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The Inhibitory Effect of Saliva on the Adherence of Streptococcus Mutans and Streptococcus Sanguis

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THE INHIBITORY EFFECT OF SALIVA ON THE ADHERENCE OF

Streptococcus mutans and Streptococcus sanguis

by

Elliot H. Kimmel

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

April

1978

DEDICATION

To my wife and parents

ACKNOWLEDGMENTS

I would like to express my deep appreciation to the following people.

To Andrew M. Chludzinski, Ph.D. my friend and advisor who made research a lively educational pursuit.

To John V. Madonia, D.D.S., Ph.D. whose advice and confidence has sustained all my educational pursuits.

To Ioannis S. Scarpa, Ph.D. whose advice and interest are most appreciated.

To Jamie D. Nonnenmann, B.S. my close friend and fellow student who always had time and encouragement.

VITA

Elliot Kimmel was born in Boston, Massachusetts, February 13, 1947. He attended elementary and junior high schools in Baldwin Park, California. He graduated from Los Altos High School, Hacienda Heights, California, June, 1964. He received a Bachelor of Arts degree from California State University, Los Angeles in 1968 with a major in International Relations. He entered the graduate program in Oral Biology at Loyola University in 1974. He will graduate from Loyola University Dental School in 1980.

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CHAPTER I

INTRODUCTION

The etiological agent and the mechanism for the formation of the carious lesion has been studied since the nineteenth century. In recent years the cariogenic organisms involved in this process as well as specific dietary requirements have been described. Currently, investigators are attempting to understand the mechanism of cell adherence to smooth surfaces which is a primary requirement of the colonization on the tooth surface and plaque formation. Research concerning the pellicle formation, structure of enamel, cell surface binding sites, sucrose hydrolytic enzymes, as well as polysaccharide and saliva compositions are necessary to understand this adherence mechanism.

It is the purpose of this study to determine the in vitro effect of whole saliva and its individual fractionated components on the adherence of selected cariogenic streptococcal microorganisms to a smooth surface.

CHAPTER II

REVIEW OF THE LITERATURE

Dental caries is among the most prevalent diseases known to man.^{35,97} As early as 1883, Miller⁶¹ noted that in most cases, microorganisms play an important role in the formation of dental caries. It was concluded that the fermentation of carbohydrates and the subsequent synthesis of acids by these bacteria, was responsible for the decalcification of tooth enamel.⁶ McIntosh⁵⁷ in 1922 identified a variety of bacilli that were capable of producing sufficient acid to decalcify teeth.

The oral cavity contains many microorganisms with streptococci being predominant, especially in plaque.^{11,39} With the aid of a scanning electron microscope, Connor et al.,⁷ have demonstrated that the thickness of the plaque is related directly to the number of microorganisms present. They also found that the total streptococci increased proportionally with the thickness of the plaque. Loesche et al.,⁴⁷ have demonstrated that S. mutans represents an average of 19% of the cultivable oral flora, while 71% of carious fissures contained these organisms. They also found that 70% of the caries-free fissures were S. mutans free. Jordan et al.,³⁵ also found a similar relationship between S. mutans and plaque samples in a study of cariogenic plaque in South American natives. Streptococcus mutans is commonly found

at the site of occlusal fissures on the tooth surface and the plaque interface.^{22,27,34} Edman et al.,¹⁰ have shown that S. mutans is limited to the area of recovery and that there is little natural transfer of the organism. While Lactobacilli have been found in dental caries, Ikeda et al.,³⁴ note that it does not initiate but is rather preceded by S. mutans in caries initiation. Streptococcus sanguis is found extensively in plaque and is usually the first to colonize on the tooth surface.^{71,88-90,40,84,86} Streptococcus salivarius is found extensively on the tongue but is not a significant organism in dental plaque.^{27,88,89} Streptococcus mitis is found in 76-89% of the total cultivable flora from the cheek, lip, and ventral surface of the tongue. It adheres far better to the buccal mucosa than any other streptococcal species.⁴⁵

Conclusive evidence that dental caries result from the bacterial fermentation of carbohydrates was first obtained from experimentation with germ-free rats. As early as 1954, Orland⁶⁹ established that rats entirely free of microorganisms did not develop dental caries despite a caries-promoting diet. Fitzgerald¹⁶ confirmed this work and found that a carious lesion could be developed in germ-free rats with the inoculation of a single strain of oral streptococcus. In a more recent study of gnotobiotic hamsters, Fitzgerald¹⁵ observed that S. mutans HS-6 actively induced caries while S. sanguis and S. salivarius produced extracellular dextrans but were caries inactive.

Sucrose in the presence of cariogenic organisms is a key dietary component for caries and plaque formation. Investigation has shown that plaque cannot be initiated by replacing sucrose with glucose, fructose, galactose, lactose, sorbitol, or a mixture of glucose and fructose.^{17,36,95} Gibbons and Van Houte²⁶ corroborated that while S. mutans can metabolize other sugars, only the hydrolysis of sucrose and the subsequent formation of dextran polymers will effect attachment. Gold²⁸ also demonstrated that sucrose is a major requirement for decay by showing that its removal from the diet effectively reduced caries.

Cariogenic streptococci produce a constitutive extracellular enzyme, dextransucrase or glucosyltransferase (GTF) which is capable of hydrolyzing sucrose.^{21,55,73,95} The enzyme hydrolyzes sucrose and synthesizes the glucose polymer dextran.^{26,24,67,21,60} The synthesis of the dextran polysaccharide from sucrose is a major contributor to plaque, accounting for 10% of its dry weight and 70% of its volume.^{95,44,63} Both soluble and insoluble dextrans are formed with primarily α -(1-6) and α -(1-3) linkages respectively.^{9,20,64} Ebisu et al.,⁹ have indicated that the insoluble property of the dextran polysaccharide is due to the linear α -(1-3) linked backbone to the polymer. There have been reports of the existence of multiple GTF activities.^{43,38} Ciardi et al.,⁵

have resolved GTF into two major fractions. They proposed that one enzyme would function for the production of water soluble glucan and the other for the formation of the insoluble glucan. Chludzinski et al.,⁴ have further corroborated that one form of the enzyme forms the α -(1-6) and another form the α -(1-3) linkages.

The ability of cariogenic organisms to aggregate as well as adhere to a variety of surfaces is dependent on the formation of the dextran polymer.^{9,23,24,59} Gibbons and Fitzgerald²³ have shown that S. mutans is very sensitive in that it will agglutinate in the presence of sucrose with a very small amount of high molecular weight (2×10^6) dextran. It is postulated that the process requires a specific receptor site on the surface of the organism which can bind dextran molecules. Agglutination occurs when the dextran molecules are large enough to accommodate many organisms simultaneously to produce aggregate forms. It is generally accepted that the coating of dextran on a surface increases the adherent ability of cariogenic streptococci.^{42,91,60} Researchers have also noted that the selective ability of certain streptococci to adhere preferentially to both saliva-coated and dextran-coated hydroxyapatite.^{33,46} Gibbons and Banghart²¹ also showed that this polysaccharide can bind to both untreated and saliva-treated hydroxyapatite.

The mechanism for adherence is similar to that of aggregation in that the organisms attach to the polysaccharide which binds to

the hydroxyapatite.²¹ McCabe⁵³ proposed that the mechanism of aggregation and adherence involved the same glucan receptor site. He postulated that GTF did not mediate the binding of cells to glucan, but rather the glucan binds directly to the cell binding site for both aggregation and adherence. Mukasa and Slade⁶² suggest that the requirements for aggregation and adherence are different since heat-treated cells, in the presence of dextran, will adhere but will not aggregate. They hypothesized that the binding sites required for adherence remained intact while those sites necessary for aggregation were destroyed. The nature of these binding sites necessary for adherence might be proteinaceous in that trypsin interferes with the adherence process. Liljemark and Schauer⁴⁶ demonstrated that trypsin treated S. sanguis had a reduced ability to adhere to hydroxyapatite and that S. mutans was similarly unable to adhere well to dextran coated hydroxyapatite. Mukasa and Slade^{63,81} suggest that trypsin remains within the cell in an active state and inhibits the synthesis of the polysaccharide or inhibits another factor associated with the adsorption of the enzyme.

The adherence of bacteria in the oral cavity appears to be selective and is probably determined in part by the cell surfaces and the acquired pellicle. The acquired pellicle is an acellular, bacteria-free structureless layer (0.1 to several microns thick) deposited after tooth eruption and is largely derived from the

selective adsorption of salivary glycoproteins.^{26,41,44,83} Among the more important constituents of the pellicle are the proteins IgA, lysozyme, amylase, and virus inhibiting glycoproteins.^{70,74,94} Sonju and Rolla⁸³ reported that there were no apparent differences among the pellicles of the upper molars, upper incisors and the lower anterior teeth. The only essential factor for the formation of the pellicle is the presence of saliva.⁵⁸ The pellicle consists of salivary glycoproteins which attach their free carboxyl groups to the calcium ions of the hydroxyapatite.⁷⁴ Gibbons and Van Houte²⁶ observed that calcium ions are specific for certain salivary components which adsorb to bacterial surfaces. They suggest that calcium ions are involved in the matrix formation and cohesiveness of plaque. It is the acquired pellicle that enhances microorganisms via the cell surfaces to attach and aggregate to form the organic plaque matrix.^{44,71} Orstavik et al.,⁷¹ demonstrated that low numbers of S. sanguis, S. mutans, and S. salivarius attach to uncoated enamel, however, in the presence of the acquired pellicle, the attachment of all strains tested was enhanced.

Considerable interest in the components of saliva has been generated in the last twenty years. In an early study on the separation of saliva by electrophoresis, Kinersly³⁷ reported that most of the organic constituents of saliva were proteinaceous in nature with mucin as the major component. Geller¹⁸ separated saliva by electrophoresis into six components. The three major

bands were identified as mucoid, albumin, and β -globulin, while three minor bands were found to be α -globulin, lysozyme and sialoprotein. Among the fractions consistently separated from parotid saliva by Mandel and Ellison⁵² were albumin, α -globulin, β -globulin, γ -globulin, amylase, and glycoproteins. Fisher et al.,¹⁴ found that by dialyzing or electro-dialyzing out amylase and ions, they were able to separate whole saliva into 13 protein bands. Ericson¹² separated the glycoproteins of saliva into several fractions by hydroxyapatite chromatography. He found that glycoproteins rich in sialic acid adsorbed well to the hydroxyapatite possibly implicating these proteins in pellicle formation. Ben-nick and Connell² utilizing electrophoresis found 30-40 protein bands with amylase being the most prominent. Shrager and Oates^{78,77} characterized a principal glycoprotein in saliva and found blood group specificity as well as the characteristic amino acid sequence and content.

