Levels of the Immunoglobulins IgG, IgA and IgM in the Inflamed Human Gingiva

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LEVELS OF THE IMMUNOGLOBULINS IgG, IgA
AND IgM IN THE INFLAMED HUMAN GINGIVA

by

CRAIG W. BYERS

A Thesis Submitted to the Faculty of the Graduate
School of Loyola University in Partial Fulfillment
of the Requirements for the Degree of
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I dedicate this thesis to my parents, Mr. and Mrs. William J. Byers, with deep appreciation for their encouragement and assistance throughout my education. And to my wife Nancy - my love.
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CHAPTER I
INTRODUCTION

It is generally agreed that most forms of periodontal disease are of microbial origin. However, the mechanisms by which microorganisms induce destruction of the periodontal tissues are unknown. Two general possibilities that have received considerable attention are: 1) direct initiation of the inflammatory response by injurious microbial metabolites and 2) initiation of periodontal inflammation by antigens of oral organisms setting immunopathologic processes into action. Bacteria isolated from dental plaque have been shown to produce many products which may damage periodontal tissues. Recent studies have shown a cause and effect relationship between the onset of gingival inflammation and the application of antigen to the gingiva of hyperimmunized experimental animals. Immunoglobulins specific for oral bacteria have been demonstrated to be present in human gingiva and human serum. The immune response has been described by some authors as a defensive mechanism for local tissues against injurious agents and by others as a mechanism that could perpetuate disease processes and contribute to the pathogenesis of periodontal disease.
It is the purpose of this study to investigate the humoral aspects of the body's immune response to the antigenic stimulus within the gingival sulcus. The goal, more specifically, is to determine quantitatively the levels of immunoglobulins IgG, IgA and IgM present in inflamed gingival tissue.
CHAPTER II
REVIEW OF LITERATURE

Daley, Healy, and Sweet\textsuperscript{(13)}, in 1936, first described bacterial allergy in relation to "parodontal disturbances". They performed a Wright's stain on a drop of blood exuded from inflamed periodontal tissue and examined it for eosinophils which they considered indicative of an active case of bacterial allergy. The patient was then tested to ascertain the strain of organism and then desensitized by administering gradually increasing doses of the particular organism to which the patient was sensitive. While the majority of their patients were classified as having "chronic Vincent's infection", Daley suggested that bacterial allergy may be an underlying factor and should not be overlooked in instances of "chronic parodontal disease".

Fish\textsuperscript{(18)}, in 1939, reported on a series of experiments in which he implanted \textit{Staphylococcus aureus} on cotton pellets into holes drilled into the mandibles of guinea pigs. The reaction which he described histologically was divided into four zones: 1) central zone of infection - only zone in which viable organisms were present and were being controlled by polymorphonuclear leukocytes (PMN'S); 2) zone of contamination - free of infection but poisoned by toxic products of the infection, all normal cells were dead and were replaced by round cells; 3) zone of initiation - some normal cells survived but a heavy round cell
infiltrate was present, active phagocytosis occurred with typical cells
being the osteoclast and the histiocyte; 4) zone of stimulation - few
round cells in this area where the "poison" was most dilute, active
fibroblasts and osteoblasts were present. He compared the last three
zones to the histologic picture of a chronic periodontitis and first
zone to the "germs" themselves which were confined to the debris of the
pocket with only their soluble toxic products invading living tissue
and setting off the reactive process of periodontal disease.

Orban (27), in 1940, suggested that the immune response played a
role in the mechanism of periodontal disease. In a histologic exami-
nation of human jaws, he observed increased numbers of esoinophils in
areas of inflammatory periodontitis and theorized that they may have
been part of an allergic response. He viewed the presence of the
esoinophils as possibly part of an autoimmune response of the body to
breakdown products of the periodontium caused by the surrounding
inflammatory process.

Weinmann (55) (56), in 1941 and later in 1952, in a histologic
study of human autopsy material with clinically evident periodontal
disease, reported the following findings: Chronic inflammation of the
gingiva, consisting mainly of a lymphocytic and round cell infiltrate.
Progression of the inflammatory response along the course of the blood
vessels into the bone marrow spaces and onto the periosteal side of the
bone; only in exceptional instances did it penetrate into the periodontal
membrane. The age of the fifteen specimens at time of death ranged from eleven to seventy-four years.

Rosebury (38), in 1947, described infection as an essential factor in periodontal disease. The infection was postulated as being endogenous, an overgrowth of certain resident parasites. Although harmless when in contact with healthy tissues, the parasites proliferate and in greater or lesser degree invade the tissues only after periodontal tissues were previously damaged by other causes. Rosebury felt that the inflammation, suppuration or ulceration was contributed directly by infection.

Fagraeus (16), in 1948, when studying tissue response in the spleen of rabbits hypersensitized to horse serum albumin noted splenic tissue possessed a capability to form antibodies in vitro only after the appearance of transitional cells with their increased basophilia from reticulo endothelial cell precursors. The further differentiation of these cells to immature plasma cells resulted in a considerable rise in the tissues ability to produce antibody. With the appearance of mature plasma cells the antibody titer declined in vitro. Fagraeus concluded that the development of antibodies takes place during that differentiation of the reticulum cells into plasma cells.

Ramfjord (33), in 1952, felt that on ulcerated surfaces, inflammation may be produced by bacteria directly or by their toxins. He suggested that bacterial toxins and enzymes are active irritants in the spread of periodontal inflammation. He pointed out that a group of
French investigators had suggested the occurrence of a local or fixed allergic reaction the gingival tissues by the bacterial toxins and enzymes.

In 1952, Waerhaug(53) instilled pure cultures of pathogenic bacteria into bacteria-free pockets of three young dogs with clinically normal gingiva. Histologic examination of the specimen revealed necrosis of the lining epithelium of the pocket and inflammation of the connective tissue with formation of exudate in the pockets. A bacteria-free condition was regained after forty-eight hours. The author alluded to bacteria as a possible etiologic factor in periodontal disease.

