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Specific Bacterial Antibodies in the Inflamed Human Gingiva

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SPECIFIC BACTERIAL ANTIBODIES IN THE INFLAMED HUMAN GINGIVA

by

Thomas F. Schneider

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

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CHAPTER I
INTRODUCTION

It has been common knowledge for many years that recovery from certain of the infectious diseases is accompanied by the development of an enhanced resistance, and recurrent attacks of a disease, once overcome, are not common. This enhanced resistance, or immunity, is specific, i.e. an individual resistant to one disease may be no more than ordinarily resistant to others, and is variable from one disease to another. Some give rise to a solid immunity of long duration while in others the immunity is imperfect or partial and transient. The specific immune state supplements the complex of factors which make up non-specific reactions or resistance to infection, and in some instances even a solid immunity may be broken down by fatigue, malnutrition, and similar factors which are not consistent with a state of physiologic well being.

Specific immunity is a consequence of the reaction of the host to the microorganism and/or its products and provides the basis for prophylaxis of disease or infection, and its development during the course of the disease is the primary determining factor in the outcome of the infection.

Historically, immunity or immunology was concerned primarily with disease, but it became clear that the basic principles have general biologic application that as yet has been limited.
Immunologic specificity appears to be intimately related to the basis of biologic individuality, and makes possible a precise and sensitive characterization of the larger molecular constituents of protoplasm. As such, the immunologic methods become unique analytical methods applicable to problems such as protein synthesis, blood and other tissue incompatibilities, phylogenetic relationships, as well as to certain kinds of non-infectious diseases or manifestations of disease such as the hypersensitivities.

Although it is not the intent of this study to demonstrate an immune response in the human gingiva, one can certainly see the possibility that this may some day be seen. It is intended to describe a method of demonstrating a local defense mechanism of the gingival tissue to the bacteria present in the adjacent sulcus or pocket. This mechanism will be shown as an antigen-antibody reaction.
CHAPTER II
REVIEW OF LITERATURE

The inflammatory reaction in the human gingiva or of other supporting structures of the teeth is not different from that of other regions of the body. Erich (1953) looked upon inflammation as a disturbance in homeostasis, and the reaction as an attempt to overcome this disturbance and restore equilibrium. According to Menkin (1950), inflammation is a complex vascular, biochemical, lymphatic and local tissue reaction elicited in higher animals by the presence of microorganisms or by the presence of non-viable irritants. It represents a basic or elemental reaction to injury whereby the deleterious agent tends to be localized and ultimately destroyed. He goes on to say that one of the characteristics if inflammation is an increase in the capillary permeability and an escape of plasma proteins into the tissue fluid. Selye (1953) considers inflammation as a local adaptation syndrome consisting of the following: 1) The local alarm reaction - tissue damage or edema, 2) Cellular reaction - mobilization of the cellular elements, 3) Local stage of resistance - granulation formation, 4) Local stage of exhaustion - breakdown, necrosis, suppuration, or healing. Grant et al. (1963) has enumerated the responses seen in the inflamed gingival tissue. The progression of the
inflammation to the periodontal structures has been reported by Weinman (1941, 1952) and Goldman (1957).

With regard to the etiology of periodontal disease, many studies have been done to reveal that bacteria are definite factors causing and maintaining the disease process. Among these studies are Rosebury (1947), Waerhaug (1952), Ramfjord (1952), Bibby (1953), Shultz-Haudt (1956), MacDonald (1960, 1962), Wentz (1960), and Arnlm (1964). Although no single organism has been found to be responsible for the disease, there is strong evidence for a causal relationship between the microorganisms and the disease. Ramfjord (1952) states that bacteria are so frequently associated with tooth loss that they may be considered the prime etiologic factors in inflammatory periodontal disease.

