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The Etiology of Periodontal Disease

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THE ETIOLOGY OF PERIODONTAL DISEASE

by

Raymond Joseph Loiselle

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Oral Biology.

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# THE ETIOLOGY OF PERIODONTAL DISEASE

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INTRODUCTION

Periodontal disease consists of the destruction of the periodontal tissues. These tissues include the gingiva, the alveolar bone, the supporting bone, the periodontal ligament and part of the tooth. The inflammation of the gingiva and underlying connective tissue spreads apically to involve the alveolar bone and process. These are attacked resulting in resorption of the bone. The result of the reactions ultimately causes the loosening and migration of teeth. Eventually, it can result in loss of teeth.

Statistical studies performed in this country between the years 1960 to 1962 revealed the following data: Of this country's approximately 110,000,000 adults, more than 20,000,000 no longer had any teeth. Approximately 25% of the remaining 90,000,000 had marked periodontal disease. Fifty percent of the 90,000,000 had gingivitis. Only about 25% of the adults exhibited little or no signs of gingivitis or periodontitis. In the United States, only about 10% of men or women aged 18-24 have a severe form of periodontal disease. With aging, the disease develops more rapidly. At 45-54 years of age 37% of the men and 30% of the women exhibited periodontitis while at 65-74 years, 58% of men and 33% of women showed signs of periodontitis.

Studies in England revealed the steady increase in the incidence of gingivitis from early childhood to late adolescence. The incidence of periodontal lesions increases from 10-29% at 19-25 years of age up to 97-100% in persons aged over 45 years. Although severe manifestations
may not appear until middle age, the early lesion is frequently present
during childhood. Statistical studies indicate that periodontal disease
is the major cause of tooth loss. Although caries is responsible for most
tooth removal prior to age 35, after this age over two-thirds of tooth
loss is due to periodontal disease.

At the 1966 World Workshop in Periodontics, it was stated that
practically all human beings have some form of periodontal disease.
Periodontal disease, per se, does not usually cause acute discomfort until
it enters into the terminal stages. Its public health significance lies
in its ultimate consequences, tooth loss. Thus, it becomes mandatory to
investigate the causes of the disease and the contributing factors to its
severity. It has been generally assumed that microorganisms or their
soluble components and/or products play a role in the etiology of perio-
dontal disease. Some of the evidence for and against this are as follows:

Several authors have indicated that some of the bacteria in the
oral flora produce enzymes that are capable of disrupting gingival tissue
components (Schultz-Haudt and Lundquist 1962, Lucas, R. B. and Thonard, J.C.
Schultz-Haudt, S. D., Bibby, B. G. and Bruce, M. A. 1954, Rapp, G. W.
and Orzolek, L. M. 1966). However, several investigators have since
pointed out that these same destructive enzymes are also produced by
polymorphonuclear leucocytes, which have been found in inflamed tissue.
S. W. Leung (1962) has made the statement that "the initial injury which starts the chain of events is thought to be physical damage caused by such substances as calculus and impacted food and debris. This leads to inflammation and subsequent bacterial invasion of the injured tissues."

However, dental calculus deposition has been shown to take place in germ-free rats. Baer and Newton demonstrated in both Swiss germ-free and conventional mice (1960) there was downgrowth of the epithelial attachment and loss of alveolar bone. Inflammation and the presence or absence of bacteria did not seem to play a role, initially, in these periodontal changes.

Keyes and Jordan (1964) have found filamentous organisms capable of producing an active type of periodontal disease in albino hamsters. These filamentous organisms have been recovered from subgingival plaque of susceptible strains of hamsters.

There have been conflicting reports concerning the penetration of bacterial into the gingival tissue. Gibson and Shannon (1965) demonstrated that if tooth debridement was performed in the presence of carbon particles, the particles appeared to penetrate the underlying connective tissues as seen in stained tissue sections. This effect did not appear in the biopsies and sections from the non-debrided group. This may tend to refute some histological studies which purported to demonstrate bacteria in gingival tissues (Beckwith et al 1925, Beckwith et al 1927, Box 1950, Vargas, B. et al 1959). It has also been reported that microorganisms have been forced into the tissues by the sectioning procedures, thus creating the artifact of bacterial presence in the tissues in the finished histological
Diffusable bacterial products are also agents in the etiology of periodontal disease. (Bibby, BG 1960). One example of this is the demonstration by Cobb and Brown that a sterile, filterable substance from gingival sulci and periodontal pockets is toxic to cells in tissue culture. A greater effect was observed with the material from the periodontal pockets (Periodontics 5:5, 1967). Rizzo (1967) demonstrated that hydrogen sulfide is produced in periodontal pockets.