In 1953, Bibby(4) reported that there was no reason to believe that any one type of organism of the oral flora was more important than the other in causing periodontitis or marginal gingivitis. He suggested the possibility of an allergic response to bacteria which are in contact with gingival margins and to which the tissues could at some time have become sensitized. Bibby also attempted to demonstrate that bacteria did not invade the gingiva by topically treating inflamed gingiva with penicillin and noting that there was no evidence of continued bacterial proliferation supra-gingivally or within the gingiva where he reasoned the antibiotic would be unable to affect the organisms.

Schultz-Haudt et al(11), in 1954 described agents present in the gingival sulcus, that were capable of attacking hyaluronic acid, chondroitin sulfate, and collagen preparations. The agents were
present in bacteria-free filtrates prepared from gingival deposits and
pure cultures obtained from individuals with various degrees of gingival
inflammation. The active agents were not found in filtrates from gingival
deposits of patients with clinically normal gingiva. Schultz-Haudt
concluded that the presence of such agents together with various forms
of trauma or local initiations were exogenous predisposing factors in
the development of gingivitis.

Coons, Leduc, and Connolly (10), in 1955, reported antibody in
lymphocytes present in regional lymph nodes of rabbits which had been
challenged with an injection of diptheria toxoid four weeks following
the initial exposure in a hind foot-pad. The greatest amount of
antibody present was found in cells of the plasma cell series. The
investigators were able to localize the antibody by employing
fluorescein labeled antibody as a histochemical reagent. Coons also
concluded that it appeared as if the plasma cell differentiates
specifically in response to antigenic stimulation.

Orban et al (28), in 1958, in a histopathologic study of peri-
dontal disease in humans, described a lymphocytic and plasmacytic
infiltration of the deeper tissue areas as being the predominant
feature of the disease. The function of these cellular elements of
the inflammatory defense reaction being the production of antibodies.
Orban held that the presence of the above elements in cases of peri-
odontitis of long duration was an indication of the body's attempt
to neutralize the toxic effects of bacteria and the products of tissue breakdown.

Askonas and Humphrey(1), in 1958, demonstrated antibody formation at the site of antigenic challenge as well as at regional lymph nodes, spleen and other immunoresponsive organs. Using tissue slices from rabbits that had been previously immunized with intramuscular injections of ovalbumin in Freund's adjuvant, the investigators studied antibody production by the amount of radiolabeled amino acids incorporated into specific antibody. They further demonstrated that the majority of the antibody produced in the animal was in the local granuloma (injection site), following the intramuscular injection.

Brill and Bromestam(7), in 1960, showed gammaglobulin to be present in fluid recovered from human gingival pockets. They were able to demonstrate this in an electrophoretic examination of the fluid which was obtained by inserting strips of filter paper into gingival pockets. They also speculated that antibodies may be present in the fluid as well.

Mergenhagen(25), in 1960, showed that soluble endotoxins of oral bacteria could elicit hemorrhagic and necrotic lesions in the rabbit when injected intracutaneously. The soluble endotoxins were prepared by the tryptic digestion of acetone dried Gram-negative oral bacteria. Mergenhagen felt that the endotoxic properties of certain oral bacteria may be an important factor in periodontal disease.
Toto\(^{(50)}\), in 1961, described plasma cells arising directly from undifferentiated connective tissue reserve cells and not lymphocytes, inflamed oral mucosa. Employing histochemical methods, he found undifferentiated perivascular cells showing increased synthesis of RNA prior to differentiation into plasma cells. Toto suggested that with the onset of RNA synthesis and accompanying basophilia the peak rate of antibody manufacture would be reached. This stage of development was earlier classed as the immature plasma cell stage by Fagraeus\(^{(16)}\).

Scherp\(^{(42)}\), in 1962, described bacteria as being the prime etiologic factor in periodontal disease. He raised the point that there may be some specific transmissible microorganism involved in chronic periodontitis although none had been demonstrated to that time. He also held out the possibility that periodontal inflammation may be due to allergy to antigens of the crevicular flora and the need for research along that line.

Socransky et al\(^{(44)}\), in 1963, observed that gingival debris is composed almost entirely of bacteria. In a total microscopic, total aerobic and anaerobic study of periodontal patients, he noted that the majority of the bacterial populations from periodontal pockets were obligately anaerobic. Socransky suggested that there is probably a delicate balance between the host and its indigenous flora, and that any change in this balance in the direction of an increase in numbers or pathogenicity of the flora, or a decrease in the resistance of the host, will lead to periodontal breakdown.
Shannon and Gibson\textsuperscript{(43)}, in 1964, in analysis of the sera of two hundred males, age seventeen to twenty-two, noted no correlation between serum total protein, albumin and globulin and periodontal disease. They concluded that periodontal disease does not manifest itself systemically in the above parameters. The investigators did not study specific globulin fractions at this time, only total globulin was evaluated.

Toto \textit{et al}\textsuperscript{(51)}, in 1964, studying periodontal disease in humans, described a proliferation of loose connective tissue leading to plasma cell differentiation and the accommodation for such activity by loss of mucopolysaccharides from the gingiva and alveolar bone. Special histochemical stains were employed in the study of inflamed human gingiva and human jaws showing periodontitis. The author postulated that the quantity of granulation tissue in periodontitis serves as a defense against irritants.

Rizzo and Mergenhagen\textsuperscript{(34)}, in 1965, observed a plasmacytosis in hypersensitivity reactions in the palatogingiva of rabbits. The abundance of plasma cells in human gingivitis and in hypersensitivity reactions, produced in the rabbit gingival mucosa with known antigens, had a highly significant histologic resemblance to one another. The authors used subcutaneous injections of horse serum and human tubercle bacilli to sensitize twenty white rabbits and challenged the animals in palato-gingival sites with horse serum and Tuberculin-Purified
Protein. Because of the similarity of the induced lesion and human gingivitis, Rizzo and Mergenhagen speculated that the plasma cell component of human gingivitis might be induced by antigens from indigenous gingival bacteria.