To a considerable extent the importance of bacteria in the initiation of periodontal disease has been stimulated by observations reported on the periodontal state of germ-free animals. Baer (1960), and Baer and Newton (1959, 1960) were not able to detect any significant difference between the degree of pocket formation and bone loss found in thirty-eight germ-free mice and that occurring in their controls. At the age of six months all of the germ-free mice had developed demonstrable periodontal disease; these mice were of a strain previously observed to be susceptible to periodontal disease. MacDonald (1960), while
admitting that evidence for the part played by bacteria is necessarily circumstantial, was not entirely satisfied that the periodontal destruction seen in Baer's germ-free mice is strictly comparable with that seen in man. However the rat studies of Cohn (1960) show a pattern of destruction similar to that seen in Baer's mice, and at the same time revealed many similarities to the histopathologic features of pocket formation seen in humans. One conspicuous difference concerned the part played by dietary consistency in relation to the initial lesion. Cohn showed that by feeding soft diets to rats the rate of pocket formation could be greatly diminished, a finding subsequently confirmed by Baer and White (1960) on their susceptible mice and by Pearson (1961) using albino rats.

Whether or not the existence of periodontal disease in germ-free mice is accepted, there can be no denying that the disease under consideration is modified and doubtless aggravated by oral microorganisms gaining access to the periodontal tissue once pocket formation has commenced. MacDonald (1960) has suggested the possible importance of Bacterioides melaninogenicus in this connection. This Gram negative pigment producing coccobacillus is the first and only organism from the oral flora of man which has been shown to produce an enzyme capable of hydrolyzing native collagen; in addition it produces a high concentration of amonia, which is known to have a lytic effect on epithelium of the mucous membrane.
The existence of enzymes capable of destroying tissues has also been reported by Dewar (1958) who was able to isolate several such substances from gingival debris of patients with periodontal disease. Once bacteria have gained ingress to the periodontal tissues the progressive destruction of periodontal disease is easily accounted for; how they reach the internal tissues however, is still a matter of controversy. Wentz (1960), dismissing hematogenous dissemination of bacteria lodging within the periodontium as a rarity of negligible significance, subscribed to the belief that bacteria do not invade the tissues but thrive on the surface and affect the underlying tissues by the seepage of toxins which they produce. Bibby (1953) analyzed the possibility of actual invasion of the tissues and concluded that the appearance of periodontal disease is more suggestive of an accumulation of organisms externally, presumably exerting pathologic effects by the diffusion of tissue destructive bacterial products into the periodontium. Weill and Bossard (1960) have demonstrated the permeability of oral mucosa by autoradiography.

The Expert Committee of the World Health Organization (1961), which dealt with these and many other details in full, concluded that bacterial plaque is a factor of paramount importance in periodontal disease. Rough surfaces in contact with the gingiva are injurious because they contribute to plaque retention rather than for any mechanical irritation they may inflict. Onishi,
quoted by Imagawa (1960), found that greater surface accumulations of bacteria occurred on non-hornified than on hornified epithelium. Important contributions to the understanding of the plaque in periodontal disease have been provided by Swiss investigators. Muhlemann and Schneider (1959) placed mylar strips into gingival crevices and found that plaque formation commenced with deposits of predominantly Gram negative organisms and degenerating leukocytes. Then Muhlemann and König (1959) demonstrated the importance of bacteria in plaque formation by topical applications of penicillin, stannous fluoride, and water (as a control) to the molars of rats. They found that plaque formation was inhibited in those rats treated with penicillin and stannous fluoride; heavy plaque formation was present in the control group within ten days. Minimal gingival damage could be detected in the plaque-free rats, whereas the controls had conspicuous pocket-like lesions after five weeks.

With reference to the plasma cells seen in this study, Oretga and Mellors (1957) showed that gamma globulin was formed in the germinal centers of lymphatic nodules and in the cytoplasm of mature and immature plasma cells of two types; those with and those without Russell bodies. Toto (1961) showed that plasma cells arise directly from undifferentiated mesenchymal cells or connective tissue reserve cells and not lymphocytes in inflamed oral mucosa. When the reserve cells begin to make RNA and then
gamma globulin, the cell changes in structure into a plasma cell. He also pointed out that as plasma cells degenerate they yield spheroid hyalin structures that may contain RNA that makes atypical gamma globulin. De Petris et al. (1963) localized antibodies in plasma cells utilizing the electron microscope.

