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Identification of Lactoferrin in the Saliva of Patients with Gingivitis

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IDENTIFICATION OF LACTOFERRIN IN THE SALIVA OF PATIENTS WITH GINGIVITIS

by

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DEDICATION

To my parents and my wife, whose loving, devotion, encouragement, and sacrifice made this all possible.

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VITA

The author, Hwu-Rang Lin, B.D.S., is the son of Hwu-Zong Lin and Shou-Ching (Chen) Lin. He was born in Hsin-Chu, Taiwan, R.O.C. on July 22, 1952.

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On September 24, 1984, Jeffery Gee-Jay Lin the first child of Hwu-Rang and Marie Lin was born.

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CHAPTER I

INTRODUCTION

In 1939 a red protein was discovered in bovine milk by Sorensen and Sorensen. This protein has been given various names, such as lactoferrin, lactotransferrin, lactosiderophilin or red milk protein.

Because of its absence in serum, lactoferrin has been regarded as a specific milk protein. However, by using immunological methods it has also been possible to demonstrate the presence of lactoferrin in various external fluids. Elevations in the level of lactoferrin in secretions have been reported in several infectious diseases, such as mastitis, pancreatitis and salivary gland diseases. Therefore, lactoferrin might play an important role in protection of epithelial tissues from infection.

The presence of lactoferrin in saliva suggests that it may be important in the control of oral disease processes, such as gingivitis, periodontitis and dental caries.

The purpose of this study was to determine if there is any differences in the concentrations of lactoferrin in saliva between patients with gingivitis and clinically healthy individuals without gingivitis, using the enzyme-linked immuno-sorbent assay (ELISA) technique. Changes in the level of lactoferrin after the treatment for the patients with gingivitis was also determined.

CHAPTER II

REVIEW OF LITERATURE

Iron and Infection

Iron is required by all living cells, including bacteria. According to Weinberg (1978), bacteria need approximately 0.4 to 4.0 uM iron for growth. In the context of bacterial growth in man iron not freely available to microorganisms. Iron content of an average man is 4.5 gm (Bell et al., 1953). It is tightly bound to hemoglobin, myoglobin, ferritin and hemosiderin. Although the total iron concentration in mammalian body fluids is 2×10^{-5}M (Griffiths et al., 1980), almost all of the iron is associated with iron-binding and transport proteins such as transferrin in plasma and lactoferrin in mucosal secretions. Only 10^{-18}M free-iron is available in body fluids (Bullen et al, 1978; Bullen et al, 1981). This concentration is far lower than the bacteria need for growth. Furthermore, mammals, birds, reptiles and vertebrates can make metabolic adjustment during infection to withhold the essential iron from invading bacteria. Such invaded hosts promptly become hypoferrimic by halting intestinal assimilation and by increasing the quantity in storage of iron in the liver (Weinberg, 1974).

Prior stationing of host iron-binding proteins at potential sites of bacterial invasion and increased synthesis of host iron-binding proteins are also two possible antibacterial protective mechanisms (Weinberg, 1978). The ability of the host to withhold iron from microbes is called "nutritional

immunity". The term was first used by Kochan in 1973. Therefore, it is the ability of the microbes to acquire the iron essential for their growth and metabolism that may determine the nature of the host-parasite interactions. One strategy to obtain iron by the successful pathogens is that they must rapidly adapt to the low-iron environment. The successful pathogens produce powerful iron-chelating agents that can remove iron from iron-binding proteins. Because of extreme insolubility of ferric ion at neutral pH, aerobic and facultative microbes can synthesize either phenolates, which are derivatives of 2,3-dihydroxybenzoic acid, or hydroxamates, which are derived from hydroxamic acid. Phenolates and hydroxamates in bacteria serve to solubilize and assimilate the iron. These compounds are known as "siderophores". Anaerobic bacteria do not need to produce such iron-binding compounds, as they produce transport ligands that have a selective affinity for ferrous ion (Lankford, 1973).

Many studies on experimental infection have shown that iron enhances bacterial virulence (Weinberg, 1978; Bullen, 1981; Findelstein et al., 1983; Hill, 1978; Bullen et al., 1968) in such strains as Pseudomonas aeruginosa, Escherichia coli, Vibrio cholerae, Neisseria meningitidis, Neisseria gonorrhoeae, Bacteroides melaninogenicus and Staphylococcus aereus.

Bornside et al. (1968) have shown that both E. coli and hemoglobin are necessary to produce lethal effect in infections of the peritoneal cavity. Although E. coli is a common inhabitant of the gut, it usually is not regraded as a highly virulent pathogen. They also found that lysed red cells were as effective as hemoglobin with E. coli in producing a lethal effect.

Bullen et al. (1968) further demonstrated that it is the iron in hemoglobin that is responsible for stimulating bacterial growth. They reported that E. coli possess a binding site for the iron binding heme molecule from which it gains selective growth advantage, when hemoglobin is available.

Based upon a number of clinical studies there is evidence of a correlation between the increase in the hemoglobin concentration in serum and susceptibility to infection. Bullen (1981) concluded that it is the liberation of heme compounds that can enhance clinical infections.

Gingival bleeding commonly accompanies gingivitis and periodontitis. In such diseases the excess hemoglobin that was released could enhance the virulence of the bacteria in the gingival sulcus.

Buhler and Muhlemann (1975) recorded the degree of gingivitis and collected the stimulated mixed saliva from 50 subjects, before and after a 10-day period of intensified oral hygiene and measured the iron content in the saliva. They found that the use of methods to improve oral hygiene can reduce gingival inflammation and salivary iron content. Gingival inflammation was significantly but not strongly ($r = 0.51$) correlated with salivary iron content.

Mukherjee (1985) measured the concentration of iron in crevicular fluid from sites in gingivitis and periodontitis in human subjects. The results showed that the concentrations of iron in crevicular fluid collected from periodontitis sites were significantly higher than those collected from gingivitis sites (5.196 vs. 3.042 mg/L). These concentrations (5.196 and 3.042 mg/L) were higher than the concentration in serum (about 1.26 mg/L) as

reported by Skypeck and Joseph (1981). Mukherjee concluded that this high iron concentration in crevicular fluid might play an important role in the growth and virulence of microorganisms of subgingival plaque and the initiation of active periodontitis.

Becroft et al. (1977) performed in vitro studies to investigate the relationship between the administration of intramuscular iron-dextran for prevention of iron deficiency and the susceptibility to E. coli infection. They found that an increased incidence of E. coli meningitis occurred after iron-dextran treatment. They reported in these cases the sepsis was due to excess iron concentration in serum. Excess iron causes inhibitory effects on leukocytes chemotaxis and markedly reduced bacteriostatic effects against E. coli.

Bullen and Wallis (1977) also have demonstrated that such reversal of the bactericidal effect of polymorphonuclear leukocytes occurs by a ferritin-antibody complex. They found that there is no effect on abolishing the bactericidal power of polymorphonuclear leukocytes by exposing them to iron salts unless high concentrations of iron are used. They further reported that if the iron is presented in the form of a ferritin-antibody complex it is readily phagocytosed by neutrophils, and that the introduction of iron in this form, results in a viable bacterial count 346 times that of the controls at 8 hours.

Sources of Lactoferrin

Lactoferrin was first described in bovine milk by Sorensen and Sorensen in 1939. Johansson in 1958 found the similar iron-binding protein in

human milk. Montreuil et al. in 1960 described a method of isolating and pruifying lactoferrin from human milk.

Biserte et al. in 1963 first demonstrated lactoferrin in sputum and could not identify it in saliva. They considered lactoferrin to be bronchial origin.

Using immunohistochemical method by staining sections of biopsy specimens with fluorescein-labelled antiserum against lactoferrin, Masson et al. (1965, 1966) further demonstrated the presence of lactoferrin in all bronchial glands. Both serous and mucous cells appeared to be involved in the production of lactoferrin.

Masson et al. (1965b) reported the same iron-binding protein in human saliva. A bronchial origin for the salivary lactoferrin is not at all impossible, since bronchial mucus is known to reach the pharynx owing to the propulsory activity of the ciliated respiratory epithelium. However, Masson et al. (1966) have obtained further immunohistochemical evidence for the occurrence of lactoferrin in the salivary glands.

Masson et al (1966, 1966b) demonstrated that lactoferrin is present in a large number of human secretions: (1) respiratory secretions, e.g. nasal and bronchial fluids; (2) tears; (3) secretions from the glandular appendages of the digestive tract, e.g. saliva , bile and pancreatic juice; (4) genital secretions, e.g. seminal fluid and cervical mucus and (5) urine.

The immunohistological localization of lactoferrin in the mucosa of a variety of normal human tissues was investigated using specific fluoresceinated antisera by Tourville et al. (1969). They found that the lactoferrin was much less ubiquitous in the epithelial cells of the various

tissues studied and appeared to be restricted primarily to the acinar epithelium of the bronchial mucosa, parotid and submaxillary salivary glands (serous acinar cells only); and was also found in renal tubular cells.

Reitano et al. (1980) used an immunoperoxidase technique to study the distribution of lactoferrin in human salivary glands from autopsy tissues. Such tissues were fixed in Carnoy's fluid for the optimal preservation of lactoferrin antigenicity. They found that specific staining was seen in the intralobular ducts of all salivary glands but never in the interlobular ducts. Also, acinar lactoferrin was detected in most serous demilunes of the mixed glands and in some, but not all, acinar cells of the pure serous glands. However, no lactoferrin was detected in the acinar cells of the pure mucous glands. They also found that polymorphonuclear leukocytes were the only additional cells containing lactoferrin in the oral tissues studied.

Moro et al. (1984) used an immunofluorescence technique to examine the distribution of lactoferrin in human minor salivary glands. Lactoferrin was found in serous acini, demilunes, intercalated and intralobular ducts. They concluded that in addition to major salivary glands and gingival crevicular fluid, the minor salivary glands are also sources of lactoferrin in whole saliva. The reported findings cited above have created the impression that lactoferrin is chiefly an epithelial secretion product.

Masson et al. (1968) studied the human uterine cervix and found that lactoferrin originated from a particular cell type adjacent to the epithelium, possibly white cells. Also, their investigations on guinea pig tissues have shown that the spleen and bone marrow also are rich sources of lactoferrin. They concluded that glandular epithelia are not the only

sources of lactoferrin in mucosal and external secretions, but certain blood cells may also be implicated in the production of this protein.

Masson et al. (1969) have demonstrated that lactoferrin is one of the major proteins present in human and guinea pig neutrophilic polymorphonuclear leukocytes. This identification was based on a comparison of its electrophoretic, antigenic, and iron-binding properties with the corresponding properties of the same protein isolated from human and guinea pig milk. Immunochemical quantitations showed that lactoferrin occurs in human neutrophilic leukocytes at the concentration of 3 ug per 10^6 cells. Immunohistochemical data indicated that lactoferrin first appears in myeloid cells at the stage of the promyelocyte.

Baggiolini et al. (1970) further demonstrated that the lactoferrin of rabbit heterophil leukocytes has an intracellular distribution almost identical to that of alkaline phosphatase. The parallelism between lactoferrin and alkaline phosphatase was observed both in the sedimentation rate and in equilibrium density. They concluded that lactoferrin in the heterophil leukocytes belongs to the same granules as does alkaline phosphatase. They are specific (secondary) granules in polymorphonuclear leukocytes.