Loe(23), in 1965, demonstrated that it was possible to produce a gingivitis in humans by withdrawing all oral hygiene practices for a period of ten to twenty-one days. He noted a gross accumulation of soft debris and the development of a marginal gingivitis in all twelve persons he studied, all of whom initially had clinically normal gingiva. Concurrent bacteriological examination showed an increased number of microorganisms in the gingival area and distinct change in the relative composition of the flora. From a predominance of coccal forms, the microflora changed to a more complex population in which first, filamentous bacteria, and later vibrios, spirochetes and gram negative cocci are predominant.

Mergenhagen et al(24), in 1965, were able to demonstrate a rather widespread occurrence of haemolytic antibody to Leptotrichia buccalis, which is indigenous to man, in the sera of adult individuals. The investigators employed sheep erythrocytes sensitized with the L. buccalis antigen incubated with human sera and guinea pig complement for the haemolysis tests. Mergenhagen felt this demonstrated that man may develop antibodies to the somatic antigen of L. buccalis or alternatively indicate that the antibodies to L. buccalis may represent
a response to heterogenetic antigens.

Brandtzaeg and Kraus\(^{(6)}\), in 1965, reported that the most striking difference between clinically healthy and inflamed gingival specimens when studied with the immunofluorescence method was the frequent marked increase in the number of plasma cells containing IgA. The investigators observed that the proportion of IgA containing to IgG containing plasma cells in many of the inflamed specimens reversed the proportion usually seen in clinically healthy specimens i.e., the IgA containing plasma cells greatly outnumbered the others. Brandtzaeg related that since IgA was probably the carrier of reaginic allergic antibody activity, the finding of an apparent IgA globulin production in some inflamed gingiva suggested that allergy of the immediate type may be a component of gingival inflammation. The most probable allergens being the products of the oral microbiota diffusing into the gingival connective tissue. No direct quantitative measures of the globulin fractions were presented.

Brandtzaeg\(^{(5)}\), in 1965 employing micro-double diffusions tests developed by Ouchterlony\(^{(29)}\), determined that the proteins IgG, IgA, IgM, albumin and fibrinogen of gingival pocket fluid were present in proportions and concentrations comparable to that of plasma. He concluded that these findings suggested that the fluid was an inflammatory exudate. The plasma proteins of the pocket fluid possibly exerting antibody activity and thus they affect the host-parasite relation in the gingival area.
Thonard and Dalbow\(^{(49)}\), in 1965, determined that antibody production, associated with indigenous cells, can take place in suitably stimulated oral mucosa. Rats and guinea pigs, employed as experimental models, were antigenically stimulated by intragingival injection of whole sheep erythrocytes. Upon sacrifice, cell suspensions of spleen and gingiva were made and assayed for antibody production according to the plague technique of Jerne\(^{(22)}\). Antibody forming cells were found in approximately fifty per cent of the inoculated gingiva. The authors pointed out that the origin of the immunocompetent cells remains a matter for conjecture.

Thonard \textit{et al.}\(^{(49)}\), in 1966, detected the presence of IgM and IgA in gingiva excised from patients with periodontal disease. They were unable to detect the presence of IgG in the inflamed periodontal tissue. Both direct and indirect immunofluorescent techniques were employed in the study which did not provide a quantitative measure of the globulins present.

Schneider \textit{et al.}\(^{(40)}\), in 1966, showed that the binding of oral bacterial flora to gingival tissue sections was usually in an area of globulin concentration. Plasma cells were also shown to be a site of binding. The investigators used gingival biopsies of eighteen adult males which were sectioned and reacted with bacteria stained with fluorescent dye which, following incubation and washing, were incubated with a contrasting specific fluorescein conjugated antibody to
demonstrate the presence of globulins in the tissues. The authors concluded that the antibodies could produce their effect either by being liberated into the sulcus and there effecting a control in numbers of the population of the flora and/or by reacting within the connective tissue with the soluble antigens produced by the organisms.

Evans et al\(^{(15)}\), in 1966, noted bactericidal antibody in various mammalian serums to human oral strains of *Veillonella* and *Fusobacterium*. Both mice and rabbits, which were immunized to *V. alcaescens* in complete Freund's adjuvant intraperitoneally, developed high titers of specific bactericidal antibody. Sera from adult human patients with periodontal disease when compared to normal adult sera showed increases in bactericidal antibody to *F. polymorphum*.

Courant and Bader\(^{(11)}\), in 1966, detected the presence of *Bacteroides melaninogenicus* or its products in the subepithelial connective tissue of the gingiva in six patients. The microorganisms were located by the use of the indirect fluorescent antibody technique.

Rizzo and Mitchell\(^{(35)}\), in 1966, demonstrated that by repeated antigen deposition in rabbit gingival pockets, it was possible to produce a local and systemic immune response. Egg albumin on cotton pellets, was deposited in the gingival pockets of mandibular incisors of rabbits in one group and topically applied to the mucolabial fold of another group for the seven to twenty-four week experimental period. In rabbits whose pockets were treated with egg albumin, the gingiva showed
chronic inflammation with numerous plasma cells and the sera contained intermediate levels of anti-egg albumin antibody. None of the pockets showed ulceration. Tests of sera for antibody to egg albumin in rabbits treated by egg albumin deposition in the mucolabial fold were negative. Rizzo and Michell concluded that the antigen was absorbed into the gingiva and caused both a local and systemic immune response. They felt that their finding lent support to the concept that the gingival plasmacytosis of chronic periodontitis is brought about by local absorption of bacterial antigens.

Cochrane and Dixon(9), in 1967, described local tissue damage which may result following antigen-antibody interaction. The authors employed fluorescent staining techniques to study the response. After local injection, the antigen diffuses though the tissues until it meets the antibody, at which point an antigen-antibody complex occurs, plasma complement is bound, polymorphonuclear leukocytes (PMN) are attracted and severe vasculitis ensues. Upon lysis, the PMN's release lysosomes which contain numerous enzymes such as acid and alkaline phosphatase, ribonuclease, deoxyribonuclease, beta glucuronidase, lysozyme and acid proteases. These enzymes may bring about cellular damage and local tissue destruction.