A review of the authors that have used fluorescent compounds as labeling agents begins with the work of Hopkins and Wormall (1933) who attached phenyl isocyanate to protein molecules and then studied their immunologic reactions. Later Fiser and Creech (1939) conjugated amino acids with the isocyanates of the anthracene and 1, 2 benzanthracene series. The next step was that of labeling various proteins, including serum proteins, such as albumin, with the isocyanates of aromatic polynuclear hydrocarbons as was done by Creech and Jones (1941) in two different studies. Shortly thereafter Coons et al. (1941) described the immunologic properties of an antibody protein containing a fluorescent group. The literature dealing with alteration of antibody proteins by attachment of chemical molecules is not extensive. Some of the publications pertaining to this are Eagle (1936), Marrack (1934), Pressman (1948, 1949), and Reiner (1930). In general the observations made in these studies were that antibody molecules can be chemically linked with simple compounds and dyes without destroying the specific reactivity of the antibody with its antigen. Many different
chemical compounds have been employed for modification of antibody proteins. The resulting changes have been measured by noting differences in the native and altered proteins demonstrable by chemical analysis for the presence of the label, isoelectric point, electrophoretic determinations, serologic reactions, radioisotopes, and observation of fluorescence.

Coons has stated (1961) that the idea of employing fluorescent antibodies for localization of antigen first occurred to him in connection with testing the hypothesis that rheumatic fever was due to an antigen-antibody reaction in certain tissues of the body. He reasoned that antigen might be detected histologically by means of an antibody, if the latter were labeled with a colored substance. When it was found that the color of such conjugates was not intense enough, the idea of using a fluorescent label was evolved. The first application of fluorescent antibody techniques in microbiology was the demonstration by Coons et al. (1942) that the soluble pneumococcal polysaccharide could be stained in sections of tissue from infected mice. During the subsequent World War II era there was little activity in the fluorescent antibody field. Beginning in 1950 papers began to appear with increasing frequency, particularly those by Coons. One of these (1950) extended previous studies on the pneumococcus to a study of the distribution of polysaccharides from types II and III in the tissues of mice. These
findings were based on improved methods for the detection of antigen in tissue cells by means of fluorescent antibodies (Coons, 1950). This latter paper presented the basic information and technique on which subsequent fluorescent antibody work has been built.

Earlier work with fluorescent antibodies was concerned primarily with an investigation of the fate of foreign proteins and polysaccharides, viruses and rickettsia in tissues of the animal body. This was a natural development for two reasons; first, the workers who originated the techniques were interested in the study of fundamental problems concerned primarily with soluble antigens and viruses, and second there was a paucity of techniques available for study of these antigens as contrasted to bacteria and protozoa. Consequently most of the earlier work was not directed toward the solving of practical diagnostic problems.

The work of Goldman (1953, 1954) who employed fluorescent antibody techniques for the differentiation of Entamoeba histolytica and Entamoeba coli, suggested the potential application of fluorescent antibody techniques in the diagnostic fields. Subsequently, fluorescent antibody studies in the field of diagnostic bacteriology were initiated and the first publications by Moody et al. (1956) and Thomason et al. (1956) appeared.

The only fluorescent antibody study with the human gingiva
was done by Billen (1964) when he demonstrated the presence of antigens in tissues taken from patients with dilantin sodium hyperplasia.
CHAPTER III
MATERIALS AND METHODS

Bacterial samples were obtained from gingival sulci of eighteen adult males ranging in age from 22 to 42 years. All areas selected were clinically inflamed, ranging from a marginal gingivitis to a periodontitis. The samples were procured by gentle passage of a dull curette within the depth of the sulcus. If hemorrhage occurred another site was selected. Each sample was placed in a few drops of a 1:1,000 solution of acridine orange dye which had been placed on a glass slide. The sample was allowed to remain in this solution from two to ten minutes. The solutions were then diluted by flushing with sterile saline until the excess dye was washed off leaving the bacteria in a clear saline solution.

Gingival biopsies were then taken from the same area as the bacterial samples and were immediately frozen on an International-Harris cryostat at -20°C, utilizing a carbon dioxide freezing unit. The frozen specimens were sectioned at six microns, placed on clean glass slides and made to adhere to the slide by gentle warmth of the underside with a finger to prevent the use of a tissue adherent which may interfere in the fluorescein dye reaction. A section from each biopsy was reacted with the fluorescein conjugated anti-human globulin solution for one minute. Each section was then photographed using Ektachrome high speed 12.
film. A standard exposure time of fifteen seconds and a magnification of 100X was used. This procedure was done in order to demonstrate the specific staining of the globulins present in the tissues and also to observe the relative amount of fluorescence in each of the specimens.