Gibbons and Spinell³⁰ have found a specific factor in whole saliva that causes the aggregation of oral bacteria. This factor is a glycoprotein which adsorbs to the hydroxyapatite surface. It has a high molecular weight and a non-dialyzable component which can be isolated from dental plaque. They also found that this factor was not IgG and that its agglutinating activity was diminished more in phosphate buffer than in acetate buffer.²⁵ McGaughey and Stowell⁵⁶ have noted that phosphate ions in saliva inhibit sal-

ivary proteins from adsorbing to hydroxyapatite and that the adsorption activity is restored with the extraction of phosphate.

It has been reported that whole saliva or salivary components have the ability to inhibit adherence. Orstavik et al.,⁷¹ found whole saliva to inhibit the adherence of S. salivarius and S. mutans to the pellicle while the effect on S. sanguis was variable. Olson⁶⁵ noted that the antibacterial agents in saliva include lysozyme, lactoferin, antibodies, and salivary peroxidases. The adherence inhibition observed from saliva was dependent upon both dialyzable and non-dialyzable molecules.⁶⁵ Although the components of adherence inhibition have not yet been identified, the activity, according to Olson is heat labile and does not stimulate an antibody formation. There was also an absence of adherence inhibition in the IgA fraction of an active oral secretion.

Another factor which may be involved in plaque formation are the extracellular dextranases produced in the oral cavity. Some investigation has been done with dextranases as a method to enzymatically reduce plaque formation. Dextranase has been isolated from plants, soil, Bacteroides, Penicillium, intestinal and oral organisms.^{8,31,32,72,79} From the oral cavity, many strains of streptococci have been isolated which are able to produce α -(1-6)-glucan glucohydrolases.^{8,29,85} When dextranase is incubated with GTF of S. mutans and S. sanguis, a reduction in the dextran synthesis or α -(1-6) branch formation was observed.^{8,9} The dextran polymer is more resistant to hydrolysis with increas-

ing amounts of branching, thus, other linkages found in dextran such as α -(1-2), α -(1-3), and α -(1-4) are resistant to the α -(1-6)-glucan glucohydrolases. Recent studies suggest that dextranase inhibits polymer production by blocking the formation of the initial α -(1-6) linkages, thereby also reducing adherence.^{17,46,76}

CHAPTER III

METHODS AND MATERIALS

Maintenance of cultures:

Streptococcus mutans strains HS-6, 6715, BHT, AHT, GS-5, OMZ-176, and Streptococcus sanguis OMZ-9 were obtained from Dr. Hutton D. Slade, Department of Microbiology, Northwestern University. All organisms were grown anaerobically in Trypticase soy broth (BBL) supplemented with 2% glucose, 0.5% yeast extract, and 0.1 M potassium phosphate buffer pH 6.8. Unless otherwise stated, all buffers used in this work are phosphate buffers, pH 6.8. After 18 hours growth at 37C, the cells were centrifuged (Beckman Model J-21 Centrifuge, Beckman Instruments, Inc., Palo Alto, Calif.) at 11,400 x g at 4C for 20 minutes. The same centrifugation procedure is used throughout this work except where noted. After centrifugation, the cells were re-suspended in a storage medium composed of equal volumes of defibrinated sheep blood and 3% Todd Hewitt broth (BBL). The cultures were rapidly frozen in dry ice and acetone and stored at -52C (Revco Ultra-Low Temperature, Revco, Inc., West Columbia, S.C.).

These organisms were also maintained as lyophilized (Virtis Freeze-Mobile Model #10-140BA, Virtis Co., Gardiner, N.Y.) cultures in 10% skim milk (BBL) and stored at -52C. Cultures for

routine use were maintained on Mitis Salivarius (Difco) plates in an anaerobic jar (10% H₂, 10% CO₂, 80% N₂) at 37C.

Collection and Preparation of Saliva:

Saliva was collected on two separate occasions. Saliva sample I consisted of 210 ml of paraffin-stimulated and non-stimulated whole saliva which was collected and pooled from approximately 35 students. This saliva was filtered with Whatman #1 filter paper, centrifuged, lyophilized, and stored at -52C. This saliva was reconstituted to one tenth its original volume in 0.01 M buffer for adherence assays and fractionation.

Saliva sample II consisted of 610 ml of paraffin-stimulated whole saliva pooled from 135 dental students. The saliva was filtered, centrifuged, lyophilized, and reconstituted in the same manner as the first sample. The protein content for both samples was determined by Lowry method using bovine serum albumin (Sigma) as a standard.⁴⁸

Isoelectric Focusing:

A 2.5 ml aliquot (40.3 mg of protein/ml) of reconstituted saliva sample I was incorporated into a 10%-69% sucrose density gradient in a 100 ml capacity LKB isoelectric focusing (IEF) columns. A pH gradient of 3.5 to 10 was established using 1% (vol/vol) carrier ampholytes (LKB, Stockholm, Sweden). The protein was focused at 300 V for 48 hours, after which 2 ml fractions

were collected. The pH of each fraction was determined by means of a micro pH electrode. The protein elution profile was obtained by measuring each fraction in a Beckman spectrophotometer (Beckman Model DB-GT, Fullerton, Calif.) at 280 m μ . The fractions were dialyzed against three, two liter changes of 0.10 M buffer for 24 hours. All fractions were preserved at -52 C.

Isoelectric focusing was also performed on a 2.0 ml aliquot (57.1 mg of protein/ml) of reconstituted salivary sample II. The procedure was the same as described for sample I except a pH gradient of 2.0 to 4.0 was utilized. This protein was focused at 350 V for 46 hours and 1.5 ml fractions were collected. Determination of pH and the protein separation profile, as well as dialysis and storage of each fraction were performed as described for sample I.

Preparation of Cells for Adherence Assay:

Cells were grown 18 hours anaerobically in Todd Hewitt broth (BBL) supplemented with 1.8% glucose, 0.3% NaCl, 0.8% NaHCO₃, and 0.15% Na₂HPO₄.⁶³ Cells used for the adherence assay were consistently grown in this THG media unless otherwise stated. Labeled cells were grown as described above in the presence of [³H]-thymidine (2.4 uCi/ml, New England Nuclear Corp., Boston, Mass.). The bacteria were collected by centrifugation, resuspended and washed twice in an equal volume of 0.05 M buffer to an optical density of 0.7 at 540 nm (Coleman Junior II, Spectrophotometer Model #6/35).

Other media used for the growth of labeled cells were as follows: TGY consisted of 0.5% yeast extract, 2.5% glucose solution, 3% trypticase, 1.0 mM magnesium sulfate, 1.0 mM manganous sulfate, 0.1 mM ammonium sulfate, 3.2 mM cysteine hydrochloride, and 0.1 M potassium phosphate buffer; TSGYF consisted of 3% Trypticase soy broth (BBL), 0.5% yeast extract, 9% fructose, 2% glucose, and 0.1 M potassium phosphate buffer; THF consisted of 3% Todd Hewitt and 9% fructose.

Radioactive Adherence Assay:

The assay mixture contained 4 μ l of 1% merthiolate, 0.08 ml of the radiolabeled cell suspension, 0.08 ml of 5% sucrose (when indicated), 0.1 ml salivary sample (when used) and 0.05 M buffer to give a final volume of 0.5 ml. The salivary samples were usually diluted 1:2 with 0.05 M buffer except where noted. The solution was gently mixed and then incubated aerobically at 37 C for 8 hours. After incubation, the unattached bacteria were removed by tilting the vials and carefully aspirating off the assay mixture. Residual free bacteria were removed by washing the vials with three 2.0 ml aliquots of 0.05 M phosphate buffer. The buffer was slowly added down the side of the vial and then gently rotated on a flat surface. The wash was removed by careful aspiration.⁷⁶ The vials were dried under a heat lamp and 10 ml of scintillation fluid (100 mg of 1,4-bis-2(5 phenyloxazoly1)-benzene, and 4 g 2,5-diphenyloxazole per liter of toluene)⁷⁵ was added to each vial.

Adherence was estimated by measuring the tritiated thymidine which remained in the vials after washing, with a liquid scintillation counter (Packard Tri-Carb Liquid Scintillation Spectrometer, Model #3320, Zurich, Switzerland.). All vials were cleaned by soaking in a detergent solution for 24 hours after which they were immersed in chromic acid for 3 minutes. The vials were then soaked again in detergent for 24 hours and thoroughly washed and dried.

Trypsin Treatment of Cells:

Radiolabeled S. mutans HS-6 and S. sanguis OMZ-9 were treated with trypsin (1 mg trypsin/ 5 mg cells⁶³) for 3 hours at 24 C. After the trypsin treatment, cells were centrifuged and washed three times with 0.05 M buffer.

Heating of Cells and Salivary Samples:

All heat treatments of salivary samples or cells were performed in sealed tubes at 100 C for 20 minutes.

Immunodiffusion Test of Salivary Samples:

The adherence inhibiting salivary fractions from saliva sample II were pooled (fractions #58-67) and tested on immunodiffusion plates (Hyland, Travenol Laboratories, Inc., Costa Mesa, Calif.) for the presence of IgG, IgA, IgM, and albumin.

Chemicals:

Potassium phosphate (monobasic and dibasic), sodium hydroxide, sodium chloride, glucose, sucrose, sodium phosphate, and ammonium

sulfate were obtained from Mallinckrodt Chemical Works, St. Louis, Mo. Yeast extract and trypticase were purchased from BBL, Cockeysville, Md. Dextran T-10 was obtained from Pharmacia Fine Chemicals AB, Uppsala, Sweden. Fructose and sodium carbonate were purchased from Matheson Coleman and Bell, Norwood, Ohio. POPOP was obtained from Amershan/Searle, Des Plaines, Ill. PPO and mangamous sulfate were purchased from J. T. Baker Chemical Co., Phillipsburg, N.J. Trypsin was acquired from General Biochemicals, Chagrin Falls, Ohio. Magnesium sulfate and phenol reagent were obtained from Fisher Scientific Co., Fair Lawn, N.J. Merthiolate was purchased from Eli Lilly and Co., Indianapolis, Ind. and BSA was acquired from Sigma Chemical Co., St. Louis, Mo.