Using direct and indirect immunofluorescent staining, Green et al. (1971) found that lactoferrin was located in the nuclei and/or nuclear membrane of mature human polymorphonuclear neutrophilic leukocytes. However, it is absent from lymphocytes, monocytes and eosinophiles. While Masson et al. (1969) have reported that lactoferrin was located in the cytoplasm of human polymorphonuclear leukocytes. Green et al. explained the discrepancy in the localization of lactoferrin in the nucleus and cytoplasm could

be due to using different fixative and lactoferrin from different species.

Leffell and Spitznagel (1972) also found that lactoferrin is contained in cytoplasmic granules of human polymorphonuclear leukocytes. Upon centrifugation, it sediments in a band of granules that also contain 50% of the lysozyme activity. Their findings accorded with those of Masson et al. and Baggiolini et al. (1970).

Properties of Lactoferrin

Lactoferrin has a single-chain structure with the molecular weight 75,000 - 80,000 (Querinjean et al. 1971; Castellino et al., 1970). Each molecule has two specific metal-binding sites. One bicarbonate ion will bind tightly for each metal ion complexed to the protein (Masson and Heremans, 1968b). Teuwissen et al. (1972) by means of a spectrophotometric titration method, found that the binding of Fe^{3+} by apolactoferrin or apotransferrin involves three tyrosyl residues per metal ion.

Gordon et al. (1963) had determined the amino acid composition of lactoferrin in bovine milk, and found that the basic character of the protein is evidenced by an excess per molecule of 27 cationic residues over anionic residues. Some investigators have insisted on the resemblance between lactoferrin and the iron-binding protein from serum, transferrin, because both proteins have a single-chain structure with the same molecular weight (Querinjean, 1971). They also have the same metal-chelating properties, as both are able to bind two atoms of iron. For each Fe^{3+} taken up, one moleucle of bicarbonate is incorporated (Masson and Heremans, 1968b) and three protons are released (Querinjean, 1971). However, the two proteins

appear to differ widely from one another, since no immunologic cross-reactivity has been demonstrated between serum transferrin and lactoferrin of the same species (Montrenil et al., 1960; Blanc and Isliker, 1961). Amino acid compositions have also failed to show similarities (Gordon et al., 1963; Blanc et al., 1963). Furthermore, the iron-binding sites of lactoferrin are more stable at pH values below 4.0 than those of transferrin (Johansson, 1960). Aisen and Leibman (1972) had shown that the binding constant for iron at pH 6.4 - 6.7, was 300 times larger for lactoferrin than for transferrin.

The Function of Lactoferrin

It has been reported by Schade (1946) that iron-free serum transferrin displays marked bacteriostatic effect on microorganisms. In order to assess whether lactoferrin has the similar properties, Masson et al. (1966b) performed a number of experiments in which solutions of lactoferrin of various degrees of saturation with iron were tested for their inhibiting capacity. They found a clear inhibitory effect by unsaturated lactoferrin on the Staphylococcus albus, Staphylococcus aureus and Pseudomonas aeruginosa. This inhibitory activity had disappeared when ferrous ammonium sulfate had been added to the lactoferrin. They suggested that iron-unsaturated lactoferrin is bacteriostatic in action.

Reiten and Oram (1967) found that lactoferrin is very heat stable (90°C for 60 minutes) but is inactivated by trypsin. They also reported that purified lactoferrin inhibited the growth of Bacillus stearothermophilus and Bacillus subtilis and that its inhibitory activity is suppressed by

ferrous ions.

Oram and Reiter (1968) further extended their study and again have shown that the bacteriostatic action of lactoferrin was suppressed by Fe^{2+} and enhanced by Zn^{2+}, Co^{2+}, Ni^{2+}, and Cu^{2+}.

Similar findings were reported by Kirkpatrick et al. (1971), by using radial immunodiffusion method to measure the content of lactoferrin in parotid fluids and leukocytes from patients with chronic mucocutaneous candidiasis and normal subjects. They found that there were no differences in the concentrations of lactoferrin in the salivas from two groups. The iron-unsaturated protein markedly impaired replication of the yeast, and the growth-inhibitory property was lost when lactoferrin was saturated with iron.

Bullen et al. (1972) found that human milk with unsaturated iron-binding lactoferrin had a powerful bacteriostatic effect on Escherichia coli. The bacteriostatic properties of milk were abolished if the iron-binding proteins were saturated with iron. They also studied guinea pig milk, and found that newly born guinea pigs fed on an artificial diet and dosed with E. coli had higher counts of E. coli in the intestine than normally suckled animals. They suggested that iron-binding proteins in milk may play an important role in resistance to infantile enteritis caused by E. coli.

Reiter et al. (1975) found two strains of E. coli that were not inhibited by undiluted colostral whey or milk. However, the colostral whey became bacteriostatic for E. coli after dialysis or dilution in Kolmer saline and the addition of precolostral calf serum or lactoferrin. The lack of inhibition of E. coli in undiluted whey is due to the high

concentration of citrate in colostral whey and milk. It is suggested that citrate competes with the iron-binding proteins for iron and makes the citrate-iron complex available to the bacteria for growth.

Bishop et al. (1976) reported in their in vitro study that as little as 0.02 mg of apo-lactoferrin per ml deionized distilled water in marked inhibition of all mastitis-causing coliform bacteria. Growth inhibition was lost if iron-saturated lactoferrin or iron plus apo-lactoferrin was added to the synthetic medium. They also found that apo-lactoferrin at 20 mg/ml was bactericidal for E. coli. The addition of apo-lactoferrin plus citrate resulted in loss of growth inhibition, however, the molar ratio (citrate to apo-lactoferrin) was found to be more important than the absolute concentration of either component. Because a ratio of 75 resulted in 50% growth inhibition of E. coli, whereas ratios of 300 and greater resulted in less than 10% growth inhibition.

The antimicrobial action of lactoferrin has been attributed to the ability of depriving the essential iron. And the reversibility of this antimicrobial effect, when an excess of iron is supplied to the nutritionally deprived organisms, suggests a simple bacteriostatic effect for lactoferrin. But Arnold et al. (1977) showed Streptococcus mutans and Vibrio cholerae, but not Escherichia coli, were killed by incubation with purified human lactoferrin. The resistance of E. coli to the bactericidal action of lactoferrin in their study, could be due to the production of iron chelators (enterochelins). The ability to synthesize iron chelators has been suggested as a significant virulence factor for several enteropathogenic strains of E. coli. The investigation by Bullen et al. (1972) and Bishop et al.

(1976) have suggested that synthesis of enterochelins allows the bacteria to utilize lactoferrin.

Bullen and Armstrong (1979) reported rabbit polymorphonuclear leukocytes contain the iron-binding protein lactoferrin and can rapidly phagocytose and destroy Pseudomonas aeruginosa. If the cells are exposed to a ferritin-antibody complex, large amounts of this are phagocytosed by polymorphonuclear leukocytes and appear in the cytoplasmic granules and phagosomes. This leads to saturation of the cellular iron-binding protein with Fe, the bactericidal power of the cells is greatly reduced with the result that some phagocytosed bacteria survive and eventually grow and destroy the cells. An apoferritin-antibody complex used as a control is also phagocytosed by polymorphonuclear leukodytes but has no effect on the bactericidal power of the cell.

Arnold et al.(1980) extended their study on bactericidal activity of human lactoferrin. The sensitivity of a variety of miroorganisms to human lactoferrin was investigated. Some of the microorganisms were clinical isolates from 4-day supragingival plaque samples. They found that susceptible microorganisms includes Gram-positive and Gram-negative microbes, rods and cocci, facultative anaerobes, and aerotolerant anaerobes. Also, they found organisms of the same species and even of the same strain can differ in susceptibility to lactoferrin. They suggested that accessibility of the microorganisms to the lactoferrin target site may account for differences in susceptibility.

Arnold et al. (1981) studied the influence of the metabolic state of the bacteria, the incubation temperature, pH, and the presence of metal

ions on the kinetics of killing Streptococcus mutans by lactoferrin. They found that after exposure to lactoferrin, a 15-minutes lag period occurred before the initiation of killing, indicating that a two step process is involved in lactoferrin killing. Cultures harvested during the early exponential phase were very sensitive to lactoferrin, whereas cultures harvested in the early stationary phase were markedly more resistant. Also, the rate of killing was dependent on temperature. At 37°C, there were no detectable colony-forming units (CFU) after 1 hour of incubation. However, when the incubations were carried out at 23°C or at 4°C, the rate of killing was markedly reduced. Additionally, bactericidal activity was enhanced under the slightly acid environment. This indicated that the reduction of the killing activity at neutral pH and the loss of killing under slightly alkaline condition might be due to the significant conformational changes of lactoferrin that occur with increasing pH. Furthermore, exogenous ferrous or ferric ions did not reverse or prevent lactoferrin killing.

Arnold et al. (1982) compared the bactericidal effects of lactoferrin on Streptococcus mutans with the bacteriostatic effects on iron deprivation. They found that growth and macromolecular synthesis (DNA, RNA and protein) were inhibited by incubation of S. mutans with purified human lactoferrin. Their early studies (1980, 1981) have shown that lactoferrin binds to the surface of susceptible bacteria, and this binding could result in the inhibition of growth by blocking some essential transport sites, resulting in nutritional deprivation of the bacteria. If this is true, then removal of the surface-bound lactoferrin should reverse this inhibition. But, in the experiments (1982), even after using a variety of washing agents to remove

the bound lactoferrin from the cell surface, the viability could not be restored. They concluded that this inhibition of metabolism and rapid loss in viability observed with lactoferrin treatment suggest that lactoferrin has a direct bactericidal effect on S. mutans that can not be attributed to simple iron deprivation. An alternative possible suggestion was that the binding of lactoferrin to the bacterial cell surface triggers a second process that results in the irreversible cell death.

Oseas et al. (1981) found that PMNs released the specific granule product lactoferrin more rapidly in response to chemotatic stimuli, which correlated with promotion of PMNs aggregation and enhanced PMNs adherence. They suggested that PMN lactoferrin serves an autoregulatory role to retain PMNs at inflammatory sites to amplify the inflammatory response.

Ambruso and Johnston (1981) reported that iron-saturated lactoferrin from human colostrum and human neutrophils enhances the generation of the bactericidal hydroxyl radical. This product may result from the competetive ability of lactoferrin to obtain iron from bacteria. They suggested that in addition to antimicrobial function, lactoferrin could play an important role as regulator of hydroxyl radical production during phagocytosis.

Alteration of Lactoferrin Levels

Estevenon et al. (1975) used an immunodiffusion technique to test the presence of lactoferrin in the duodenal juice of patients with chronic calcifying pancreatitis (CCP). They found that almost all patients with CCP and 7% of control subjects without CCP have shown lactoferrin in duodenal juice. They suggested that the lactoferrin test could have a certain value

in the diagnosis of CCP, especially in the differential diagnosis between CCP and other pancreatic diseases.

Colomb et al. (1976) further extended their study in 1975. By using the immunodiffusion techniques, lactoferrin was present in the pathological pancreatic juice only. However, when indirect immunofluorescence method was used, the fluorescence was found in all samples, including normal pancreatic tissue. The difference between these two techniques were explained by the different sensitivity and these results indicated that lactoferrin is a protein of pancreatic secretions.