Serial sections from each of the biopsies were placed on other glass slides and the above prepared acridine orange stained bacterial suspension was flowed over each section. The slides with the stained bacterial suspension in place, were then put in a wet chamber which consisted of a petri dish containing moist filter paper. They were allowed to incubate at 37°C for 45 to 60 minutes. After incubation, each specimen was washed thoroughly in physiologic saline solution as described by Coons (1958), in order to remove the free bacteria and any debris that may have been obtained with the bacterial sample. The same sections were then reacted for one minute with a prepared solution of rabbit anti-human globulin globulin conjugated to fluorescein isothiocyanate a fluorescent dye*. This was done by placing a drop of the solution over each of the specimens. The sections were washed again in a saline solution to remove the excess dye. A glass

* Sylvana Company, Millbourn, N.J., lot #0905636; Prior to conjugation the material is adjusted to a protein content of 1%, and in conjugating, 20mg. of fluorescein isothiocyanate is used per 100mg. of protein, After conjugating, the material is dialized to remove the excess dye.
cover slip was mounted over each section with a drop of buffered glycerine for microscopic examination.

Serial sections from representative specimens were stained with hematoxylin and eosin for an examination of the inflammatory process.

Sections from three of the specimens were incubated with bacteria from different subjects and were processed and studied as above. In addition, serial sections from the three specimens were first stained with the fluorescein conjugated rabbit anti-human globulin for one minute and then allowed to incubate with the acridine orange stained bacteria from their homologous sites for 45 to 60 minutes.

A Reichert Zetopan large research microscope was used with the Binolux twin lamp unit with an HBO high pressure mercury vapor lamp for use with fluorescence microscopy. There is a permanent heat absorption filter mounted directly in front of this lamp. In addition to this filter, the E1 exciter filter was used. This serves both as a UV pass and a red absorption filter. The E1 filter has a peak transmission of 28% at a wavelength of 360 to 370 millimicrons. A neutral filter was placed next in order to reduce the intensity of illumination to a greater degree. Either a light, medium, or dark neutral filter was used. These filters have respective transmission values of 10%, 1%, and 0.1%. A barrier filter was placed between the
specimen and the eyepiece in order to absorb the ultraviolet light and thereby prevent it from reaching the eye. The sections were photographed immediately using exposure values of 15 to 30 seconds.

Six of the bacterial samples were cultured in both fluid thioglycollate medium for growth of anaerobic bacteria and on brain-heart infusion agar slants for growth of aerobic bacteria. Samples of each such cultured bacteria were stained using the Gram method.
CHAPTER IV
FINDINGS

The reaction to local injury by the gingival tissue was first characterized by physical changes in the stratified squamous epithelium as seen in the H & E sections. The epithelium appeared atrophic and was accompanied by varying degrees of intracellular edema. Also there were microscopic ulcerations in the epithelium lining the sulcus with a loss of such cells into the gingival sulcus. Conversely, the epithelium sometimes showed hyperplasia characterized by long epithelial ridges extending deep into the lamina propria. Where periodontal pockets were present in the specimens the epithelium was seen to cover the underlying granulomatous connective tissue. The lining epithelium was not keratinized.

The connective tissue was generally edematous. In the subepithelial region of the lamina propria, especially in the areas of atrophy or ulceration of the epithelium, there was an infiltration of polymorphonuclear leukocytes. These cells were seen to migrate through the epithelium onto the surface, in ulcerated areas fibrin formation also was seen accompanying the PMNs, forming a fibrino-purulent exudate seen in acute inflammation.

The collagenous fibers around the periphery of the blood vessels appeared swollen. There was perivascular connective tissue proliferation. Many loose connective tissue cells showed
an aggregate of dense chromatin material at the nuclear membrane. There also were foci of differentiating plasma cells found in such loose perivascular connective tissue. Some degenerating plasma cells were seen liberating spheroid hyalin structures into the connective tissue ground substance. These structures are pyroninophilic.