Taichman(17), in 1968, suggested that the role of the inflammatory process in the mediation of tissue destruction in the periodontium merited consideration. He stressed that stimuli capable of precipitating
an inflammatory reaction may in themselves possess negligible tissue destroying potentials - citing allergic inflammations as perhaps the best example. He pointed out that bacterial metabolites activate the pharmacologic effects of various humoral factors, such as catecholamines, complement, and kinins. Cytotoxic bacterial agents could also lead to the liberation of biologically active compounds from cells of the inflammatory exudate. Taichman also brought out that the presence of numerous plasma cells, lymphocytes and macrophages in inflamed gingiva and the synthesis of immunoglobulins in these tissues suggested that immediate or delayed hypersensitivity reactions play a role in the destructive process.

Steinberg and Gerhoff(45), in 1968, noted a higher titer of circulating antibody to Treponema microdentium in patients with moderate periodontal disease than in patients with severe periodontal disease who demonstrated none of the specific circulating antibody. Tanned-cell hemagglutinations using whole spirochetal cells were employed to measure antibody titer. The authors speculated that the absence of the specific antibody in the sera of patients with severe periodontal disease may have been due to the increased number of spirochetes in the gingival crevice hence their immediate complexing with any available antibody or that the host may have been rendered immunologically tolerant due to the continued "challenges".
In 1969, Wittwer, Dickler and Toto\(^{(58)}\), in a histologic study of gingival biopsies from fifty patients with gingivitis, described an overwhelming preponderance of plasma cells. The authors held that the plasma cells were locally produced in response to some antigenic substance. They assumed that since plasma cells are associated with antigen-antibody reactions, that an immunologic reaction was present in gingivitis.

Sussman, Bartels and Stahl\(^{(16)}\), in 1969, failed to observe bacteria within intact epithelial tissue in gingival or col specimens. Gingival biopsies from thirty-nine patients with periodontal disease were studied histologically for microorganisms present in these areas. Approximately fifteen percent of the specimens with ulcerated epithelium showed bacteria in the lamina propria while there was no bacterial penetration in areas of intact epithelium. The authors held that their work supported the contention that gingival inflammation may be the response to bacterial products rather than microbial penetration.

Crowley\(^{(12)}\), in 1969, was unable to demonstrate the presence of IgA, IgM and IgG by immunofluorescent techniques in plasma cells found in the inflamed gingiva of dogs. He was able to demonstrate large quantities of acid hydrolases in the plasma cells which he speculated could be of major importance in the breakdown of gingival tissue.

Saito et al\(^{(39)}\), in 1969, noted increased levels of IgG in the sera of eighteen of thirty periodontal patients when compared to
normals. Serum IgA was found to be increased in only eight of the thirty cases studied. The authors employed immuno-electrophoresis for the analysis of the globulin fractions and did not present a quantitation of the levels of IgG and IgA, only whether they appeared to be increased or decreased with reference to the "normals". Saito suggested that the increase in IgG and IgA suggested that some immunologic mechanism may have been involved in producing the periodontal disease in the subjects studied.

Berglund, Rizzo and Mergenhagen\(^3\), in 1969 determined that low doses of an antigen administered intramucosally stimulated an immune response limited to the local lymph nodes. New Zealand white rabbits were injected in the palatal mucosa with *Escherichia coli* somatic antigen. Five days later, the granuloma at the site of injection, regional lymph nodes, and spleen were assayed quantitatively for cells producing antibody to the *E. coli* antigen in the manner described by Jerne \(^{22}\). Antibody activity was detected in the granuloma at the injection site as well as in the regional lymph nodes. The authors proposed that the immune response provided both beneficial and deleterious affects. On one hand affording protection by antibodies against active bacterial infection while on the other setting up immune reactions which mediate the inflammatory process resulting in tissue damage.
Rizzo and Berglund (36), in 1969, demonstrated that endotoxin concentrations as low as one hundred ug/ml could produce an inflammatory response in the gingiva. The endotoxin was introduced into rabbits sulci, whose epithelial lining had been scratched, and the response studied histologically and quantitatively for antibody-forming cells. The low concentrations of endotoxins that produced the reaction, and similar to those found in plaque, lend support to the possibility that bacterial endotoxins are of significance in the etiology of periodontal disease.

Hartzer (21), in 1969 demonstrated antibody activity in the gingiva of women, to sonified Streptococcus mitis. Gingival biopsies from thirty-eight women were prepared for histologic and radiographic study. Fragmented S. mitis, labeled with the radioisotope I-131 was employed in the autoradiographic technique. Inflamed gingiva was found to contain significantly more antibody activity to S. mitis than clinically normal gingiva in both pregnant and non-pregnant women. The author concluded that the above findings point to the close relationship between the immune response and periodontal disease in the human gingiva.

Mergenhagen, Tempel and Snyderman (26), in 1970, noted that endotoxins may act directly on the C5 fraction of serum resulting in the generation of chemotactic factors for PMN's. Using modification of the Boyden chamber technique, the authors were able to study the
unidirectional migration of PMN's, in *vitro*, in response to the chemotactic factor generated by the incubation of *Veillonella alcalescens* with guinea pig serum. The authors speculated that with the activation of the complement system, biologically-active products would be released with host neutrophils being drawn to the area and cell lysis occurring. With the lysis of PMN's, the release of tissue damaging enzymes would occur. Should the challenge of bacterial antigens continue, the process could become chronic and lead to the tissue changes noted in periodontal disease.