CHAPTER IV

RESULTS

In order to properly evaluate the effects of saliva on the adherence of streptococci to smooth surfaces, it was necessary to develop an accurate, reproducible assay. A radioactive cell adherence assay was designed to determine the optimal parameters for adherence. Adherence was measured by determining the relative number of radioactively labelled streptococci that attached to glass scintillation vials under varying conditions. All vials contained merthiolate to inhibit growth of cells in the assay vials.

A primary requirement for the adherence of streptococci to smooth surfaces and plaque formation is the presence of sucrose as a substrate for extracellular dextran polymer formation. Optimum adherence for S. sanguis OMZ-9 and S. mutans HS-6 was assayed at sucrose concentrations varying from 0.5% to 10%. It was determined that 0.5% and 1.0% sucrose concentrations resulted in maximal adherence for S. sanguis and S. mutans respectively (Fig. 1&2). Higher concentrations of sucrose were found to be slightly inhibitory to cell adherence.

In addition to the optimum concentration, it was necessary to determine the time of incubation for the assay. Adherence of S. mutans HS-6 and S. sanguis OMZ-9 was measured at various time

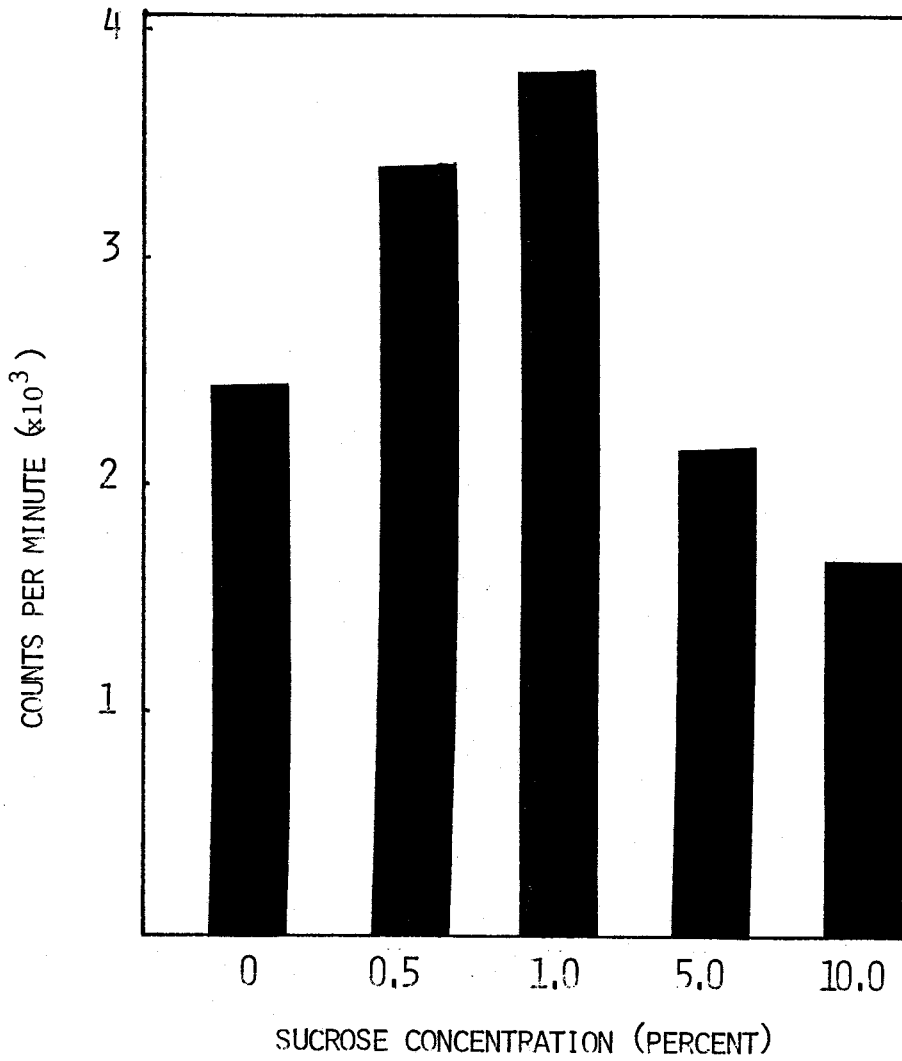


FIGURE 1 Effect of sucrose concentration on adherence of S. mutans HS-6.

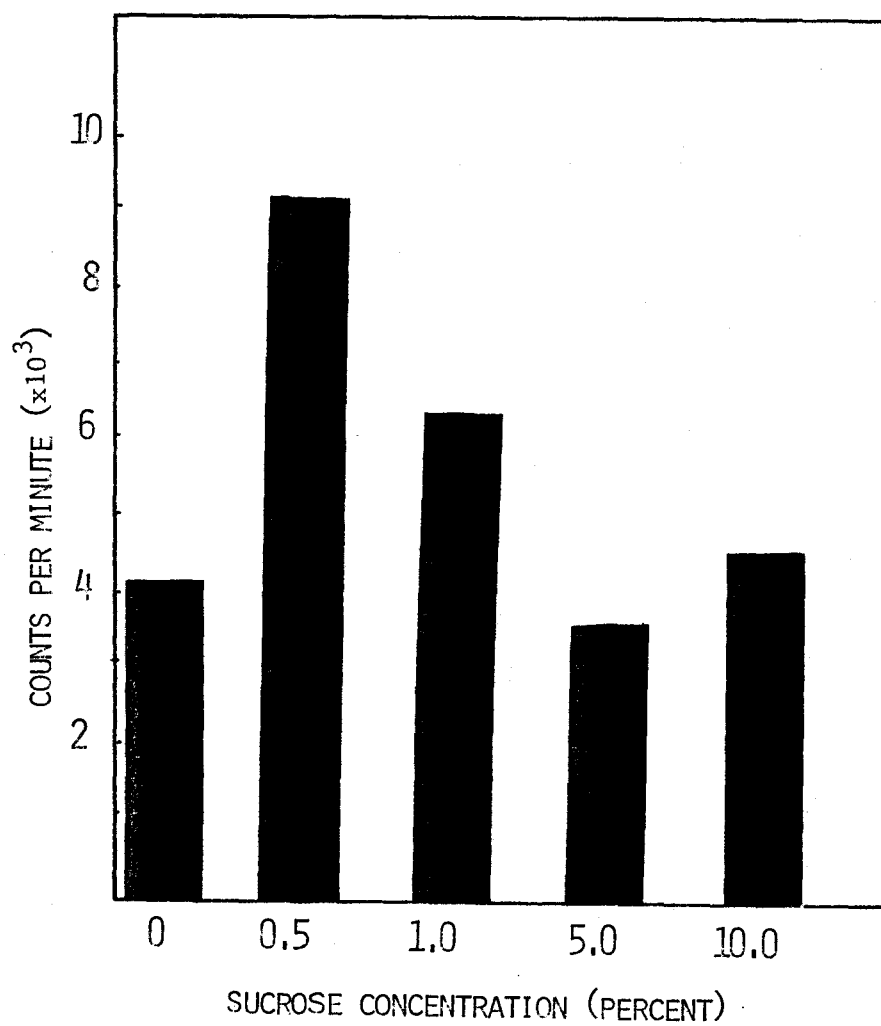


FIGURE 2 Effect of sucrose concentration on the adherence of *S. sanguis* OMZ-9.

intervals during a 16 hour incubation period (Fig. 3). Streptococcus mutans showed very little adherence in the first two hours of incubation, however, this was followed with rapid binding of cells to glass which continued to increase even at 16 hours, however, S. sanguis demonstrated a consistent increase in adherence with maximum attachment occurring at 12 hours.

Whole saliva was tested next to determine if this complex mixture of proteins could stimulate or inhibit cellular adherence. The effect of whole saliva (sample I) on adherence was determined for S. mutans strains HS-6, 6715, BHT, AHT, GS-5, OMZ-176, as well as S. sanguis OMZ-9. Whole saliva was observed to be effective in reducing adherence of all strain tested (Fig. 4). This unfractionated saliva inhibited the adherence of S. mutans HS-6 by 71% and S. sanguis OMZ-9 by 86%. The remaining S. mutans strains were inhibited as follows: 6715, 93%; OMZ-176, 79%; GS-5, 55%; AHT, 50%; BHT, 2%.

Since whole saliva demonstrated the ability to inhibit cell adherence (Fig. 4), it was necessary to separate and characterize the components involved in this process. In order to obtain partially purified adherence inhibiting salivary components, whole saliva was fractionated by isoelectric focusing (IEF). This technique was utilized in order to separate salivary proteins by their overall charge in a pH gradient. Proteins differing by only 0.02 units in their isoelectric point could be separated by this method.¹