The sections which were reacted with the rabbit anti-human globulin globulin conjugated to fluorescein isothiocyanate showed an "apple green" fluorescence when exposed to an ultraviolet light source. This fluorescence was seen predominantly in the connective tissue which stained intensely. There were three distinct intensely stained areas seen; the collagen fibers, the perivascular ground substance, and the connective tissue cells themselves. These reacted connective tissue cells were seen predominantly around the blood vessels but were found dispersed throughout the connective tissue. The intercellular ground substance was stained to a lesser degree than the cells and appeared in varying degrees of intensity.

There was also fluorescence present in the epithelial tissue. This was of a lesser intensity and was seen only intercellularly. There was no apparent increase in the amount of fluorescence from the basal layer to the surface cell layers.

A bacterial attraction to the connective tissue was seen in each section of the specimens studied. None of the sections showed a bacterial affinity for the epithelial tissue as all of
the reactive sites were within the connective tissue. There was a difference in the amount of reactive bacteria in some of the specimens, but all of the reactive sites on the sections were always the same. These sites always showed intense "apple green" fluorescence. The bacteria appeared as contrasting orange fluorescing plaques or aggregates of cells directly over these green stained areas. The morphology of the fluorescing bacteria could be identified as bacilli, cocci, and in a few instances filamentous forms. The bacteria were seen clumped together in most instances but in each of the sections individual plasma cells were seen reacted with the bacteria. In these instances the bacteria could be seen as single cells lined up around the periphery of the cell and also overlying the cytoplasm of the cell. These connective tissue cells had the appearance of the typical plasma cell with a large rounded non-staining nucleus eccentrically placed, and a highly fluorescein stained cytoplasm. Many times plasma cells were obscured only because of the large amounts of orange fluorescing bacteria which were present. In these cases an intense fluorescein stained area could be seen around the periphery of the groups of bacteria.

There were many discernable plasma cells which showed no presence of bacteria after incubation. There was therefore a random pattern seen in the reaction of the bacteria to the gingival connective tissue except that wherever the bacteria had
reacted with the tissue, it was always directly related to an intensely fluorescein stained area.

The sections which were incubated with the stained bacteria from sulci of inflamed gingiva of different subjects and then reacted with the fluorescein conjugated rabbit anti-human globulin globulin, showed the presence of bacteria in the same random manner as did the previous sections.

All of the sections which were reacted with the fluorescein conjugated rabbit anti-serum prior to incubation with the acridine orange stained bacteria, showed the complete absence of bacteria after washing with saline.

The aerobic growths on all six agar slants appeared in four basic types of colonies. The first colony consisted of moderately raised elevations which were not larger than 1mm. in diameter. They were opaque and had a smooth surface with a regular margin. The second colony seen was larger, varying from 1 to 3mm. in diameter with a hemispheric elevation and a heaped appearance. The margins were regular with few indentations. The third type colony seen varied from 2 to 10mm. in diameter and had an ulcerated appearance. The surface of the center of the colony was smooth and the borders raised. The entire border of some of these colonies was not raised but ended in a smoothly rounded edge. The fourth type appeared much the same as the third except for the raised borders. These colonies were from 3 to 10mm. in
diameter with a smooth but irregular surface. The border was regular with a rounded edge that sloped gradually from the center of the colony.

The anaerobic growths within the fluid thioglycollate medium appeared in three types of colonies. The first was merely a cloudy solution with no distinct gross morphology. The second colony was spherical and appeared within the medium at different levels. Many of these colonies had irregular surfaces with filamentous projections on all surfaces. The third type colony had long finger-like projections from the surface of the medium extending down to a length of 10mm. These projections were filamentous in appearance and in some cases arose from small spheroid colonies within the medium.

The microscopic examination of both the aerobic and the anaerobic growths after staining by the Gram method revealed similar morphologic structures. The majority of the forms seen were Gram positive bacilli and cocci. These appeared singularly and in chains. There were also Gram positive diplococci, curved bacilli, short bacilli, filamentous forms, and spirochetes. There were Gram negative cocci, bacilli, and filamentous forms. These cocci and bacilli appeared also in diploid forms and in chains.
CHAPTER V

DISCUSSION

The fluorescent antibody method can be understood best by considering it to be a new way of detecting conventional antigen-antibody reactions. Smears or sections of tissue containing antigen are examined with the aid of the fluorescence microscope after having been treated with appropriate solutions of fluorescein labeled antibody. Fluorescent, rather than non-fluorescent dyes are used in this method because it is much easier to detect a minute amount of light against a dark background than to detect a slight amount of color against a bright background.

