTS) reached a maximum under slow growth conditions on limited concentrations of glucose. Conversely, excess glucose with maximum growth rates and a low pH repressed the transport of glucose. In other words, the glucose-PTS accounted for transport only at slower growth rates

and limited glucose concentrations. They concluded that the transport of glucose at faster rates of growth and excess glucose required a second transport system. In 1982, Ellwood and Hamilton (1982) showed that growth of Streptococcus mutans Ingbritt on sucrose at faster growth rates required the activity of a second transport system as well. During fast growth, the major end product was lactic acid. Growth at slower rates produced acetic and formic acids as well as ethanol along with lactic acid.

2. Sugar Substitutes

The non-caloric sweeteners (cyclamate, saccharin and aspartame) are classified as non-cariogenic because they are not metabolized to acid by the oral microorganisms. Sugars other than sucrose (fructose, glucose and particularly lactose) appear to have a lower cariogenic potential but are generally not available on a wide scale at the present time. Sugar alcohols (sorbitol and xylitol) are caloric sweeteners that have been shown to be non-cariogenic (Glass, 1983; Scheinin and Makinen, 1975). A recent study showed that the surface area of buccal white spot lesions decreased over a two year period during which xylitol was substituted for sucrose in the diet (Rekola, 1986). Birkhed et al. (1978), however, reported on an adaptation of the plaque flora with sorbitol use and subsequent acid production.

F. PROCESS OF WHITE SPOT DEMINERALIZATION

1. Caries "Triad"

The "chemo-parasitic" theory proposed by Miller (1973) forms the basis for the present day "microflora-host-substrate" theory of caries development.

a. Oral Microflora

Orland et al (1954) and others (Fitzgerald and Keyes, 1960; Keyes, 1960; Orland et al., 1955) showed that microorganisms with cariogenic potential must be present for the initiation of caries in animal models. Although longitudinal clinical studies in humans have confirmed the presence of S. mutans and lactobacilli prior to and after the detection of carious lesions (Boyar and Bowden, 1985; Hemmens et al., 1946; Ikeda et al., 1973), the results of other investigations point to the heterogeneous nature of the plaque microflora associated with the carious lesion (Bowden et al., 1975; Hardie et al., 1977). Milnes and Bowden (1985) found that the levels of S. mutans, Lactobacillus and Veillonella were significantly greater at the carious sites than at the control sites in caries-active children. However, there were no significant differences between the flora of susceptible sites which developed caries and those which remained caries-free. These findings support the concept that caries is a multifactorial process.

b. Host

Numerous studies involving humans from birth have supported the concept that cariogenic microorganisms require a suitable host. Although S. salivarius may become established in the mouth a few hours after birth, S. mutans doesn't appear usually until after

eruption of the primary dentition, and the presence of Lactobacillus (principally L. casei) is correlated with the occurence of dental caries (Berkowitz et al., 1975; Carlsson et al., 1975; Catalanotto et al., 1975; McCarthy et al., 1965).

c. Substrate

Stephan (1944) used electrodes to measure pH changes in dental plaque on the labial surface of human anterior teeth. He noticed that decreases in pH occurred in all subjects following ingestion of a glucose solution but the drop in pH was greatest in individuals with extensive caries activity. Geddes (1974) found that high concentrations of lactic acid rapidly accumulated in human dental plaque following exposure to sucrose. She also noted that "resting" plaque had an acidic pH due to the presence of a number of volatile acids in addition to lactate. Others have shown that not only were the quantities of acid produced significantly greater in plaque from carious enamel than from sound enamel, but the rate of acid production was also faster (Igarashi, 1987; Minah and Loesche, 1977).

2. White Spot Macro-structure

The white spot lesion on the smooth surface of enamel results from an increase in tissue porosity and is observed clinically as a decrease in translucency. The cervical border of the lesion follows the gingival contour and reflects the usual location of plaque accumulation. From an epidemiological standpoint, the distinction between white spots (opacities) and horizontal white lines (mottling) is important (Curzon and Spector, 1977). However, mottled enamel due to fluorosis is comprised of areas of diffuse

hypomineralization or porosity in the subsurface enamel with a well mineralized surface layer just as in the enamel carious lesion (Fejerskov et al., 1977).

Most would agree that the enamel surface is highly variable, not only between individuals but also from tooth to tooth as well as within small areas of the same tooth (Thylstrup et al., 1983). Scanning electron micrographs reveal some of the common surface characteristics of an active white spot lesion which include deepened, irregular Tomes' processes pits, widened intercrystalline spaces and even microcavities corresponding to enamel dissolution (Fejerskov and Thylstrup, 1986; Haikely and Voegel, 1983). Detectable incipient smooth surface caries have been produced experimentally in humans twenty-three days after discontinuing oral hygiene and adding daily sucrose rinses to the diet.(von der Fehr et al., 1970). Boyar et al. (1989) found evidence of enamel dissolution under orthodontic bands after forty-eight hours.

3. White Spot Infrastructure

With the aid of polarized light microscopy, distinct areas or zones have been described which correspond to the loss of mineral (Silverstone, 1973). The translucent zone is located at the apex or advancing front of the lesion where initial dissolution of enamel occurs. The dark zone is located external to the translucent zone. In vitro studies suggest that remineralization occurs within the dark zone. The body of the lesion exists between the dark zone and the surface zone. Here mineral loss is most pronounced in contrast to the relatively intact enamel surface.

Scanning electron microscopy (SEM) has demonstrated the presence of gaps at the enamel rod periphery indicating that mineral dissolution spreads along the rod boundaries (Johnson, 1967). The orientation of the apatite crystals with respect to acid attack influences the differential dissolution that occurs (Poole and Johnson, 1967; Sharpe, 1967).

4. Interactions in the Caries Process

The clinically detectable white spot lesion represents a relatively late stage in the natural caries process, according to Thylstrup et al. (1983). Interactions between the enamel surface and the surrounding oral environment occur from the time of eruption. The caries process consists of a dynamic series of events, with phases of both demineralization and remineralization, which ultimately determine the content of the enamel matrix (Silverstone, 1973).

a. Ion Concentration of Enamel and Surrounding Oral Fluids

The integrity of the enamel apatites (hydroxyapatite and fluorapatite) is directly influenced by the pH and the concentrations of calcium, phosphate and fluoride in the surrounding fluids (plaque and saliva). Equilibrium equations for the different ions indicate that under normal physiologic conditions saliva and especially plaque are supersaturated with respect to both apatites (Dawes and Jenkins, 1962; Gron, 1973). In other words, plaque, in particular, is capable of functioning as remineralizing fluid for enamel.

Koulourides (1966) developed an <u>in vivo</u> system to study the dynamic equilibrium between the tooth surface and oral fluids. He concluded that both the organic and inorganic components of the

tooth surface, through interactions with the surrounding oral fluids, are involved in the formation, growth and dissolution of apatite crystals.

A review of recent in vitro investigations on the diffusion dynamics in dental plaque by Dibdin (1984) indicated that plaque has an absolute permeability as well as a permeability-selective nature for ions and molecules. Thus, the diffusion characteristics of dental plaque probably play an important role in the interactions between the tooth surface and the surrounding oral fluids taking place during the caries process.

b. pH of Oral Fluids

Enamel apatite solubility is inversely related to the pH of the oral fluids. With an increase in hydrogen ion concentration, the hydroxyl (and fluoride) ion concentration decreases and apatite phosphate (PO_4^{3-}) is converted to HPO_4^{2-} and $H_2PO_4^-$ (Brown, 1974).

The pH at which saliva (or plaque) is exactly saturated with respect to enamel apatite has been termed the "critical pH".This value depends on the calcium and phosphate concentrations in the oral fluids, and is lower for fluorapatite (pH 4.5) than for hydroxyapatite (pH 5.2-5.5). Larsen (1973) and others (Larsen and Fejerskov, 1977; Moreno and Zahradnik, 1974) developed in vitro models based on the critical pH. They were able to produce subsurface demineralization in the presence of a buffer solution (pH 4.0-5.0) which was unsaturated with respect to hydroxyapatite and supersaturated relative to fluorapatite.

c. Enamel Dissolution

The investigations of numerous authors (Featherstone et al., 1979; Gray and Francis, 1963; Moreno and Zahradnik, 1979; van Dijk et al., 1979; Zahradnik and Moreno, 1977) support the basic concept that organic acids produced by plaque microorganisms diffuse inward and react with the apatites to form soluble calcium and phosphate ions (or complexes) which diffuse outward leaving a porous inner matrix Subsequently a concentration gradient is created between the enamel surface and the surrounding plaque fluid which ultimately determines the extent of de- and remineralization within the white spot lesion.

d. Surface Zone of the White Spot Lesion

A number of mechanisms have been proposed to explain the presence of the surface zone in the white spot lesion. The enamel surface itself has a higher mineral content (including fluoride) than subsurface enamel. Hallsworth et al. (1971) examined sections of white spot enamel and discovered that the fluoride content was elevated throughout the lesion particularly within the surface zone where there was more fluoride than in adjacent sound enamel.

Silverstone (1968) and others (Featherstone et al., 1978; Moreno and Zahradnik, 1974) suggested that the surface layer is continuously regenerated by precipitation of calcium and phosphorous ions dissolved from the subsurface enamel. Larsen (1973) maintained that both the surface and subsurface layers are dissolved by acid. Supersaturation of the plaque fluid with fluoride ions results in their reprecipitation as fluorapatite in the surface layer. Another theory advanced by Christofferson and Arends (1982)

claimed that inhibitors in the oral environment (salivary proteins, fluoride ions) are adsorbed onto the outer surface of enamel. These factors protect against direct surface dissolution but simultaneously allow the diffusion of acid into the enamel matrix. Thus, dissolution of calcium and phosphate from the body of the lesion and reprecipitation in the surface layer maintains a relatively intact outer enamel surface.

5. Fixed Orthodontic Appliances and White Spot Lesions

The literature supports the idea that a linear relationship exists between plaque accumulation and white spot formation in orthodontic patients. The presence of bands and brackets create new sites for plaque retention which are predisposed to demineralization. Brackets bonded to anterior teeth favor white spot formation in the gingival one-third of the labial surface (Gorelick et al., 1982; Mizrahi, 1983; Zachrisson and Zachrisson, 1971).

Artun and Thylstrup (1986, 1989) evaluated the surface changes of incipient carious lesions longitudinally following removal of the bonded brackets. Clinical examinations, color slides and SEM of positive surface replicas were used to assess the surface characteristics. Almost immediately after debonding, they noted a decrease in the surface opacity and by three years only remnants of opaque enamel remained. Microcavities present at debonding were practically smoothed out at thirty-six months. The surface features of these inactive lesions as seen under SEM suggested wear and abrasion of the outermost enamel rather than remineralization and repair.

G. MEASUREMENTS OF CARIES ACTIVITY

Nikiforuk (1985) makes the distinction between caries activity and caries susceptibility. Activity refers to the increment of active lesions during a stated period of time, while susceptibility is the inherent propensity of the tooth toward cariogenesis. Currently, there is no test for caries susceptibility although predictions are often based on past dental history.

1. Indices

a. Dental Plaque

Epidemiological surveys utilize indices to quantitate dental health. Indices recorded longitudinally might reveal a particular trend with regards to caries susceptibility and predictions about subsequent lesion development may be based on these trends. The Oral Hygiene Index (OHI) introduced by Greene and Vermillion (1960) and later modified (Greene and Vermillion, 1964) measures the fraction of the buccal or lingual surface area covered by debris and calculus in fully erupted permanent teeth in particular segments of the mouth. Silness and Loe (1964) proposed the Plaque Index (PII) for scoring the accumulated soft debris in the gingival area of a tooth.

b. White Spot Lesions

A number of different classification systems have been devised to describe enamel opacities. von der Fehr (1961) categorized human enamel in vitro based on color changes and appearance of the perikymata. The Caries Index proposed by Zachrisson and Zachrisson (1971) scored the gingival enamel area in contact with an orthodontic band for changes in color and surface integrity. Another classification based on the percentage of labial

surface enamel affected by opaque flecks, spots or patches was introduced by Curzon and Spector (1977). A cross-sectional study of white spot incidence following a multibonded appliance graded enamel appearance according to the surface structure and the extent of the lesion in the gingival area on the labial surface (Gorelick et al., 1982).

2. Microbial Tests

Specific microbiological tests may be used to quantitate particular groups of bacteria known to be important in the caries process. Organisms from saliva and plaque are cultured on selective media, and the colony count expressed as colony forming units per milliliter of saliva and total colony forming units, respectively (Gold et al., 1973; Rogosa et al., 1951). Jordan et al. (1987) recently introduced a commercially prepared dip-slide test (Cariescreen SM, APO Diagnostics Inc.) for salivary levels of S. mutans. Growth of reference strains of S. mutans was equivalent on Cariescreen SM dip-slides and on MSB agar plates. Previously, Crossner and Hagberg (1977) had evaluated a dip-slide test for salivary lactobacilli. Their results showed that the accuracy and reliability of the Dentocult (Dentocult, Orion Diagnostica) dip-slide test were comparable to Rogosa SL agar.

H. CARIES PREVENTION

1. Fluorides

Epidemiological surveys have demonstrated the anticaries effect of fluoride (Dean, 1942). Backer Dirks (1974) found that whereas the overall caries rate declined by sixty percent following long term water fluoridation, caries inhibition was even greater on the buccolingual surfaces approaching eighty-five percent. The concentration of fluoride in the water supply differs throughout the world. In areas where elevated levels of fluoride are found, endemic dental fluorosis or mottled enamel occurs due to the ingestion of excessive amounts of fluoride during the period of early tooth development (Council on Dental Therapeutics, 1982).

Fluoride supplementation may occur either systemically or topically. Systemic administration of fluoride is usually through the community water source. Fluoride in a more concentrated form is applied topically and available in a variety of chemical compositions and forms (Shannon, 1981; Zachrisson, 1975).

a. Anticaries Mechanism(s)

The mechanism(s) by which fluorides impart caries resistance to the tooth surface is not fully understood. Several theories have been proposed to explain the anti-caries effect of fluoride on various components of the tooth-plaque interface.