Fedail et al. (1978) used radioimmunoassay to measure the concentration of lactoferrin in pure pancreatic juice from the patients with chronic pancreatitis and control subjects. They found that mean concentrations of lactoferrin were 2310 mg/ml (range 870 - 11,4000) in the patients with chronic pancreatitis and 128 mg/ml (range 30 - 390) in control subjects. They suggested that the measurement of lactoferrin concentration in pure pancreatic juice may be useful in the diagnosis of pancreatic diseases. Lactoferrin seems is a sensitive marker of chronic pancreatitis because lactoferrin concentrations in pancreatic juice are specifically elevated in these patients.

Harmon et al. (1975) determined the lactoferrin concentration in milk by electroimmunodiffusion assay from normal lactating cows and cows with mastitis. They found that the mean concentration of lactoferrin in normal group was 0.35 mg/ml. In contrast, that found in the mastitis group from 0.55 mg/ml on day 1 to 1.89 mg/ml by day 3. Harmon et al. (1976) also found that experimentally induced Escherichia coli infection of bovine mammary

gland resulted in a 30-fold increase in lactoferrin concentration in mammary secretion by 90 hours post-inoculation, and 4-fold increase in total daily production of lactoferrin by 264 hours post-inoculation. They suggested that the increased lactoferrin production may be a result of a specific response of secretory tissue to inflammatory agents.

Tabak et al. (1978, 1978b) examined the levels of lactoferrin in the parotid secretions of groups of patients (a) with chronic recurrent parotitis, (b) with complaints of dry mouth but no evidence of disease, (c) with suspected or confirmed Sjogren's disease, (d) with sarcoidosis and (e) with diabetes. They found that lactoferrin values in parotid saliva during the active phase of parotitis were elevated from two to twenty times normal. The values for the recovery phase of parotitis were lower than in the active phase, but still two to ten times normal. Elevations in lactoferrin were also noted in five of six patients with Sjogren's disease, but not in subjects with sarcoidosis, diabetes or "dry mouth" without sialographic changes.

In 1971, Kakizaki et al. reported a direct relationship between experimentally induced pancreatic disorders and alterations of histology and function of the parotid glands. Later, they developed a new test on parotid saliva and emphasized the usefulness of the saliva test for the diagnosis of pancreatic diseases (Kakizaki et al., 1976).

In order to find out if lactoferrin levels in parotid saliva are elevated in chronic pancreatitis, Durr et al. (1982) and Benini et al. (1983) measured the concentration of lactoferrin in parotid saliva from patients with pancreatitis and controls. They found that there was no difference in the lactoferrin between these two groups. They concluded that the elevation

of lactoferrin concentration is confined to the exocrine pancreas and do not affect salivary glands in chronic pancreatitis.

Fridman et al. (1983) using rocket immunoelectrophoresis to study the concentrations of lactoferrin and lysozyme in the crevicular fluid from patients with gingivitis, adult periodontitis, localized juvenile periodontitis and normals. They found that lactoferrin in the normal group was significantly lower than those in gingivitis group (0.63 vs. 1.5 ug/ul) but did not show significant differences between gingivitis, adult periodontitis and localized juvenile periodontitis.

Konttinen et al. (1984) studied the concentration of salivary lactoferrin in patients with Sjogren's syndrome by radioimmunoassay. The concentration of lactoferrin in unstimulated mixed saliva in Sjogren's syndrome group was 16.28 ± 3.05 ug/mg of protein (range 7.42 - 33.64) and that in sex and age matched normal group was 2.82 ± 0.82 ug/mg of protein (range 0.59 - 7.35). They demonstrated that by using the peroxidase-antiperoxidase staining method for labial salivary gland biopsy, a strong staining of lactoferrin was found in intralobular and interlobular ducts. Their results indicated that the concentration of unstimulated, mixed salivary lactoferrin is a useful diagnostic tool for the diagnosis of Sjogren's syndrome. They suggested that abnormal salivary duct secretion and/or reabsorption is the reason for high salivary lactoferrin values.

Muratsu and Morioka (1985), in order to study the mechanisms of increased susceptibility to oral infection in diabetics, examined the level of salivary antibacterial factors; including lysozyme, lactoperoxidase and and lactoferrin in diabetic hamsters. They found that although lysozyme

activity decreased by 56% and lactoperoxidase activity decreased by 53% in diabetic hamsters, there was no significant difference between the streptozotocin induced diabetic and non-diabetic hamsters in the amount of salivary lactoferrin. Also, before insulin treatment the ratio of lactoferrin to total protein increased to double the amount of that of the control hamsters. After the insulin treatment, the ratio of lactoferrin to total salivary protein reverted to normal values.

The Role of Bacteria in Gingivitis

Withdrawal of tooth-brushing in healthy human experimental subjects results in the accumulation of microbial plaque on the teeth and the development of gingivitis (Loe et al., 1965; Theilade et al., 1966). If the bacterial plaque is removed by scaling, root planing and gingival curettage, the gingivitis is reversible. It indicates that bacteria are casually related in gingivitis (Krygier et al., 1973).

Listgarten (1976) in a study with the electron microscope found that in healthy gingival sulcus a thin microbial layer approximately 60 um thick adhered to the enamel surface of teeth. The cells were predominantly coccoid in shape with a majority exhibiting cell wall features compatible with those of Gram-positive microorganisms.

According to Socransky (1977), healthy periodontal tissues appear to be associated with a scanty microbial flora that is located almost entirely supragingivally on the tooth surface. Microbial cell accumulations are usually 1 to 20 cells in thickness and are composed mainly of Gram-positive coccal forms.

The microorganisms commonly encountered in such sites include Streptococcus mitis, Streptococcus sanguis, Staphylococcus epidermidis, Rothia dentocariosa, Actinomyces viscosus, Actimyces naeslundii and occasionally species of Neisseria and Veillonella (Listgarten, 1976; Listgarten et al., 1975; Socransky et al., 1977; Slots, 1977).

According to Slots (1979), a scant microbial flora dominated by Gram-positive microorganisms (85%), usually Streptococcus and facultative Actinomyces species is found in the healthy gingival sulcus.

In the gingivitis sites on teeth, Listgarten (1976) found that bacterial deposits appeared to be thicker than that found in the normal samples, about 0.4 mm in thickness. The bacterial samples comprised a wide variety of microorganisms, including coccoid as well as filamentous forms, and those with Gram-positive and variety of Gram-negative cell wall patterns. Also, flagellated bacteria and spirochetes were not uncommon in gingivitis samples.

According to Socransky (1977), in experimental gingivitis there is an increase in the total mass of plaque and cell layers which often extend to 100 to 300 cells in thickness. There is an increase in proportions of members of the genus Actinomyces. This group of microorganisms tends to be the dominant genus associated with supragingival plaque, frequently comprising 50% or more of the isolates (Listgarten et al., 1975; Loesche and Syed, 1975; Syed et al., 1975). In long standing gingivitis, approximately 25% of the microbial flora may be Gram-negative, including species of Veillonella, Vibro (Campylogacter) and Fusobacterium. The Gram-negative cells appear to be located primarily on the surface of the bacterial plaque in subgingival sites (Listgarten, 1976; van Palenstein Helderman, 1975).

According to Slots (1979), the development of gingivitis is accompanied by a marked increased in the total number of Gram-negative microorganisms. Fusobacterium nucleatum, Bacteriodes melaninogenicus ss. intermedius, Haemophilus species, and other Gram-negative microorganisms comprised about 45% of the total gingivitis isolates. Streptococcus and facultative and anaerobic Actinomyces species constituted the majority of the Gram-positive gingivitis isolates.

According to Page and Schroeder (1981), gingivitis and periodontitis in man and other animals, without exception, is caused by bacteria. However, the mere presence of bacteria, even the presence of specific pathogens is insufficient. They emphasized that an interaction of bacteria with the host's response systems is essential of the disease is to become established and progress. This indicates that a healthy gingiva is the result of an equilibrium between pathogenic factors from the gingival crevice flora and the natural defense of the adjacent tissues. If the nature of gingival crevice flora alters either by an increase in the number of organisms or by a shift to a more virulent bacterial population, the equilibrium might be broken and inflammation and destruction of tissues will follow.

Enzyme-linked Immunosorbent Assay

Antibodies and antigens labeled with fluorescent dyes or isotopes have been used extensively for immunodiagnosis over the past two decades. However, they do have some disadvantages. Immunofluorescence, usually depends on subjective assessment of the end result, is time-consuming and is not easily automated so it can be used for only small batches of tests.

Radioimmunoassay is particularly suitable for large-scale operations but the short shelf-life of the reagents, the expensive equipment, and because it carries some risks, has tended to exclude radioimmunoassay from many small laboratories. These considerations have led workers to search for alternative labels for antiboides or antigens (Boller et al., 1976; Voller et al., 1978).

Enzyme labeled reactants are safe, have long shelf life and yield objective results with the same sensitivity as radioimmunoassay, yet can be used with relatively cheap, simple equipment. According to Engvall et al. (1971), by using enzyme-linked immunosorbent assay (ELISA) 1 - 100 ng/ml of the antigen could be determined.

The basic ELISA test depends on two assumptions: (1) that antigen or antibody can be attached to solid-phase support yet retain immunological activity and (2) that either antigen or antibody can be linked to an enzyme and the complex to retain both immunological and enzymatic activity. According to Voller et al. (1976) the double antibody "sandwich" for detection of antigen and the indirect method for detection of antibody are the most useful in practice.

It is clear from the work of Cantarero et al. (1980), Fields, et al. (1983) and Kemeny et al. (1985) that immobilization of proteins to plastic material is dependent on the nature of the protein and the conditions used for coating. Kemeny et al. (1985) found that prolonged incubation of the antibody with the plates resulted in an increased capacity for binding antigen. Also, the rate of antigen bound to the antibody-coated plates was

much faster than that for the antibody alone bound to the plates, and the amount of antigen bound to the antibody-coated plates did not increase after 1 hour.

A serious problem limiting the measurement of specific antigen is the high non-specific antibody binding to the plates. Urbanek et al. (1985) found that the rate of antigen bound to antibody-coated plates showed down after 3 hours incubation while the non-specific binding increased progressively with longer incubation. Also, the rate of this binding was higher at 37°C than 21°C or 4°C, but this was largely due to increased non-specific binding at this temperature. They suggested that the best separation of specific antibody binding from non-specific binding was to incubate the plates at 4°C.

Thus, in this study we reduced the incubation time to 1 hour and reduced incubation temperatures to 4°C. Also, we used 0.1% bovine serum albumin (BSA) and 1% normal rabbit serum (NRS) as blocking agent. Also, Tween-20 was included in all washing steps to prevent unspecific binding.

CHAPTER III

MATERIAL AND METHOD

Selection of Patients

Ten adult clinical patients with gingivitis, three females and seven males, ranging in age from 20 to 31 were selected from the Department of Periodontics, School of Dentistry, Loyola University of Chicago, based on the following clinical criteria described by Listgarten in 1976: (1) Gingival Index (GI): 2 - 3 ; (2) Pocket depth less than 5 mm; (3) Absence of radiographic evidence of bone loss. In addition, a group of age and sex-matched clinically normal control subjects with healthy gingiva (3 females and 7 males, ranging in age from 23 to 31) were selected from dental students at the School of Dentistry, Loyola University of Chicago.

None of the participants had received a prophylaxis within the last six months. They were not taking any medication and did not show any evidence of systemic diseases or salivary gland diseases. The female participants were neither pregnant nor taking oral contraceptives.