In the first part of this study, a specific labeled antibody, i.e. the rabbit anti-human globulin globulin, was used in order to demonstrate the specific staining of the globulins in the gingival tissue. Tissues which showed clinical and histologic signs of inflammation were chosen because the diseased state rather than the normal was to be studied. This does not preclude the fact that the reaction could take place in normal gingival tissue which histologically shows some signs of inflammation. For example, few plasma cells "normally" are found in the lamina propria of the normal gingival tissue.

The reaction of the specific antibody with the gingival tissue was positive as was shown by the intense fluorescence,
demonstrating the distribution of globulins present. The differences in the intensity of stain in different areas of the gingival tissue shows that the globulins are present in varying amounts.

It is evident that the globulins have different affinities for the different tissue elements. One of the more prominent areas exhibiting fluorescence is the perivascular connective tissue. This indicates a liberation of serum globulins since all of the free globulins are saturated with the labeled anti-serum in these sections. An abundance of globulins is expected to be seen in this area because of the increased permeability of the vessels in the inflammatory reaction and also because of the perivascular mucopolysaccharides binding the globulins in this area. It is possible that a reservoir of globulins could be present here. Another intensely fluorescing area, the collagenous fiber bundles, probably show a binding of the globulins to the mucopolysaccharides on these fibers. A further indication of the possible binding of globulins to mucopolysaccharides is the presence of fluorescence in the basement membrane. The globulins in the intercellular epithelial tissue of inflamed gingiva could further indicate a possible pathway for the liberation of these globulins into the sulcus. In gingival inflammation however, there is a loss of tonofibrillae and widened intercellular spaces, even to the point of ulceration, in the epithelium, which further
allows for the escape of globulins and other tissue fluid elements from the connective tissue.

The final area in which a concentration of fluorescence is found is within the cytoplasm of the connective tissue cells, primarily the plasma cell. Since it is the plasma cell which actively synthesizes and liberates gamma globulin, which contains antibodies, an intense positive fluorescence is expected to be seen within these cells.

The increased number of plasma cells and the globulins in inflamed gingiva is probably a function of the numbers and/or variety of the antigen producing bacteria found in the gingiva. Certainly, the antigen-antibody reaction between the various microorganisms and the gingiva suggests a spectrum of antibodies stimulated by the bacterial antigen. Since no organisms are found within the tissues we can conclude that this indicates a successful defense against bacterial invasion. The antibody therefore produces its effect either by being liberated into the sulcus or pocket and there effecting a control in the numbers of a population of the flora, and/or by reacting within the connective tissue with the soluble antigens produced by the organisms. The latter of these would seem more likely to be the case considering the alterations of the gingival tissue in inflammation. These antigens could thereby be inactivated by the diffuse-ly distributed globulins found in the connective tissue.
The bacteria which were collected from the sulci or pockets were of a comparable flora to those found by Rosebury (1947, 1950), Boyde (1953), MacDonald (1962), Danielewiczowa (1963), and Crowley and Ramfjord (1964). These studies included both normal and diseased states. Burroughs (1959) says that the oral flora includes lactobacilli, filamentous forms both branching (actinomyces), and non-branching (leptotrichia), alpha hemolytic streptococci or enterococci, miscellaneous Gram positive cocci, Neisseria, diphtheroid bacilli, fusiform bacilli and other non-sporulating anaerobic forms, pneumonia-like organisms, spirochetes, yeasts, and monillia. He goes on to say that not all of these are readily cultivable and the numbers and kinds of microorganisms cultured from saliva or tooth plaques differ from those found in direct smears, and it is probable that any single method of assay provides at best a distorted reflection of the microbial flora present. Although each of these specific organisms was not identified as such, the compatibility to the cells seen in the Gram stained smears was sufficiently demonstrated.