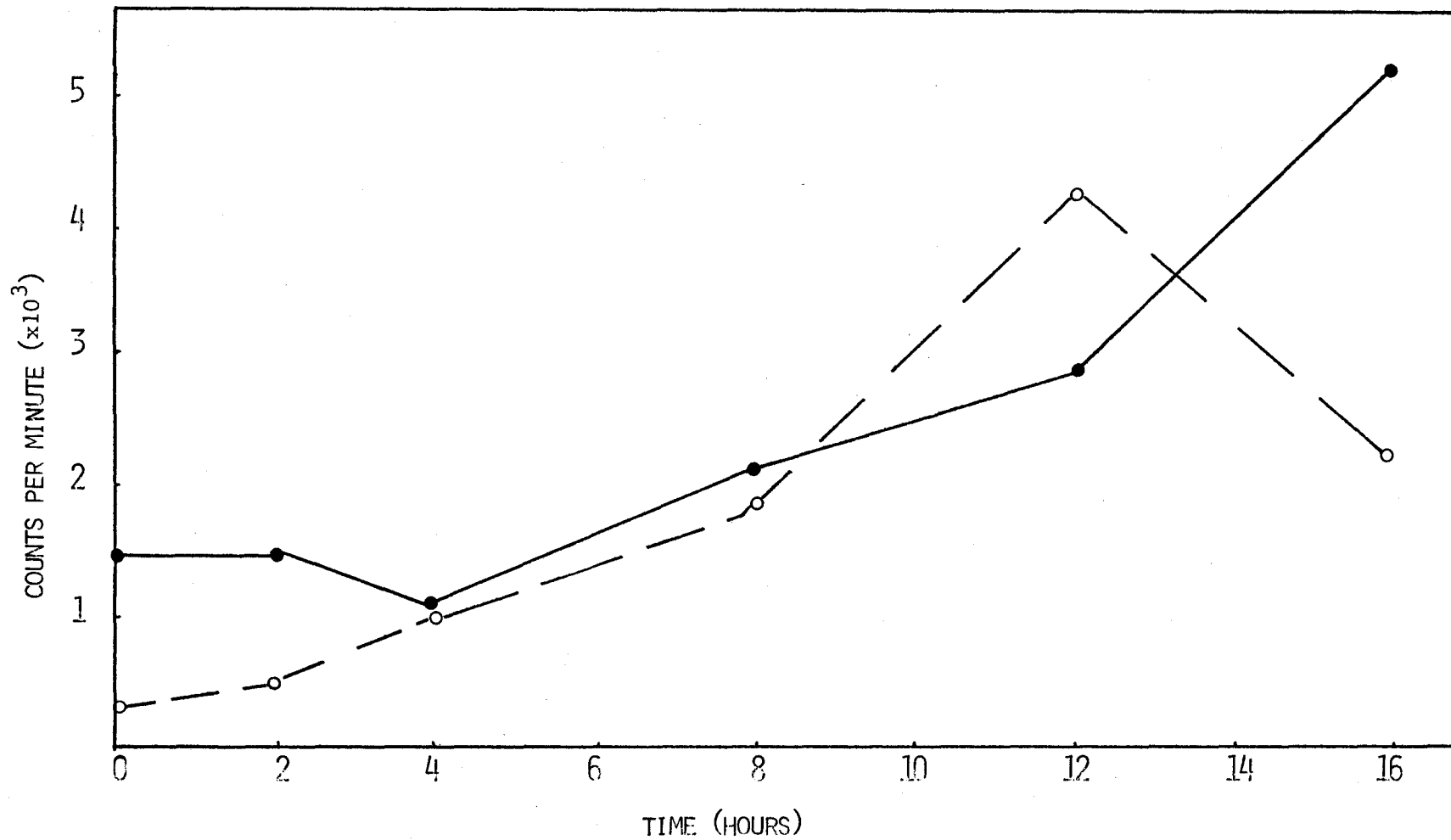


FIGURE 3 Effect of incubation time on the adherence of *S. mutans* HS-6 and *S. sanguis* OMZ-9. Cells were cultured in THG and adjusted to 0.95 at 540 nm. Symbols: (●), *S. mutans* HS-6; (○), *S. sanguis* OMZ-9.

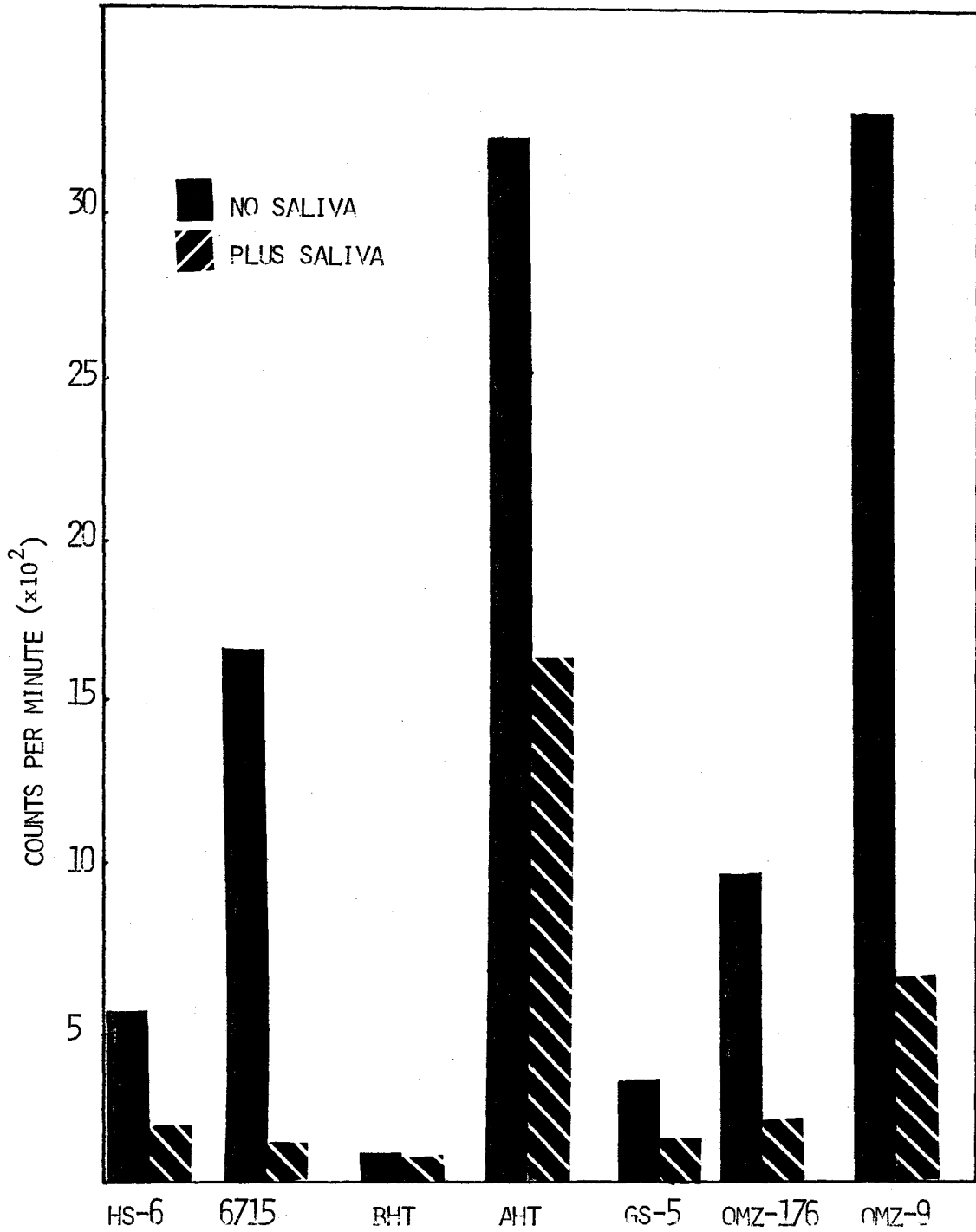


FIGURE 4 Effect of whole saliva I on adherence of streptococcus strains. Optical density of each washed culture was adjusted to 0.44 at 540 nm.

Figure 5 demonstrates the protein separation profile and the adherence inhibition activities of the salivary components of saliva sample I as fractionated by IEF. After separation, these fractions were eluted and measured for protein content (optical density at 280nm), pH and inhibitory properties. Most of the protein, as measured by absorption at 280nm appeared to be anionic in charge focusing below pH 7. These fractions were tested for their inhibitory effect on the adherence of S. sanguis OMZ-9, which was found to be significantly inhibited (86%) by those saliva fractions focusing between pH 1 and 3. The remaining fractions inhibited adherence at an average background level of 25%.

To further separate the inhibitory fractions, a narrower pH gradient (pH 2.0 to 4.0) was employed on salivary sample II. Figure 6 illustrates the IEF separation profiles of this salivary sample. Most of the protein in this sample focused between pH 3 and 5 with two minor peaks of protein below pH 3. This fractionated saliva sample II exhibited maximum adherence inhibition between pH 4.5 and 5.25, while the remaining fractions exhibited little or no inhibitory effect. The adherence inhibiting fractions (#56 thru #71) were then pooled, dialysed, and used for further adherence studies.

In order to determine if this adherence inhibition factor was an immunoglobulin, an immunodiffusion technique was utilized. The fractionated saliva sample II was tested for human IgA, IgM,

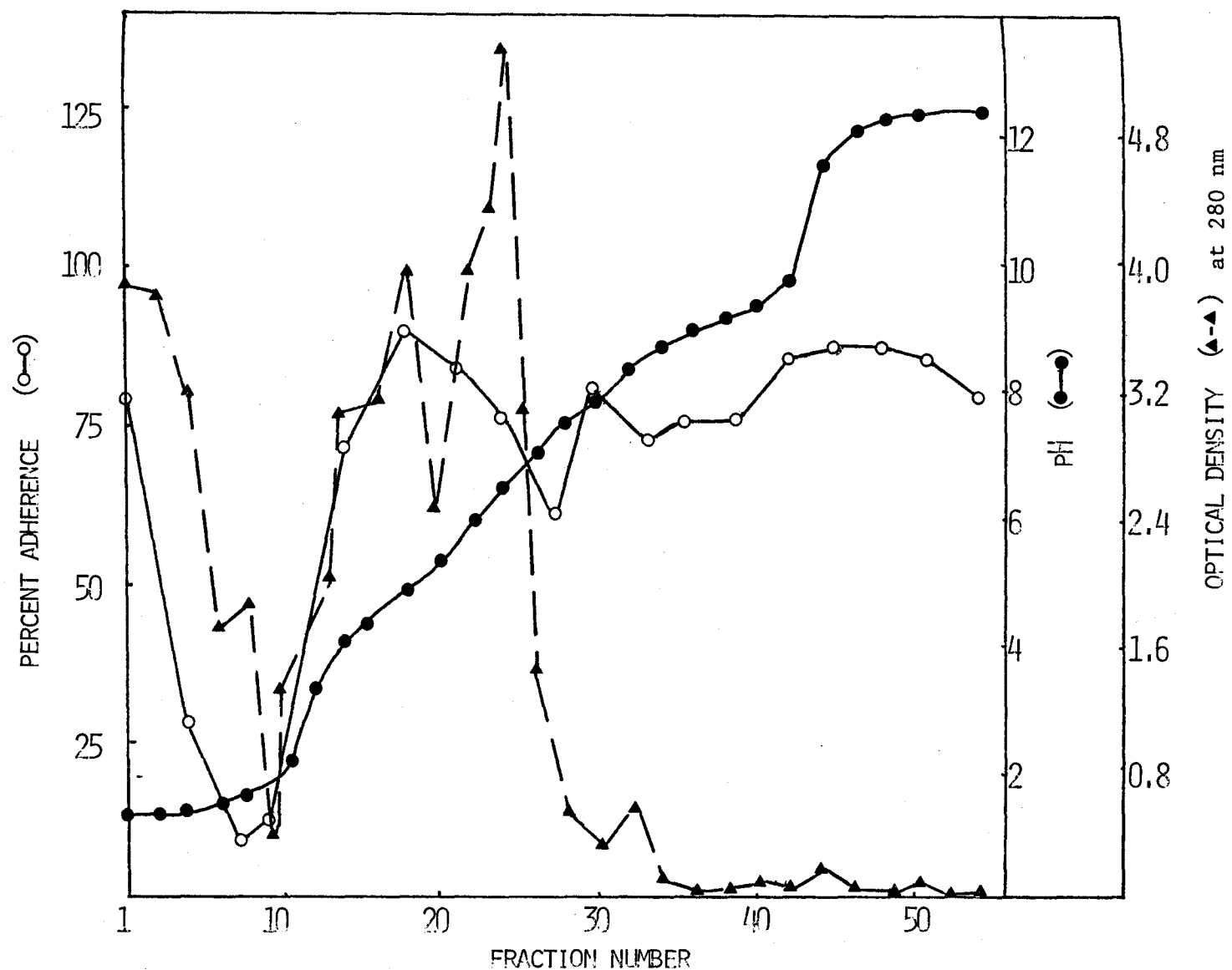


FIGURE 5 Isoelectric focusing of saliva sample I. The sample was focused in an ampholine pH gradient of 3.5 to 10. Fractions were tested for their ability to inhibit adherence as described in Materials and Methods using *S. sanguis* OMZ-9.