In 1970, Platt, Crosby and Dalbrow (30), noted the presence of specific globulin fractions in gingivectomized tissue from patients with periodontal disease. The authors employed the direct immunofluorescent technique in the histochemical study of the specimens. No quantitative determinations were made. IgG and IgM plasma cell types were most frequently detected in the acute gingivitis with the results of IgA being inconclusive. The tissue obtained from patients with severe periodontal disease show many IgM positive plasma cells, a moderate number of IgA positive cells and only a few cells positive for IgG. Intact, as well as disaggregated collagen was also IgA and IgM positive. The authors concluded that most of the bacteria persisting in the gingival crevice are inactivated by immune globulins. Also, the defensive mechanism provided by globulins are available to the host and persist even through the long periods of chronic inflammation.
Ranney and Zander (32), in 1970, reported similarities between experimentally produced hypersensitivity and human periodontal disease. Squirrel monkeys were sensitized by subcutaneous injection of ovalbumin and challenged three times a week for three months with ovalbumin soaked thread being placed in the animals' gingival crevices. Upon sacrifice and histologic examination, the periodontal tissues showed many of the features and changes characteristic of periodontal disease. Among the changes were chronic inflammation with vascular dilation, infiltration of the gingival connective tissue by lymphocytes and plasma cells, proliferation of crevicular epithelium into the underlying connective tissue, and microulceration of pocket epithelium. The authors held that their work supported the hypothesis that hypersensitivity reactions may be of etiologic significance in periodontal disease.

In 1970, Ranney (31) described cells containing specific antibody in the gingiva and submandibular glands of squirrel monkeys which had been sensitized with ovalbumin and challenged with ovalbumin soaked thread placed in the gingival crevices. Immunofluorescent methods were used to identify the specific antibodies in the tissues in the histological examination. Four unsensitized monkeys were employed as controls. Cells containing the specific antibody to the challenging antigen were discovered in the sensitized monkey's gingiva and ipsilateral submandibular nodes from the site of challenge. No
specific antibody was seen in the gingiva of the unsensitized animals although a few specific antibody forming cells were noted in the ipsilateral lymph nodes. The authors proposed that the regional lymph nodes could begin to form antibody to bacterial antigens that gain access to the gingival connective tissue. Further antigenic stimulation of the gingiva would cause antibody formation in the regional lymphoid tissues to be continued. Finally, when sensitization is completed and antigenic challenge repeated, specific antibody containing cells will appear in the gingiva, as in the model.

Rizzo (37), observed that a low molecular weight antigen was unable to pass through the intact epithelial lining of a rabbit's sulcus. Two groups of rabbits were hyperimmunized for a period of several weeks to egg albumin. Prior to the intrasulcular challenge with the egg albumin, the sulci of one group of rabbits were scratched and the sulci of the other group left intact. The rabbits with the intact sulci showed no response to the challenge whereas the rabbits with the scratched sulci, on histologic examination, showed ulceration of the sulcus epithelium and signs of an Arthus like reaction. Rizzo concluded that an altered epithelial barrier allowed the inward diffusion of antigens which contribute to the local tissue destruction.

Berglund (2), in 1971, noted that immunoglobulins in periodontally inflamed tissue originate from cells in the inflamed tissue as well as from the serum. Thin sections of inflamed gingival tissues were
combined with antigens from microorganisms of the bacterial plaque. The passive immune hemolysis assay system employed sheep red blood cells and an agar mixture with complement was used to detect antibody formation. Antibodies from each gingival specimen reacted with almost all antigen preparations. Berglund concluded that immune complexes in the gingiva may be important factors in the mediation of periodontal inflammation since such complexes were observed to activate the complement system, a mediator of the inflammatory response.

In 1971, Wilton et al. (51) noted there was no detectable difference in the level of humoral antibodies to oral bacterial antigens between patients with acute ulcerative gingivitis (AUG) and controls (clinically normal). The humoral antibodies were estimated using the tanned red cell hemagglutination test. The bacterial antigens prepared by ultrasonic disintegration included Veillonella alcalescens, Bacteroides melaninogenicus, and Fusobacterium fusiforme. Employing the lymphocyte transformation test with the same antigens, the investigators observed a significant increase in activity of the lymphocytes of the AUG patients when compared to that of the controls. The authors postulated that a cell mediated response may account in part for the possible immune pathogenesis of acute ulcerative gingivitis.

In 1972, Dolby (11) reported that immune complex formation does not play a part in the pathogenesis of acute ulcerative gingivitis. Immunofluorescent techniques were employed to determine the location
of the immune complexes which were classically described as occurring in the walls of small vessels. No such complexes were noted in the gingival biopsy specimens from patients with AUG.

The immune response has been proposed as a mechanism of protection, a mediator of injury, and a necessity for repair in human periodontal disease. Immune globulins have been previously described as being present in inflamed human gingiva but no quantitative determinations of their relative levels has been made to date. It is the goal of the author, to describe the relative levels of immune globulins IgG, IgA and IgM in inflamed human periodontal tissues.
CHAPTER III
MATERIALS AND METHODS

Resected inflamed gingival tissue specimens were obtained from sixteen patients whose surgerized areas were clinically diagnosed as having chronic periodontal disease. The sixteen periodontal patients, ranging in age from twenty-seven to sixty-eight years, included eight males and eight females (Figure 1).

Clinically normal, non-inflamed gingiva was obtained from patients who were undergoing tuborosity reductions prior to the fabrication of complete maxillary dentures. The normal gingiva from the five patients, three females and two males, who ranged in age from thirty-three to fifty-eight years, was pooled due to the small amount obtained from each patient (Figure 1). All of the patients included in the study were in good general health and were not presently taking any medications. The procedures were performed under local anesthesia employing regional blocks.

Immediately after removal, each tissue specimen was placed in a specimen bottle containing fifty ml. of 0.85% saline. The bottle was shaken for thirty seconds. The saline was then decanted until only enough saline remained to cover the tissue specimen.

The bottles containing the specimens were tightly sealed and
stored at minus fifteen degrees centigrade until ready for use. None of the specimens were stored longer than one month.

The saline extraction of globulins from the tissue specimen was carried out in an Environ-room at four degrees centigrade. All materials used in the extraction were cooled to four degrees centigrade by overnight storage in the Environ-room.