This study has pointed out the fact that there is a definite antigen-antibody reaction taking place between the microorganisms in the sulci and the globulins in the gingival tissue. This was evidenced by the fact that bacteria were seen to be reacted with the tissue in areas of globulin concentration. Furthermore this reaction was shown to be specific by the fact that the
organisms taken from one individual also reacted with the tissue from a different individual. Another consideration for the specificity of this reaction is that it is inhibited once the gingiva had been reacted with the rabbit anti-human globulin globulin. This inhibition is based on the immunologic phenomenon of blocking specific antigen-antibody reactions by first exposing the antigen to a different aliquot of homologous antibody solution. The inhibitory reaction is important because it is strong evidence of specificity of a staining reaction as shown by Coons and Kaplan (1950). The antigen-antibody reaction as seen in single plasma cells with the bacteria found around the periphery and overlying the cytoplasm of the cell is evidence that it is the antibody producer.

The specific antigen-antibody reaction between the bacteria of the normal flora and the globulins in the gingiva plus the fact that the normal gingiva always shows some signs of inflammation, in particular, the presence of plasma cells (Anderson, 1962 and Grant, 1963), indicates that the specific antibody may always be present in response to the ever present bacteria, thus maintaining a relatively constant defense in the healthy individual. If this were to be the case, an alteration in the globulin components may help to explain the variations seen in the reactions of the gingival tissue to various irritants.

In suggesting further research along these lines, one other
point should be brought out in regard to the bacterial strains found. It was mentioned that the majority of the morphologic forms found were Gram positive rods and cocci. The individual organisms seen reacted with the plasma cell, although not discrete because of the fluorescent stain could also have been identified as either single rods, single cocci, fusiform bacilli, or small chains of coccal forms. Since no one single organism has ever been found to be the causative agent inducing periodontal disease, it may prove of interest to react pure cultures of bacteria with the gingival tissues. Different labeled specific anti-sera could also be used. This would further restrict the specificity of the antigen-antibody reaction seen here, and may lead to the eventual isolation of a particular antigen and thereby to a particular antibody also.
CHAPTER VI
SUMMARY AND CONCLUSIONS

Gingival biopsies and bacterial samples were taken from eighteen adult males. The biopsies were frozen immediately and a specific conjugated antibody was reacted with sections of each of the biopsies in order to show the presence of globulins in the tissue. The bacteria were stained with a contrasting fluorescent dye and incubated with serial sections from each specimen. After incubation and washing, the same sections were reacted with the fluorescein labeled antibody to show an attraction of the bacteria to the gingival tissue. Cross reactions of bacteria and tissue also showed a positive attraction. The specificity of the reaction was shown by an inhibition of the attraction of the bacteria when the tissues were first reacted with the specific antibody.

The bacterial flora obtained for use in this study was shown to be compatible to the oral flora found in other studies.

The globulins in the tissue were shown to be present in varying amounts in different areas. The attraction of the bacteria to the gingival tissue was always in an area of globulin concentration. Plasma cells were shown to be a site of bacterial attraction and globulin production.

The antigen-antibody reaction between the microorganisms and the gingiva suggests a spectrum of antibodies stimulated by
the bacterial antigen. The antibody could produce its effect either by being liberated into the sulcus or pocket and there effecting a control in the numbers of a population of the flora and/or by reacting within the connective tissue with the soluble antigens produced by the organisms. The presence of plasma cells in normal gingival tissue indicates that a specific antibody may always be present in the tissue in response to the ever present bacteria, thus maintaining a relatively constant defense in a healthy individual.
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Figure 1.

H & E section of gingiva showing connective tissue and epithelium of area biopsied. \(\times85\).

Figure 2.

Fluorescein section of gingiva showing distribution of globulins as evidenced by the amount of "apple green" fluorescence present. \(\times100\).
Figure 3.

Fluorescein section of epithelium showing intercellular fluorescence. X400.

Figure 4.

Fluorescein section of connective tissue showing perivascular fluorescence. X400.
Figure 5.

and

Figure 6.

Fluorescein sections of connective tissue showing the attraction of the acridine stained bacteria to the cells.

X 400.
Figure 5.

Figure 6.
Figure 7.
Gram stain of aerobic culture obtained from the gingival sulcus. X 400.

Figure 8.
Gram stain of anaerobic culture obtained from the gingival sulcus. X 400.
APPROVAL SHEET

The thesis submitted by Thomas F. Schneider has been read and approved by three members of the faculty of the Graduate School.

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: 5-17-65

Anthony W. Gargiulo, D.D.S., M.S.

Signature of Advisor