Pocket depth was measured to the closest millimeter by means of a standard periodontal probe. Bone loss was considered to be absent, meaning that the alveolar crest is within 2 mm of cementoenamel junction(CEJ). The severity of gingival inflammation was assessed by the criteria of the Gingival Index of Loe and Silness (1963) as modified by Loe (1967). Four gingival areas, buccal, lingual gingival margin and interdental papillae, of all

teeth were given a score from zero to three as follows:

0: normal (absence of inflammation)

1: mild inflammation (slight change in color, little change in texture, no bleeding on probing.)

2. moderate inflammation (moderate glazing, redness, edema and/or hypertrophy, bleeding on probing.)

3. severe inflammation (marked redness and edema, hypertrophy, spontaneous bleeding or ulceration.)

The scores from the four areas of the tooth were added and divided by four to give the GI for the tooth. Finally, by adding the indices for the teeth and dividing by the total number of teeth examined, the GI for the individual was obtained.

Experimental Design

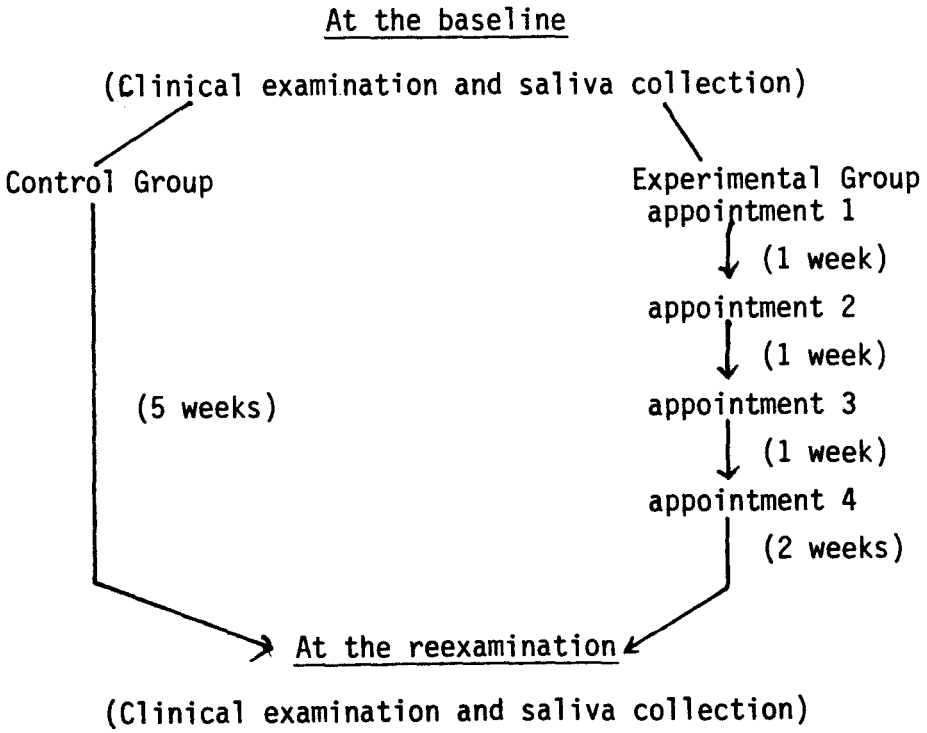
This study was reviewed by the chairman of the Loyola University Medical Center Institutional Review Board for the Protection of Human Subjects (IRB) before commencement. At the baseline, after signing the consent form, participants were given a clinical examination, and provided saliva samples as subsequently described.

Patients with gingivitis received the following treatment: oral hygiene instruction, scaling, root planing and gingival curettage. It took four appointments, with a one week interval, to complete the treatment procedures. Two weeks after the last appointment, the patients came back to clinics for re-examination. At this time, the baseline procedures were repeated. For the control group, five weeks after the initial appointment

the baseline procedures were repeated, and the study terminated.

Each subject brushed with water to remove plaque and residual debris before saliva collection. All participants spit saliva actively into a funnel connected to a graduated centrifuge tube until 3 ml were obtained. The collected saliva were clarified immediately by centrifugation at 3,675 rpm for ten minutes. 0.1% (about 3 mg) cetylpyridinium chloride (CPC) was added to supernate, and the aggregated material in the supernate was removed. The supernate samples were centrifuged at 3,675 rpm for ten minutes again. The salivary supernatant then were frozen and stored for further tests, as described by Olson et al. in 1985.

Experimental Design



ELISA Procedures

Quantitation of lactoferrin was performed by enzyme-linked immunosorbent assay (ELISA) as described below (Dipaola and Mandel, 1980; Kemeny et al., 1985; Urbanek et al., 1985):

1. Tissue culture plates (American Scientific Products 24 - 2 ml wells) were employed as solid phase.
2. The wells were coated with specific antibody to lactoferrin by passive adsorption and run in triplicate. 2 ml of rabbit anti-human lactoferrin (Pel-Freez Biological) at concentration of 10 ug/ml in 0.05 M Na_2CO_3 buffer (pH 9.6) were added to each well.
3. The coated wells were incubated at 4°C for overnight.
4. The wells were washed four times to remove unbound antibody with tris-buffered saline (TBS).
5. The coated wells were then treated with 0.1% bovine serum albumin and 1% normal rabbit serum in 0.05 M TBS at room temperature for 30 minutes.
6. The wells were washed four times with TBS.
7. 2 ml of lactoferrin, prepared from human standard serum (Calbiochem) at concentrations of 10.3 to 26.9 ng/ml in 0.05 M TBS were added to triplicate wells to establish a standard curve.
8. 2 ml of test saliva diluted 1:500 with 0.05 M TBS, pH 7.4 were added to the remaining wells.
9. The plates were incubated at 4°C for 1 hour.
10. The wells were washed four times with TBS.

11. 2 ml of an enzyme-labeled preparation of the antibody, rabbit anti-human lactoferrin-alkaline phosphatase (Pel-Freez Biological), diluted 1/200 with 0.05 M TBS and 0.2% BSA were added and incubated at 4°C for 1 hour.
12. The wells were washed four times with TBS.
13. 2 ml of p-nitrophenyl phosphate, substrate, were added to each well at the concentration of 1 mg/ml in 0.1 M Ethanolamine (2-Aminoethanol) pH 9.6 containing 10 mM $MgCl_2$.
14. The wells were incubated at room temperature for 30 minutes.
15. 0.2 ml of 1 N NaOH were added to stop the enzyme reaction.
16. 150 ul solution from each well were transferred to Dynatech plates containing 96 wells.
17. The optical density (OD) of each well was read with Minireader II (Dynatech) at 410 nm.
18. The concentration of lactoferrin was determined by comparison with a standard curve.

The ELISA procedures were performed twice, and the average concentration of each sample was obtained. The values were analysed by the two sample student t test in order to establish a statistically significant difference between the two groups.

CHAPTER IV

RESULTS

Clinical Observations

Gingival Index (GI):

At the baseline, the gingival index for the experimental group was 1.8 ± 0.2. After the periodontal treatment, the GI was reduced to 1.2 ± 0.3 (Table 1). This was significantly different at the level of 0.001 (Table 3). For the control group, at the baseline and at the reexamination, the GI were 0.2 ± 0.1 and 0.2 ± 0.2 respectively (Table 2), and did not differ significantly (Table 3).

Probing Pocket Depth:

At the baseline, the mean probing pocket depth was 2.5 ± 0.2 mm for the experimental group, and was 2.0 ± 0.2 mm for the control group (Table 4 and 5). This was significantly different at the level of 0.001 (Table 6). At the reexamination, the mean probing pocket depth was 2.4 ± 0.2 mm for the experimental group, and 2.0 ± 0.2 mm for the control group (Table 4 and 5). This was significantly different at the level of 0.001 (Table 6). However, there was no significant difference between at the baseline and at the reexamination either in the experimental or control group (Table 6).

Lactoferrin Concentrations

The value for lactoferrin from unstimulated whole saliva from ten

patients with gingivitis and ten clinically normal individuals, as quantitated by the double antibody sandwich ELISA technique, are shown in Table 7 and Table 8. For the experimental group, at the baseline the mean value was 0.57 ± 0.08 mg%, after the periodontal treatment the mean value was 0.58 ± 0.09 mg%. There was no significant difference. For the control group, at the baseline the mean value was 0.56 ± 0.21 mg%, at the reexamination the mean value was 0.55 ± 0.15 mg%. There was no significant difference. Also, no significant differences were found between the experimental and control groups either at the baseline or at the reexamination (Table 9).

Table 1

Gingival Index for the Experimental Group

Patients	At the baseline	At the reexamination
1	1.6	0.8
2	2.1	1.4
3	1.8	1.4
4	1.7	0.8
5	1.8	0.8
6	2.0	1.5
7	1.9	1.3
8	1.9	1.4
9	1.7	1.3
10	1.6	1.2
Mean	1.8	1.2
S.D.	0.2	0.3

Table 2

Gingival Index for the Control Group

Subjects	At the baseline	At the reexamination
11	0.3	0.3
12	0.2	0.2
13	0.3	0.3
14	0.2	0.2
15	0.0	0.0
16	0.5	0.5
17	0.3	0.4
18	0.2	0.1
19	0.0	0.0
20	0.2	0.2
Mean	0.2	0.2
S.D.	0.1	0.2

Table 3

Gingival Index (Mean ± S.D.), comparison between two groups

	At the baseline	At the reexamination	t	p
Experimental Gr.	1.8 ± 0.2	1.2 ± 0.3	5.83	<0.001
Control Gr.	0.2 ± 0.1	0.2 ± 0.2	0.00	N.S.*

* N.S. = not significantly different.

Table 4

Probing Pocket Depth (mm) for the Experimental Group

Patients	At the baseline	At the reexamination
1	2.4	2.2
2	2.8	2.7
3	2.6	2.6
4	2.5	2.3
5	2.6	2.2
6	2.5	2.4
7	2.6	2.5
8	2.3	2.3
9	2.7	2.6
10	2.4	2.3
Mean	2.5	2.4
S.D.	0.2	0.2

Table 5

Probing Pocket Depth (mm) for the Control Group

Subjects	At the baseline	At the reexamination
11	2.0	2.0
12	1.9	1.9
13	2.2	2.2
14	2.1	2.1
15	2.0	2.0
16	2.2	2.2
17	2.1	2.1
18	2.0	2.0
19	1.7	1.7
20	2.2	2.2
Mean	2.0	2.0
S.D.	0.2	0.2

Table 6

Probing Pocket Depth (mean ± S.D., mm), comparison between two groups

	At the baseline	At the reexamination	t	p
Experimental Gr.	2.5 ± 0.2	2.4 ± 0.2	1.43	N.S.*
Control Gr.	2.0 ± 0.2	2.0 ± 0.2	0.00	N.S.
t	7.14	5.26		
p	<0.001	<0.001		

* N.S. = not significantly different.

Table 7

Lactoferrin Concentrations (mg%) for the Experimental Group

Patients	At the baseline	At the reexamination
1	0.51	0.46
2	0.53	0.49
3	0.68	0.58
4	0.69	0.54
5	0.51	0.63
6	0.53	0.77
7	0.52	0.56
8	0.68	0.58
9	0.46	0.57
10	0.59	0.64
Mean	0.57	0.58
S.D.	0.08	0.09

Table 8

Lactoferrin Concentrations (mg%) for the Control Group

Subjects	At the baseline	At the reexamination
11	0.48	0.51
12	0.49	0.48
13	0.42	0.38
14	0.46	0.47
15	0.66	0.61
16	0.50	0.58
17	0.46	0.52
18	0.44	0.48
19	1.13	0.94
20	0.52	0.48
Mean	0.56	0.55
S.D.	0.21	0.15

Table 9

Lactoferrin Concentrations (mean ± S.D., mg%), comparison between two groups

	At the baseline	At the reexamination	t	p
Experimental Gr.	0.57 ± 0.08	0.58 ± 0.09	0.28	N.S.*
Control Gr.	0.56 ± 0.21	0.55 ± 0.15	0.125	N.S.
t	0.14	0.6		
p	N.S.	N.S.		