1) Action on Hydroxyapatite

At low concentrations, fluoride acts on the apatite to cause preferential reacquisition of calcium by calcium-deficient mineral (Ingram and Nash, 1980). Silverstone (1983) found that the solubility of apatite decreased when low levels of fluoride were

added to a calcium phosphate solution. Apatite crystals were larger, and both the rate and degree of remineralization increased. Another study analyzed the apatite crystal structure and suggested that voids can occur due to stearic interference between hydroxyl ions. Fluoride ions can occupy the occasional void and provide a more ordered crystal with lower free energy (Young and Elliott, 1966). Moreno (1974) calculated that partially fluoridated hydroxyapatite had lower free energy (and less solubility) than both unfluoridated and completely fluoridated hydroxyapatite. Brown et al. (1977) explained the effect of low concentrations of fluoride on enamel solubility by proposing that a thin layer of fluorapatite exists on the surfaces of hydroxyapatite crystals.

2) Action on Plaque Bacteria

Both plaque and the outer layer of enamel can have very high levels of fluoride. However, most of the fluoride within bacterial cells, enamel and plaque is bound and not freely available. In vitro studies by Hamilton (1977) and others (Eisenberg et al., 1980; Hamilton and Ellwood, 1978; Schachtele and Mayo, 1973) have demonstrated that fluoride inhibits glycolysis and glycogen synthesis in bacterial cells. Loesche et al. (1975) found that high levels of fluoride applied topically reduced the numbers of S. mutans isolated in occlusal but not interproximal plaque. Sharma et al. (1974) showed that fluoride had no effect on glucan synthesis in a strain of Streptococcus sanguis, whereas Rolla (1977) suggested that fluoride through its high affinity for calcium inhibits adsorption of salivary proteins by hydroxyapatite and reduces plaque formation.

Others have questioned the antibacterial effect of fluoride in vivo. Bowden et al. (1982) concluded that fluoride, at levels usually available in water, has only a limited effect on the plaque microflora even at low pH values. Klock and Krasse (1978) found that the infectivity of S. mutans and lactobacilli was not affected by various preventive measures including fluoride rinses. Another study compared the plaque flora of caries-free children exposed to different water fluoride concentrations since birth. No detectable differences in the microflora of the plaque samples were observed (Kilian et al., 1979).

b. Clinical Trials

Ogaard et al. (1986) created a cariogenic environment on the buccal surface of premolars scheduled for extraction by placing specially designed plaque-accumulating orthodontic bands. Daily mouthrinsing with a neutral 0.2% NaF solution occurred during the four week test period. Mineral loss was reduced by eighty percent and lesion depth was decreased by a factor of three in the fluoride rinse group compared to the non-fluoride control group.

Sonis and Snell (1989) demonstrated the effectiveness of a fluoride releasing, visible light-activated resin bonding system on demineralization in orthodontic patients. While over ten percent of the conventionally bracketed teeth displayed evidence of decalcification, none of the teeth bonded with the fluoride releasing resin had any clinically observable demineralization.

Hirschfield (1978) and others (Dyer and Shannon, 1982; Muhler, 1970) investigated the efficacy of daily fluoride mouthrinses in

preventing decalcification in orthodontic patients. Their results indicate a reduction in demineralization with all types of fluoride.

2. Brushing and Flossing

Toothbrushing and flossing are recognized as effective means of plaque removal from smooth surfaces of enamel (Wright, 1977). However, the methods employed are quite variable, and the amount of time devoted to daily oral hygiene is, on the average, inadequate. The latter point is particularly pertinent in view of the findings by Honkala (1984) that most children brush for less than one minute. Honkala et al. (1986) showed that plaque removal is directly related to the duration of brushing. Others have discovered a relationship between parental toothbrushing proficiency and attitudes about oral hygiene, and the dental health of their children (Blinkhorn, 1980; Sarnat et al., 1984).

a. Toothbrushing and Malocclusion

Several studies have attempted to correlate tooth irregularity with ineffective plaque removal. Shaw's (1980) review of the literature failed to establish a causal relationship. A recent cross-sectional study by Addy et al. (1988) demonstrated a small but significant positive correlation between the subjects' mean plaque and mean irregularity indices.

b. Toothbrushing and Fixed Orthodontic Appliances

The presence of bands and brackets along with archwires and attachments hinders the mechanical removal of plaque (Zachrisson, 1974). To effect adequate cleaning along the gingival margin, Zachrisson (1974) recommended horizontal brushing with either the scrub or the Bass technique for patients wearing fixed orthodontic

appliances. Williams et al. (1987) tested a specially designed toothbrush (Oral B 15/Oral B, Oral B Laboratories Ltd) for its plaque-removing effectiveness. They found a small but significant decrease in the amount of plaque remaining on the buccal surfaces of anterior teeth in a group of patients with fixed orthodontic appliances who used the orthodontic toothbrush on an alternating basis with a regular toothbrush.

A number of longitudinal studies have shown that plaque levels do not necessarily increase during orthodontic treatment if toothbrushing technique as part of an oral hygiene program is outlined prior to the placement of bands and brackets (Lundstrom et al., 1980; Pender, 1986; Zachrisson and Zachrisson, 1971).

3. Other Preventive Measures

In vitro studies have evaluated the effectiveness of resin sealants in preventing white spot lesion formation at the periphery of bonded brackets and in arresting white spot lesion progression. Ceen and Gwinnett (1981) found that chemically catalyzed resin sealants failed to prevent demineralization. The lack of protection was attributed to the sealant's low viscosity and loss of the incompletely polymerized thin film that was produced. Goepferd and Olberding (1989), on the other hand, described resin tags in sealed white spot lesions which were responsible for interfering with lesion progression.

CHAPTER 2

RATIONALE FOR PRESENT STUDY

The relationship between enamel demineralization and the oral microflora, particularly S. mutans and Lactobacillus, has been well established (Duchin and Van Houte, 1978; Fitzgerald et al., 1960; Ikeda et al., 1973; Loesche et al., 1975). Although epidemiological surveys indicate that dental decay has been declining in the pediatric population, the presence of banded/bonded appliances in orthodontic patients alters the oral environment so that certain teeth in caries susceptible individuals may develop white spot demineralization (Gorelick et al., 1982; Gwinnett and Ceen, 1979; Mattingly et al., 1983; Mizrahi, 1983; Sakamaki and Bahn, 1968; Scheie et al., 1984; Sheridan, 1988; Zachrisson and Zachrisson, 1971).

The present study was initiated to assess several parameters associated with the caries process and to determine if correlations can be made between these factors and white spot demineralization in a group of orthodontic patients wearing fixed appliances over a twelve month period. If a relationship between white spot demineralization and "cariogenic factors" (microbiology, diet, fluoride intake,etc.) can be shown, it may be possible to devise a protocol for predicting white spot formation in orthodontic patients with fixed appliances. In this way, individuals who are at risk for white spot demineralization may be identified and additional preventive measures implemented throughout the course of orthodontic treatment with banded/bonded appliances.

CHAPTER 3

MATERIALS AND METHODS

A. Subject Group

The study population consisted of patients under twenty years of age who were scheduled to begin fixed appliance therapy in the Graduate Orthodontic Clinic, Faculty of Dentistry, University of Manitoba, during the Fall of 1988. The orthodontic appliance included brackets bonded to the maxillary and mandibular incisors, and bands/brackets on the posterior teeth.

The nature and purpose of the study were explained to each patient and their parent. Following this, a parent was asked to sign a consent form permitting their child to participate.

B. Clinical Data

A history and clinical examination were completed on each patient prior to the placement of a banded/bonded appliance (time 0) and a saliva sample was obtained. The history assessed significant medical problems, exposure to fluoride, and oral hygiene practices. In addition to a DMFS and caries intensity index (DMFS/surfaces at risk x 100) (Muhlemann, 1976), the clinical examination included whole mouth plaque (Silness and Loe, 1964) and white spot (Gorelick et al., 1982) indices.

Additional clinical examinations were performed at approximately one month (time 1), five months (time 2), nine months (time 3) and twelve months (time 4) after placement of a fixed appliance. The number of bands and brackets along with the indices assessed at the baseline examination were recorded. At each of these clinical sessions plaque and saliva samples were obtained

after which routine oral hygiene instruction was outlined to the patient.

The fluoride content of each subjects' primary water source was determined. Dietary habits were evaluated in all patients at baseline and after approximately nine months of fixed orthodontic treatment using a five day diet diary.

1. Collection of Saliva

Paraffin was used to increase salivation and to remove plaque from the tooth surfaces. Approximately 5 milliliters (mL) of saliva were collected at the beginning of each clinical session. A total of five saliva samples were obtained from each subject during the study.

2. Collection of Plaque

A number of clinical studies have shown that the cervical area on the labial surface of bonded maxillary incisors has a high incidence of white spot lesion formation (Artun and Brobakken, 1986; Gorelick et al., 1982; Hirschfield, 1978; Mizrahi, 1983). In Gorelick's et al. (1982) exhaustive survey, maxillary lateral incisors had the highest incidence of white spot demineralization while mandibular incisors were infrequently affected in adolescents wearing bonded orthodontic appliances. In the present study, a plaque sample was obtained from the cervical area of the same maxillary lateral incisor ("A"/high risk site) and from the cervical area of the same mandibular incisor ("B"/low risk site) with a sterile dental explorer (Hu-Friedy no. 6DE) at each of the clinical examination sessions following placement of the fixed appliance. All subjects in the study received an oral prophylaxis prior to

banding/bonding. The sample from time 1 (one month after the teeth had been banded and bonded) thus represented the baseline plaque sample. A total of four plaque samples per tooth were collected over a twelve month period. The samples were immediately placed into 1 mL of sterile reduced transport fluid (RTF) (Loesche and Syed, 1973) and processed within 1 hour.

3. White Spot Diagnosis

All clinical examinations were performed by the same individual using an examining light, dental mirror and standard dental explorer. The cervical areas on the labial surface of an upper lateral incisor and a lower incisor in each subject were evaluated for white spot formation as part of the clinical examination. The distinction was made between existing white spot demineralization (non-developmental) and abnormal enamel calcification (developmental) during the initial clinical examination. Subsequent examinations confirmed the differentiation between developmental and nondevelopmental enamel opacties on the basis of location, shape and dimensional stability with time (Gorelick et al., 1982).

The cervical areas on the labial surface of the teeth were scored according to the following scale: 1, no white spot formation; 2, slight white spot formation; 3, excessive white spot formation; and 4, white spot formation with cavitation (Figure 3.1). All scoring was done without reference to the white spot index scores (wsis) from previous clinical examinations.

4. Diet Evaluation

Each patient recorded their total food intake during five consecutive days prior to the placement of bands/brackets and after

wearing the fixed orthodontic appliance for approximately nine months. The diet diaries were analyzed for the amount and type of foods sweetened with sugar and the frequency of their consumption (Nizel and Papas, 1989). Based on Stephan's (1944) work, the average daily period of time during which the teeth were exposed to acid demineralization was calculated.

C. Microbiological Procedures

1. Laboratory

a. Culture of Samples

All samples were processed within one hour after collection. Both the plaque samples in RTF and the non-commercial saliva samples were sonicated using a Kontes sonifier at a power setting of 8 for 60 seconds. The dispersed samples were serially diluted with RTF to 1:10, 1:100, 1:1000 and 1:5000 concentrations and plated with a Spiral Plater (Spiral System, Inc., Cincinnati, OH). The plaque samples were cultured on blood agar (Oxoid CM 271 Blood Agar Base No. 2 supplemented with haemin, Vitamin K_1 and 5% sheep's blood, Atlas Laboratories, Winnipeg, Manitoba); TYC plus bacitracin (0.2 units/mL) (Boyar and Bowden, 1985) and Rogosa SL agar (Rogosa et al., 1951) (Difco Lab.). The saliva samples were plated on TYC plus bacitracin (0.2 units/mL) and Rogosa SL agar. All plates were incubated anaerobically at 37° C for a minimum of 4 days under an atmosphere of 85% N_2, 10% H_2 and 5% CO_2.

b. Colony Counts

The agar plates were divided into pie-shaped sections using a "cookie cutter" (Spiral System Inc., Cincinnati, OH). The number of colony-forming units (CFU) for a particular organism was

determined from colonies on segments of the plate following the formulae provided with the Spiral Plater. The total cultivable flora was estimated from the blood agar plates. Colony-forming units for each identified organism were expressed as a percentage of the total cultivable flora and per milliliter of saliva in plaque and saliva samples, respectively. Representatives of each colony type were subcultured onto supplemented blood agar for 48 hours at 37° C under anaerobic conditions to confirm their identification.

c. Identification of Isolates (Table 3.1)

Initial classification of the purified isolates into Streptococcus and Lactobacillus was based on their Gram reaction, cell morphology, catalase production and atmospheric requirements.

1) Gram-stain

The isolates were identified as either Gram-positive or Gram-negative (Hucker and Conn, 1923).

2) Cell morphology

The distinction was made between cocci and rods.

3) Catalase production

The production of bubbling within 5 seconds of the addition of a drop of 30% H_2O_2 (Fisher Sci. Co., NJ) to a sample indicated a positive result (Cowan and Steel, 1974).

4) Atmospheric requirements

Isolates were tested for growth in air and under anaerobic conditions at 37° C for 48 hours.

d. Identification of Species

Additional tests were used to identify species of Streptococcus and Lactobacillus.

1) Streptococcus (Hardie and Bowden, 1976) (Table 3.2)

a) Carbohydrate fermentation

A medium consisting of 2% proteose peptone, 0.5% yeast extract, 0.5% sodium chloride, 0.1% disodium phosphate, 1.5% Bacto-Difco agar, and 0.002% bromo-cresol purple was used to assess carbohydrate fermentation (Cowan and Steel, 1974). The test carbohydrates were added to the medium as filter-sterilized solutions in a concentration of 0.5% w/v. The carbohydrates used were mannitol (man), sorbitol (sor), raffinose (raf), melibiose (melb), trehalose (tre), amygdalin (amy) and inulin (inu). Evidence of fermentation was monitored at 24 hours and 48 hours by detecting a change in the color of the medium from purple to yellow indicating a pH change from 7.6 to 5.5.

b) Arginine hydrolysis

Each isolate was grown in arginine (arg) broth for 48 hours at 37° C. The addition of Nessler's reagent (BDH Chemical, England) followed by an immediate bright orange color indicated that arginine had been hydrolyzed to ammonia (Hardie and Bowden, 1976).

c) Aesculin hydrolysis

Strains were grown on aesculin (aes) agar plates for 48 hours at 37° C to test for hydrolysis (Cowan and Steel, 1974). Blackening of the medium around the colonies and a loss of fluorescence when viewed under ultra-violet light (Ultra-Violet Prod. Inc., San Gabriel, CA) indicated aesculin hydrolysis.

d) H_2O_2 production

Colonies from the blood agar plates were placed onto a moistened reagent strip (Peroxid-Test, Merckoquant strips, Merck, Darmstadt Germany). Development of a blue color was a positive reaction.

e) Extracellular polysaccharide production (EPS)

Strains of Streptococcus were grown on TYB plates under anaerobic conditions for 48 hours at 37° C. They were examined under a dissecting microscope for colony features characteristic of isolates capable of synthesizing extracellular glucosyl and fructosyl transferases. These enzymes catalyze the production of glucans and levans from sucrose (de Stoppelaar, 1971). The colonies of enzyme-producing organisms have a hard and rubbery consistency and adhere to the agar. Soft colonies were regarded as negative for extracellular polysaccharide (eps) production.