The tissue specimens were removed from the freezer and thawed at four degrees centigrade. Fifty ml. of 0.85% saline were added to the specimen bottle which was shaken vigorously for thirty seconds and the saline decanted. The tissue was pressed dry between two by two inch gauge sponges with finger pressure to remove excess saline, then weighed on an Ainsworth type 10 V. balance.

The tissue specimen was finely sliced with a razor blade (Figure 16) and the slices placed in a porcelain mortar. Two ml. of saline (0.85%) per gram of tissue were then added to the tissue slices in the mortar (Figure 17). The tissue slices in saline were ground by hand with a porcelain pestle for five minutes. The resulting tissue homogenate was placed in a fifteen milliliter glass test tube (Figure 18) and centrifuged in a Beckman J-21 centrifuge at 2,000 g for 30 minutes at four degrees centigrade. The straw colored supernatant fluid (Figure 19) was pipetted off and stored in a sealed 5 ml. vial at -15 degrees centigrade. This procedure was repeated for each gingival specimen and the pooled normal. The tissue extracts (Figure 20) were stored no
longer than forty-eight hours before the globulin assays was performed.

The tissue extracts were assayed for immunoglobulins IgG, IgA and IgM using low-level immunodiffusion plates (Meloy Laboratories Inc., Springfield, Virginia) as described by Fahey and McKelvey (17) in 1965. The authors found a probable error of ±10% in the measurement in immunoglobulins and determined that the assay method may be used to quantify globulin concentrations as low as 3 mg%.

One-half hour prior to assay, the tissue extracts were removed from the freezer and allowed to thaw at room temperature. Serial dilutions of the standard solution (Meloy Laboratores Inc., Springfield, Virginia) containing IgG (610mg%), IgA (108mg%) and IgM (100mg%), were then made as follows using saline: 100%, 50%, 25%, 12.5% and 6.25% and plain 0.85% saline as a control (Figure 2), tissue extracts and serial dilutions of the standards were stirred for thirty seconds, then the required amount of solution, (approximately 0.1 ml) was placed in the diffusion wells by means of capillary tubes. The tissue extracts and standards were run in duplicate on separate 24-well plates with the same batch numbers (Figure 3). The plates were incubated at room temperature with the IgG and IgA plates being read at eighteen hours and the IgM plates being read at forty-eight hours (Figures 21, 22, 23). The precipitant ring diameters (Figures 4, 5, 7, 8, 10, 11) were measured with a vernier caliber, the diffusion plates being illuminated by the Immuno-illuminator (Hyland Laboratories, Costa Mesa, California).
The mean ring diameter from the duplicate runs was used for final determinations (Figures 6, 9, 12). Immunoglobulins levels were plotted on the Y-axis and mean ring diameters in 0.1 mm units on the X-axis of semi-log graph paper. Using the known serial dilutions, a standard curve for each globulin fraction was then drawn and the unknown immunoglobulin concentrations were then read from the standard curve (Figures 13, 14, 15).
CHAPTER IV
RESULTS

The concentrations of globulin fractions IgG, IgA and IgM were determined for each gingival tissue specimen. In the inflamed gingival specimens IgG had a range of 64-780 mg.% with a mean of 214 mg.%, IgA had a range of 7.1-22.0 mg.% with a mean of 12.3 mg.% and IgM had a range of 0-19.0 mg.% with a mean of 5.0 mg.%. The globulin levels in the pooled gingiva were 95 mg.% IgG, 7.1 mg.% IgA, and no detectable IgM, which are substantially lower than the mean globulin levels for IgG, IgA and IgM in inflamed gingival tissue. (Table 1 and Table 3)

The mean globulin levels in normal human serum (59) are 950 mg.% IgG, 165 mg.% IgA and 130 mg.% IgM. (Table 2)

The mean ratio of IgA to IgG was 0.07 for inflamed gingiva, 0.07 for pooled normal gingiva and 0.17 for normal human serum. (Table 2)

The mean ratio of IgM to IgG was 0.02 for inflamed gingiva, 0.0 (none-detected) for pooled normal gingiva and 0.14 for normal human serum. (Table 2)

Globulin activity in the saline control was negative in all instances. No correlation existed between the globulin levels and the age or the sex of the patient or the weight of the specimen assayed.
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<th>Specimen</th>
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<th>(mg.% (\text{IgA}))</th>
<th>IgA (\text{IgG})</th>
<th>(mg.% (\text{IgM}))</th>
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<td>IgA/IgG</td>
<td>IgM (mg.%)</td>
<td>IgM/IgG</td>
</tr>
<tr>
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<td>------------</td>
<td>------------</td>
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<td>5.0</td>
<td>0.02</td>
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<tr>
<td>Pooled Non-inflamed Gingiva</td>
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<td>0</td>
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<tr>
<td>Mean Normal Human Serum</td>
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<td>0.17</td>
<td>130</td>
<td>0.14</td>
</tr>
</tbody>
</table>
TABLE 3

![Graph showing immunoglobulin levels in mean inflamed and pooled normal gingiva.]

- **IgG**
- **IgA**
- **IgM**

- **Mean inflamed gingiva**
- **Pooled normal gingiva**

Gingiva labels: IgG, IgA, IgM.
CHAPTER VI
DISCUSSION

This study has shown that there is a marked increase in the level of IgG, IgA and IgM in inflamed gingiva when compared to the levels present in healthy gingiva. The immune globulins which had been extracted from gingival tissue with saline were assayed for with immunodiffusion plates. Previous studies employing the immunofluorescent technique also demonstrated increased levels of IgG, IgA and IgM in inflamed gingiva although their findings regarding the levels of the specific globulin fractions were not in agreement with one another. (6, 12, 30, 49, 60)