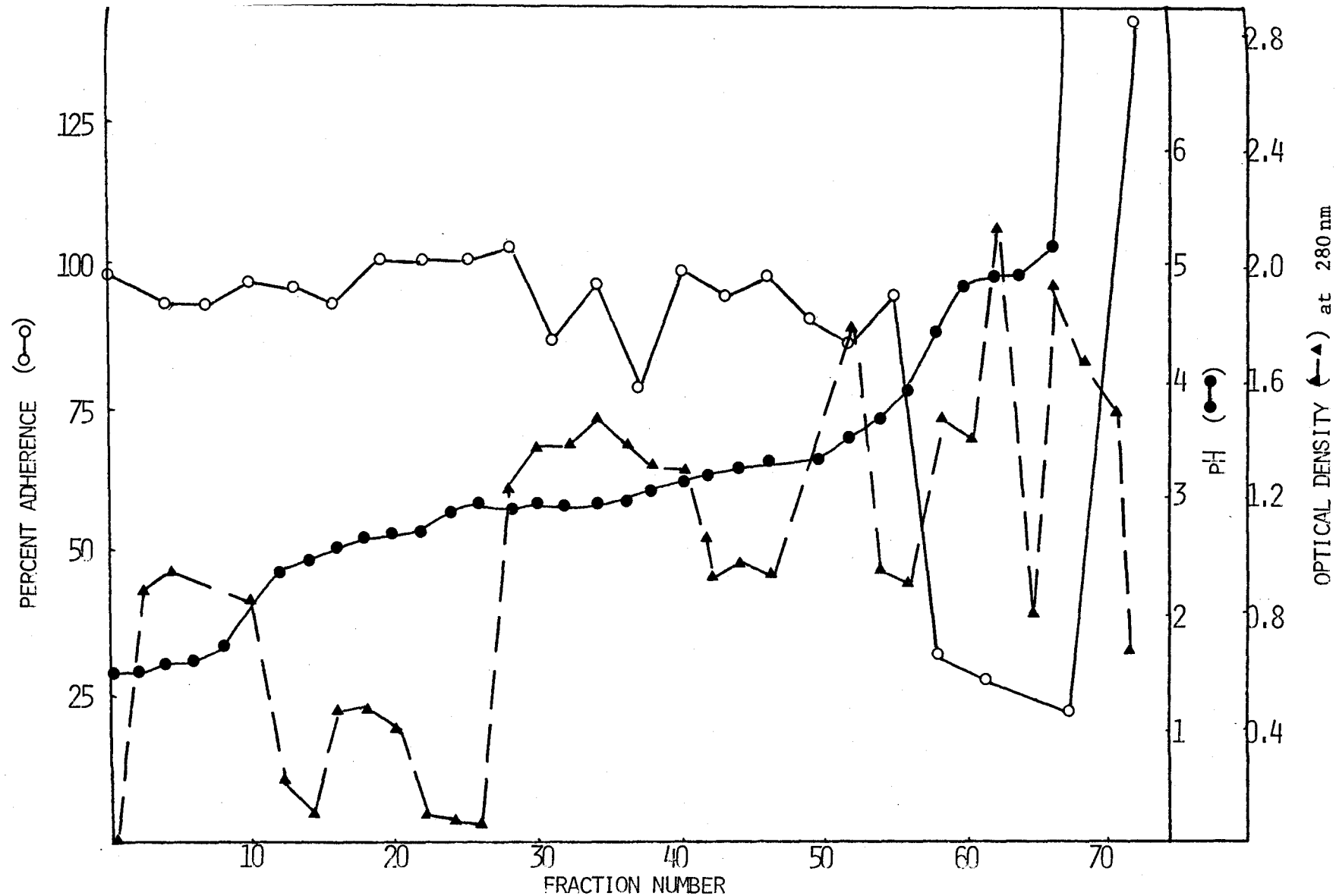


FIGURE 6 Isoelectric focusing of saliva sample II. The sample was focused in an ampholine pH gradient of 2.0 to 4.0. Fractions were tested for their ability to inhibit adherence as described in Materials and Methods using *S. sanguis* OMZ-9.

IgG and human albumin components on immuno-plates. In comparison to known immunoglobulins and albumin, the saliva fraction was shown to contain some human albumin (Table 1).

In order to determine the effect of the concentration of the inhibitory component on adherence, an assay was prepared with various dilutions of the isoelectric focused (Fig. 6) inhibitory fraction #59 as shown in figure 7. It was found that adherence of S. sanguis OMZ-9 was maximally inhibited (72%) in the presence of the undiluted saliva (0.8mg protein per ml) fraction while a two fold dilution resulted in a 57% inhibition. Adherence inhibition was observed to decrease exponentially over a hundred-fold decrease in concentration, with a 16% enhancement of adherence at the highest dilution.

In an effort to determine the nature of the adherence inhibiting material, salivary proteins were heated and the adherence inhibition was assayed both in the presence and absence of sucrose (Fig. 8). Bovine serum albumin (BSA) at a final concentration of 1mg per ml was assayed under the same conditions as a non-specific effector of cellular adherence. Figure 8A illustrates that whole saliva as well as the fractionated sample II are effective in inhibiting adherence of S. mutans in the absence of sucrose (96% and 59%). Heating the whole saliva sample did not appreciably affect adherence, however, the fractionated sample exhibited 13% more inhibition. Bovine serum albumin inhibited adher-

		COMPONENT ADDED				
		IgA	IgM	IgG	HUMAN ALBUMIN	FRACTION SALIVA SAMPLE II
IMMUNO-PLATE	IgA	2.5	2.5	3.0	0.0	0.0
	IgM	0.0	5.0	3.0	0.0	0.0
	IgG	0.0	3.3	3.5	0.0	0.0
	HUMAN ALBUMIN	0.0	0.0	0.0	1.5	5.0

TABLE 1 Immunologic relation between saliva sample II and Immunoglobulin A, G, M, and Human Albumin; (measured in mm.).

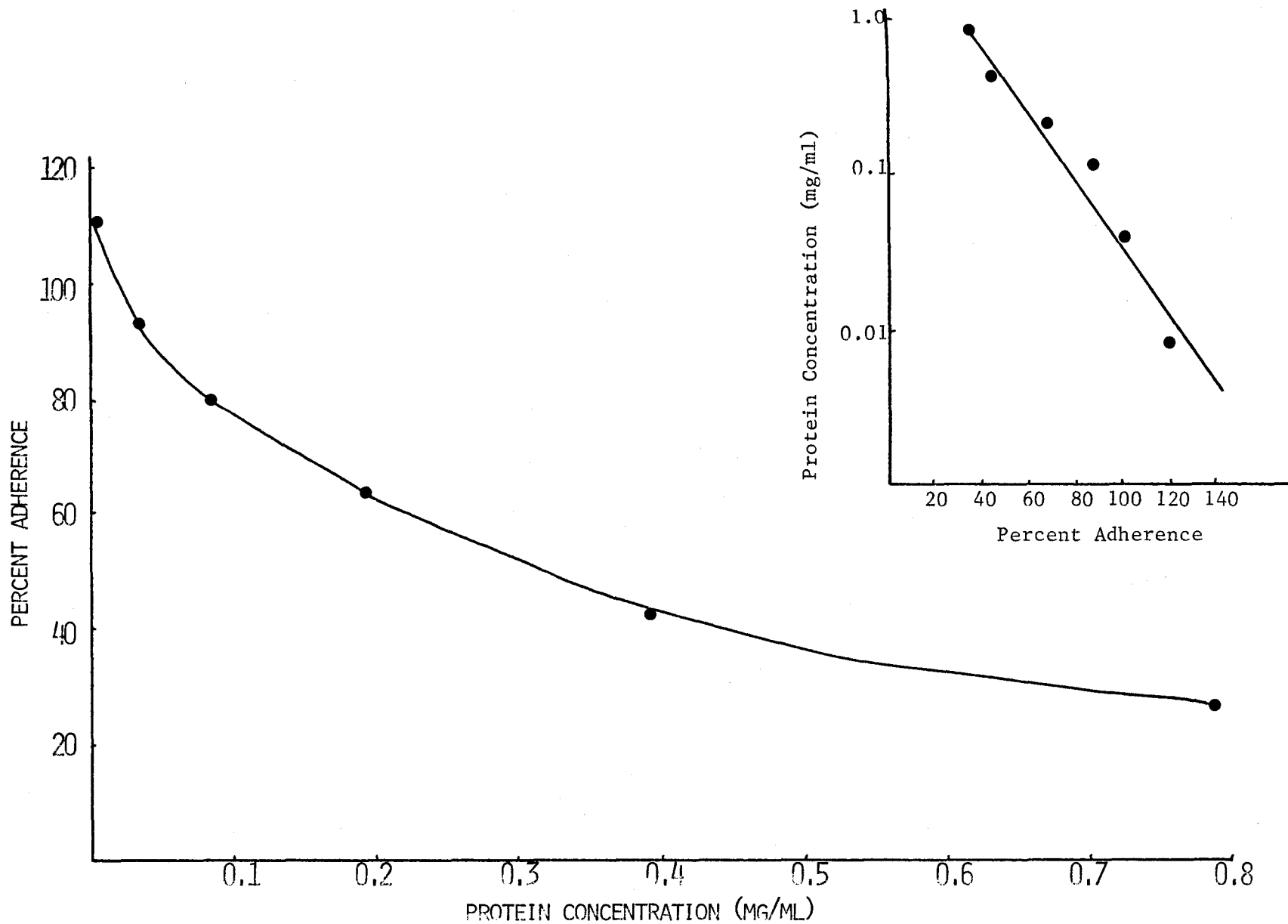


FIGURE 7 Effect of saliva protein concentration on adherence of *S. sanguis* OMZ-9. Assay contains 1.0% sucrose and salivary sample II (fraction #59). Inset, logarithmic plot.