Studies have shown the presence of fungal organisms in addition to bacteria in calculus and plaque. Howell, Rizzo, and Paul (1965) cultured calculus-plaque material and found bacteria and the fungal organisms (Actinomyces israelii, Actinomyces naeslundii and Veillonella species (schizomycetes). Wenford and Haberman (1966) cultured diseased gingiva and isolated strains of Actinomyces naeslundii and Nocardia salivae, as well as bacterial microorganisms. However, the presence of these fungal organisms is no indication of their role, if any, in the etiology of periodontal disease.

Severe periodontal disease was induced in a group of mice infected at birth with polyoma virus (1965). Shklar and Cohen described the periodontal alterations, which included apical migration of epithelial attachment, periodontal pocket formation with purulent exudate, and extensive resorption of alveolar bone. Similarly, Baer and Kilham (1964) describe the occurrence of periodontal disease in hamsters inoculated intracerebrally with rat virus.

Rizzo and Mergenhagen (1965) produced hypersensitivity reactions in
a palato-gingival site in the rabbit by sensitizing and challenging with horse serum, and by sensitizing with human tubercle bacilli and challenging with PPD. The ensuing plasmacytosis, enhanced by repeated local injections of antigen, resembled histologically that occurring in human gingivitis. In a later study Rizzo and Mitchell (1966) reported that the repeated deposition of antigenic protein into healthy gingival pockets of the rabbit caused "chronic allergic inflammation", with plasmacytosis, in the adjacent periodontal tissues. Low levels of serum antibody to the deposited antigen were evident within two weeks after treatment began and tended to remain at low levels throughout the 17 weeks of continual pocket exposure to the antigen, egg albumin. The published data indicate that antigen was absorbed into the gingiva and that it caused both a local and systemic immunologic response. Thonard and Dalbow (1965) injected sheep red blood cells into the interdental papillae of rats and guinea pigs and found antibody-forming cells in the gingival tissue by preparing cell suspensions and testing for antibody production by the antibody-plaque technique.

Dalbow and Baumhammers (1968) showed by immunofluorescent techniques the presence of major classes of immunoglobulins in periodontal tissue of both humans and animals with periodontal disease. These immunoglobulins were also present in normal human gingiva.

Platt, Dalbow and Crosby (1968) upon trying to simulate a delayed-hypersensitivity response by the injection of histo-compatible lymphoid cells into germ-free mice intraperitoneally, followed by the intragingival injection of heat-denatured collagenase in Freund's adjuvant, were able to demonstrate marked round-cell infiltration, pronounced collagen
disaggregation and fibrotic changes in the gingivae.

Ranney (1968) challenged sensitized monkeys by inserting ovalbumin into the gingival sulcus. Three hours after a single challenge, a dense infiltration of polymorphonuclear leucocytes beneath the sulcular epithelium and vascular congestion and dilatation were visible. In three days, the acute inflammation subsided and mononuclear cells were prominent in the tissue sections. When three consecutive challenges were employed the cellular infiltration was more dense and dissolution of collagen and osteoclastic activity were prominent.

Thonard, Welty, Dalbow and Platt (1968) gave germ-free mice a prolonged regimen of challenge with Clostridium histolyticum collagenase, in the native state or heat-inactivated, and showed an altered tissue response that histo-pathologically resembles chronic periodontitis. If immunosuppressive quantities of Imuran were given to the animals, no significant changes in the periodontal structures occurred, even when subjected to prolonged challenge with the enzyme.