The immunodiffusion plate assay technique employed in this study offered a number of advantages when attempting to quantitate the levels of IgG, IgA and IgM. The plates were mono-specific for each globulin fraction to be evaluated. The plates had an acceptable degree of accuracy and the results were readily reproducible. (17) The plates had a wide working range and it was possible to detect globulin concentrations as low as two mg. per cent. This technique appears to be superior to the immunofluorescent technique employed by other authors in the description of gingival antibody levels. The immunofluorescent technique is not mono-specific for immunoglobulins since disaggregated collagen which is often seen in areas of inflammation gives a positive fluorescence for
IgA and IgM. (19) Previous workers (6, 30, 49, 60) have also chosen to relate the number of mature positive fluorescing plasma cells to the levels of IgG, IgA and IgM. This application may give an inaccurate description of the actual levels of immunoglobulins since the mature plasma cell is not the cell type most active in immunoglobulin synthesis. The immature plasma cell not yet fully morphodifferentiated is the cell most active in the synthesis of the immunoglobulins. (16) It has also been shown that lymphocytes may synthesize immunoglobulin and contribute to the cell-free globulin pool. (10)

The marked increase in globulin levels in the inflamed gingival tissue when compared to the levels of normal gingiva is probably due to the greater antigenic stimulus present in the gingival pocket. (31) The antigens and toxic bacterial products of the gingival pocket are not readily accessible for removal by normal oral function nor are they readily subject to dilution or lavage by fluids in the oral cavity. (20) The prolonged exposure of the sulcarg epithelium to the antigenic and toxic bacterial products may result in the breakdown of the first line of defense afforded by the epithelium (37, 52) and allow the ingress of antigens to the underlying connective tissue. This may result in the establishment of an immune response both at a local (31) and a systemic level. Immunoglobulins with an antibody titer to oral bacteria have been demonstrated in both the human gingiva (2) and serum. (15, 24) The increased tissue level of immunoglobulins demonstrated in this study may
be due in part to the globulin synthesized by locally differentiating immunocompetent cells\(^{(51)}\) and/or the globulin produced by cells of the plasmacyte series which have migrated to the area.\(^{(57)}\) The findings of this study that the IgA to IgG and IgM to IgG ratios in inflamed gingiva were substantially different than those for normal human serum add further support to the thesis of local antibody production in inflamed human gingiva. The immunocompetent cells of the gingiva then appear to synthesize antibodies in response to the antigenic stimulus in the gingival pocket. These immunocompetent cells must continually synthesize immunoglobulins since the half-life of IgG is about twenty days and only about four or five days for IgA and IgM and may possibly be even shorter in the areas stressed by repeated antigenic challenges.\(^{(57)}\) The shorter half-life of IgA and IgM may account for other workers describing higher numbers of IgA and IgM producing plasma cells in relation to those cells producing IgG. Since the turnover rate for IgA and IgM is greater, more immunocompetent cells may be required to maintain homeostasis between the globulin levels.

It would be difficult to explain the increased levels of immunoglobulins in inflamed tissue on the basis that the assay may only be reflecting serum globulin levels due to increased vascularity and greater capillary prèmeability since the specimens were anemic in appearance due to their exsanguination at the time of removal. The saline rinse prior to tissue homogenation apparently removed most of the remaining blood as
was evidenced by the lack of heme pigmentation in the final supernatant fluid. The substantial difference between the IgA to IgG and IgM to IgG ratios for inflamed gingiva and normal human serum lends further support to the premise that the extravascular immunoglobulins were the predominate fraction being assayed.

The finding that the mean IgA to IgG ratio was the same for both inflamed and normal gingiva may be a result of the body's homeostatic mechanisms which regulate the levels of blood and tissue immunoglobulins. The overall blood and tissue immunoglobulin levels are under the control of a homeostatic mechanism which regulates the daily exchange rate of about twenty-five per cent of the total cell-free circulating immunoglobulin pool. The findings of this study show an apparent ability on the part of the body to maintain the homeostatic relationship between various globulin fractions in states of health and inflammation. The mechanism appears to maintain a base level of tissue immunoglobulins in a state of health to provide protection against the ingress of antigens and regulate the increase of the levels in instances of antigenic challenge to maintain the homeostatic relationship as well as provide a strengthened defense against the antigenic stimulus.

The finding of IgM levels in only six of the sixteen inflamed gingival specimens in this study indicates that IgM antibody is not a consistent feature of chronically inflamed human gingiva. The IgM antibody may be present in response to a recent strong antigenic challenge to the
gingiva. This would seem to be a plausible explanation since the principle protection against gram-negative bacteria such as found in the oral cavity is offered by the bactericidal activity of IgM antibodies. It also appears that one molecule of IgM antibody in an antigen-antibody system may activate the complement reaction sequence whereas in the case of IgG apparently at least two molecules of antibody properly spaced are required to activate the complement reaction. Thus, IgM is probably the more efficient molecule in killing gram-negative bacteria in concert with complement. The IgM antibodies may also be present in response to bacterial polysaccharides which are not readily degraded and may persist in amounts sufficient to maintain maximum stimulation for long periods of time. The response to a primary dose of bacterial polysaccharide may continue unabated for years. (57)

Although IgM antibody is the first serum antibody to appear following antigenization, its presence was not detected in ten of the sixteen inflamed gingival specimens or in the pooled normal gingiva. The disappearance or lack of IgM antibody in the tissues is probably due to the specific feedback suppression by IgG antibodies which follow IgM antibodies in appearance. (57) The local tissue may even be sufficiently protected by the antimicrobial action of homeostatic levels of IgG and IgA so that the bactericidal capabilities of IgM are not required. Also the lack of a strong antigenic challenge or a relatively "inactive" stage of periodontal disease may not elicit an IgM response.
The interaction of the gingival immunoglobulins and the bacterial antigens may, in the case of IgG and IgM, activate the complement system. This in turn releases biologically active products which increase capillary permeability, contract smooth muscle, and are chemotactic for PMN's. (26) The ensuing edema would allow the ingress of more bacterial products and eventual autolysis would locally liberate their tissue destructive enzymes, causing additional tissue damage and promoting the chronic inflammatory picture seen in periodontal disease.