* N.S. = not significantly different.

CHAPTER V

DISCUSSION

In the present study gingival tissues were assessed for the degree of inflammation by the criteria of the Gingival Index System (Loe and Silness, 1963; Loe, 1967). The criteria include gingival tissues pale pink in color, stippled in texture, firm in consistency, and in the absence of bleeding upon probing is considered clinically normal. This criteria received a Gingival Index score of zero. Gingival tissues displaying mild inflammation, slight change in color, slight edema, no bleeding on probing, and received a Gingival Index score one. Gingival tissues displaying moderate inflammation, redness, edema and glazing, in the presence of bleeding upon probing, and received a Gingival Index score of two. Gingival tissues which displayed severe inflammation, ulceration, and tendency to spontaneous bleeding received a Gingival Index score of three.

All participants in the control group were considered to be clinically normal, while in the experimental group, the scores were changed from 1.8 at the baseline to 1.2 at the reexamination. This significant change was apparently the result of the scaling, root planing, curettage and oral hygiene instruction as performed in the protocol.

The experimental results reported in this study do show the presence of lactoferrin in the whole saliva of the patients with gingivitis and in clinically normal individuals as measured by the ELISA technique.

Double antibody sandwich ELISA technique indeed can measure the contration of lactoferrin in the whole saliva. However, the procedures used in this study were modified by precipitation of salivary acid mucopolysaccharides with cetylpyridinium chloride (CPC) in the saliva processing. CPC is a good fixative for acid mucopolysaccharides (Williams and Jackson, 1956). Since 1 - 100 ng/ml of the antigen can be determined by ELISA (Engvall et al., 1971), ELISA is a very sensitive and useful method to measure such small amount of lactoferrin in saliva.

The levels of lactoferrin in the saliva of the patients with gingivitis and individuals with clinically normal gingiva do not have significant differences as determined by ELISA method. This suggests that gingivitis does not change the host's response with respect to the lactoferrin level in saliva. After the patients with gingivitis in this study received scaling, root planing, gingival curettage and oral hugiene instruction, the level of lactoferrin in their saliva did not change. This suggests that lactoferrin level in saliva does not respond to the treatment of gingivitis, even when there is a significant clinical improvement in the gingival inflammation.

Also, in this study, the probing pocket depth in gingivitis group was 2.5 ± 0.2 mm, and 2.0 ± 0.2 mm in control group (Table 4 and Table 5), it does not suggest a similar effect in chronic periodontitis. It is not known whether there is any change of the level of lactoferrin in the saliva in the patients with chronic periodontitis. In such cases it is observed that they show deeper probing pocket depth and more soft tissue and alveolar bone destruction. A study of salivary lactoferrin levels in patients with

chronic periodontitis in order to assess any relevance and in comparison to the findings reported in this study should be done in the near future.

A previous study by Masson et al. (1966) reported the lactoferrin values in stimulated parotid saliva as low as 0.2 to 0.3 mg% by the electrophoresis method. Brandtzaeg (1971) reported lactoferrin concentration in stimulated parotid saliva to be about 1 mg% and also noted that unstimulated salivary samples exhibited higher values than stimulated samples.

Tabak et al. (1978) used the rocket electrophoresis method and reported a range of 0.5 to 2 mg% lactoferrin in parotid saliva in their control subjects.

Dipaola and Mandel (1980) who also used the ELISA method reported the lactoferrin value 0.81 ± 0.36 mg% in the unstimulated parotid saliva and 0.54 ± 0.26 mg% in the stimulated parotid saliva, in normal human subjects. In their study, the saliva samples were diluted 1:25. In our study the saliva samples were diluted 1:500, and indicates that our ELISA techniques is more sensitive than that reported by Dipaola and Mandel. This may be due to the use of different buffer solution, incubation time and incubation temperature.

The sources of lactoferrin in human mouth has not been determined. However, it could arise in part from disrupting polymorphonuclear leukocytes and, in part, from epithelial cells that synthesize lactoferrin in the major and minor salivary glands (Masson, 1968; Tabak et al., 1978).

The majority of salivary leukocytes continuously migrate into the mouth through the gingival crevice (Wright, 1964; Schiott and Loe, 1970), and 97 - 99% of them are neutrophils (Attstrom, 1970; Raeste, 1972). The

lactoferrin concentrations found in the saliva of the patients with gingivitis and clinically normal individuals appear to be the same in this study. This was unexpected as a three-fold increase in the number of leukocytes entering the gingival crevice had been observed during the course of gingivitis (Schiott and Loe, 1970; Attstrom and Egelberg, 1971). This increased supply of lactoferrin could be balanced by the increased flow rate of gingival crevicular fluid in gingivitis (Attstrom and Egelberg, 1971; Alfano, 1974).

The saliva contains other antibacterial proteins. Lactoferrin and several other proteins in saliva have been identified as antibacterial agents, including secretory Ig A, the lactoperoxidase-thiocyanate-hydrogen peroxide system, and lysozyme. The concentration of each of these antibacterial agents alone may not be sufficient to influence the oral flora. They may need to act synergistically to accomplish that task. Arnold et al. (1979) found that although there was no significant difference in the lactoferrin values in saliva between the patients with immune dysfunction and controls, the salivary concentrations of lysozyme and lactoperoxidase tend to be elevated in the group of immune deficient patients as compared to normal controls.

There are apparently conflicting results in the measurements of salivary and sulcus fluid concentrations of antibacterial proteins. Friedman et al. (1983) found that lactoferrin in the crevicular fluid from the normal group was significantly lower than those from gingivitis group (0.63 vs. 1.5 ug/ul), but there was no differences in the lysozyme concentrations between these two groups. van Palerstein Helderman (1976) found that there were no

significant differences in lysozyme concentrations between normal subjects and those with gingivitis.

Another important consideration in future studies of salivary antibacterial proteins was that of Olson et al. (1985) who found that the saliva of smokers contained lower concentrations of lactoferrin and greater concentrations of thiocyanate than the saliva in non-smokers, and no differences in lysozyme concentrations between these two groups.

It is uncertain at present whether the levels of the other antibacterial agents in saliva have been changed in the patients with gingivitis or chronic periodontitis.

These observations may reflect differences in the methods used to measure such proteins or variabilities in the salivary secretions of such proteins in subjects with gingivitis and in smokers. The reasons for such differences remain to be explained.

CHAPTER VI

SUMMARY AND CONCLUSION

SUMMARY:

Ten adult clinical patients with gingivitis and ten clinically normal age- and sex-matched control subjects were selected for measuring the concentrations of lactoferrin in saliva by using an enzyme-linked immunosorbent assay (ELISA) technique. At the baseline, all participants were given a clinical examination, and provided saliva samples. Patients with gingivitis then received the following treatment: oral hygiene instruction, scaling, root planing and gingival curettage. Approximately five weeks after the initial appointment, both patients with gingivitis and control subjects came back for reexamination. At this time, the baseline procedures were repeated, and the study terminated.

CONCLUSION:

(1) The experimental results reported in this study do show the presence of lactoferrin in the whole saliva of patients with gingivitis and clinically normal individuals.

(2) Double antibody sandwich ELISA technique is a very sensitive and useful method to determine the concentration of lactoferrin in saliva.

(3) The levels of lactoferrin in the saliva of patients with gingivitis and individuals with clinically normal gingiva do not have significant differences as determined by ELISA technique. This suggests that gingivitis

does not change the host's response with respect to the lactoferrin level in saliva.

(4) After the patients with gingivitis in this study received scaling, root planing, gingival curettage and oral hygiene instruction, the level of lactoferrin in their saliva did not change. This suggests that lactoferrin level in saliva does not respond to the treatment of gingivitis, even though there is a significant clinical improvement in the gingival inflammation.

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APPENDIX

LOYOLA DENTAL SCHOOL DEPARTMENT OF PERIODONTICS

PERIODONTAL CASE RECORD

Patient # 1 Student Date

Residence Address Phone

Business Address Phone

Insurance Company Address Phone

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
PRE		333	323	322	323	322	322	323	323	322	222	323	323	223		
POST		323	322	323	323	322	322	323	323	212	212	323	313	212		

FACIAL / R / LINGUAL

PRE		324	423	424	423	323	323	323	323	222	222	223	322	322		
POST		323	323	313	323	323	323	323	323	212	222	322	322	322		
GI	Pre	2 / 1	2 / 1	0 / 0	1 / 0	1 / 1	1 / 0	1 / 0	2 / 1	2 / 1	2 / 1	1 / 0	1 / 0	2 / 0		Post
M-G																
PRE	223			322	222	222	212	222	212	323	323	332	323		223	323
POST	323			222	222	222	212	211	212	222	211	222	322		223	323

LINGUAL / R / FACIAL

PRE	223			322	322	222	322	223	323	323	312	322	322		223	333
POST	223			322	322	222	212	212	212	223	212	312	322		223	323
GI	Pre 2 / 2			2 / 1	2 / 1	1 / 1	2 / 1	2 / 1	2 / 0	2 / 1	1 / 1	1 / 1	1 / 1		2 / 1	2 / 1 Post
M-G																
	32	31	30	29	28	27	26	25	24	23	22	21	20	19	18	17

PRE-EXAMINATION Date: POST-EXAMINATION Date:

LOYOLA DENTAL SCHOOL DEPARTMENT OF PERIODONTICS

PERIODONTAL CASE RECORD

Patient # 2 Student Date

Residence Address Phone

Business Address Phone

Insurance Company Address Phone

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
PRE	434	423	333	322	323	333	333	323	323	323	323	323	333	323	324	
POST	334	423	333	323	323	323	333	323	323	323	323	323	333	323	324	

FACIAL

R

LINGUAL

PRE	333	333	333	323	333	323	323	323	323	323	323	323	333	333	334	
POST	333	323	333	323	333	323	313	323	223	223	222	323	323	333	333	
GI	Pre 2	3/2	2/1	2/2	1/1	2/1	2/1	1/1	1/1	2/1	2/1	2/2	2/2	2/2	2/2	Post
M-G																
PRE		323	333	323	323	323	313	323	222	323	313	323	333	333	334	
POST		333	333	333	323	323	222	322	222	323	313	323	333	333	334	

LINGUAL

R

FACIAL

PRE		434	434	323	333	313	323	323	323	333	333	333	323	334	334	
POST		323	333	323	323	322	322	323	323	323	323	333	323	323	333	
GI	Pre	3/2	3/2	2/2	2/1	2/1	2/1	2/1	2/1	2/1	2/1	2/1	2/2	2/1	3/2	Post
M-G																
	32	31	30	29	28	27	26	25	24	23	22	21	20	19	18	17

PRE-EXAMINATION Date: POST-EXAMINATION Date:

LOYOLA DENTAL SCHOOL DEPARTMENT OF PERIODONTICS

PERIODONTAL CASE RECORD

Patient # 3 Student _____ Date _____

Residence Address _____ Phone _____

Business Address _____ Phone _____

Insurance Company _____ Address _____ Phone _____

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
PRE		323	323	323	323	222	222	222	222	222	222	222	323	323	323	333
POST		323	323	323	323	222	222	222	222	222	222	222	323	323	323	333
FACIAL																
R																
LINGUAL																
PRE		323	323	323	323	222	222	222	222	222	222	222	323	323	323	333
POST		323	333	323	323	222	222	222	222	222	222	222	323	323	323	333
GI	Pre 2	2/2	2/2	2/1	2/1	2/1	1/1	1/1	2/1	1/1	2/1	2/1	2/2	2/2	2/2	2/2 Post
M-G																
PRE	333	333	333	333	323	333	323	333	333	323	333	233	323	333	333	333
POST	333	333	333	333	323	333	323	333	333	323	323	323	323	323	333	333
LINGUAL																
R																
FACIAL																
PRE	353	333	333	333	323	333	323	323	323	333	333	323	323	333	333	333
POST	343	333	333	333	323	333	223	323	323	332	323	323	323	333	333	333
GI	Pre 2	2/2	2/2	2/2	2/1	2/1	1/1	1/1	1/1	1/1	2/1	2/1	2/2	2/2	2/2	2/2 Post
M-G																
	32	31	30	29	28	27	26	25	24	23	22	21	20	19	18	17

PRE-EXAMINATION Date: _____ POST-EXAMINATION Date: _____

LOYOLA DENTAL SCHOOL DEPARTMENT OF PERIODONTICS

PERIODONTAL CASE RECORD

Patient # 4 Student Date

Residence Address Phone

Business Address Phone

Insurance Company Address Phone

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
PRE	333	332	332	332	322	222	222	222	222	222	222	223	223	233	323	333
POST	323	323	323	222	322	222	212	222	222	212	212	223	323	323	323	333

FACIAL

R

LINGUAL

PRE	333	333	332	322	222	222	222	222	222	222	223	323	323	333	333	333
POST	333	333	332	322	222	212	222	212	212	212	212	222	323	323	323	323
GI	Pre 2/1	2/2	2/0	1/1	1/1	1/0	2/0	2/1	2/1	1/1	1/1	1/1	1/1	2/1	2/1	2/1 Post
M-G																
PRE	333	333	333	332	322	222	222	222	222	222	222	223	223	333	333	333
POST	333	323	323	222	222	222	222	222	222	222	222	223	233	323	323	333

LINGUAL

R

FACIAL

PRE	333	333	333	332	332	332	222	222	222	222	233	333	333	333	333	333
POST	323	323	323	222	222	222	222	222	212	222	222	223	223	223	233	333
GI	Pre 2/1	2/0	2/1	2/1	2/0	2/0	2/1	2/2	2/1	2/1	2/0	1/0	1/0	2/1	2/2	2/2 Post
M-G																
	32	31	30	29	28	27	26	25	24	23	22	21	20	19	18	17

PRE-EXAMINATION Date: POST-EXAMINATION Date:

LOYOLA DENTAL SCHOOL DEPARTMENT OF PERIODONTICS

PERIODONTAL CASE RECORD

Patient # 5 Student _____ Date _____

Residence Address _____ Phone _____

Business Address _____ Phone _____

Insurance Company _____ Address _____ Phone _____

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
PRE		444	434	323	323	323	322	222	222	323	323	434	434	434	434	
POST		333	332	323	323	222	222	222	222	223	223	333	333	323	333	
FACIAL																
R																
LINGUAL																
PRE		323	323	323	323	323	323	323	323	323	323	333	323	323	323	
POST		322	222	222	322	322	212	212	212	212	222	222	222	223	323	
GI	Pre	2/1	3/1	2/1	2/0	2/0	1/0	1/0	2/1	2/1	2/1	1/0	2/1	2/1	2/1	Post
M-G																
PRE		323	222	223	223	323	222	222	222	222	223	323	323	323	323	
POST		322	222	222	222	222	212	212	222	222	222	323	323	323	323	
LINGUAL																
R																
FACIAL																
PRE		323	323	222	222	223	323	323	323	323	323	323	323	323	323	
POST		222	222	222	222	222	322	222	222	223	323	222	323	323	323	
GI	Pre	2/1	1/1	1/1	1/0	2/1	2/1	2/1	2/1	2/1	2/1	2/1	2/1	2/1	2/1	Post
M-G																
	32	31	30	29	28	27	26	25	24	23	22	21	20	19	18	17

PRE-EXAMINATION Date: _____ POST-EXAMINATION Date: _____

LOYOLA DENTAL SCHOOL DEPARTMENT OF PERIODONTICS

PERIODONTAL CASE RECORD

Patient # 6 Student Date

Residence Address Phone

Business Address Phone

Insurance Company Address Phone

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
PRE		434	434	434	435	322	222	222	222	233	323	334	333	323	333	
POST		333	323	323	333	322	222	222	222	222	323	333	333	323	333	

FACIAL / R / LINGUAL

PRE		323	323	323	222	222	222	222	222	222	222	222	223	323	323	
POST		322	323	323	322	222	222	222	222	222	223	222	223	323	323	
GI	Pre	2/2	2/2	2/1	2/1	2/1	2/1	1/1	1/1	2/1	2/1	2/1	2/1	2/2	2/2	Post
M-G																
PRE		323	323	334	434	222	222	222	222	222	223	322	433	433	333	
POST		323	322	332	333	222	212	212	222	212	223	323	333	333	333	

LINGUAL / R / FACIAL

PRE		322	323	222	223	222	222	222	222	222	222	222	223	323	323	
POST		323	323	222	222	222	222	222	222	222	222	222	222	323	323	
GI	Pre	2/2	2/1	2/1	3/2	3/2	3/2	3/2	3/2	3/2	3/2	3/2	2/1	2/1	2/2	Post
M-G																
	32	31	30	29	28	27	26	25	24	23	22	21	20	19	18	17

PRE-EXAMINATION Date: POST-EXAMINATION Date:

LOYOLA DENTAL SCHOOL DEPARTMENT OF PERIODONTICS

PERIODONTAL CASE RECORD

Patient # 7 Student _____ Date _____
Residence Address _____ Phone _____
Business Address _____ Phone _____
Insurance Company _____ Address _____ Phone _____

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
PRE			333	333	323	333	322	222	322	222	222	233	333	334		
POST			333	333	323	323	322	222	222	222	222	222	323	333		

FACIAL / R / LINGUAL

PRE			433	323	333	313	322	212	222	222	223	223	333	334		
POST			433	323	333	313	322	222	222	222	222	223	333	334		
GI	Pre		2/2	2/2	2/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	2/1	2/1	2/2	Post
M-G																
PRE			434	434	333	333	323	222	222	222	222	333	323	334		
POST			433	434	333	323	222	222	222	222	222	323	323	334		

LINGUAL / R / FACIAL

PRE			433	333	333	333	212	222	222	223	323	333	323	333		
POST			433	323	323	333	212	212	222	222	223	233	323	333		
GI	Pre		3/2	3/2	2/2	2/1	2/1	2/1	2/1	2/1	2/1	2/1	3/2	3/2		Post
M-G																
	32	31	30	29	28	27	26	25	24	23	22	21	20	19	18	17

PRE-EXAMINATION Date: _____ POST-EXAMINATION Date: _____

LOYOLA DENTAL SCHOOL DEPARTMENT OF PERIODONTICS

PERIODONTAL CASE RECORD

Patient # 8 Student _____ Date _____

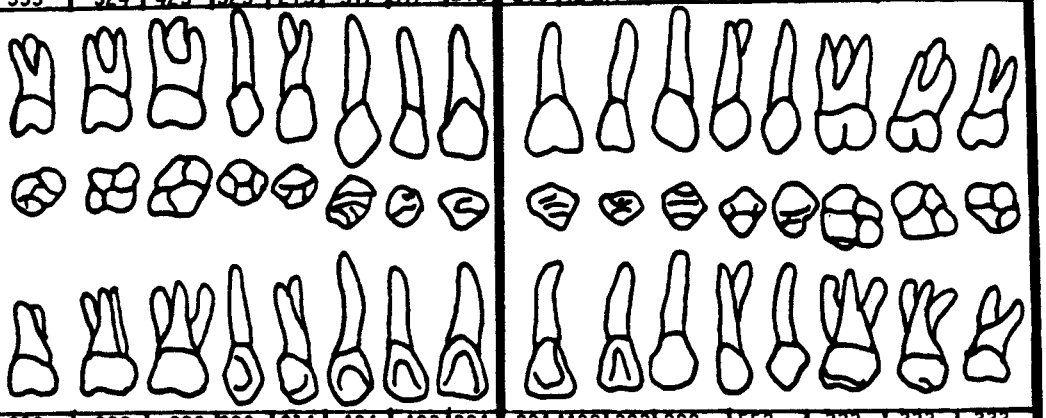
Residence Address _____ Phone _____

Business Address _____ Phone _____

Insurance Company _____ Address _____ Phone _____

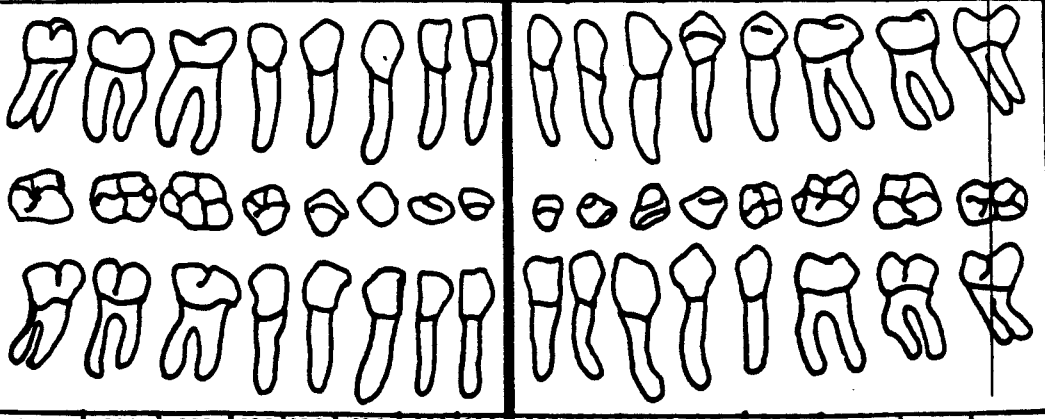
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
PRE	333	324	423	323	213	312	312	212	213	424	414	413	424	313	313	323
POST	333	324	423	323	213	312	312	212	213	424	413	412	324	313	313	323

FACIAL / R / LINGUAL



PRE	323	223	323	323	314	424	423	324	324	422	323	222	552	333	333	333
POST	323	223	323	323	314	424	423	323	324	422	323	222	442	333	333	333
GI	Pre 2/2	3/3	2/1	2/1	2/2	2/1	2/1	2/1	2/1	2/1	1/1	2/1	2/1	2/1	3/2	2/2 Post
M-G																
PRE	222	212	222	222	211	211	212	313	313	314	312	312	212	222	223	
POST	222	222	222	222	212	212	222	313	313	314	312	312	222	222	233	

LINGUAL / R / FACIAL



PRE	323	222	222	212	213	313	423	313	223	314	412	213	313	223	333	
POST	323	222	222	212	213	313	423	313	223	314	413	213	313	223	333	
GI	Pre 2/2	1/1	2/1	2/1	1/1	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/1	2/1	2/2	Post
M-G																
	32	31	30	29	28	27	26	25	24	23	22	21	20	19	18	17