Thus, immunological reactions have been shown to cause inflammation and tissue alterations in experimental animals. The nature of these reactions has not been identified, nor have their relations, if any, to human periodontal disease. Although proper oral hygiene will alleviate some of the symptomatology of periodontitis, the relationship of bacteria and fungi to the disease have not been satisfactorily explained.
SPECIFIC AIDS

The presence of immunoglobulins in the crevicular fluid of human gingiva as reported by Brill and Bronnestam (1960) and confirmed by other investigators coupled with the occurrence of immunoglobulins in the cytoplasm of gingival epithelial cells raised the following questions: (1) What is the nature of the antigen or antigens responsible for stimulating the local antibody production? (2) Is this local production of immunoglobulin associated with a protective or an allergic reaction on the behalf of the host? It may be that the globulins are directed against bacterial antigens or against tissue components. When viewed in this context, the problem of cross-reactivity of antibodies to bacterial and tissue antigens may play a major role in chronic periodontal disease. In other words, antibodies initially formed against bacteria may ultimately cross react tissue in the periodontal structure. The patients utilized in this clinic study were selected from the population examined by the Dental Service, Veterans Administration Hospital, Hines, Illinois. The patients utilized were those showing no acute cause for periodontitis. The lack of the acute phase of the disease at the time of sampling did not imply it did not exist, but merely that it had been treated and eliminated and what was now seen was a chronic disease state after the removal of acute initiating factors. The diagnosis of chronic periodontitis was confirmed by histological studies of biopsy specimens taken after cultures of the gingival crevice were obtained.

This particular study is designed to examine one aspect of periodontal
disease - chronic, non-suppurative periodontitis. I wish to determine whether there is a relationship between the bacterial organisms present in the gingival sulcus of patients with chronic, non-suppurative periodontitis and the reaction which these patients have to periodontal therapy.
EXPERIMENTAL METHOD

Gingival culture specimens were taken in a standardized fashion from twenty patients. The technique used utilized pre-packaged individual sterile trays for each patient. The cultures were taken by carefully inserting sterile absorbant paper points into the gingival crevice with sterile cotton forceps. The paper points were then placed in test tubes containing enriched nutrient broth and transported in this media to the bacteriology laboratory. In the bacteriology laboratory the points were removed from the test tube and plates streaked and incubated for the purpose of detecting all anaerobic and aerobic organisms present in the initial specimen taken from the gingival crevice. In addition, thioglycolate broth was also inoculated as a further means of checking the effectiveness of culturing the organisms in the aerobic and anaerobic techniques. Using selective media and environment all organisms found growing on any of the plates were identified.

The organisms routinely cultured from all of the patients sampled were streptococcus viridans. The Streptococcus viridans were then further subcultured and identified. Identification of Streptococcus mitis and Streptococcus salivarius was made by the use of selective Streptococcus mitissalivarius medium. Neissena catarrhalis were recovered from 30% of the patients. The distinction between S. salivarius and S. mitis was made on the basis of morphology, growth in selective media and ability to ferment and utilize added nutrients. S. salivarius are spherical or ovoid cells less than 1 micron in diameter and form either long or short chains. Normally
an indifferent or gamma hemolytic reaction on horse blood agar is seen but a few strains will produce greening. Optimal growth occurs at 37° and no growth occurs at 10° or 47° C. When grown on agar media containing sucrose or raffinose, large characteristic mucoid colonies are formed. These colonies may be rough or smooth. Variation between rough and smooth types occurs spontaneously in broth cultures. S. salivarius will not grow in broth containing 6.5% sodium chloride or at a pH 9.6 or on 30% bile blood agar or in milk containing 0.1% methylene blue. S. Salivarius ferments salicin, inulin, raffinose, sucrose, maltose and glucose. Gelatin in starch is not hydrolized by S. salivarius nor can it produce ammonia from arginine.

Streptococcus mitis forms slightly smaller cells than S. salivarius, seldom exceeding 0.8 microns in diameter. Only a few strains of S. mitis produce mucoid colonies on sucrose agar. True rough and smooth variants occur regularly in solid or liquid media and the rough form tends to revert to the smooth form in liquid media. S. mitis consistently ferments glucose, maltose and sucrose, usually ferments lactose and salicin, and rarely ferments raffinose. Gelatin is not hydrolized by S. mitis.

The large number of organisms necessary for further stages of this work were obtained by propagating pure cultures of S. salivarius ATCC9222, S. mitis ATCC903 and N. catarrhalis ATC8176 on selective media plates. The colonies were then harvested mechanically by means of sterile loop from the plate into sterile BHI broth where they were re-suspected. The organisms were then recovered from the broth by centrifugation. Specific antibodies to the bacteria were then developed in New Zealand rabbits.
Additional quantities of the bacteria were grown for conjugation with fluorescein isothiocynate.