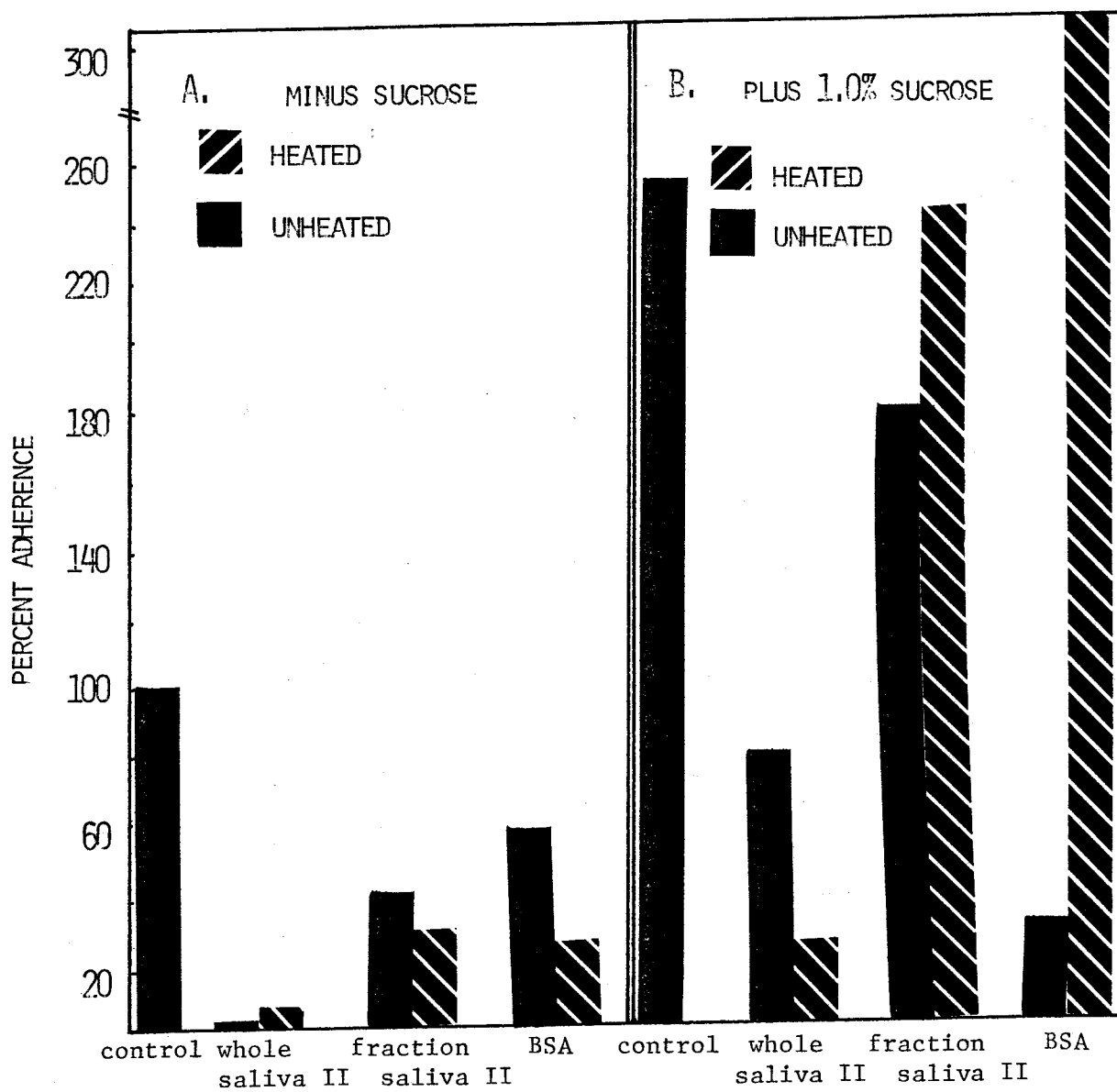


FIGURE 8 Adherence of *S. mutans* HS-6 in the presence of heated and unheated salivary protein.

ence by 43%, but the heated protein inhibited adherence by 75%. Figure 8B illustrates the same assay conditions with the addition of sucrose. Under these conditions, whole saliva was not an effective inhibitor of adherence unless heated. On the other hand, the fractionated saliva stimulated adherence by 179%, while heating this sample increased adherence by 151%. Bovine serum albumin decreased adherence by 70% but enhanced it by 218% when heated.

The effect of whole and fractionated saliva on adherence (*S. sanguis*) was also tested under the conditions described above for *S. mutans*. In the absence of sucrose (Fig. 9A), adherence was inhibited by whole saliva (96%) as well as the fractionated salivary sample II (74%). Heating these samples further increased adherence. Bovine serum albumin proved to be 70% inhibitory only when heated. In the presence of sucrose (Fig. 9B), the adherence of *S. sanguis* was greater in all assays, especially with the fractionated saliva and BSA. Whole saliva inhibited adherence by 96% while the fractionated sample enhanced it above the control without sucrose. The heating of the fractionated sample enhanced adherence by 199% while the heating of the whole saliva remained unaffected. Bovine serum albumin inhibited adherence by 265% as compared to the sucrose control, however, the inhibiting activity was increased by 206% after the heat treatment.

Cells were also cultured in a variety of different media in an attempt to determine if nutritional growth conditions had

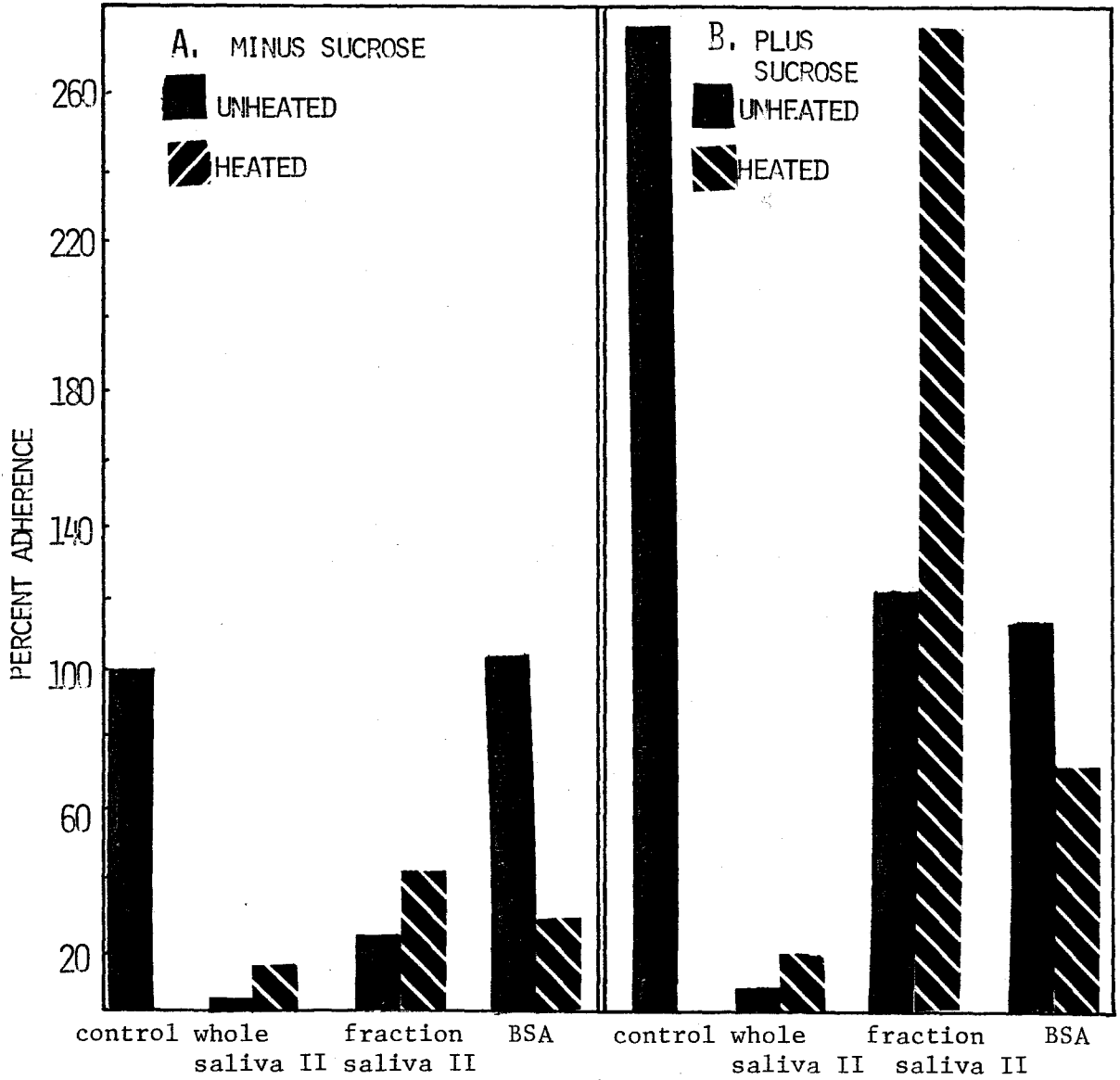


FIGURE 9 Adherence of *S. sanguis* OMZ-9 in the presence of heated and unheated salivary protein.

any effect on the ability of the cells to adhere to glass surfaces. The growth and adherence of S. mutans (Table 2) cultured in various media (Todd-Hewitt-fructose, THF; trypticase-glucose-yeast, TGY; trypticase soy broth-glucose-yeast-fructose, TSGYF) were compared to the normal growth medium (Todd-Hewitt-glucose, THG). The control conditions utilized THG grown cells assayed in the absence of sucrose. Viable THG grown cells adhered maximally in the presence of sucrose (171%) and minimally when boiled and assayed in the presence of sucrose (62%). Viable and non-viable S. mutans adhere maximally (192% and 219%) in the presence of sucrose when cultured in TSGYF. Cells grown in TGY adhere the least under both viable and non-viable conditions. The presence of sucrose in the assay with TGY grown cells increased adherence slightly for both the viable and non-viable assays. The presence of sucrose did not significantly effect the adherence of viable cells grown in THF, however, non-viable cells with sucrose adhered 62% more than the same assay without sucrose. In general, cells grown in TSGYF demonstrated the greatest adherent capabilities while those cells cultured in TGY demonstrated the least. Cells grown in THF consistently demonstrated less adherence than the control with the exception of the non-viable plus sucrose assay (159%).