PRE-EXAMINATION Date: _____ POST-EXAMINATION Date: _____

LOYOLA DENTAL SCHOOL DEPARTMENT OF PERIODONTICS

PERIODONTAL CASE RECORD

Patient # 9 Student _____ Date _____

Residence Address _____ Phone _____

Business Address _____ Phone _____

Insurance Company _____ Address _____ Phone _____

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
PRE		434	433	333	323	313	323	323	323	323	323	333		333	334	
POST		433	333	333	323	313	323	323	323	323	323	333		333	334	

FACIAL / R / LINGUAL

PRE		434	334	333	323	323	323	323	323	323	323	333		434	434	
POST		433	334	333	323	323	323	323	323	323	323	333		434	434	
GI	Pre	3/2	3/2	2/1	1/1	2/1	2/1	2/1	1/1	1/1	2/1	2/1		2/2	2/2	Post
M-G																
PRE		323	333	332	322	222	222	222	222	223	222	223	323	323	333	
POST		323	323	323	322	222	222	222	222	223	222	223	323	323	333	

LINGUAL / R / FACIAL

PRE		323	333	323	322	212	222	222	222	222	223	323	323	323	333	
POST		323	333	323	322	212	222	222	222	222	223	323	323	323	333	
GI	Pre	3/2	2/2	2/1	2/1	1/1	1/1	1/1	1/1	1/1	1/1	2/1	1/1	1/2	2/2	Post
M-G																
	32	31	30	29	28	27	26	25	24	23	22	21	20	19	18	17

PRE-EXAMINATION Date: _____ POST-EXAMINATION Date: _____

LOYOLA DENTAL SCHOOL DEPARTMENT OF PERIODONTICS

PERIODONTAL CASE RECORD

Patient # 10 Student _____ Date _____

Residence Address _____ Phone _____

Business Address _____ Phone _____

Insurance Company _____ Address _____ Phone _____

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
PRE		333	323	222		422	323	332	313	314	314		414	324	323	
POST		333	323	222		322	323	332	222	313	313		424	312	323	

FACIAL

R

LINGUAL

PRE		323	333	222		313	313	313	213	312	313		323	323	323	
POST		323	333	222		313	313	323	323	323	323		323	323	323	
GI	Pre	2 2	2 2	2 1		2 1	1 1	2 1	2 1	1 1	1 1		2 1	2 1	2 1	Post
M-G																
PRE		323	324	223		322	312	212	212	313	313		313	212	323	
POST		322	324	323		322	322	222	212	313	313		323	222	333	

LINGUAL

R

FACIAL

PRE		323	323	312		322	212	313	312	212	212		213	323	323	
POST		323	223	323		322	222	312	312	212	222		223	323	323	
GI	Pre	2 2	2 2	2 2		1 1	1 1	1 1	1 1	1 1	1 1		2 1	1 1	3 2	Post
M-G																
	32	31	30	29	28	27	26	25	24	23	22	21	20	19	18	17

PRE-EXAMINATION Date: _____ POST-EXAMINATION Date: _____

LOYOLA DENTAL SCHOOL DEPARTMENT OF PERIODONTICS

PERIODONTAL CASE RECORD

Patient # 11 Student _____ Date _____

Residence Address _____ Phone _____

Business Address _____ Phone _____

Insurance Company _____ Address _____ Phone _____

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
PRE		313	323	212	222	222	222	222	222	212	222	313	323	323	323	
POST		313	323	222	222	212	222	222	222	212	222	313	323	323	323	

FACIAL

R

LINGUAL

PRE		313	323	322	212	222	212	212	222	222	223	222	313	323	323	
POST		313	313	322	212	222	212	212	222	222	223	212	313	323	323	
GI	Pre	1/1	1/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1/1	1/1	Post
M-G																
PRE		313	313	312	222	212	212	212	212	212	312	312	312	323	323	
POST		313	313	312	222	212	212	212	212	212	312	312	312	323	323	

LINGUAL

R

FACIAL

PRE		323	323	312	312	222	211	111	212	212	212	212	213	323	323	
POST		323	323	312	312	222	211	212	212	212	212	212	213	323	323	
GI	Pre	1/1	1/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1/1	1/1	Post
M-G																
	32	31	30	29	28	27	26	25	24	23	22	21	20	19	18	17

PRE-EXAMINATION Date: _____ POST-EXAMINATION Date: _____

LOYOLA DENTAL SCHOOL DEPARTMENT OF PERIODONTICS

PERIODONTAL CASE RECORD

Patient # 12 Student _____ Date _____
Residence Address _____ Phone _____
Business Address _____ Phone _____
Insurance Company _____ Address _____ Phone _____

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
PRE	323	222	222	212	212	212	212	222	222	212	212	111	222	323		
POST	323	322	222	211	212	212	212	222	222	212	212	112	222	323		
FACIAL																
R																
LINGUAL																
PRE	333	323	323	222	222	222	222	222	222	212	222	212	323	333		
POST	333	323	323	222	222	222	222	222	222	212	222	212	323	333		
GI	Pre 1	0 0	0 0	0 0	0 0	0 0	0 0	1 1	1 1	0 0	0 0	0 0	0 0	0 0		Post
M-G																
PRE		333		323	222	322	212	111	111	111	112	212	333		323	
POST		323		323	222	322	211	111	111	111	111	212	333		323	
LINGUAL																
R																
FACIAL																
PRE		323		323	212	222	212	111	111	111	112	212	323		333	
POST		323		322	212	222	212	111	111	111	112	212	323		323	
GI	Pre 0 1			0 0	0 0	1 1	0 0	0 0	0 0	0 0	1 1	1 1	0 0		0 0	Post
M-G																
	32	31	30	29	28	27	26	25	24	23	22	21	20	19	18	17

PRE-EXAMINATION Date: _____ POST-EXAMINATION Date: _____

LOYOLA DENTAL SCHOOL DEPARTMENT OF PERIODONTICS

PERIODONTAL CASE RECORD

Patient # 13 Student _____ Date _____
Residence Address _____ Phone _____
Business Address _____ Phone _____
Insurance Company _____ Address _____ Phone _____

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
PRE	323	323	323	212		312	222	212	212	222	212	223	223	313	323	
POST	323	323	323	222		322	222	212	212	222	212	223	223	313	323	

FACIAL / R / LINGUAL

PRE	333	323	323	322		222	222	222	212	222	212	212	323	323	323	
POST	333	323	323	322		222	222	222	212	222	212	212	323	323	323	
GI	Pre 1 / 1	1 / 1	0 / 0	0 / 0		0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	1 / 1	Post
M-G																
PRE	333	323	313	312	212	222	212	211	212	222	212	212	323	323	323	
POST	333	323	323	312	212	222	212	211	212	222	222	212	323	323	323	

LINGUAL / R / FACIAL

PRE	333	323	323	312	212	212	212	212	212	212	212	213	313	323	323	
POST	333	323	323	312	212	212	212	212	212	212	222	213	313	323	323	
GI	Pre 2 / 2	1 / 1	1 / 1	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	1 / 1	1 / 1	1 / 1	Post
M-G																
	32	31	30	29	28	27	26	25	24	23	22	21	20	19	18	17

PRE-EXAMINATION Date: _____ POST-EXAMINATION Date: _____

LOYOLA DENTAL SCHOOL DEPARTMENT OF PERIODONTICS

PERIODONTAL CASE RECORD

Patient # 14 Student _____ Date _____

Residence Address _____ Phone _____

Business Address _____ Phone _____

Insurance Company _____ Address _____ Phone _____

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
PRE	323	222	323	222		322	222	222	212	222	212		222	333	333	323
POST	323	222	323	222		322	222	222	212	222	212		222	333	333	323

FACIAL

R

LINGUAL

PRE	323	323	323	222		222	212	212	212	212	222		213	323	323	323
POST	323	323	323	222		222	212	212	212	212	222		213	323	323	323
GI	Pre 1	0	0	0		0	0	0	0	0	0		0	1	1	1 Post
M-G																
PRE		323	222	212		212	212	212	212	212	212		223	323	323	
POST		323	222	212		212	212	212	212	212	212		223	323	323	

LINGUAL

R

FACIAL

PRE		323	222	212		212	212	212	212	212	212		222	222	323	
POST		323	222	212		212	212	212	212	222	212		222	222	323	
GI	Pre	1	0	0		0	0	0	0	0	0		0	0	1	Post
M-G																
	32	31	30	29	28	27	26	25	24	23	22	21	20	19	18	17

PRE-EXAMINATION Date: _____ POST-EXAMINATION Date: _____

LOYOLA DENTAL SCHOOL DEPARTMENT OF PERIODONTICS

PERIODONTAL CASE RECORD

Patient # 15 Student _____ Date _____

Residence Address _____ Phone _____

Business Address _____ Phone _____

Insurance Company _____ Address _____ Phone _____

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
PRE		322	322	222	222	212	222	212	212	212	212	222	222	213	323	
POST		322	322	222	222	212	222	212	212	212	212	222	223	313	323	

FACIAL

R

LINGUAL

PRE		323	312	212	222	212	212	212	212	212	222	222	323	323	323	
POST		323	312	212	222	212	212	212	212	212	222	222	323	323	323	
GI	Pre	0/0	0/0	0/0	0/0	0/0	1/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	Post
M-G																
PRE		322	323	322	212	222	212	212	211	112	222	223	323	323	323	
POST		322	323	322	212	222	212	212	211	112	222	223	323	323	323	

LINGUAL

R

FACIAL

PRE		323	323	222	322	222	212	212	212	212	212	212	222	323	323	
POST		323	323	222	322	222	212	212	212	212	212	212	222	323	323	
GI	Pre	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	Post
M-G																
	32	31	30	29	28	27	26	25	24	23	22	21	20	19	18	17

PRE-EXAMINATION Date: _____ POST-EXAMINATION Date: _____

LOYOLA DENTAL SCHOOL DEPARTMENT OF PERIODONTICS

P E R I O D O N T A L C A S E R E C O R D

Patient # 16 Student _____ Date _____
Residence Address _____ Phone _____
Business Address _____ Phone _____
Insurance Company _____ Address _____ Phone _____

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
PRE		323	323	323	222	222	222	222	222	222	222	222	323	323	323	
POST		323	323	323	222	222	222	212	222	222	212	222	323	323	323	

FACIAL

R

LINGUAL

PRE		323	323	323	222	222	222	212	212	222	222	223	323	323	323	
POST		323	323	323	222	222	222	222	212	222	222	223	323	323	323	
GI	Pre	1 / 1	0 / 0	1 / 1	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	1 / 1	1 / 1	1 / 1	0 / 0	Post
M-G																
PRE		323	333	323	222	212	212	212	212	212	212	222	323	323	333	
POST		323	333	323	222	212	212	212	212	212	212	222	323	323	333	

LINGUAL

R

FACIAL

PRE		333	333	323	222	222	222	222	212	212	222	222	222	323	333	
POST		333	333	323	222	222	212	212	212	212	212	222	222	323	333	
GI	Pre	2 / 2	2 / 2	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	1 / 1	1 / 1	2 / 2	Post
M-G																
	32	31	30	29	28	27	26	25	24	23	22	21	20	19	18	17