2) Lactobacillus (Table 3.3)

Gram-positive, catalase-negative rods grown on Rogosa SL agar under anaerobic conditions after 48 hours were identified as Lactobacillus. Additional tests were performed to classify the species (Bowden et al., 1976). All cultures were incubated anaerobically for 48 hours at 37° C.

a) Gas production

Sodium gluconate (glucn) and glucose (gluc) broths with a Durhams tube were used to determine gas production.

b) Aesculin hydrolysis

Aesculin (aes) broth was inoculated and incubated at 37° C for 48 hours. Aesculin hydrolysis was detected when the medium turned black after the addition of 1% (w/v) ferric chloride.

c) Carbohydrate fermentation

The medium consisted of Lactobacillus sugar base agar to which 2% (w/v) of the test carbohydrate and bromocresol purple were added. Ribose (rib), cellobiose (cel), mannitol (man), melibiose (melb), raffinose (raf) and melezitose (melz) were tested for fermentation by the different strains. All of the carbohydrates except ribose, which was filter sterilized, were autoclaved for 20 minutes at 115° C and added to sterile agar base. Acid production was indicated by a color change of the medium from purple to yellow.

2. Commercial Tests (Saliva Counts)

a. Culture of Samples

The saliva samples were also cultured on commercially available selective media (Bactotest LB, APO Diagnostics Inc., Markham, ONT, and Cariescreen SM, APO Diagnostics Inc., Markham, ONT) at the initial clinical session (time 0) and at the final clinical examination (time 4).

All saliva samples were collected and mixed with the agar dip-slide (Cariescreen SM and Bactotest LB, APO Diagnostics Inc., Markham, ONT) according to the manufacterer's instructions. Each vial was stored in an upright position for 48 hours at 37° C.

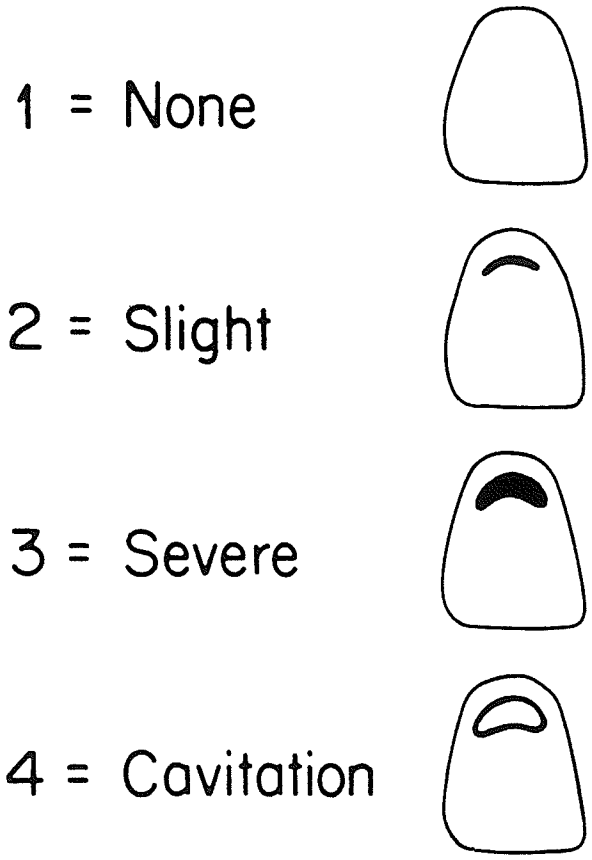
b. Colony Counts

The counts of S. mutans and Lactobacillus per milliliter of saliva were derived from the colony density charts (Cariescreen SM and Bactotest LB, APO Diagnostics Inc., Markham, ONT) after identification of the colony type by light microscopy.

D. Statistical Analysis

The age, DMFS/CI index, fluoride concentration and DEApH (daily exposure to acid pH) were averaged for subjects within each group, and the mean plaque and white spot score for the teeth in the groups were measured. Comparisons were made between groups for statistical significance using paired t tests. The mean concentrations of S. mutans and Lactobacillus in saliva were compared at times 0, 1, 2, 3 and 4 using a two-way analysis of variance. Correlation coefficients were used to compare mean salivary concentrations of S. mutans and Lactobacillus in commercial and laboratory cultures. Differences in the mean percentages of S. mutans and Lactobacillus between site A and B and at times 1, 2, 3 and 4 were analyzed with a two-way analysis of variance. Comparisons between the salivary concentrations and the mean percentages at site A and B of S. mutans and Lactobacillus were made using correlation coefficients. Paired t tests were used to compare the mean percentages of S. mutans and Lactobacillus in plaque from sites A and B at times 1, 2, 3 and 4 with the plaque and white spot indices. Statistical significance was assigned to p values less than 0.05.

(Figure 3.1) White Spot Index Scale[a)]



a) adapted from Gorelick et al., 1982

(Table 3.1) Identification of Isolates

Genus	Cell Morphology	Gram Stain	Catalase Production	Aerobic Growth	Anaerobic Growth
Streptococcus	coccus	+	-	+	+
Lactobacillus	rod/filament	+	-	+	+

(Table 3.2) Identification of Mutans Streptococci Species[1)]

Species	man	sor	raf	melb	tre	amy	inu	arg	aes	h_2o_2	eps
S. mutans	+	+	+	+	+	+	+	-	+	-	H[3)]
S. rattus	+	+	+	+	+	NA[2)]	+	+	+	-	H
S. cricetus	+	+	+	+/-	+	NA	+/-	-	+/-	-	H
S. sobrinus	+	+	+/-	+/-	+/-	NA	+/-	-	+/-	+	H
S. ferus	+	+	-	-	NA	NA	NA	-	+	-	H

1) adapted from Coykendall and Gustafson (1986)
2) NA = results not available
3) H = hard colony on TYB agar

(Table 3.3) Identification of Lactobacillus Species[1)]

Strain	gluc	glucn	aes	rib	cel	man	melb	raf	melz
Betabacterium									
L. fermentum	+	+	-	+	-				-
L. cellobiose	+	+	+	+	+				-
L. buchneri	+	+	+	+	-				+
L. brevis	+	+	+	+	-				-
Streptobacterium									
L. casei	-	+		+			-	-	
L. plantarum	-	+		+			+	+	
Thermobacterium									
L. acidophilus	-	-	+	-	+	-			
L. salivarius	-	-	-	-	-	+			

1) adapted from Bowden et al. (1976)

CHAPTER 4

RESULTS

A. Sample Size

The final sample size was forty-five subjects; twenty-nine females and sixteen males, with an average age of 13.6 years (10.7 to 17.6 years). Two teeth per subject (an upper lateral incisor/UI and a lower incisor/LI) were examined for white spot lesion (wsl) formation in the gingival third of the labial surface, and plaque samples were obtained from the same surfaces during each clinical session. A total of 450 enamel surface evaluations were made, and 360 plaque and 225 saliva samples analyzed throughout the 16 month (Aug., 1988 to Dec., 1989) study period.

B. General Clinical Findings (Table 4.1)

1. DMFS/Caries Intensity

The initial clinical examination revealed a mean DMFS of 8.6 for the subject population. The caries intensity index (CII) ranged from 0.0% to 25.0% for all subjects. Those subjects who developed a wsl in an upper lateral incisor had a mean DMFS of 8.7 while those with wsl formation in a lower incisor averaged 12.6 DMFS.

2. Fluoride Analysis

The fluoride content of the subjects' drinking water averaged 0.65 micrograms/liter (parts per million/ppm) with a range of 0.18 ppm to 1.25 ppm. The drinking water for nine of the patients (three were subjects with white spot formation) contained less than 0.3 micrograms/liter of fluoride while twenty of the remaining subjects were exposed to fluoride levels which exceeded 0.7 ppm.

The drinking water of subjects with wsl formation in an upper lateral incisor or a lower incisor had a fluoride content of 0.62 micrograms/liter and 0.81 micrograms/liter, respectively.

3. Diet Assessment

Thirty-seven subjects completed a five day diet record at the beginning of the study and a second one near the end of the sixteen months. The daily mean period of time during which the teeth were potentially exposed to acid (DEApH) was 63.5 minutes with a range of 16 minutes to 188 minutes.

Subjects who developed a wsl in the upper lateral incisor averaged less (52 minutes) potential acid exposure on a daily basis while those individuals with wsl formation in the lower incisor averaged slightly more (65 minutes) than the overall group.

4. Plaque Accumulation

Plaque scores averaged 1.2 (UI) and 1.1 (LI) in subjects without white spot lesions. The mean values for individuals with white spots in the upper lateral incisor were 1.3 (UI) and 1.3 (LI), and 1.4 (UI)/1.4 (LI) in subjects with lower incisor white spots.

5. White Spot Formation (Table 4.2)

White spots developed in both upper lateral incisors and lower incisors throughout the study period. Overall, approximately 22% of the incisors that were evaluated developed a white spot lesion. However, the incidence as well as the prevalence of ws development was larger in site A than in site B (Figure 4.1).

An upper lateral incisor in one subject and a lower incisor in a different subject each exhibited a white spot lesion at the initial clinical examination. A white spot index score (wsis) of 2 was given

to these incipient lesions, and they were categorized separately from white spot lesions appearing after placement of the fixed appliance. Neither of the white spot lesions present at time 0 appeared clinically to progress during the study. Interestingly, in both of these individuals a white spot lesion developed in the alternative site; i.e., the subject with a wsl in the upper lateral incisor developed a wsl in the lower incisor, and the patient with a wsl in the lower incisor formed a wsl in the upper lateral incisor during the period of fixed appliance wear.

Two of the subjects (one female and one male) in the study developed a wsl in both an upper lateral incisor and a lower incisor. In other words, the total number of white spot lesions exceeded the total number of subjects with white spot lesions by two.

a. A Site/Upper Lateral Incisor/ High Risk

Of the remaining forty-four subjects, eight females and six males (or 32% of the study population), with an average age (13.7 years) slightly greater than the mean for the entire group, showed evidence of wsl formation in the cervical third on the labial surface of an upper lateral incisor during the study. Development of the incipiencies occurred as follows: three (approximately 21%) of the white spot lesions were present by one month and another three after five months of fixed appliance wear; four wsl's were detected at nine months; and four more (or 29% of the total in the upper incisors) were observed clinically twelve months after fixed appliances had been placed. A single wsl developed by five months after the initiation of fixed appliance therapy in an upper lateral incisor which hadn't yet been bracketed. One of the fourteen white

spot lesions progressed to a wsis of 3 by twelve months after the placement of bands and brackets.

b. B Site/Lower Incisor/Low Risk

The onset of wsl formation in the lower incisors similarly occurred throughout the entire observation period. A total of five teeth (11% of the lower incisors) in one female and four males (mean age of 14.2 years) displayed a wsl in the gingival third on the labial surface. Three of the lesions developed prior to bracket placement on the tooth with two of them becoming clinically apparent by one month after the initiation of fixed appliance therapy. All lesions in the lower incisors maintained a wsis of 2.0 for the duration of the clinical study.

C. General Microbiological Data

1. Streptococcus

A total of 850 isolates were identified. 420 or 49% were S. mutans and 45 (8%) were S. sobrinus. The remainder of the isolates were identified as S. sanguis, S. mitis and S. salivarius. No correlation existed between the species isolated from the saliva samples and white spot formation.

a. S. mutans

Data for the presence of S. mutans in the different groups of subjects over time are shown in Table 4.3. The majority of strains of S. mutans were isolated from TYB agar (> 90%). The other streptococci were isolated from the blood agar plates. These streptococci were selected on the basis of their colony form which resembled S. mutans.

1) Percentage Isolation Frequency

a) Upper lateral incisor

Data for the upper lateral incisors are shown in Table 4.4. The mean and range of isolation frequency for S. mutans in all subjects over a twelve month period were 2.4 and 0 to 4, respectively. Twelve patients (27%) gave four of four positive samples while eleven (24%); eleven (24%) and seven (16%) gave 75%, 50% and 25% positive samples, respectively. Those fourteen subjects who developed a wsl during the study showed isolation frequencies of 100% in three patients (21.3%), 75% in three patients (21.3%), 50% in three patients (21.3%) and 25% in four patients (29%).

b) Lower incisor

The lower incisor values are presented in Table 4.5. The mean and range of isolation frequency for S. mutans in all subjects during their first twelve months of fixed appliance wear were 1.4 and 0 to 4, respectively. One patient out of forty-five (2%) gave four of four positive samples while five (11%); twelve (27%) and twenty-one (47%) were positive for S. mutans in 75%, 50% and 25% of their plaque samples, respectively. The five subjects who developed a wsl during the study had isolation frequencies of 75% in three patients (60%), 50% in one patient (20%) and 25% in one patient (20%).

c) Saliva

The saliva sample data are shown in Table 4.6. Values for the mean and range of isolation frequency of S. mutans in all subjects during the entire study period were 4.1 and 0 to 5, respectively. Twenty-two patients (49%) were positive for S. mutans in five of five samples whereas fifteen patients (33%); two patients (4.5%);

three patients (7%) and one patient (2%) gave 80%, 60%, 40% and 20% positive samples, respectively. Those seventeen subjects who developed a wsl during the study showed isolation frequencies of 100% in eleven patients (65%), 80% in four patients (23%) and 0% in two patients (11%).

2) Mean Percentage Contribution and Mean Concentration

The mean percentage contribution of S. mutans to the total cultivable bacterial flora in the various samples is given in Table 4.7. S. mutans comprised 5.5% (0 to 77%) and 2.5% (0 to 56.5%) of the microflora in samples from upper lateral incisors and lower incisors, respectively. In the seventeen subjects who developed a wsl in an incisor (14 upper and 5 lower), the mean percentage contribution of S. mutans in each of the samples was 6.3% (upper lateral incisor) and 3.1% (lower incisor).