The adherence of S. sanguis OMZ-9 to glass scintillation vials was observed after culturing in THG, THF, TGY, and TSGYF media (Table 3). Cells grown in the THG control medium demon-

ADDITIONAL ASSAY COMPONENTS		RELATIVE PERCENT ADHERENCE			
		THG	THF	TGY	TSGYF
VIABLE CELLS	NONE	100	69	22	122
	SUCROSE	171	72	46	192
BOILED CELLS	NONE	105	97	24	139
	SUCROSE	62	159	35	219

TABLE 2 Effect of culture medium on the ability of *S. mutans* HS-6 to adhere to glass.

ADDITIONAL ASSAY COMPONENTS		RELATIVE PERCENT ADHERENCE			
		THG	THF	TGY	TSGYF
VIABLE CELLS	NONE	100	50	112	175
	SUCROSE	119	90	77	123
BOILED CELLS	NONE	214	76	274	200
	SUCROSE	162	96	161	158

TABLE 3 Effect of culture medium on the ability of S. sanguis OMZ-9 to adhere to glass.

strated maximum adherence after boiling and without sucrose (214%), while the addition of sucrose increased adherence by 62%. Viable S. sanguis in the presence of sucrose adhered 19% above the control. Viable cells grown in THF and assayed with sucrose adhered 40% more than a similar assay without sucrose. Non-viable cells harvested from THF adhered 20% more with the addition of sucrose than in its absence. Generally, cells grown in THF demonstrated less adherence when compared to other media. The maximum adherence of S. sanguis grown in TGY was found without sucrose after boiling (274%). The addition of sucrose diminished adherence to 161%. Cells grown in TSGYF adhered better than the THG control with maximum adherence demonstrated without sucrose for both viable and non-viable organisms. Similar relationships between viable and non-viable cells are noted for cells grown in TGY and TSGYF. Streptococcus sanguis cultured from both these media adhered better without sucrose.

The adherence variability of S. mutans HS-6 and S. sanguis OMZ-9 when grown in THG and TGY media was assayed in the presence and absence of sucrose and saliva. Todd-Hewitt-glucose and trypticase-glucose-yeast extract media differ substantially in their composition especially with respect to salt composition and vitamins. Viable S. mutans grown in THG adhered maximally in the presence of sucrose (Table 4). Viable HS-6 adherence is inhibited (72%) in the presence of saliva and an additional 5% more effec-

ADDITIONAL ASSAY COMPONENTS		RELATIVE PERCENT ADHERENCE			
		HS-6		OMZ-9	
		THG	TGY	THG	TGY
VIABLE CELLS	NONE	100	22	100	112
	SUCROSE	171	46	119	77
	SUCROSE SALIVA	72	23	82	50
	SALIVA	67	17	60	31
BOILED CELLS	SALIVA	24	-	145	-
	SUCROSE SALIVA	27	-	141	-

TABLE 4 Effect of saliva sample II on adherence of streptococci cultured in TGY and THG media. Assay utilized fractionated saliva with S. sanguis OMZ-9.

tive when sucrose is absent from the assay. Cells grown in TGY and assayed without sucrose or saliva demonstrated less than 25% of the control THG adherence whereas sucrose raises adherence to 46% of the control. The presence of saliva again inhibits adherence activity (23%). Non-viable S. mutans grown in THG both with and without sucrose and in the presence of saliva demonstrate approximately one-fourth the adherence of the control while the presence of sucrose increases adherence by only 3%. Streptococcus sanguis OMZ-9 demonstrate maximal attachment in all assays when cultured in THG as compared to TGY medium. The adherence of non-viable OMZ-9, like S. mutans is unaffected by the addition of sucrose. The adherence of S. sanguis is 145% whereas the S. mutans adhere only 25% of the control. Both S. mutans and S. sanguis adhere well when assayed as viable cells from THG in the presence of sucrose without saliva (171% and 119%). Saliva is an effective adherence inhibitor in both viable and non-viable assays for S. mutans, while adherence inhibition for S. sanguis is only demonstrated among the viable cells.

It is recognized that dextran formation is an integral part of the adherence complex of streptococci. For this reason it was necessary to observe the effect of preformed dextran which has been reported to act as a primer for the polymer chain. Table 5 demonstrates the effect of Dextran T-10 on the adherence of S. mutans HS-6. Cells assayed in the presence of dextran adhered

ADDITIONAL ASSAY COMPONENTS		PERCENT ADHERENCE			
		HS-6		OMZ-9	
		W/O DEXTRAN	PLUS DEXTRAN	W/O DEXTRAN	PLUS DEXTRAN
VIABLE CELLS	NONE	100	86	100	101
	SUCROSE	167	146	65	86
BOILED CELLS	NONE	108	90	90	69
	SUCROSE	73	62	85	73

TABLE 5 Effect of dextran on the adherence of *S. mutans* HS-6 and *S. sanguis* OMZ-9. Dextran T-10 was utilized at a final concentration of 8.2×10^{-5} M and was assayed for 8 hours. (w/o=without).

from 11% to 21% less than those without it. Viable S. mutans adhered maximally with sucrose (167%), while dextran inhibited adherence by 21%. Viable S. sanguis (Table 5) in the presence of dextran adhered 21% more with sucrose than in a similar assay without dextran. Non-viable cells both with and without sucrose adhered 12% to 21% less than the control in the presence of dextran.

Table 6 indicates the ability of S. mutans to adhere to a glass surface both with and without previously attached S. sanguis. Streptococcus mutans preferentially adhere to surfaces with previously attached S. sanguis in the absence of sucrose (120%). An assay containing both saliva and sucrose resulted in adherence enhancement regardless of previously attached or unattached S. sanguis. Minimal attachment was demonstrated when saliva was the only additional assay component.

While the precise mechanism for adherence is still unknown, it is believed that cells induce enzymes such as glucosyltransferase which initiates the dextran formation and is involved with cell wall sites for adherence and aggregation. Table 7 illustrates the results of an attempt to interfere with cell structure with the use of trypsin. In all cases, regardless of assay composition or cell viability, the presence of trypsin dramatically decreased adherence of S. mutans 6715, HS-6, and S. sanguis OMZ-9.

ADDITIONAL ASSAY COMPONENTS	PERCENT ADHERENCE	
	W/O OMZ-9	PLUS OMZ-9
NONE	100	120
SUCROSE	81	96
SALIVA	27	22
SUCROSE SALIVA	138	125

TABLE 6 Adherence of S. mutans HS-6 to previously attached S. sanguis OMZ-9. Both organisms were cultured in THG. Streptococci sanguis was allowed to adhere to the vials for 8 hours in the presence of 1.0% sucrose. Radio-labelled S. mutans were assayed by introducing them into the scintillation vials both with and without previously attached S. sanguis. All vials contained both buffer and merthiolate and were incubated for 8 hours. (W/O=without).

ADDITIONAL ASSAY COMPONENTS		PERCENT ADHERENCE					
		HS-6		6715		OMZ-9	
		W/O TRYP	PLUS TRYP	W/O TRYP	PLUS TRYP	W/O TRYP	PLUS TRYP
VIABLE CELLS	NONE	100	8	100	14	100	24
	SUCROSE	171	4	104	7	119	10
BOILED CELLS		105	4	284	13	214	24
	SUCROSE	62	8	204	14	162	26

TABLE 7 Effect of Trypsin on the adherence of S. mutans HS-6, 6715, and S. sanguis OMZ-9. Cells were treated with 1 mg trypsin per 5 mg cells for 3 hours at 24 C before assaying. (W/O= without; TRYP= Trypsin).

DISCUSSION

CHAPTER V

Dental plaque is an amorphous mass of bacterial cells aggregated in an organic matrix on the tooth surface. This matrix consists of pellicle, salivary proteins, dextran, levan, enzymes, and sugars such as sucrose, glucose and fructose. Salivary glycoproteins play an important role in plaque formation by the deposition of a thin pellicle on tooth surfaces which enhance bacterial accumulation.⁵⁸ This pellicle is bacteria-free and is 0.1 μ to several microns thick.²⁶ Leach⁴⁴ describes these selectively adsorbing glycoproteins as long protein chains covalently linked to carbohydrates. Further analysis has shown IgA, IgM, IgG, albumin, amylase and fibrinogen to be among the proteins found in the pellicle.⁴¹ Following the deposition of the pellicle, microorganisms begin to adhere to the adsorbed salivary glycoproteins. While both S. mutans and S. sanguis are frequently in this adsorption and plaque formation, numerous studies have demonstrated that S. mutans is the primary etiological agent associated with dental caries.^{7, 11, 34, 47}

Bacterial accumulation on tooth surfaces requires two distinct processes, adherence and agglutination. Streptococcus mutans produces the extracellular enzyme glucosyltransferase (GTF) which hydrolyzes sucrose and forms the dextran polymer.

This dextran polymer binds to a protein site on the bacterial cell wall and mediates agglutination of oral streptococci. The significance of agglutination is reported by Magnusson et al.,⁴⁹ who found that a relatively high amount of agglutination is inversely related to plaque formation. Williams and Gibbons⁹⁴ found that certain microorganisms that became aggregated in the presence of salivary glycoproteins were also inhibited in their ability to attach to human epithelial cells.

Dextran polymers produced by oral streptococci may be of a soluble or insoluble nature. The soluble dextrans are made up primarily of α 1,6 linkages whereas the insoluble dextrans consist mostly of α 1,3 linkages. Current research has indicated that GTF isoenzymes may be responsible for the different dextran linkages.^{4, 5, 9} Inasmuch as some organisms found in plaque do not bind GTF or insoluble dextran, these organisms may exhibit an alternative mode of attachment to the tooth. These organisms according to Slade⁸² may also be "trapped" as dextran is being synthesized by other cells.