PRE-EXAMINATION Date: _____ POST-EXAMINATION Date: _____

LOYOLA DENTAL SCHOOL DEPARTMENT OF PERIODONTICS

PERIODONTAL CASE RECORD

Patient # 17 Student _____ Date _____

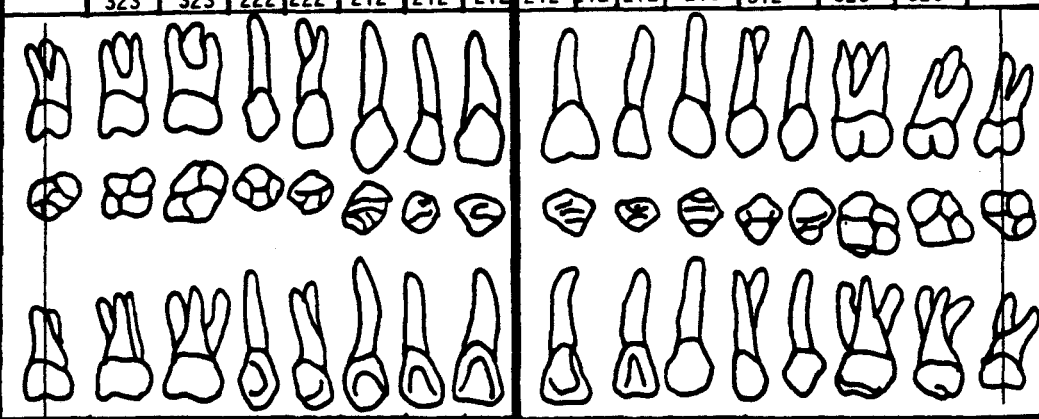
Residence Address _____ Phone _____

Business Address _____ Phone _____

Insurance Company _____ Address _____ Phone _____

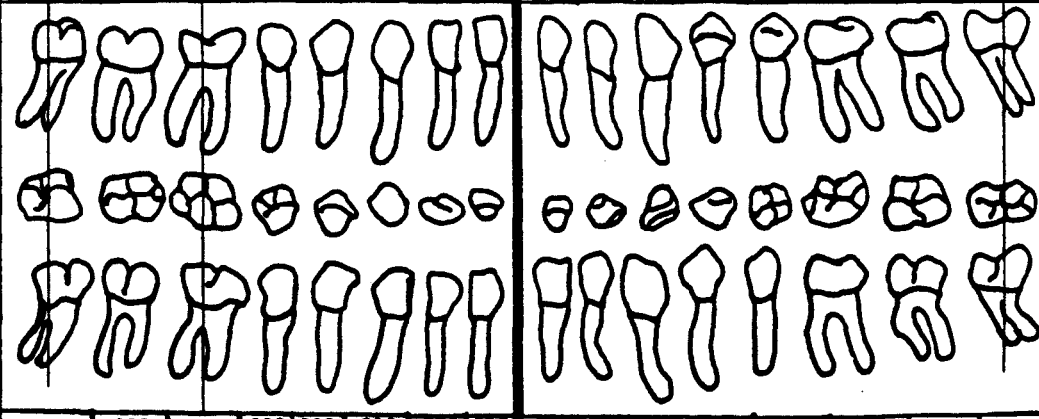
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
PRE		323	323	222	222	212	212	212	212	212	212	213	312	323	323	
POST		323	323	222	222	212	212	212	212	212	212	213	312	323	323	

FACIAL / R / LINGUAL



PRE		323	323	222	222	212	212	222	222	212	212	213	323	333	333	
POST		323	323	222	222	212	212	222	222	212	212	213	323	323	333	
GI	Pre	1/1	1/1	0/0	0/0	0/0	0/0	1/1	1/1	0/0	0/0	0/0	0/0	1/1	1/1	Post
M-G																
PRE		323		323	322	212	222	212	212	212	212	222	323	323	333	
POST		333		323	322	212	222	212	212	212	212	222	323	323	333	

LINGUAL / R / FACIAL



PRE		323		323	322	212	222	212	212	212	212	222	323	323	333	
POST		323		323	322	222	222	212	212	212	212	222	323	323	333	
GI	Pre	0/0		0/0	0/1	0/0	1/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1/1	Post
M-G																
	32	31	30	29	28	27	26	25	24	23	22	21	20	19	18	17

PRE-EXAMINATION Date: _____ POST-EXAMINATION Date: _____

LOYOLA DENTAL SCHOOL DEPARTMENT OF PERIODONTICS

PERIODONTAL CASE RECORD

Patient # 18 Student _____ Date _____

Residence Address _____ Phone _____

Business Address _____ Phone _____

Insurance Company _____ Address _____ Phone _____

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
PRE		222	222	212		212	212	212	212	212	212		222	223	323	
POST		222	222	212		212	212	212	212	212	212		222	223	323	

FACIAL

R

LINGUAL

PRE		322	322	212		212	212	212	212	212	212		222	223	323	
POST		323	322	212		212	212	212	212	212	212		222	223	323	
GI	Pre	0 / 0	0 / 0	0 / 0		0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0		0 / 0	0 / 0	0 / 0	Post
M-G																
PRE		333	323	212		222	222	111	222	212	222		222	223	323	
POST		333	323	212		222	222	212	222	212	222		222	223	323	

LINGUAL

R

FACIAL

PRE		333	323	212		222	222	212	212	212	111		222	222	323	
POST		333	323	212		222	222	212	212	212	212		222	222	323	
GI	Pre	1 / 1	0 / 0	0 / 0		0 / 0	2 / 1	0 / 0	0 / 0	0 / 0	0 / 0		0 / 0	0 / 0	0 / 0	Post
M-G																
	32	31	30	29	28	27	26	25	24	23	22	21	20	19	18	17

PRE-EXAMINATION Date: _____ POST-EXAMINATION Date: _____

LOYOLA DENTAL SCHOOL DEPARTMENT OF PERIODONTICS

PERIODONTAL CASE RECORD

Patient # 19 Student _____ Date _____

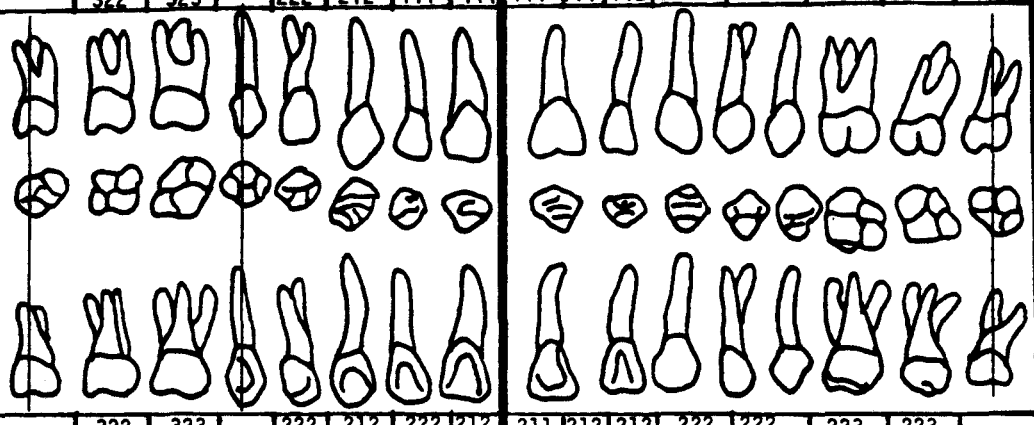
Residence Address _____ Phone _____

Business Address _____ Phone _____

Insurance Company _____ Address _____ Phone _____

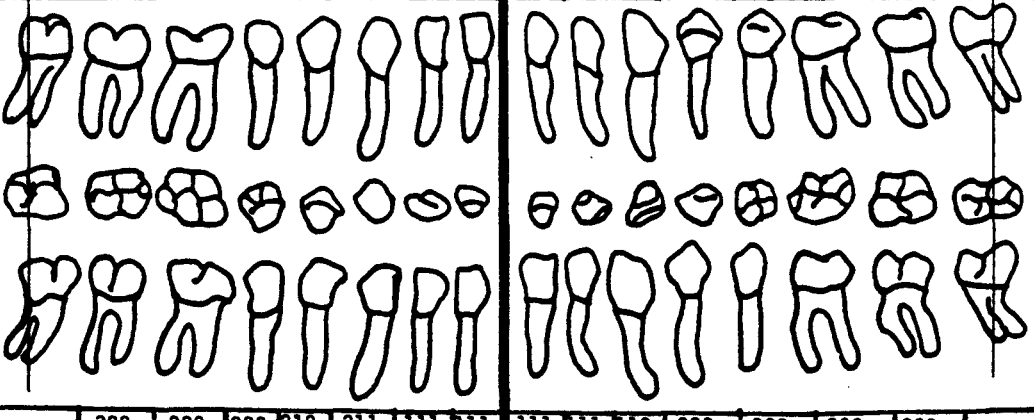
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
PRE		322	323		222	212	111	111	111	11	112	212	222	222	222	
POST		322	323		222	212	111	111	111	11	112	212	222	222	222	

FACIAL / R / LINGUAL



PRE		322	323		222	212	222	212	211	212	212	222	222	223	223	
POST		322	323		222	212	222	212	211	212	212	222	222	223	223	
GI	Pre	0 0	1 1		1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	Post
M-G																
PRE		322	222	222	211	111	111	111	111	111	111	111	212	223	323	
POST		322	222	222	211	111	111	111	111	111	111	111	212	223	323	

LINGUAL / R / FACIAL



PRE		322	222	222	212	211	111	111	111	111	112	222	222	223	323	
POST		322	222	222	212	211	111	111	111	111	112	222	222	223	323	
GI	Pre	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	Post
M-G																
	32	31	30	29	28	27	26	25	24	23	22	21	20	19	18	17

PRE-EXAMINATION Date: _____ POST-EXAMINATION Date: _____

LOYOLA DENTAL SCHOOL DEPARTMENT OF PERIODONTICS

PERIODONTAL CASE RECORD

Patient # 20 Student _____ Date _____
Residence Address _____ Phone _____
Business Address _____ Phone _____
Insurance Company _____ Address _____ Phone _____

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
PRE	323	323	323	322	222	212	222	212	212	222	212	322	223	323	323	333
POST	333	333	323	322	222	212	222	222	222	222	222	322	223	323	323	333
FACIAL																
R																
LINGUAL																
PRE	323	323	323	222	223	222	222	222	222	222	222	223	322	323	323	333
POST	323	323	313	212	223	222	222	222	222	222	222	223	322	323	323	333
GI	Pre 1	1 1	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 1 Post
M-G																
PRE		323	323	322	222	312	212	212	212	212	212	322	313	323	323	
POST		323	323	322	222	312	212	212	212	212	212	322	323	323	323	
LINGUAL																
R																
FACIAL																
PRE		323	323	212	222	212	212	212	212	212	212	222	223	333	333	
POST		323	323	212	222	222	212	212	212	212	222	222	223	333	333	
GI	Pre	1 1	1 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 1	Post
M-G																
	32	31	30	29	28	27	26	25	24	23	22	21	20	19	18	17

PRE-EXAMINATION Date: _____ POST-EXAMINATION Date: _____

APPROVAL SHEET

The thesis submitted by Hwu-Rang Lin has been read and approved by the following committee:

Dr. Patrick Toto, Director
Professor, Gen/Oral Pathology, Loyola

Dr. Anthony Gargiulo
Professor, Periodontics, Loyola

Dr. James Hagen
Associate Professor, Microbiology, Loyola

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 by the Committee with reference to content and form.

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

10-14-86
Date

Director's Signature