The mean concentration of S. mutans in saliva samples from all subjects was 2.6×10^6 /ml at time 0, 3.1×10^6 CFU/ml at time 1, 8.3×10^6 CFU/ml at time 2, 4.1×10^6 CFU/ml at time 3 and 5.4×10^6 CFU/ml at time 4. Overall, the mean concentration of S. mutans was 4.7×10^6 CFU/ml of saliva in the entire population and 5.6×10^6 CFU/ml of saliva in subjects with white spot formation (Tables 4.8 and 4.9).

2. Lactobacillus

Data for the presence of Lactobacillus in the different groups of subjects over time are presented in Table 4.10. The species of 360 isolates of Lactobacillus were identified as follows: L. casei (58%); L. fermentum (17%); L. plantarum (11%); L. buchneri (6%); L. cellobiosus (6%) and L. acidophilus (2%). There was no obvious

relationship between the species isolated in saliva and white spot formation.

a. Percentage Isolation Frequency

1) Upper lateral incisor

Data for the upper lateral incisors are shown in Table 4.4. The mean and range of isolation frequency of Lactobacillus in all subjects over the twelve month period were 0.7 and 0 to 4, respectively. One patient (2%) gave four of four positive samples. Nine patients (20%) and ten patients (22%) gave 50% and 25% positive samples, respectively. There were twenty-five patients (56%) who gave no positive samples. The fourteen subjects who developed a wsl during the study exhibited isolation frequencies of 50% in one patient (7%), 25% in two patients (14%) and 0% in eleven patients (79%).

2) Lower incisor

Values for the lower incisors are given in Table 4.5. The mean and range of isolation frequency of Lactobacillus in all subjects during the first twelve months of fixed appliance therapy were 0.3 and 0 to 2, respectively. None of the subjects gave either 100% or 75% positive samples, whereas three patients (7%) and nine patients (20%) gave 50% and 25% positive samples, respectively. Thirty-three patients (73%) were negative for Lactobacillus in all four samples. Those five subjects who developed a wsl during the study showed isolation frequencies of 50% in one patient (20%), 25% in one patient (20%) and 0% in three patients (60%).

3) Saliva

Salivary values for Lactobacillus are shown on Table 4.6. The mean and range of isolation frequency of Lactobacillus for all subjects during the entire study period were 4.2 and 0 to 5, respectively. Twenty-one subjects (47%) were positive for Lactobacillus in all five samples while seventeen (38%) and five (11%) gave 80% and 60% positive samples, respectively. Those seventeen subjects who developed a wsl in an incisor during the study showed isolation frequencies of 100% in ten patients (59%), 80% in five patients (29%) and 60% in one patient (6%).

b. Mean Percentage Contribution and Mean Concentration

The mean percentage contribution of Lactobacillus to the total cultivable flora in samples from the different sites is presented in Table 4.7. Lactobacillus comprised 0.4% (0 to 55%) and 0.01% (0 to 0.54%) in samples from the upper lateral incisors and lower incisors, respectively. The seventeen subjects who developed a wsl in an incisor (14 upper and 5 lower) during the study gave a mean percentage contribution of Lactobacillus in each of the samples of 0.01% (upper incisor) and 0.02% (lower incisor).

The mean concentration of Lactobacillus in saliva samples for the entire population was 0.13×10^6 CFU/ml at time 0, 0.22×10^6 CFU/ml at time 1, 0.39×10^6 CFU/ml at time 2, 0.25×10^6 CFU/ml at time 3 and 0.26×10^6 CFU/ml at time 4. Overall, the mean concentration of Lactobacillus was 0.25×10^6 CFU/ml of saliva in all subjects and 0.32×10^6 CFU/ml of saliva in white spot subjects (Tables 4.8 and 4.9).

D. Data Analysis

1. Presence of Microorganisms

a. Saliva (Figures 4.2 and 4.3)

The mean concentration of S. mutans in saliva was significantly greater at time 2 (five months after band/bracket placement) than at times 0 (8.3 vs. 2.5, p=0.002), 1 (8.3 vs. 3.1, p=0.005) and 3 (8.3 vs. 4.1, p=0.02). There were no statistically significant differences in the mean concentration of Lactobacillus over time.

1) Comparison between commercial and laboratory cultures (Figures 4.4 and 4.5)

At time 0, a statistically significant positive linear relationship existed between the salivary concentration of S. mutans in the commercial cultures and the laboratory cultures (p=0.013). The two cultures were unrelated in terms of S. mutans concentrations at time 4. The concentrations of Lactobacillus in the different cultures, on the other hand, were directly related at time 4 (p=0.003) but not at time 0. At both times, the colony counts of the organisms were less on the commercial culture than on the laboratory culture.

b. Plaque (Figures 4.6 and 4.7)

A significant difference in the mean percentage of S. mutans on site A (upper lateral incisor) was found between times 1 and 4 (3.2 vs. 8.2, p=0.03). Also at time 4, the mean percentage of S. mutans on sites A and B (lower incisor) were different (8.2 vs. 1.7, p=0.006). There were no statistically significant differences in the

mean percentage of Lactobacillus in plaque over time, between sites or between site and time.

2. Concentration of Microorganisms in Saliva and Mean Percentage of Microorganisms in Plaque (Figures 4.8 and 4.9)

The salivary concentration of Lactobacillus was directly related to the mean percentage of Lactobacillus in plaque from site B at time 1 ($p=0.0001$), whereas the salivary concentration and mean percentage of S. mutans in plaque at site A showed a positive linear relationship at time 4 ($p=0.04$).

3. Plaque Index and Mean Percentage of Microorganisms in Plaque

a. A Site/Upper Lateral incisor

The mean percentage of S. mutans in subjects with a mean plaque index of 1.0 or less in the upper lateral incisor was significantly smaller than in subjects whose mean plaque index was 2.0 or greater at time 4 (3.0 vs. 16.4, $p=0.01$).

b. B Site/Lower Incisor

There was no significant relationship between the mean plaque index and the mean percentage of Lactobacillus and/or S. mutans on the lower incisor.

4. Mean Percentage of Microorganisms in Plaque and White Spot Index

a. A Site/Upper Lateral Incisor (Figures 4.10 and 4.11)

Both at time 1 and at time 2 the mean percentage of S. mutans in plaque from site A was greater in subjects with a wsi of 1.0 than in subjects with a diagnosed wsl (wsi $\geq$ 2.0) ($p=0.04$).

b. B Site/Lower Incisor (Figures 4.12 and 4.13)

The mean percentage of S. mutans in site B plaque at time 1 was less in subjects with a wsi of 1.0 than in subjects with a wsi of 2.0 or greater (0.7 vs. 18.8, p=0.0002).

(Table 4.1) General Clinical Findings

Subjects	N	Age* (yrs)	DMFS/CII(%)*	F^{-}* (ppm)	PI* UI/LI	DEApH* (mins.)	WSI* UI/LI
All subjects	**45**	**13.6**	**8.6 / 7.2**	**0.65**	**1.2/1.1**	**63.5**	**1.2/1.1**
Female	29	13.5	8.1 / 6.8	0.69	1.2/1.2	59.9	1.1/1.0
Male	16	13.7	9.6 / 7.9	0.58	1.3/1.2	69.4	1.3/1.2
Subjects without wsl	**28**	**13.6**	**8.2 / 6.9**	**0.68**	**1.2/1.1**	**67.7**	**1.0/1.0**
Female	21	13.6	8.4 / 7.1	0.71	1.1/1.1	68.2	1.0/1.0
Male	7	13.5	7.7 / 6.4	0.59	1.1/1.1	66.6	1.0/1.0
Subjects with wsl in UI	**14****	**13.7**	**8.4 / 6.7**	**0.57**	**1.3/1.3**	**56.5**	**1.5/1.6**
Female	8	13.3	6.6 / 5.3	0.64	1.3/1.4	40.9	1.4/1.3
Male	6	14.2	10.7 / 8.5	0.49	1.3/1.2	67.2	1.5/1.1
Subjects with wsl in LI	**5****	**14.2**	**12.6 / 10.4**	**0.81**	**1.4/1.4**	**65.3**	**1.5/1.6**
Female	1	17.5	24 / 18.8	1.08	1.4/1.4	NA	1.6/1.2
Male	4	13.4	9.8 / 8.2	0.75	1.4/1.4	65.3	1.4/1.6

* (mean values)

** (one female and one male developed a wsl in both their UI and LI)

(Table 4.2) White Spot Formation

Tooth	N	Time 1	Time 2	Time 3	Time 4
Upper lateral incisor (UI)	14	3	3	4	4
Lower incisor (LI)	5	2	1	1	1

(Table 4.3) Presence of S. mutans

		Sample Times				
		0	1	2	3	4
Organism/Site	N[1)]	Subjects with the organism				
S. mutans/UI (all subjects)	45	NS[2)]	26	27	34	22
S. mutans/UI (subjects with wsl)	17	NS	9	11	13	8
S. mutans/LI (all subjects)	45	NS	11	17	25	11
S. mutans/LI (subjects with wsl)	17	NS	5	7	11	6
S. mutans/Saliva (all subjects)	45	38	35	37	33	39
S. mutans/Saliva (subjects with wsl)	17	14	15	14	12	15

1) N = total number of subjects
2) NS = not sampled

(Table 4.4) Percentage Isolation Frequency/Upper Lateral Incisor

		Positive Samples				
Samples	N=4	0	1	2	3	4
S. mutans (all subjects)	N=45	9%	16%	24%	24%	27%
S. mutans (subjects without wsl)	N=31	10%	10%	26%	26%	29%
S. mutans (subjects with wsl)	N=14	7%	29%	21.3%	21.3%	21.3%
Lactobacillus (all subjects)	N=45	56%	22%	20%	0%	2%
Lactobacillus (subjects without wsl)	N=31	45%	26%	26%	0%	0%
Lactobacillus (subjects with wsl)	N=14	79%	14%	7%	0%	0%

(Table 4.5) Percentage Isolation Frequency/Lower Incisor

		Positive Samples				
Samples	N=4	0	1	2	3	4
S. mutans (all subjects)	N=45	13%	47%	27%	11%	2%
S. mutans (subjects without wsl)	N=40	15%	50%	28%	5%	2%
S. mutans (subjects with wsl)	N=5	0%	20%	20%	60%	0%
Lactobacillus (all subjects)	N=45	73%	20%	7%	0%	0%
Lactobacillus (subjects without wsl)	N=40	75%	20%	5%	0%	0%
Lactobacillus (subjects with wsl)	N=5	60%	20%	20%	0%	0%

(Table 4.6) Percentage Isolation Frequency/Saliva

		Positive Samples					
Samples	N=5	0	1	2	3	4	5
S. mutans (all subjects)	N=45	4.5%	2%	7%	4.5%	33%	49%
S. mutans (subjects without wsl)	N=28	0%	4%	10%	7%	36%	43%
S. mutans (subjects with wsl)	N=17	12%	0%	0%	0%	23%	65%
Lactobacillus (all subjects)	N=45	0%	0%	0%	11%	38%	47%
Lactobacillus (subjects without wsl)	N=28	4%	0%	0%	10%	43%	43%
Lactobacillus (subjects with wsl)	N=17	0%	0%	0%	6%	29%	59%

(Table 4.7) Mean Percentage Contribution

Organism	Number of subjects	Upper incisor	Lower incisor
S. mutans (all subjects)	N=45	5.5%	2.5%
S. mutans (subjects with wsl)	N=17	6.3%	3.1%
Lactobacillus (all subjects)	N=45	0.4%	0.01%
Lactobacillus (subjects with wsl)	N=17	0.01%	0.02%

(Table 4.8) Mean Concentration

Organism	Number of subjects	Saliva (x 10^6 CFU/ml)
S. mutans (all subjects)	N=45	4.7
S. mutans (subjects with wsl)	N=17	5.6
Lactobacillus (all subjects)	N=45	0.25
Lactobacillus (subjects with wsl)	N=17	0.32

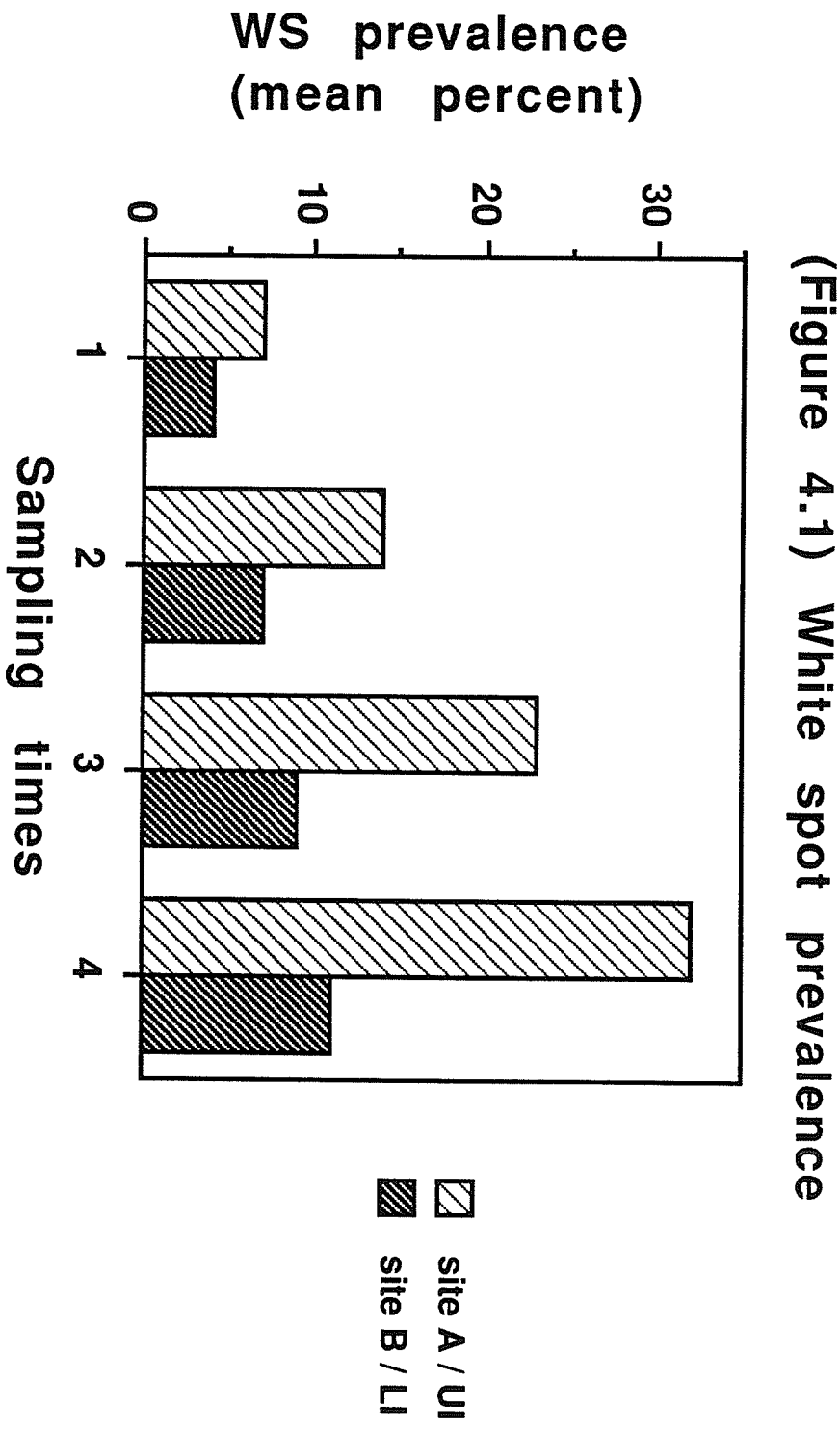
(Table 4.9) Mean Concentration (x 10^6 CFU/ml)/Saliva