Saliva is ubiquitous in the oral cavity and its components are found in the pellicle and dental plaque. Because of the importance of saliva in the oral cavity, the current investigation was initiated to observe what effect saliva has on the adherence of cariogenic streptococci. Whole saliva was found to be effective in decreasing the adherence of streptococci. Adherence on the other hand, required the synthesis of insoluble dextran by

the GTF enzymes which are bound to both a cell wall polysaccharide and a separate protein site.⁸² Mukasa and Slade⁶² have shown that adherence and aggregation are distinct processes. They found that both viable and heat killed S. mutans cells were capable of adherence with the addition of GTF, however, only viable cells were able to agglutinate in the presence of dextran. This indicated that the protein site to which dextran binds was heat labile and was destroyed by the boiling thereby resulting in a loss of agglutination. These results indicate separate sites for both adherence and agglutination. The proteinaceous nature of the GTF binding site has been demonstrated by the ability of the hydrolytic enzyme trypsin to destroy its normal function. The current study (Table 7) is in agreement with other investigators who found that trypsin-treatment decreased the ability of the cell to bind enzymes for both adherence and agglutination.^{46,63,81,84} Mukasa⁶³ suggests that trypsin remains within the cell and inhibits the synthesis of the polymer.

The salivary factor may inhibit adherence by its presence at the interface of the tooth structure and the streptococcal cell wall. Ericson and Magnusson¹³ note that adherence inhibition may be due to the concentration of an inhibiting factor in saliva as well as the affinity of that factor to the tooth structure. It has further been observed that resting whole saliva is more effective in reducing the rate of plaque formation than stimulated whole saliva.⁴⁹

In order to measure adherence and how it is related to a saliva, a sensitive, quantifiable and reproducible in vitro assay was necessary. An assay was designed to observe the effects of culture media, dextran, cell viability, and saliva on streptococcal adherence. Adherence assays have been performed on epithelial cells, tooth surfaces, glassware, enamel powder, scintillation vials, and on wire suspended in a medium.^{33,54,62,63,71,76} The procedures for the measurement of adherent microorganisms include microscopic enumeration, optical density, and scintillation counting of radioactively labelled cells. The present study utilized H³-thymidine to radiolabel streptococcal cells and liquid scintillation counting to measure the attachment to glass scintillation vials.

In an effort to further characterize these adherence inhibitory factors in saliva, it was necessary to separate them from other salivary components which might promote adherence. In the past, salivary proteins have been separated by paper electrophoresis and chromatography on hydroxyapatite.^{12,14,51} Geller et al.,¹⁸ found mucoid, albumin, and β -globulin most frequent in whole saliva when separated by electrophoresis. The present study utilized isoelectric focusing because of its precision of separation in a pH gradient. Using an ampholine carrier with a pH range of 3 to 10 most of the protein focused below pH 7 (Fig. 5). Similarly, Beeley¹ found most of the salivary protein

had an isoelectric point between pH's 5 and 8 using the same ampholine carrier (pH 3-10). To further separate this protein, an ampholine carrier with a narrow pH gradient of pH 2.0 to 4.0 was utilized (Fig. 6). By assaying the effect of each fraction on adherence, it was observed that saliva focusing between pH 1 and 3 significantly inhibited adherence. This observation is corroborated by the results noted in figures 5 and 6.

After partial purification of the adherence inhibiting factors by IEF, it was necessary to further characterize these salivary fractions. Recent data has shown IgA in saliva to have a significant inhibitory effect on bacterial adherence and aggregation. Considerable experimentation has been done in this area using animals which have been immunized with dextrans from various strains of oral streptococci.^{32,67,87,93} Olson et al.,⁶⁷ found that immune sera to dextrans produced by S. mutans were capable of inhibiting agglutination of these bacterial suspensions even in the presence of either dextran or sucrose. Williams and Gibbons⁹³ found IgA isolated from parotid saliva to be an effective inhibitor of bacterial adherence. Taubman and Smith⁸⁷ found IgA able to interfere with S. mutans 6715 colonization and growth on dental surfaces.

Since immunoglobulins found in saliva have been shown to be effective in inhibiting adherence it was necessary to test the inhibitory fractions used in this study for IgG, IgA, IgM, and human albumin. It was found that the adherence inhibiting

fractions contained only human albumin (Table 1). The presence of human albumin may suggest a non-specific inhibition since BSA was also found to be effective as an adherence inhibitor of both S. mutans and S. sanguis (Figs. 8 & 9). A protein such as BSA may attach to the GTF binding site thus eliminating it for future adherence.

Figure 7 further characterized the effect of the salivary protein concentration on adherence. The results demonstrate that as the protein concentration increases, the adherence decreases.

The inhibitory protein was also found to be labile as demonstrated in figures 8 and 9. Adherence was particularly variable when the assay included sucrose with the fractionated saliva sample II. The adherence of both S. mutans and S. sanguis increased significantly in the presence of the fraction. Perhaps the heat-treatment inactivated the agglutinating proteins leaving only the adherence-promoting proteins available for assaying. It is possible that adherence inhibition requires multiple salivary factors for total activity. Separation of some of these components by IEF or destruction by heat-treatment could result in diminished inhibitory activity. This may be the case since whole saliva samples demonstrate the greatest inhibitory activity. Olson⁶⁵ observed that adherence inhibition from saliva was dependent on both dialyzable and non-dialyzable molecules which were heat labile. From the evidence of the current study, the adherence inhibition activity does not seem to be related to IgA,

IgG, or IgM.

In order to determine if cell surface components are heat labile, adherence assays were performed after the cells were boiled. Liljemark⁴⁶ found that boiling S. sanguis did not decrease adherence. The current study (Table 3) is in agreement with these findings. The ability of an organism to adhere even after boiling further indicates that some component involved in adherence is not heat labile. The results for the adherence of S. mutans were variable after boiling. Non-viable S. mutans in the presence of sucrose and grown in THF medium adhered better than THG cultured cells.

The medium in which an organism is cultured may have an effect on the adherence of that organism since the cell surface components may change depending on the available nutrients. It has been demonstrated that even trace amounts of sucrose or preformed soluble dextran in a culture medium is sufficient to promote cell-associated GTF activity and increase the dextran-binding capacity of the organism. Spinell⁸⁴ found that cells grown in trypticase-soy broth in the presence of even 0.01% sucrose resulted in cell bound enzymatic activity. The present investigation found that cells cultured in trypticase-soy broth-glucose-yeast-fructose provided maximum adherence for both S. mutans and S. sanguis (Tables 2 and 3). The addition of sucrose to the assay components further increased this adherence. A possible explanation for the maximum adherence of cells grown in TSGYF might be

related to cell wall components. Cells which are cultured in TSGYF may produce altered cell wall binding sites which are capable of binding more GTF than normal thus increasing its adherence capability.⁷⁴

Fructose in a culture medium generally has an inhibitory effect on adherence. Mukasa and Slade⁶³ reported that fructose inhibits the adsorption of GTF enzyme responsible for the synthesis of insoluble dextran. They suggest that fructose inactivates the dextran-sucrase enzyme resulting in adherence inhibition. Chludzinski⁴ states that fructose is a competitive inhibitor of sucrose and causes a decline in dextran formation. The results of the present investigation indicate that the addition of fructose to Todd-Hewitt broth with a single exception decreased adherence of both S. mutans and S. sanguis below that of the control (Tables 2 and 3). Inasmuch as no glucose is present in the THF medium, sucrose contamination is unlikely, and cellular adherence is observed to be less than both THG and TSGYF media. These results indicate that non-specific adherence is increased as the fructose competes with sucrose for the glucosyltransferase binding site.

In the absence of sucrose as a substrate, it should be evident that enzymatic activity cannot take place and that S. mutans is unable to effectively colonize teeth.⁴⁵ Michalek et al.,⁶⁰ found that 5.0% sucrose is the optimum concentration for caries

production. The current study found 1.0% sucrose to promote maximum adherence of S. mutans HS-6 (Fig. 1). The current research consistently observed adherence in the absence of the sucrose substrate (Figs. 1,2,8,9; Tables 2-5,7). These results indicate that non-specific adherence may take place without the sucrose-dextran mechanism. The non-specific adherence factor seems to be of a non-proteinaceous nature in that heating of the cells does not consistently inhibit adherence. It should also be noted however that the addition of sucrose generally increases adherence and induces the previously described GTF-dextran-adherence mechanism.

As was previously reported, dextran is an important component of the adherence complex. An assay was performed to observe the effect on adherence by the addition of Dextran T-10 in the presence of sucrose. The results (Table 5) indicate a decrease in adherence (12-21%) of S. mutans. These results are in general agreement with Slade who reports a 15% reduction in adherence.⁸² Mukasa and Slade⁶³ found that insoluble polysaccharide synthesis was inhibited by soluble linear dextran. They report that soluble dextran may terminate the elongation of the insoluble polymer chain resulting in decreased cellular adherence.

The sources of variability seen in this paper may to some extent be attributed to cellular and salivary differences. Different strains of streptococci adhere to saliva-treated enamel, hydroxyapatite, and the pellicle with variable affinities.^{13, 33, 71}

The outer surface of a cell may vary with the conditions of its growth and assays. As the outer surface of the cell changes, its enzyme-binding and adherence properties are changed.

Because of the variable influences on saliva such as type and intensity of stimulation, time of day, sex and health state of the individual, variable adherence results are to be expected.^{3,50,68} To limit salivary variations, components of saliva capable of adherence inhibition should be isolated and characterized. Once identified, the presence of these salivary factors which inhibit adherence may be related to individuals with different caries susceptibilities. All sources of variability must be studied more carefully in an effort to more clearly define the cause and mechanism of adherence.

SUMMARY AND CONCLUSION

CHAPTER VI

Saliva samples were collected, pooled and assayed for effective adherence activity on S. mutans HS-6 and S. sanguis OMZ-9. Samples were separated by isoelectric focusing and assayed for their effect on cellular attachment to glass scintillation vials. Human saliva found to be inhibitory was pooled and used for further study. The adherence inhibitory factors as separated by IEF contained human albumin but was devoid of immunoglobulins.

Assays were performed to observe viable and non-viable cells as well as the effect of different culture media. The enzyme trypsin was utilized to observe its effect on adherence. These results indicate that a cell-associated protein is involved in adherence inasmuch as adherence was significantly inhibited after such treatment.

The evidence suggests that both specific and non-specific factors are involved in adherence. Heat-treatment of cells did not necessarily diminish adherence, and the heat-treatment of inhibitory factors did not necessarily inactivate the inhibitory activity. Adherence was observed to some extent regardless of the assay conditions, however, saliva was generally found to diminish adherence.

CHAPTER VII

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APPROVAL SHEET

The thesis submitted by Elliot H. Kimmel has been read and approved by two members of the Graduate School faculty.

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

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

April 16, 1978
Date

Robert M. Chelmsford
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