Gingival biopsies and biopsies of masticatory mucosa from edentulous regions were performed in the patients from whom the cultures were taken. Frozen sections of these tissues were cut for microscopic and immunologic examination.

It was of interest to know if there were streptococcal antigens or antibodies to these antigens in the tissue sections. The sections were cut, frozen and mounted on a glass microscope slide. The tissue was treated with rabbit antiserum to the specific microorganisms (mitis and salivarius) and then stained with fluorescein conjugated goat or sheep anti-rabbit serum.

To reduce the non-specific binding of the rabbit anti-mitis and anti-salivarius serum to the tissue, each step was preceded by incubation with normal serum. Fluorescein tagged microorganisms were also used to determine the presence of antibodies to S. mitis and S. salivarius in the tissue sections.

The results obtained in the two variations above with human gingival tissue was compared with that seen in masticatory mucosa from the edentulous tuberosity from the same patients. Thus, the tissue from the edentulous region should not demonstrate the same immunological reactions as the gingival tissue, not having been subjected to the same microbiological and trauma stresses as the gingival tissue. The alternative to using human tissue as a control would be to use the gingival tissue of an immunized and a nonimmunized, non-exposed rabbit. This would assure both positive and negative controls, especially for the determination of antibodies in the
tissue but would not necessarily be of value in relation to human periodontal disease.

The immunological procedures used for staining tissue are as follows:

(1) tissue → rabbit antiserum → normal sheep serum → fluorescent sheep antiserum to rabbit;

(2) tissue → fluorescent microorganisms.
DATA

A coupling of the specific anti-sera was noted in the gingival sections while none was present in the tissue specimens of masticatory mucosa from edentulous regions. Figures 1 through 4 show fluorescent staining with anti-neisseria and anti-streptococcus sera. Figure 5 shows binding of fluorescein tagged bacteria (S. mitis) to the tissue section. Histologic examination of comparable sections stained with H & E show a severe plasmacytosis. The same patients masticatory mucosa tissue sections did not show a marked plasmacytosis nor did they bind the specific anti-sera or fluorescein coupled Streptococcus mitis.
Figure 1

Gingival tissue from a patient with periodontal disease stained with rabbit antiserum to Neisseria catarrhalis, followed by fluorescein-conjugated sheep, anti-rabbit-globulin serum. This shows fluorescence in cells below the keratin layer. Magnification about 100X.
Gingival tissue from a patient with periodontal disease stained with rabbit antiserum to Neisseria catarrhalis, followed by fluorescein-conjugated sheep, anti-rabbit-globulin serum. This shows fluorescence in cells below the keratin layer. Magnification about 100X.
Figure 3

Gingival tissue from a patient with periodontal disease stained with rabbit antiserum to *Streptococcus mitis*, followed by fluorescein conjugated sheep anti-rabbit-globulin serum. This shows fluorescence exhibited by a single cell in the field. Magnification = 250X.
Gingival tissue from a patient with periodontal disease stained with rabbit antiserum to *Streptococcus salivarius*, followed by fluorescein-conjugated sheep anti-rabbit-globulin serum. This fluorescence is seen in the sub-keratin layer. Magnification = 400X.
Figure 5

Gingival tissue from a patient with periodontal disease stained with fluorescein-conjugated Streptococcus mitis. Fluorescence is seen throughout the whole epithelial layer. Magnification = 25.
SUMMARY AND CONCLUSIONS

1) Organisms cultured from the gingival crevice of patients with non-superative, chronic periodontitis were predominently Streptococcus viridans. Neisseria catarrhalis were recovered from 30% of the patients.

2) Further identification of the S. viridans showed them to be S. mitis and S. salivarius.

3) Anti-sera to S. mitis and S. salivarius and the bacteria themselves could be bound to sections of mucosa from the gingiva of the patients but not to the sections of masticatory mucosa from edentulous regions of the same patients.

From these points I concluded that the bacteria in the gingival sulcus plays a role in chronic periodontal disease. This role would appear to be in the realm of immunologic reactions rather than a toxic reaction.
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APPROVAL SHEET

The thesis submitted by Dr. Raymond Joseph Loiselle has been read and approved by members of the Department of Oral Biology.

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

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

Date: Jan 23, 1962

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