		Sample Times				
Organism	N	0	1	2	3	4
S. mutans (all subjects)	45	2.6	3.1	8.3	4.1	5.4
Lactobacillus (all subjects)	45	0.13	0.22	0.39	0.25	0.26

(Table 4.10) Presence of Lactobacillus

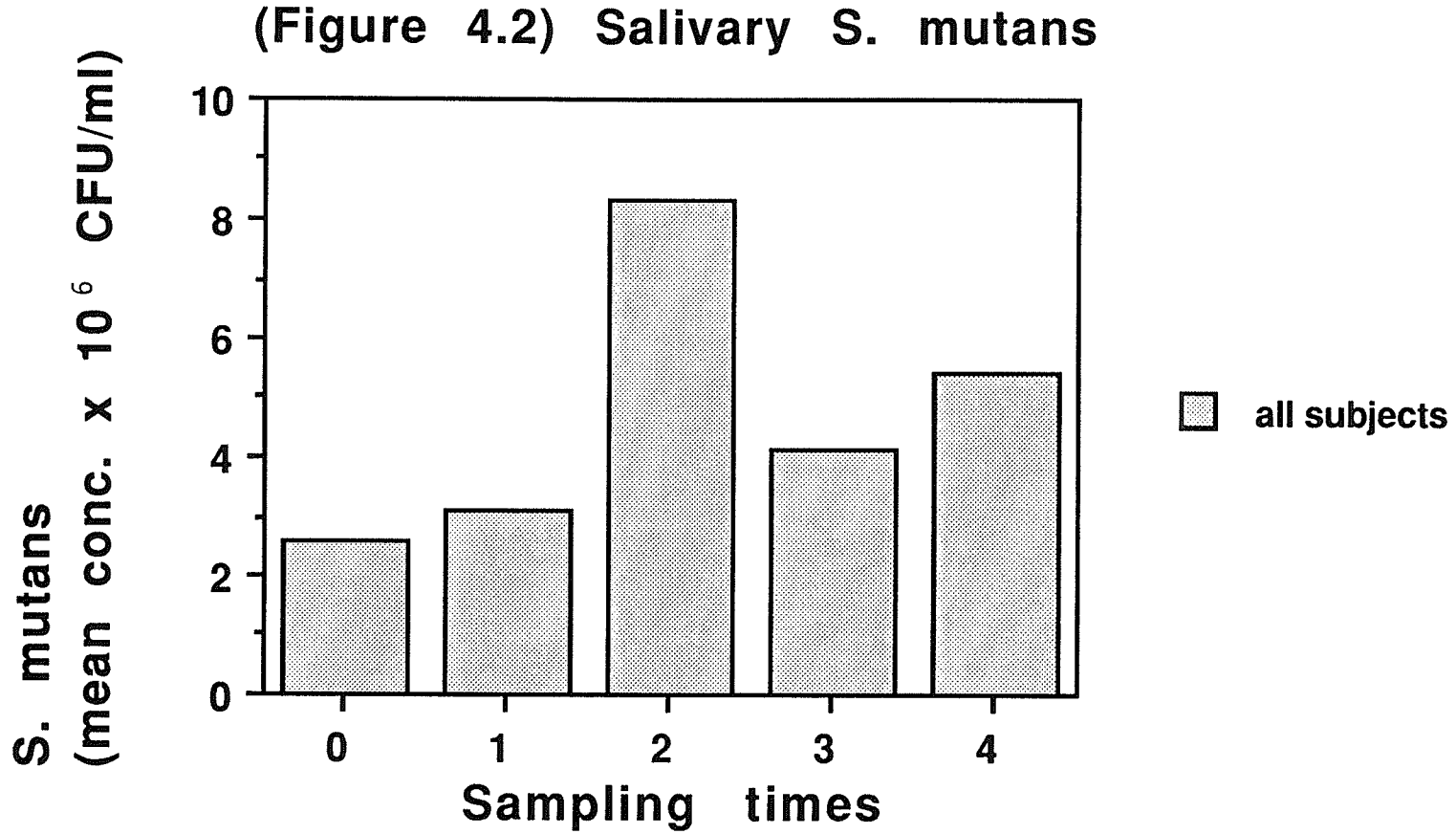
		Sample Times				
		0	1	2	3	4
Organism/Site	N[1)]	Subjects with the organism				
Lactobacillus/UI (all subjects)	45	NS[2)]	11	7	7	6
Lactobacillus/UI (subjects with wsl)	17	NS	3	2	2	3
Lactobacillus/LI (all subjects)	45	NS	2	5	4	3
Lactobacillus/LI (subjects with wsl)	17	NS	1	2	2	1
Lactobacillus/Saliva (all subjects)	45	30	37	39	39	43
Lactobacillus/Saliva (subjects with wsl)	17	13	15	13	14	16