This possible model for periodontal destruction should not be taken to imply that the limited local tissue injury produced by such reactions is an overall disadvantage to the host since inflammation normally provides for the localization, destruction and ultimate removal of injurious agents. The tissue responses to natural antigen-antibody interaction are by and large of more benefit than harm to the host and exert a profound effect on the histopathology and course of a lesion due to foreign agents. (57)

Further study is needed to correlate clinical impressions with histologic and quantitative measure of immunoglobulin levels in the inflamed gingiva and to investigate the role of delayed hypersensitivity in the disease process. Work must also be directed towards the detection of specific antigens responsible for eliciting the immune response in the gingival tissues of the periodontal patient. These studies would provide further insight into the mechanisms involved in periodontal disease.
CHAPTER VII
SUMMARY AND CONCLUSIONS

Resected inflamed gingival tissue was obtained from 16 patients, 8 males and 8 females, ranging in age from 27 to 68 years, whose surgerized areas showed signs typical of chronic periodontal disease. Clinically normal gingiva was obtained from the edentulous maxillary ridges of 5 patients, 3 females and 2 males ranging in age from 33 to 58 years. The clinically normal gingiva was pooled for the assay due to the small amount obtained from each patient. All patients included in the study were in good general health and not taking any medications at the time of surgery.

Upon removal, the tissue specimens were washed with 0.85% saline, frozen and stored at -15° C until ready for use. Following thawing of the tissue specimens, the saline extraction of globulins from the tissue was carried out in an Environ-room at 4° C. The tissue homogenate was centrifuged at 2,000 g. for 30 minutes. The supernant fluids were then stored for no longer than 48 hours at -15° C. before thawing. The supernant fluids along with a control of saline were then assayed for globulin fractions IgG, IgA and IgM employing low level immunodiffusion plates for the assay. Precipitant ring diameter were measured following incubation and immunoglobulin concentrations read from the standard curve plotted with the values obtained from the serial dilutions of the known standard.
Globulin activity in the saline control was negative in all cases.

The mean concentrations of IgG, IgA and IgM were substantially greater in the inflamed gingiva when compared with the levels present in the pooled normal gingiva. This indicates the apparent existence of an immune response in the inflamed human gingiva with the local synthesis of immune globulins.

The ratio of IgA to IgG was the same for the mean inflamed gingiva and the pooled normal gingiva which points to the existence of an unknown homeostatic mechanism that may regulate tissue globulin levels in states of both health and inflammation. The ratio of IgA to IgG for the mean inflamed gingiva and the pooled normal gingiva differed considerably with the IgA to IgG ratio for normal human serum. This indicates that the predominate globulin fraction being assayed in this study was extravascular.

IgM was detected in only 6 of the 16 inflamed gingival specimens. This demonstrates that IgM is not a consistent feature of inflamed gingival tissue but may be present only in areas of recent antigenic challenge or in response to certain types of antigens.

IgM was not detected in the pooled normal human gingiva. This indicates that IgM is not present in the human gingiva in levels detectable by the assay method used in this study.
The following conclusions can be drawn from this study:

1) IgA and IgG are present in both inflamed and normal gingiva and although their levels are substantially higher in the inflamed gingiva, their ratio, one to another, remains the same.

2) IgM can not be consistently demonstrated in inflamed gingiva with the assay technique employed.

3) No IgM was demonstrated to be present in normal human gingiva with the assay technique employed.

4) A local immune response exists in the inflamed gingiva of humans with chronic periodontal disease.

5) There is an unknown homeostatic mechanisms which regulates the globulin levels in states of health and inflammation.

6) The saline extraction technique and immunoplate assay method used in this study are valuable tools in accessing the extravascular globulin levels in soft tissue.
<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex of</th>
<th>Age of</th>
<th>Specimen Wt. (G)</th>
<th>0.85% Saline Added</th>
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Figure 1.
### Dilutions of Standard

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<th>(mg.%) IgA</th>
<th>(mg.%) IgM</th>
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<td>50</td>
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<td>1:15</td>
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**Figure 2.**

### Plating Design

on Immunodiffusion Plates:

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<td>25%</td>
<td>12.5%</td>
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<td>100%</td>
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<tr>
<td>B</td>
<td>#1</td>
<td>#2</td>
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<td>#4</td>
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<tr>
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<td>#8</td>
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<td>#14</td>
<td>#15</td>
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ring diam. in mm.

**Figure 3.**
### IgG - Plate 1

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*Figure 4.*

### IgG - Plate 2

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*Figure 5.*
IgG - Mean ring diam.

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Figure 6.
### IgA - Plate 1

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**Figure 7.**

### IgA - Plate 2

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**Figure 8.**
**IgA - Mean ring diam.**

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*Figure 9.*
### IgM - Plate 1

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*Figure 10.*

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*Figure 11.*

### IgM - Mean ring diam.

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*Figure 12.*
Figure 13.

IgG

mean ring diam. (mm.) plates 1 and 2

Figure 13.
Figure 1h.

IgM

mean ring diam. (mm.) plates 1 and 2
Figure 15.

IgA

mean ring diam. (mm.) plates 1 and 2
Figure 16. Finely sliced resected gingival tissue.
Figure 17. Tissue slices in mortar with 2 ml. of saline per gram of tissue.
Figure 18. Resected gingival tissue homogenate prior to centrifugation.
Figure 19. Gingival tissue centrifugate with straw-colored supernatant fluid.
Figure 20. Saline extract of gingival tissues prior to assay for immunoglobulin levels.
Figure 21. Representative IgG immunodiffusion plate.
Figure 22. Representative IgA immunodiffusion plate.
Figure 23. Representative IgM immunodiffusion plate.
CHAPTER IX

REFERENCES


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36. Rizzo, A. A. and Berglund, S. E.: Response of Rabbit Gingiva and Regional Lymph Nodes After Prolonged Contact Exposure to Bacteria Endotoxins. IADR Abst. #69, 1969.


APPROVAL SHEET

The thesis submitted by Dr. Craig W. Byers 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 requirement for the Degree of Master of Science.

5-18-73
Date

[Signature]
Signature of Advisor