1) N = total number of subjects
2) NS = not sampled

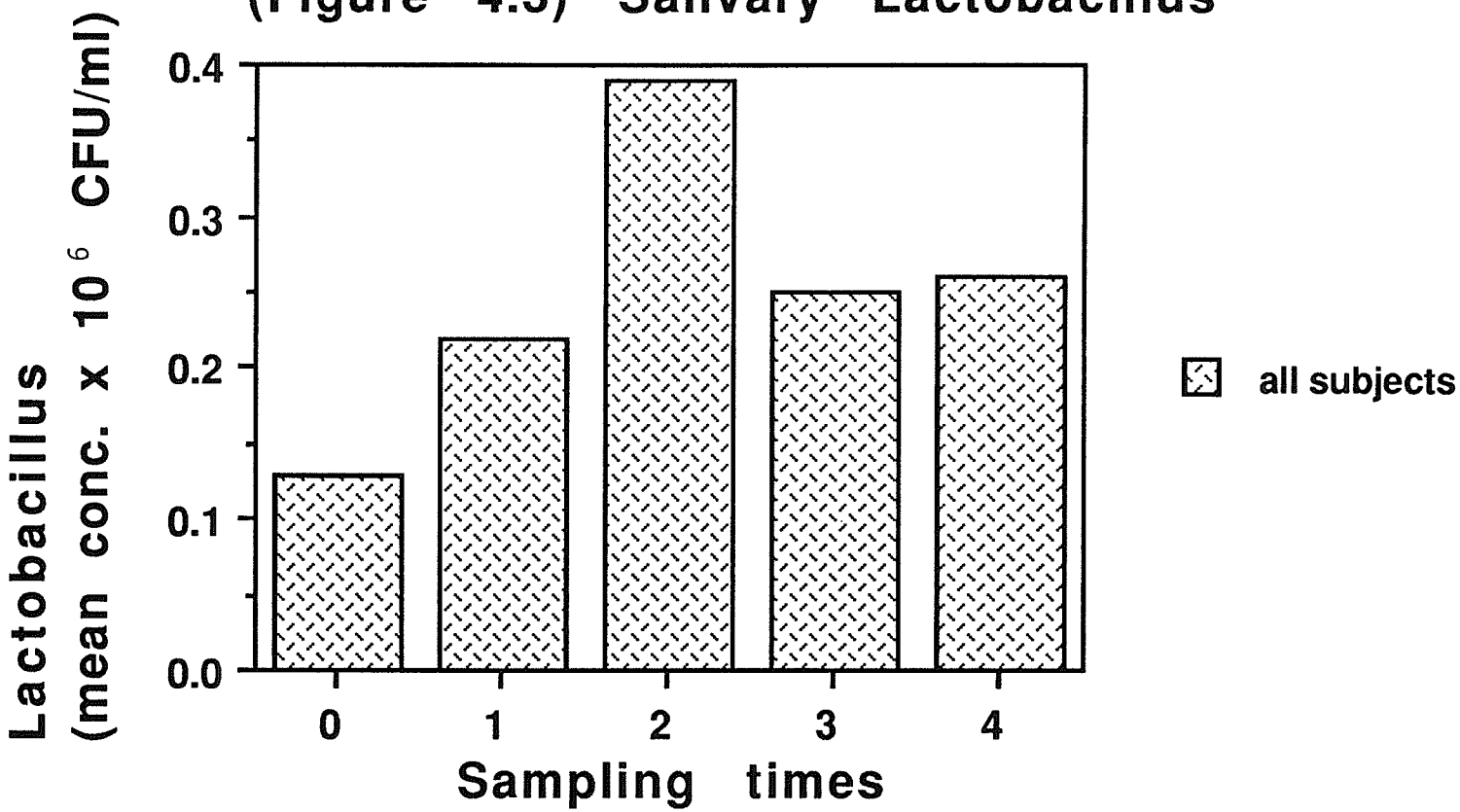


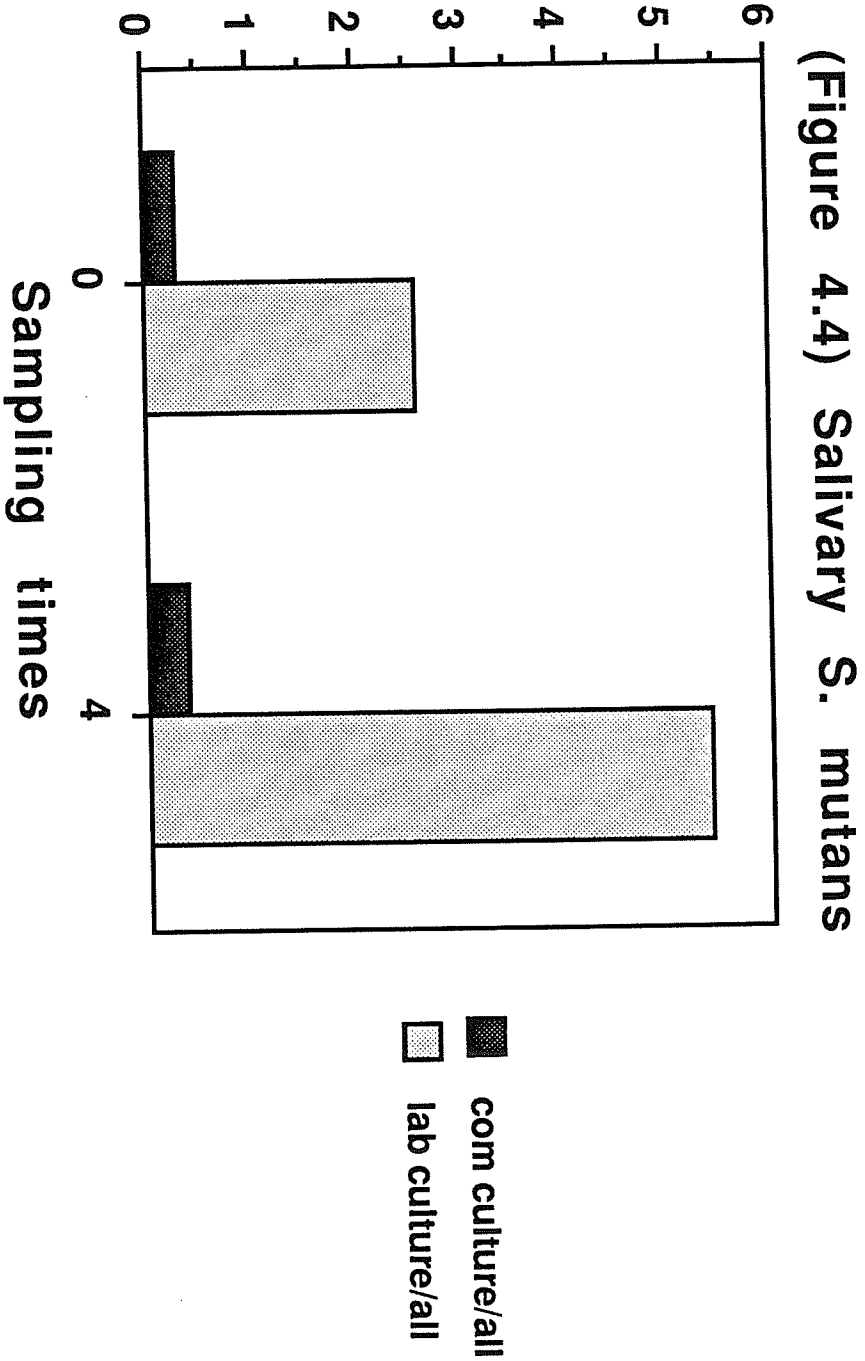
(Figure 4.1) White spot prevalence

(Figure 4.2) Salivary S. mutans

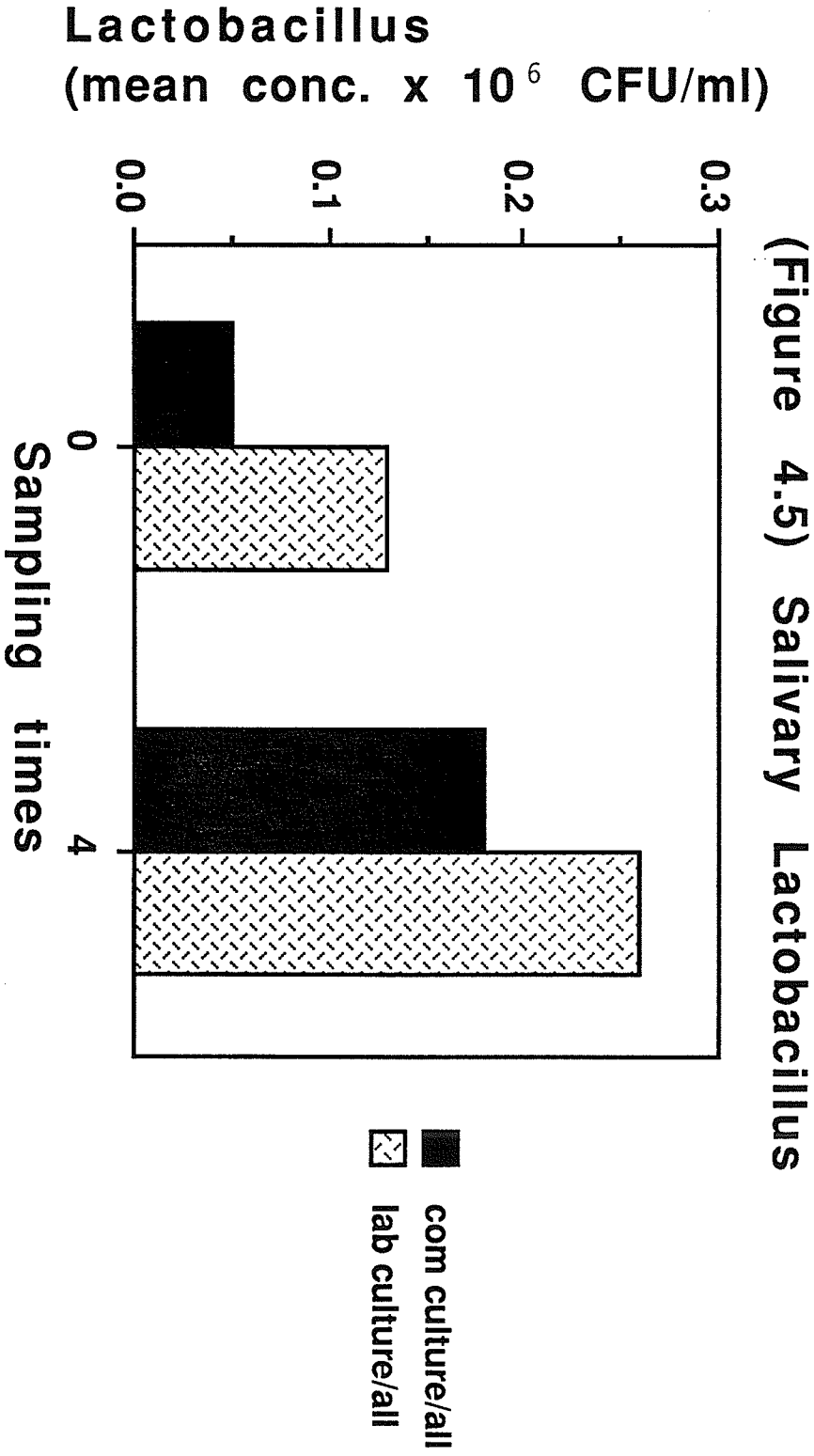


(Figure 4.3) Salivary Lactobacillus



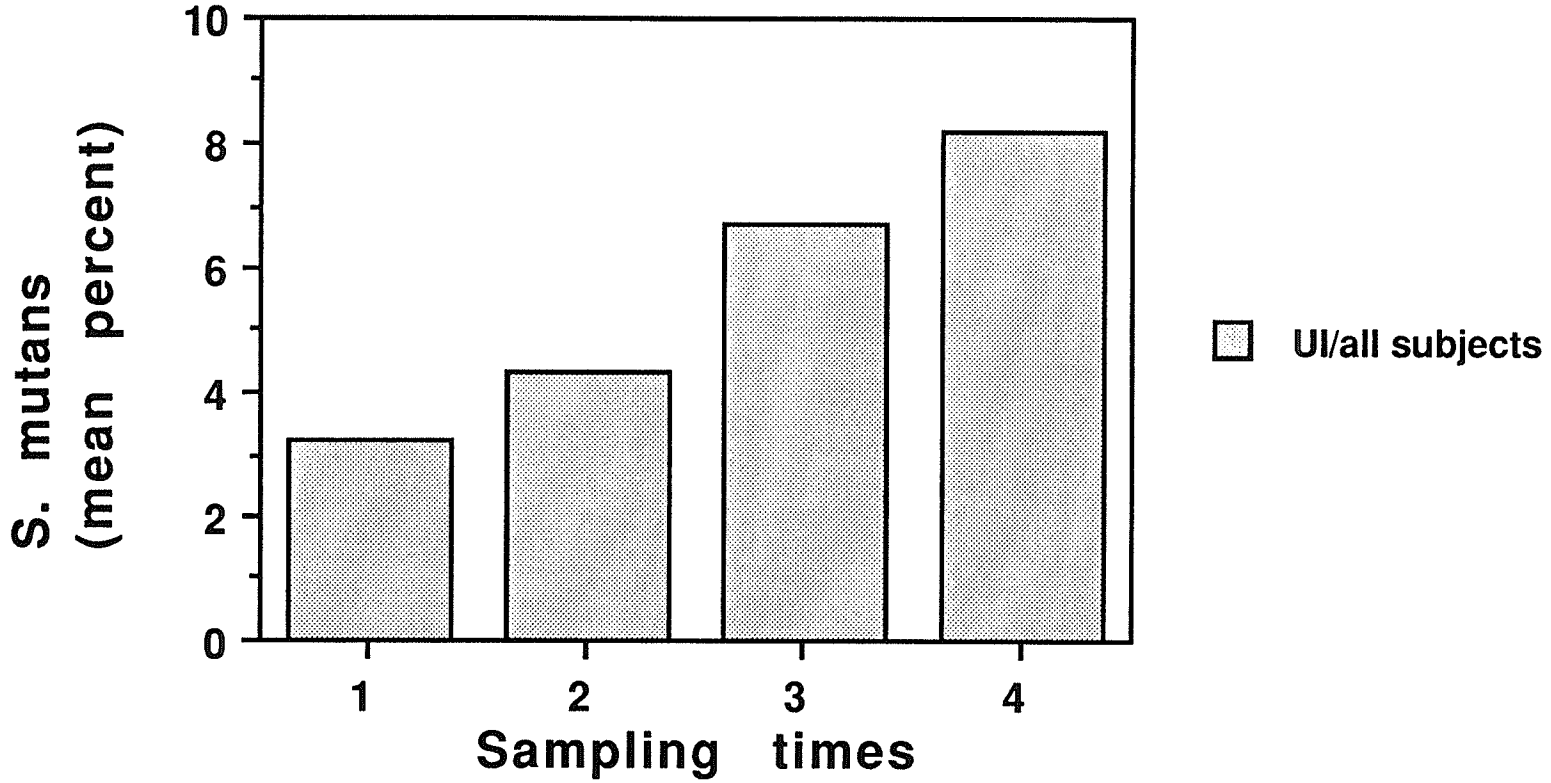


(Figure 4.4) Salivary S. mutans

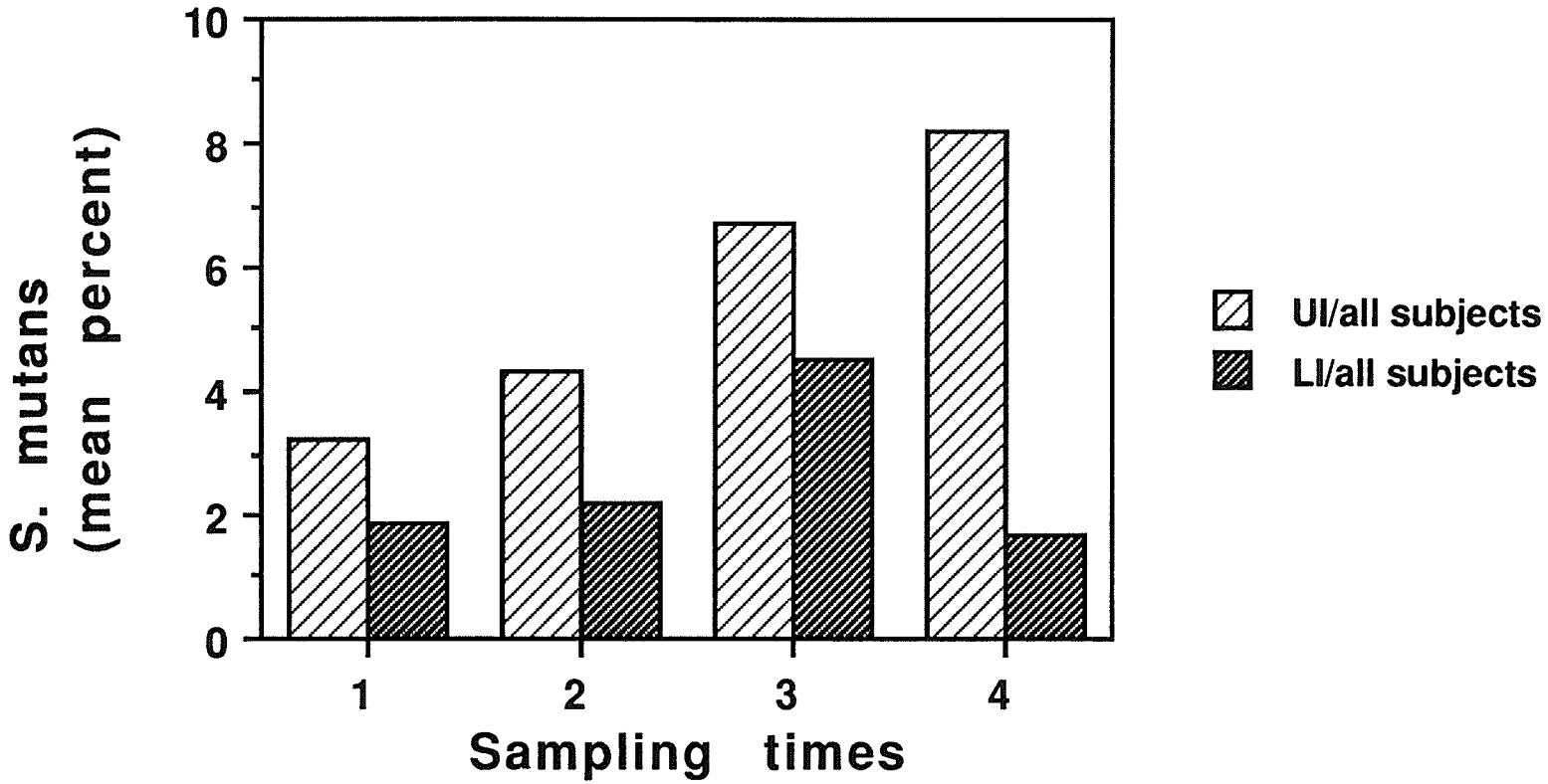


(Figure 4.5) Salivary Lactobacillus

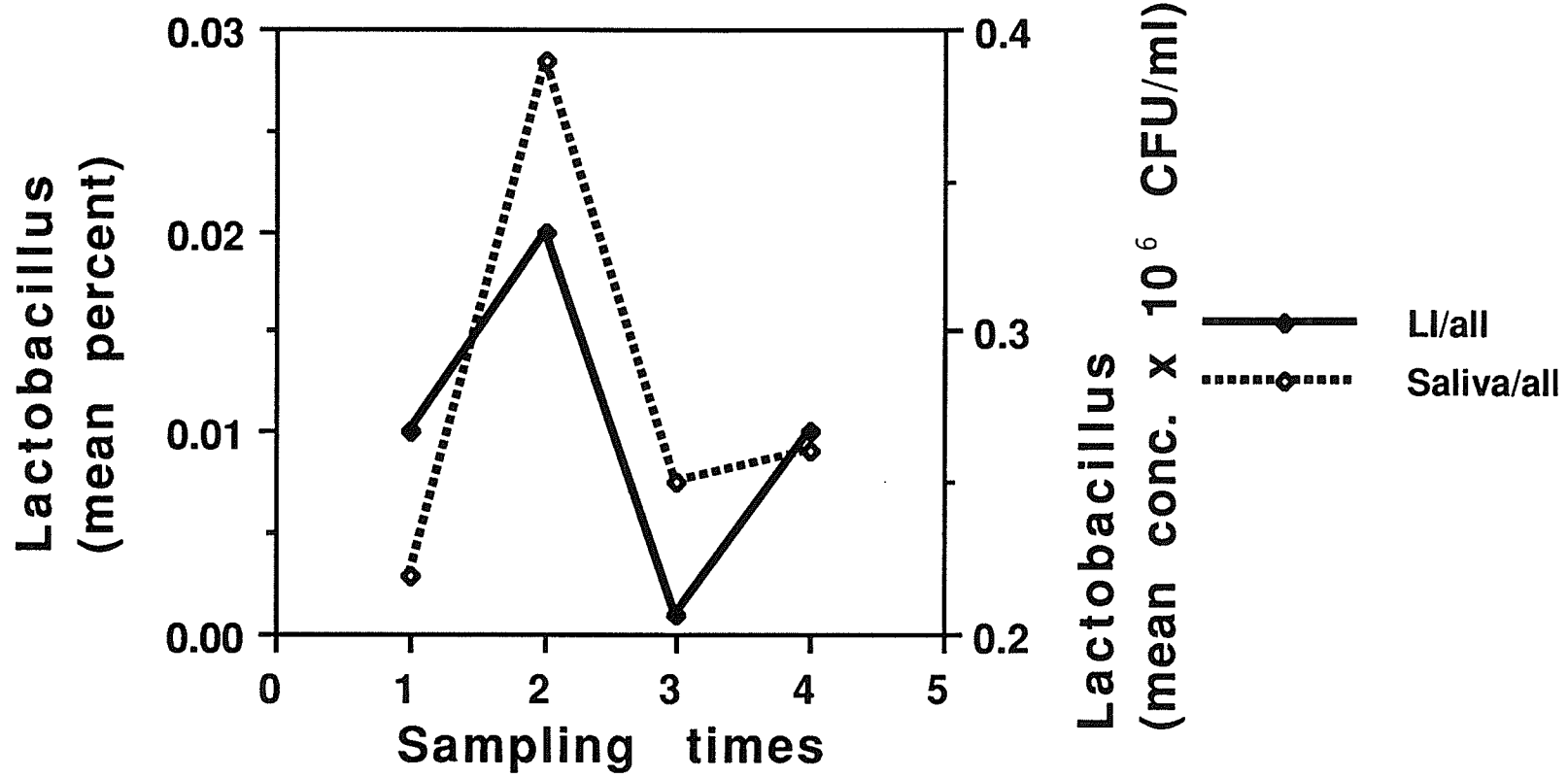
(Figure 4.6) S. mutans / UI



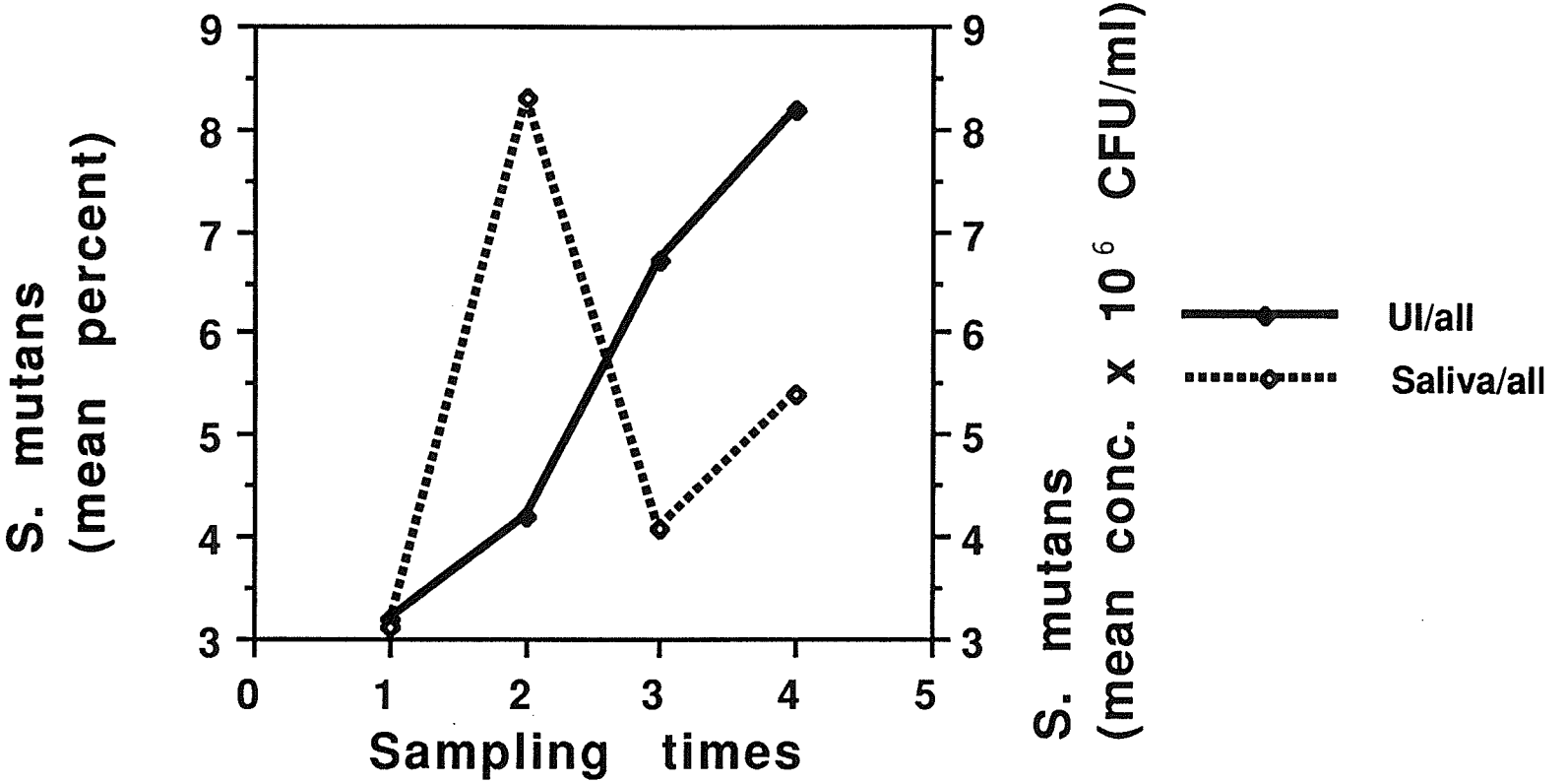
(Figure 4.7) S. mutans / UI vs. LI

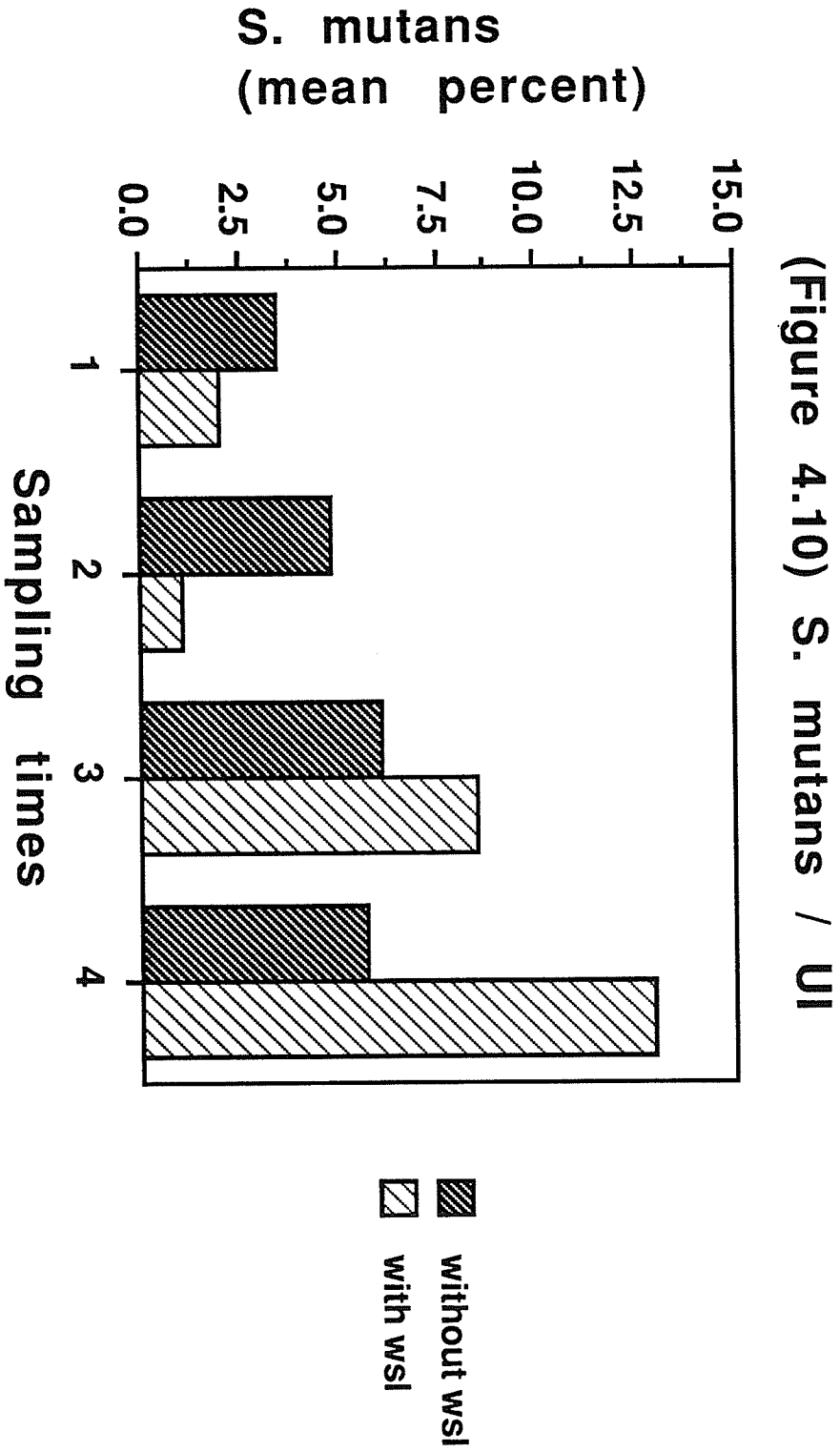


(Figure 4.8) Lactobacillus / LI vs. Saliva



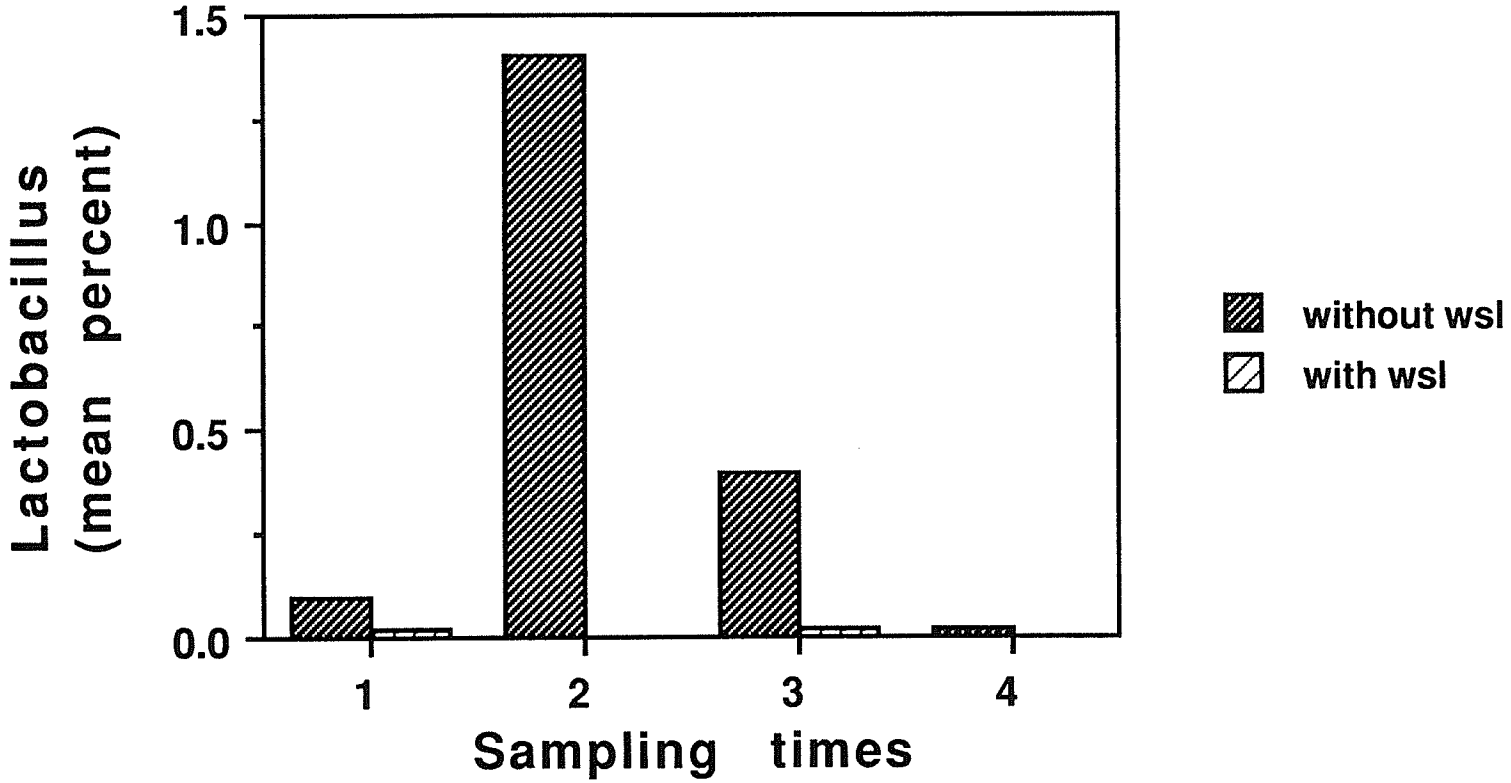
(Figure 4.9) S. mutans / UI vs. Saliva



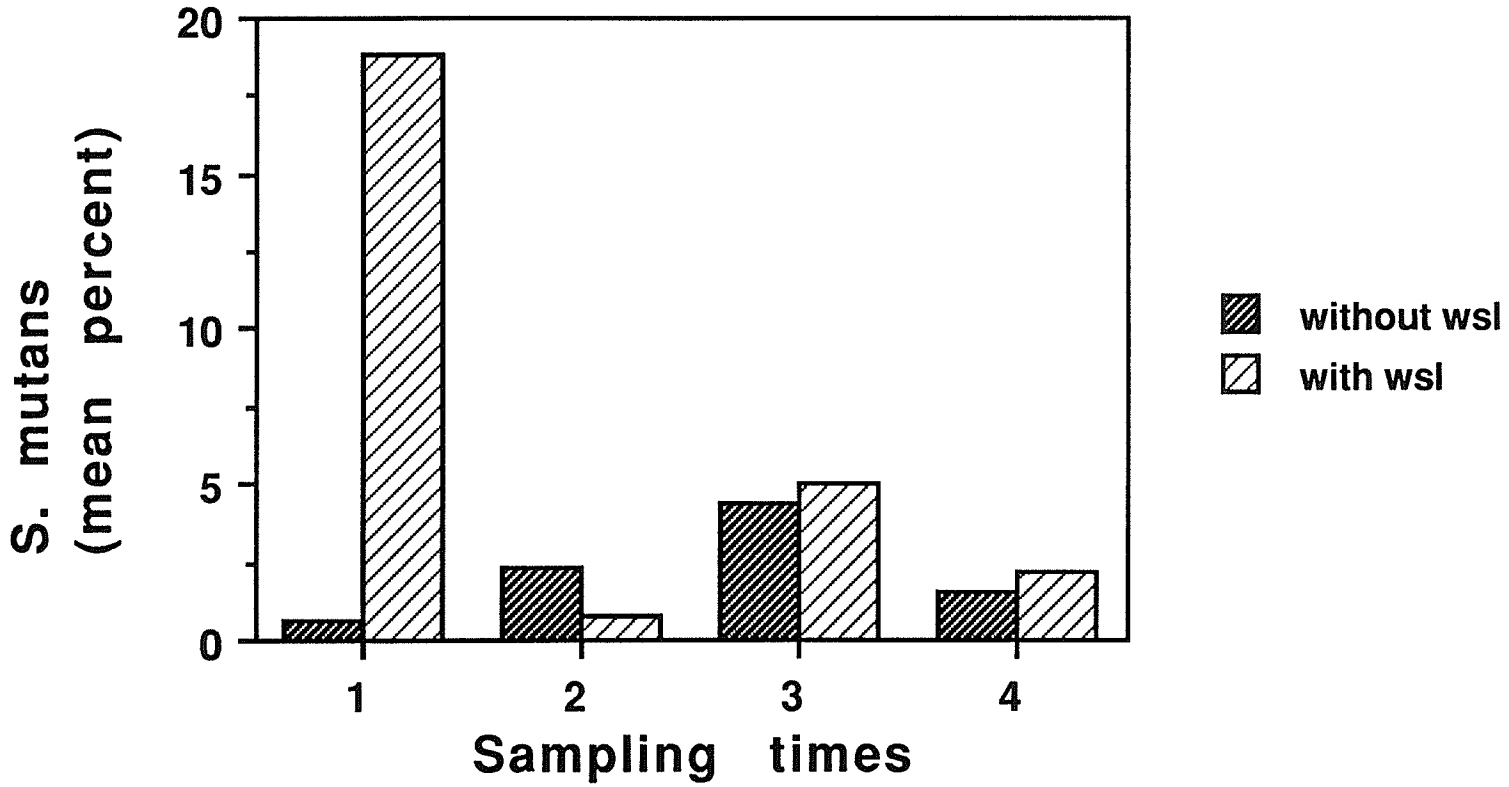


(Figure 4.10) S. mutans / UI

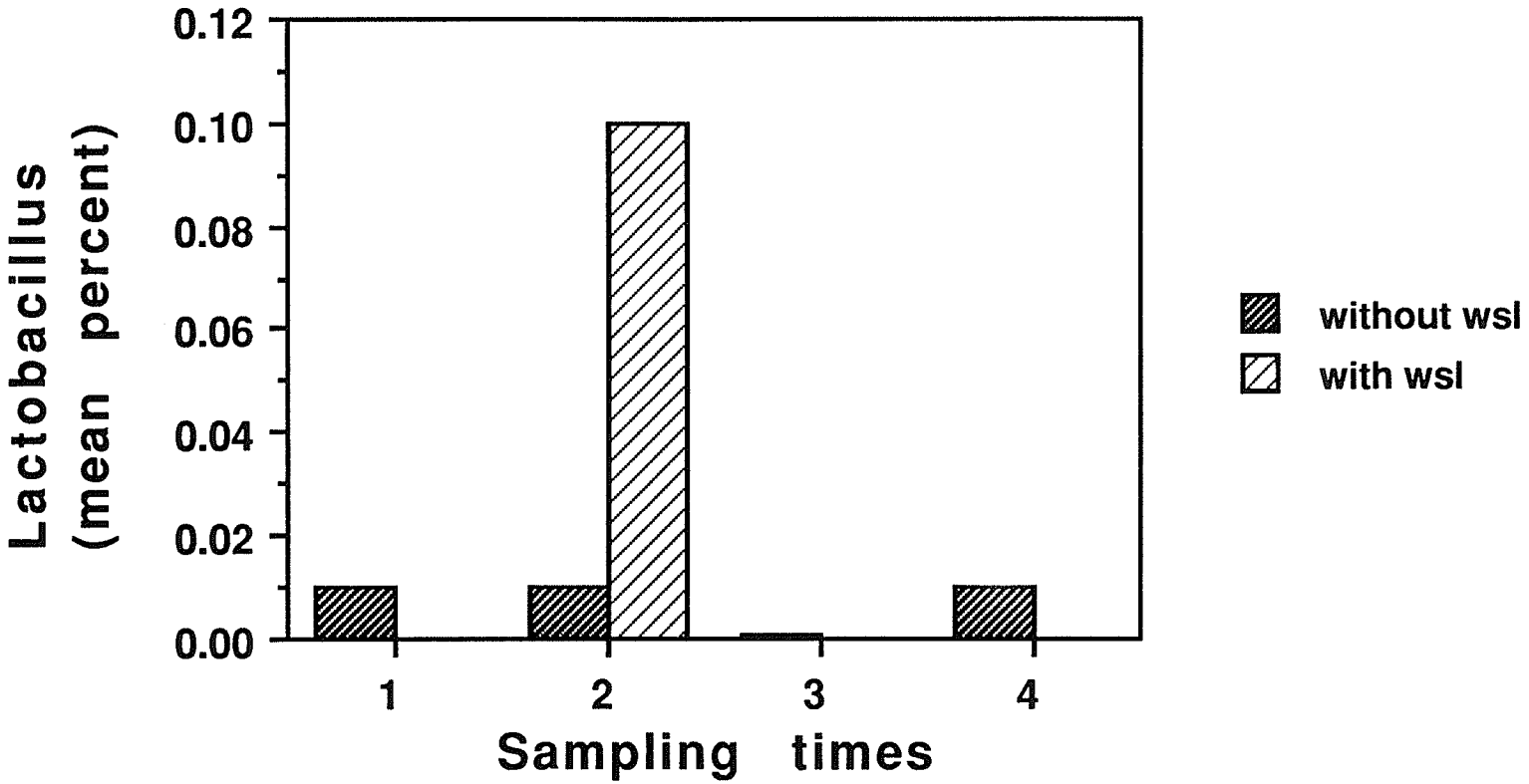
(Figure 4.11) Lactobacillus / UI



(Figure 4.12) S. mutans / LI



(Figure 4.13) Lactobacillus / LI



CHAPTER 5

DISCUSSION

A. Previous Studies

The methods used in this study were similar to those found in other clinical studies of white spot formation in orthodontic patients (Artun and Brobakken, 1986; Bloom and Brown, 1964; Gorelick et al., Mattingly et al., 1983; Zachrisson and Zachrisson, 1971). Whereas these previous investigations examined either the clinical or the microbiological changes associated with the presence of fixed appliances, this study measured both clinical and microbiological parameters and determined their relationship to white spot formation in adolescents undergoing orthodontic treatment with banded/bonded appliances. Furthermore, the microbiological tests included identification of individual species of microorganisms.

Tooth surfaces with either a high or a low risk for white spot development, based on the findings of Gorelick et al. (1982) and others (Artun and Brobakken, 1986; Mizrahi, 1983), were selected. In this way longitudinal clinical and microbiological comparisons could be made between enamel surfaces which developed white spots and those which did not.

B. White Spot Prevalence

Over twenty percent of the incisors in the study developed a clinically detectable white spot lesion in the gingival one-third of the labial surface. The white spot prevalence was greater in upper laterals than in lower incisors (32% vs. 11%) which substantiates the results of Gorelick et al. (1982).

Differences in white spot prevalence could be attributed to various factors: 1) increased accessibility to salivary flow in the lower incisor region; 2) mesiogingival concavity in the upper lateral incisor; 3) upper incisor bonded before lower due to malocclusion with a deep overbite; 4) loops and auxiliaries usually located between lateral incisors and canines in the upper archwire, and 5) less effective plaque removal in the upper lateral incisor region (Artun and Brobaken, 1986; Gorelick et al., 1982).

C. Clinical Data and White Spot Development

The clinical variables differed very little between white spot subjects and non-white spot individuals. The DMFS/CI index tended to be slightly higher for subjects with a lower incisor white spot as well as for males with white spot formation in an upper lateral incisor.

Fluoride levels in the drinking water of those with white spot formation in a lower incisor actually exceeded the concentration below which supplementation is recommended. It should be noted that the fluoride exposure for each subject was determined from the measured concentration in their drinking water at home. A water sample was collected and analysed once and did not include other sources of water (school, work). Although the data seem to support the conclusions of Bowden et al. (1982), differences in the frequency of fluoride exposure among subjects may have accounted for the positive correlation between fluoride concentration and white spot formation in lower incisors.

There were slight increases in the mean values of the plaque index for subjects with white spot lesions. According to Dibdin

(1983), the diffusion characteristics of dental plaque influence the exchange of ions between enamel and the surrounding oral fluids. Other authors (Dawes and Jenkins, 1962; Gron, 1973) have reported that the ion concentration of plaque is normally supersaturated with respect to enamel apatite which allows the plaque to function as a remineralizing fluid for enamel. However, the relationship between plaque mass and the physiologic properties of plaque are unknown.

That there was no difference in daily exposure to an acid pH between the white spot and non-white spot groups most likely reflects the inaccuracy of diet records (Nikiforuk, 1985). Alternatively, the similarity in the mean values for DEApH (daily exposure to acid pH) may well indicate an increased buffer capacity and/or rate of sugar clearance in the saliva of subjects who did not form white spots (Dawes, 1983; Frostell, 1979).

D. Microbiological Data and White Spot Development

1. Saliva

A significant increase in salivary concentration of S. mutans had occurred by five months after placement of the fixed appliance. This supports the conclusions of Scheie et al. (1984) that fixed appliances may be associated initially with a drop followed by an increase in the salivary levels of S. mutans.

The stable levels of Lactobacillus over time contradict the results of other clinical studies (Bloom and Brown, 1964; Dikeman, 1962). However, the orthodontic appliance used in earlier studies consisted entirely of bands which may have provided a more suitable environment for Lactobacillus colonization than bonded brackets.

Comparison between the commercial and laboratory cultures indicated a positive correlation for the salivary concentration of S. mutans and Lactobacillus at the beginning and at the end of the study, respectively. This provides further evidence for the reliability of dip-slide tests in quantitating salivary levels of S. mutans and Lactobacillus (Crossner and Hagberg, 1977; Jordan et al., 1987). However, the colony counts were less on the commercial culture at both times which indicates that laboratory cultures are more accurate in determining the salivary concentrations of S. mutans and Lactobacillus.

2. Plaque

In this study, the isolation frequency of S. mutans and Lactobacillus were virtually the same for both groups of upper lateral incisors. However, the plaque levels of S. mutans increased whereas the mean percentage contribution of Lactobacillus to the total cultivable microflora remained unchanged in plaque from upper lateral incisors with white spot formation. Both the salivary concentration of S. mutans and the plaque index reflected this change in the number of S. mutans at site A after twelve months of orthodontic treatment with fixed appliances. Duchin and van Houte (1978) also reported a higher proportion of S. mutans in samples from incipient lesions. However, multiple plaque samples from a single lesion in their study tended to be quite heterogeneous with respect to the levels of S. mutans.

The similarity in isolation frequency between white spot and non-white spot sites in upper lateral incisors may have been due to sampling technique. Shlair et al. (1972) showed that the isolation

frequency of S. mutans was highest in plaque samples taken directly from the carious lesion and decreased in samples obtained from noncarious areas on the enamel surface. In the present study, areas known to be susceptible to white spot formation were sampled prospectively, and thus the sample sites may not have coincided in every case with the sites which developed white spot lesions.

Plaque from lower incisors which developed white spots showed an increased isolation frequency and contained particularly high levels of S. mutans shortly after orthodontic treatment began. Three of these incipient lesions appeared before the placement of a bonded bracket on the tooth and two were apparent one month into treatment. These findings indicate that white spot formation is associated with elevated levels of S. mutans as has been shown by others (Duchin and van Houte, 1978). At five months, a decrease in the S. mutans levels was accompanied by an increase in the numbers of Lactobacillus. The mean percentage of S. mutans and Lactobacillus in subsequent samples from lower incisors with white spot lesions increased and decreased presumably in response to successional changes (Alexander, 1971).

E. Assessment of White Spot Risk

The upper lateral incisor was identified as high risk for white spot formation and, as predicted, the mean percentage of S. mutans increased from low levels initially to high levels by nine months and higher levels still by twelve months. Almost sixty percent of upper lateral incisor white spot formation occurred in conjunction with these elevated levels of S. mutans.

An unexpected, and possibly more important, finding was the very high level of S. mutans initially in plaque from lower incisors which developed white spots. According to Gorelick et al. (1982), only about one percent of untreated lower incisors form white spot lesions. Artun and Brobakken's (1986) clinical study of demineralization around an edgewise appliance similarly reported that the mandibular incisors had a low risk of white spot formation. Although only five of the lower incisors studied developed white spots, almost one-half of the lesions were diagnosed during the period when S. mutans levels were elevated. Another interesting result was that more than one-third of the subjects with white spot demineralization in an upper lateral incisor had higher than average levels of S. mutans in plaque from the lower incisor.

These findings suggest that low risk rather than high risk sites on the enamel surface may provide more pertinent information regarding an individual's susceptibility to white spot development. Elevated levels of S. mutans in plaque reflect an imbalance in the microenvironment which may result in white spot formation. Based on the results from previous studies (Artun and Brobakken, 1986; Gorelick et al., 1982; Mizrahi, 1983; Zachrisson and Zachrisson, 1971), an increase in the salivary and plaque levels of S. mutans was predicted for the upper lateral incisors in subjects wearing an edgewise appliance. However, the salivary concentration and mean percentage of S. mutans in these subjects did not reach significant levels until the end of the study. As a matter of fact, at both one and five months the upper lateral incisor plaque levels of S. mutans were significantly greater in subjects without a white spot lesion

than in subjects who had developed a white spot. The lower incisors which underwent white spot formation, on the other hand, exhibited significantly higher levels of S. mutans early in the study. This increase in the levels of S. mutans on a tooth surface with low caries potential would seem to indicate that the individual has an increased susceptibility to white spot lesion development. The fact that forty percent of the subjects with lower incisor white spots also developed white spots in the upper lateral incisor tends to support this statement.

The low levels of Lactobacillus in both saliva and plaque weren't unexpected (London, 1976). Except for an increase in its mean percentage contribution in plaque from white spot lower incisors at five months, the plaque samples of Lactobacillus showed no measurable differences between incisors with and without white spots. This apparent similarity may be due to the fact that Lactobacillus has a low affinity for the tooth surface and is normally associated with lesion progression rather than caries initiation (Boyar and Bowden, 1985; Boyar et al., 1989; van Houte et al., 1972). A white spot which hasn't progressed to the cavitation stage of lesion formation would have essentially the same surface characteristics as a noncarious smooth surface (Silverstone, 1973).

F. Conclusions

1) Both clinical and microbiological measurements are essential in evaluating risk of white spot lesion formation.

2) Upper lateral incisors are more likely to develope white spot lesions than lower incisors in adolescents wearing an edgewise appliance.

3) Elevated levels of S. mutans are associated with white spot lesion formation.

4) Lactobacillus doesn't appear to be active in white spot development.

5) Low risk enamel surfaces may give a better indication of caries susceptibility than high risk surfaces.

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CONSENT FORM

I,_____ do hereby consent to participate in the experimental study conducted by Dr. C. Morin, Dr. R. Baker, Dr. G. Bowden and Dr. A. Milnes.

I understand that my gums and teeth will be evaluated for changes while I am wearing braces and samples of my plaque (soft deposits on the surfaces of the teeth) and saliva will be collected periodically during the study. The study will begin with my first appointment in the orthodontic clinic at the Faculty of Dentistry, University of Manitoba, and continue for approximately twelve months from the date that I receive my braces.

The nature and purpose of this study have been explained to me to my satisfaction.

I understand that I have the right to revoke this consent and discontinue my participation in the study at any time without penalty.

Signed:
(parent or guardian if participant is younger than 18 years of age)

Date:

Witness: