The Presence of Immunoglobulin IgG and Complement Factor C3 in Inflammatory Papillary Hyperplasia

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Loyola University Chicago

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THE PRESENCE OF IMMUNOGLOBULIN IgG
AND COMPLEMENT FACTOR C3
IN INFLAMMATORY PAPILLARY HYPERPLASIA

BY
Ilze Irene Eglitis

A Thesis Submitted to the Faculty of the Graduate School
of Loyola University of Chicago in Partial Fulfillment
of the Requirements for the Degree of
Master of Science
April
1980
DEDICATION

To my loving and wonderful parents

especially for their years
of support, encouragement, and inspiration

Arnolds Vladimirs Klavins

Maija Skaidrite Klavins
ACKNOWLEDGEMENTS

The author would like to express her appreciation and offer special recognition to Dr. William Malone, thesis director, for his guidance throughout this investigation and for his continued faith during her periods of extreme doubt. Special recognition is also offered to Dr. Patrick D. Toto, who provided the impetus to this study and invaluable help throughout. The author is grateful to Dr. Rinert Gerhard for serving as a member of the thesis committee.

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The assistance of Mr. John Corliss in coordinating the statistical analyses was very appreciated.

I thank my parents for their encouragement and support during the years of my lengthy education. This education was possible only with their unfailing inspiration and personal sacrifice.

Finally, I thank my dear friends for their understanding and moral support throughout these long years of attempting to maintain a balance between education and the ‘other life.’
VITA

The author, Ilze Irene Eglitis, is the daughter of Arnolds Vladimirs Klavins and Maija Skaidrite (Galovska) Klavins. She was born June 23, 1951, in Oregon, Illinois.

Her elementary education was obtained in the public schools of Chicago, Illinois. She graduated Von Steuben High School, Chicago, Illinois, in June, 1968.

In September, 1968, she enrolled at Loyola University, Chicago, Illinois. The degree of Bachelor of Science magna cum laude with honors was awarded June, 1972, with a major in biology. During her undergraduate years, she was a member of Beta Beta Beta Biological Society and was elected to the Blue Key National Honor Fraternity.

In September, 1972, she entered the freshman class of Loyola University School of Dentistry. She graduated in June, 1976, with the degree of Doctor of Dental Surgery summa cum laude and valedictorian of her class. Her efforts were recognized by election to Omicron Kappa Upsilon Honorary Dental Fraternity and Alpha Sigma Nu National Jesuit Honor Society. She received awards from the Academy of General Dentistry, American Society of Pedodontics and Alpha Omega Fraternity. She represented Loyola in the American Dental Association Student Table Clinic Program presenting 'Esthetics for Tetracycline Stained Teeth.'
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In January, 1979, she entered Loyola University to continue graduate dental education towards a Certificate of Specialty in Prosthodontics, began the pursuit of a Master of Science degree in Oral Biology and entered private practice.

She was awarded a Dental Teacher Training Fellowship from the American Fund for Dental Health for the 1978/1979 and 1979/1980 academic years.
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CHAPTER I

INTRODUCTION

The exact nature of the etiology of inflammatory papillary hyperplasia remains controversial. A major emphasis has been placed on physical factors such as occlusal problems associated with dentures and movement of the denture base in function. Among biologic factors cited are Candida albicans, inadequate oral hygiene measures and allergy to denture base components. The relationship of inflammatory papillary hyperplasia in edentulous patients and inflammatory periodontal disease in dentulous patients has not been previously suggested.

It is generally agreed microorganisms of dental plaque comprise the primary etiologic agent responsible for chronic periodontal disease. Component reactions of the immune system may be a factor in the wide variation noted in patient susceptibility.

During recent years it has become increasingly apparent there is hardly any pathologic process going on in the body that does not involve the immune apparatus. The pattern seen in any one human disease is often complex, involving not just one, but several pathways or responses of the immune system. The effects of the immune process may be both protective and destructive to host tissues. Complement, a series of nine serum proteins, is intimately associated with the immune system. The activation of the complement sequence produces fragments and complexes with various biologic activities amplifying the inflammatory process and effects of immune
reactions.

A multifactorial etiology has been described for inflammatory periodontal lesions. The hypothesis of the present study is that a similar multifactorial etiology complicated by component reactions of the immune system exists for inflammatory papillary hyperplasia. The intaglio of the denture base and the tissues supporting it provide an area for dental plaque to develop. A hyperplasia of the palatal mucosa in response to the irritants occurs. Numerous papillary projections, closely arranged, are separated by clefts which may harbor plaque as do periodontal pockets, complicating oral hygiene measures and aggravating the lesion. A dense infiltration of lymphocytes and plasma cells in the underlying connective tissue is seen histologically.

The purpose of the present investigation is to determine whether selected factors of the immune response are present in palatal tissues exhibiting inflammatory papillary hyperplasia.
CHAPTER II

LITERATURE REVIEW

A. PROSTHODONTIC STUDIES RELATING TO DENTURE-INDUCED INFLAMMATORY SOFT TISSUE LESIONS

The etiology of inflammatory soft tissue lesions underneath denture prostheses has remained a controversy in the dental literature. The treatment has remained subjective and the cure elusive.

Black (1886) referred to inflammatory lesions of the palate and attributed the condition to contact with old and unhygienic dentures.

Cahn (1936) described palatitis under dentures as “denture sore mouth.” The subepithelial inflammation was attributed to infection of the mucosa in which Candida albicans was the predominant factor. He eliminated allergic sensitivity to denture base materials as a cause. In his opinion, “denture sore mouth” was seen in its severest form in debilitated patients. He used antiseptics on both dentures and tissues in treating the condition.

Bartels (1937) also attributed “denture sore mouth” to yeastlike organisms.

Hecht (1939) reported five cases of chronic denture irritation. One case of palatitis of the nodular type was ascribed to trauma, ill-fitting dentures and debris. The lesion was treated with surgical removal of the involved tissue.

In reviewing 560 cases of epidermoid carcinoma, Hobaek (1949) reported a causal connection between irritation caused by denture prostheses and cancer development in 86 cases. In his opinion, palatal stomatitis should be
considered a precancerous condition in some cases. However, his opinion must be considered in the light of the state of the art at that time. This was demonstrated by viewing tobacco as relatively tissuephilic with respect to cancer induced lesions.

Nyquist (1952) divided cases of “denture sore mouth” into three classes:

1. diffuse, fairly smooth, hyperemic, edematous mucosa under the entire denture;
2. discrete, inflamed, sometimes granular mucosa with certain predilection sites particularly under vacuum chambers and suction disks;
3. a combination of the above.

The chief conclusion he drew from his investigation demonstrated trauma from occlusion as the predominant cause of “denture sore mouth.” Allergic and toxic effects of denture base constituents were also considered possible.

Inflammatory papillary hyperplasia was first described as a separate disease entity by Fisher and Rashid (1952). They associated the condition with wearing non-hygienic artificial dentures. In most cases, the denture bases were not satisfactorily adapted to the oral tissues. The fundamental process appeared to be inflammatory in nature caused by chronic excessive retention of food debris and stagnant oral secretions. They suggested discarding unsatisfactory dentures as appropriate therapy. The regression of the lesion with improved oral hygiene refuted a neoplastic process. They emphasized the importance of distinguishing between inflammatory papillary hyperplasia and true papillomas.

Thoma (1952), however, was of the opinion all papillomas were potential
malignancies and should be removed completely by wide excision.

Van Huysen, Fly and Leonard (1954) presented nine case reports of untoward responses of the oral mucosa to dentures. Three cases exhibiting nodular hyperplasia were not resolved when new dentures were fabricated. The accompanying inflammation was reduced however.

Halperin (1957) reported inflammatory papillary hyperplasia tended to occur in the midline of the hard palate. The tissue appeared as elevated, reddish, lobulated or papillary lesions attached to a broad base. On the other hand, true neoplastic papillomas could be seen anywhere on the hard or soft palate, were frequently white in color, attached by a narrow or broad base, and an obvious local irritant was lacking. He quoted a review of 51 cases of palatal carcinoma reported by Sharp, Bullock and Hazlet. They reported mechanical trauma of negative pressure created by excessive relief of palatal denture areas had a remarkably low relationship to carcinoma. In treating inflammatory papillary hyperplasia, Halperin advised eliminating the irritating denture, then a short observation period and excision of any portion which failed to resolve.

Lyon and Chick (1957) found *Candida albicans* could be cultured far more frequently from “denture sore mouth” patients than from controls. Sodium caprylate, a fungicide, was used to treat the lesions. They hypothesized denture bases exhibiting a rough intaglio or porosity could harbor organisms. Such foci could be responsible for relapses after cessation of treatment.

Robinson (1957) considered inflammatory papillary hyperplasia a “precancerous” condition even though in his experience he encountered only occasional malignancies. He classified inflammatory papillary hyperplasia as a *pseudo-epitheliomatous hyperplasia*, resembling malignant
change but lacking invasive features. He reported the lesion usually showed histologic dyskeratosis.

Campbell (1961) discussed relief chambers in maxillary dentures. These had been advocated due to differences in resiliency of palatal and ridge tissues. The central area of the palate might act as a fulcrum for rocking the denture base. Other theories for relief over the median raphe included compensation for (a) anticipated resorption of the alveolar process, (b) the greater pressure incurred in impression materials in this region, and (c) curing shrinkage of acrylic resin bases which cause the palatal area to be high. Campbell reported relief chambers resulted in an increased fluid film and less retention. They reduced the area of support from the hard palate, increasing force on the residual ridges. Negative pressure in relieved areas may stimulate proliferation of tissue.

Newton (1962) divided “denture sore mouth” into three stages according to clinical signs:

1. Pinpoint hyperemia: small areas of inflammation in otherwise normal tissue which are usually found around the ducts of palatal mucous glands;
2. Diffuse hyperemia: generalized inflammation of the entire denture bearing area;
3. Granular: a nodular, hyperemic mucosal surface which may be present over the entire denture bearing area but commonly restricted to central areas, especially under relief areas and suction disks.

He suggested “denture sore mouth” may be due to the lateral spread of saliva.
into the tissues following occlusion of palatine gland duct orifices. He related this proposed etiology to the sweat retention syndrome.

Mullins (1962) dismissed palatal glands as an etiologic factor since the lesions of inflammatory papillary hyperplasia are denser than the mucous glands at the site of occurrence. He attributed inflammatory papillary hyperplasia to rocking or ill-fitting dentures complicated by stagnant saliva, debris or suction.

Kuller and Kutscher (1963) described various treatments for a case of inflammatory papillary hyperplasia. They had no success in using Orahesive, Kenalog 0.1% in Orahesive, Steri-Sol mouthwash, Dianabol 0.037% in Orahesive, and Mucoplex. Use of Kenalog 0.1% in Orahesive for 10 days promoted Candidiasis lesions. Resolution of inflammation and fibrous connective tissue healing occurred after a 6 months’ use of Cepacol as a mouthwash and denture soaking solution.

Shafer, Hine and Levy (1963) described papillary hyperplasia or papillomatosis to be of an unknown etiology, though associated with ill-fitting dentures and poor oral hygiene. It was rarely seen in dentulous patients. Clinically, it presented with numerous closely arranged papillary projections, often involving nearly all of the hard palate. Histologically, numerous small vertical projections each composed of stratified squamous epithelium and a central core of connective tissue were seen. There was an inflammatory cell infiltration of the connective tissue with hyperkeratosis and dyskeratosis occasionally present. They alluded to the possibility of malignant transformation.

Yrastorza (1963) presented a review of 64 cases of inflammatory papillary
hyperplasia. Dentures with relief areas outnumbered "unrelieved" dentures by a ratio of 4:1. Three of the 64 patients wore vulcanite dentures, and 60 had acrylic resin dentures. One patient had a partially edentulous maxilla, wore no prosthesis, and had extremely poor oral hygiene. The papillae in all these patients persisted despite prolonged periods (6 weeks to 3 years) of not using dentures. The inflammation appeared to resolve, however. Examination of biopsy specimens showed hyperplasia of the epithelium with multiple papillary projections from the surface and digit-like extensions of rete pegs. A chronic inflammatory infiltrate in submucosal layers was composed of lymphocytes, some plasma cells and a few histiocytes. Areas of hyperkeratosis and parakeratosis were seen in several specimens. 10% of the specimens exhibited dyskeratotic changes in the form of intraepithelial keratinization, hyperchromatism of the basilar layer and altered cell polarity. No instances of malignancy were seen. Clinical behavior and histopathology are compatible with inflammatory disease. Yrastorza, however, felt the irreversibility of the lesion combined with occasional dyskeratosis suggested true neoplasia. He emphasized early recognition, surgical treatment, and a biopsy follow-up.

Schmitz (1964) conducted a study of 125 patients exhibiting lesions of inflammatory papillary hyperplasia. He concluded the proliferation of hyperplastic tissue was due to a frictional irritation caused by a skidding, plunging motion of ill-fitting dentures. Since complete rest or pressure atrophy methods did not result in regression of the lesions, he recommended surgical excision.

Smith (1964) reported regression of inflammation, decrease in tissue thickness, and return to normal tissue color when triamcinolone acetonide was
placed in the dentures of 6 patients with inflammatory papillary hyperplasia for 3 to 12 weeks. One case exhibited dyskeratotic changes. The dentures were remade and no recurrence of the lesion was seen six months after treatment.

Bolender, Swenson and Yamane (1965) evaluated the effects of fabricating new dentures combined with and without surgical removal of the lesion in 20 patients with inflammatory papillary hyperplasia. All patients had maxillary full dentures with palatal relief. They suggested using an air syringe to aid in the diagnosis of inflammatory papillary hyperplasia. The papillary projections became more evident after a jet of air was directed to the palate. Except for a moderate reduction in erythema in 5 patients, virtually no change was seen clinically or histologically in 10 patients after 1 year of wearing new dentures without relief chambers. Supraperiosteal excision of the lesions by a high-frequency cutting current and fabricating new dentures effectively eradicated the lesions for the 10 patients in this group.

Guernsey (1965) conducted a clinical investigation of 99 cases of inflammatory papillary hyperplasia. Two general types of lesions were seen: a diffuse papillary one and a nodular or polypoid one. A review of 5,892 dental records showed the incidence to be 2.9% among denture wearers and 0.2% among non-denture wearers. Inflammatory papillary hyperplasia occurred in the age range from 20 to 50, but most frequently in the 20s. Among dentulous subjects, the shape of the palatal vault seemed significant. A high, narrow vault with a tendency toward outfolding tissues seemed to prevent normal cleansing by the tongue. Etiologic factors noted in the survey were:

ill-fitting prostheses,
mechanical irritation with the perpetuation of the lesion in successive denture bases,
continuous wearing of the prosthesis,
poor oral hygiene,
chemical irritation from denture cleansing agents,
putrefaction of food,
decreased salivary flow,
smoking,
and allergy.

However, Guernsey felt predisposing factors to be most important since many patients with ill-fitting prostheses and poor oral hygiene exhibited no lesions. Among the differential diagnoses, he listed midline pyogenic granuloma, fungal granulomas, capillary hemangiomas, allergic lesions and exophytic squamous cell carcinoma. He recommended surgical removal. Among 59 biopsy specimens, no evidence of dyskeratosis was found. The lesion was considered benign, reactive and inflammatory.

After an initial biopsy and removal of possible irritative factors, O'Driscoll (1965) advised careful observation of inflammatory papillary hyperplasia supplemented by exfoliative cytological examination. Ucellani (1965) described many factors, systemic and local, which affect tissue tolerance to dentures. Though a thorough dental, medical, dietary and personal history may aid in comprehending tissue tolerance, additional investigations are needed to provide more meaningful evaluations of the oral mucosa antecedent to complete denture construction.

After examining 25 patients with clinical evidence of inflammatory papillary hyperplasia, Lambson (1966) found 24 of them wore their upper dentures continuously. Only one of 24 dentures met all requirements of adaptation, stability and retention. Twenty dentures had relief areas. One
dentulous subject exhibited extremely poor oral hygiene. He described the lesions as predominantly nodular, papillary, or mossy. Papillary and nodular forms were located at the center of the lesion while border areas were mossy. Mossy forms may be the earliest diagnosable lesions. Lambson viewed inflammatory papillary hyperplasia as a complication arising from dentures which have been in use too long.

Stephens, Cox and Sharry (1966) investigated diurnal variations in palatal mucosal thickness in 13 dentulous patients. The average diurnal change was 0.0045 in. Their results indicated palatal tissues were thickest when lying in bed after a full night's sleep. An immediate and rapid shrinkage occurred upon arising, which tapered off in the afternoon. A slight increase occurred throughout the evening before retiring and when lying down to sleep. Posture seemed to be the outstanding factor. Other factors considered were: the balance of fluid intake and output and forces transmitted to tissues during eating and swallowing. Though carelessness in impression technique cannot be condoned, Stephens et al felt the magnitude of change in tissue thickness was great enough to suggest attempts at securing extremely accurate impressions and final denture bases as unrealistic, inordinate clinical procedure goals.

Fairchild (1967) examined 36 full denture and 16 partial denture patients with inflammatory papillary hyperplasia. A bacteriologic study was terminated after cultures from 25 patients revealed a normal oral flora. Alginate tests for voids between the denture base and palate were done. 80% of the prostheses had voids. 18 full dentures and 1 of 7 partial dentures with palatal coverage had palatal relief chambers. Since dimensional change occurs in processing heat-cured acrylic resins, and metal shrinks upon solidification, Fairchild
concluded denture resins and materials create their own relief between the palate and denture base material. He indicated the etiology of inflammatory papillary hyperplasia may not be bacterial, but may be due to behavior of denture base materials during processing, to impression techniques designed to provide palatal relief, and to the improper use of impression materials. He felt the void between the denture base material and palatal tissue may be of etiological significance.

Lambson and Anderson (1967) conducted a survey of 301 denture wearers. 48% wore their dentures only during waking hours and exhibited no inflammatory papillary hyperplasia. 52% wore their dentures continually; 20% had inflammatory papillary hyperplasia. Palatal relief failed to offer conclusive evidence of etiology. They suggested the wearing of a denture past its useful lifespan of 5 to 6 years increased the possibility of developing inflammatory papillary hyperplasia.

Questionnaires for 522 denture patients were completed and examined by Love, Goska and Mixson (1967). The incidence of severe inflammation and inflammatory papillary hyperplasia was twice as great in the age group under 29 than in the group over 50. It was 10 times greater for those patients who wore their dentures while sleeping. The incidence was twice as great for those who did not soak their dentures in a cleanser or who did not use a mouthwash to rinse and clean the supporting mucosa. Inflammation and inflammatory papillary hyperplasia occurred 5 times more often under resin bases compared to metal bases. No incidence of inflammatory papillary hyperplasia occurred among patients who combined brush stimulation with the removal of dentures at night.
Amaral, Frost, Howard and Cheatham (1968) suggested cryosurgery in the form of liquid nitrogen applications as a treatment of inflammatory papillary hyperplasia. Advantages they cited were ease of application, absence of pain, and opportunity for repeated applications.

McClatchey (1968) attributed the etiology of inflammatory papillary hyperplasia to “ill-fitting” dentures. He recommended a conservative treatment using tissue conditioning materials. If the lesion persisted, surgery was then the treatment of choice.

Ritchie and co-workers (1969) examined 100 patients with denture stomatitis. 93 dentures had traumatic factors. 74 patients wore their dentures at night. Apparently, 21 patients had palatal hyperplasia previously. Denture stomatitis appeared to begin in the anterior one-third of the palate and spread posteriorly and then finally into the residual ridges. This progression threw doubt on the etiology proposed by Newton (1962), which supposed it commenced at the posterior portion of the hard palate and spread forward. However, occlusion of gland orifices could be responsible for spread of the condition. Exfoliative cytology revealed a differential count consisting entirely of nucleate cells. Bacteria, leukocytes and yeast hyphae were present in all. Normal palates had a predominant anucleate pattern and no yeasts. Ritchie recommended a combination drug and prosthetic therapy. He implicated six main etiologic factors in denture stomatitis: trauma, bacterial and fungal infection, allergy, malnutrition, hormonal imbalance, and the use of oral antibiotics.

Budtz-Jorgensen and Bertram (1970) also included papillary hyperplasia in their study of denture stomatitis or “denture sore mouth” lesions, as some
other researchers had also done. Their population consisted of 58 patients with full dentures and denture stomatitis and 58 patients with full dentures and clinically normal palatal mucosa. The mean of denture age and years of experience with dentures were higher in the denture stomatitis group. However, these differences were not statistically significant. Occlusal relations and denture cleanliness tended to be better in the control group. Denture cleanliness was evaluated by using a disclosing solution. The quality of denture-bearing tissues was poorer in the denture stomatitis group as judged by the degree of atrophy of alveolar processes and compression and mobility of the overlying mucosa. Yeast colonies were found in greater numbers in cases with denture stomatitis. The colonies were found in areas of localized simple inflammation and papillary hyperplasia. They covered the total maxillary denture-bearing area in cases of generalized simple inflammation. A pronounced statistical significance was found between poor denture cleanliness and clinical evidence of heavy inflammation. They concluded trauma and Candida infection were involved in the etiology of inflammatory papillary hyperplasia. They contended Candida infection may be verified by the presence of hyphal structures in a direct palatal smear. According to the investigators, it is not possible to determine whether Candida are present in the parasitical mycelial phase or in the saprophytical blastospore stage by cultivation methods. Antifungal therapy was therapeutically effective for patients with verified Candida infections. Relapses did occur after treatment was terminated. A therapeutic effect from a combined prosthetic treatment including occlusal equilibration, tissue conditioning and new dentures was noticed primarily in patients with a non-verifiable Candida infection. No patients showed
regression of the papillary hyperplasia with either treatment modality.

An evaluation of 341 surgical specimens of inflammatory papillary hyperplasia was conducted by Bhaskar, Beasley and Cutright (1970). The occurrence of inflammatory papillary hyperplasia was directly related to oral hygiene. It was 10 times more frequent in patients who slept with their dentures. 20% of patients who wore their dentures continually exhibited inflammatory papillary hyperplasia. It was 5 times more common in patients wearing acrylic dentures than metallic dentures. Inflammatory papillary hyperplasia apparently showed a 10% prevalence rate among denture wearers and comprised 1.5% of biopsy specimens taken in a dental office. In their case population, the age range was 7 to 86 years, the majority from 20 to 50 years of age. The average age was 42. It was slightly more common in the male, had no race predilection and more than 92.7% of the cases occurred on the palate. A histologic examination of the cases resulted in the following profile:

<table>
<thead>
<tr>
<th>Condition</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>inflammation</td>
<td>100.0%</td>
</tr>
<tr>
<td>pseudoepitheliomatous hyperplasia</td>
<td>99.7%</td>
</tr>
<tr>
<td>parakeratosis</td>
<td>95.3%</td>
</tr>
<tr>
<td>intraepithelial keratinization</td>
<td>27.3%</td>
</tr>
<tr>
<td>keratinized</td>
<td>11.4%</td>
</tr>
<tr>
<td>myxomatous degeneration</td>
<td>5.8%</td>
</tr>
<tr>
<td>sialadenitis</td>
<td>2.3%</td>
</tr>
<tr>
<td>microcysts</td>
<td>1.7%</td>
</tr>
<tr>
<td>ulceration</td>
<td>1.5%</td>
</tr>
</tbody>
</table>

In no case was there any evidence of dyskeratosis or malignant change. They recommended conservative curettage, fabrication of a better adapted prosthesis and a regime of meticulous oral hygiene as treatment.

Allergy as a cause of "denture sore mouth" was investigated by Danilewicz-Stysiak (1971). 24 of 40 patients had ill-fitting dentures and symptoms of
"denture sore mouth" were alleviated after new dentures were made. 16 patients exhibited a positive allergy to denture resins based on patch tests with standard substances. Of the 16, only 4 patients were sensitive to filings from their own dentures and their symptoms disappeared when the dentures were left out of the mouth for a given period of time. Patch and mucosal contact tests of these 4 patients implicated pigments and the catalyst hydroquinone. Danilewicz-Stysiak implied allergies may be due to an interaction between compounds produced during polymerization and the oral flora.

Miner (1973) examined and photographed sections of polished and impression basal surfaces of polymethyl methacrylate, vulcanite and cast gold denture base materials using the scanning electron microscope. Polymethyl methacrylate had the roughest surface and had the most intricate and diverse surface contours. Though vulcanite was observed to be smoother, it absorbs more oral fluids. Gold had a relatively fine scanning electron microscope topography. It does not absorb oral fluids and deforms less than polymethyl methacrylate or vulcanite bases during use. Transitional topographic changes seen across the surfaces ranged from large craters, ridges and projections to small pittings, porosities and striations. These areas are capable of providing traps for debris, microorganisms and allergens. Those same surfaces may act as plungers or suction cups which traumatize oral tissue.

Various microbiologic studies of denture stomatitis were conducted by Van Reenen (1973). Microscopic examination of smears taken from denture stomatitis lesions demonstrated the gram-positive cocci as the predominant organism and the number of bacteria increase with years of denture wearing and with increased severity of denture stomatitis. Examination of
microorganisms cultured from palates of denture wearers showed the palatal mucosa supported a mixed flora containing a number of potential pathogens. No specific organism was associated with the lesions; the infection was caused by a community of pathogens. Gram-positive bacteria predominated in the cultures. Van Reenen demonstrated a nonselective attachment to palatal epithelial cells occurred with most bacteria isolated from denture stomatitis. He thought metabolic changes occurring in palatal epithelium as a result of wearing dentures may favor attachment of microorganisms to surface cells. A relative absence of bacteria on epithelial surfaces of non-denture wearers was seen from an examination of palatal epithelia under the scanning electron microscope. Epithelia from denture wearers exhibited surface bacteria and microulcers. Van Reenen proposed irregularities of cell surfaces could favor bacterial attachment by increasing surface area and restricting fluid movement. Desquamation of surface cells may be an important protective mechanism of the epithelium. Trapped by wearing dentures, the surface cells accumulate and aid in increasing bacterial numbers in the salivary film under the denture. Bacteria could be demonstrated on epithelial surfaces, intracellularly and subepithelially by means of phase contrast microscopy of sections of tissues of denture stomatitis and papillary hyperplasia stained by the Gram method and albumin-OsO₄. Raised antibody titers to microorganisms isolated from denture stomatitis lesions suggested a number of microorganisms participate in the pathogenesis of the disease. Nystatin was found to be inhibitory on the growth of fungi, streptococci and pneumococci. A two-week penicillin regimen cleared the denture stomatitis condition in five patients. If yeasts were the main etiology the condition was expected to
deteriorate. A negative pressure of 5 in. Hg stimulated the growth of *Escherichia coli* and *Candida albicans*. *Diplococcus pneumoniae* was enhanced by a negative 15 in. Hg. Reduced pressure may increase the permeability of bacterial cell membranes, enabling growth factor to pass through more readily. Reduced pressure may affect tissue permeability and metabolic products are likely to diffuse more readily through the epithelium. Therefore, Van Reenen proposed the force of retention of the maxillary denture is a possible factor in promoting infection under a denture.

Tucker and Heget (1976) found a high correlation between the incidence of inflammatory papillary hyperplasia and the continuous wearing of dentures.

Bauman (1977) considered the failure of dentists to adequately impress upon their patients the importance and nature of proper home care as a factor contributing to the development of inflammatory papillary hyperplasia. 54 patients with inflammatory papillary hyperplasia were interviewed. 96% wore their dentures continuously while 65% stated they received no instructions.

Inflammatory papillary hyperplasia was considered irreversible by Miller (1977). Inflammation could be controlled by leaving the denture out of the mouth. He recommended removal of nodules by surgical curettage and a biopsy, since he mentioned the possibility of malignancy exists. A new prosthesis with no relief chamber should be made when healing is complete. He recommended the patient should leave his denture out 8 hours daily.

The following treatment for inflammatory papillary hyperplasia was described by Cameron (1978): Half of the papilla should be smoothed off the master model and half from the denture intaglio. A denture with excellent occlusion and no contact in the incisor region should be made. He claimed the
tissue recovers in one month. Elimination of tobacco, alcohol and bruxing aids treatment.

From a clinical study of 34 patients, Pearson (1979) formulated the following classification of etiologic factors of inflammatory papillary hyperplasia. They are listed in order of offense.

1. Excessive palatal relief
2. Coexistent conditions:
   - continuous denture-wearing
   - poor oral hygiene
   - improperly cleaned dentures
3. Incorrect coordination of CO with CR
4. Denture bases poorly adapted to supporting tissues
5. Numerous microbes, including *Candida albicans*

In most patients, all five factors are found. Pearson outlined a treatment methodology based on an oral hygiene regimen and controlled pressure over the lesion with tissue conditioning material in a new denture with a properly coordinated functional occlusion.
B. STUDIES RELATED TO THE EFFECTS OF DENTURES ON MUCOSA OF EDENTULOUS RIDGES

The oral epithelium is composed of stratified squamous cells and its function is to provide protection to the underlying tissues against physical, chemical and biologic irritants. Keratinization is an example of the protective mechanism against functional forces. The response of oral mucous membrane can be inflammatory, degenerative, or hyperplastic, depending upon the nature and severity of injury and the individual tissue response.

The oral mucous membrane must function in a new environment when it must support a complete denture. It is difficult to predict the nature of tissue changes even if the stresses created by the dentures are within the biologic limits of tissue tolerance. Tissue response is also a function of the compatibility of denture base material.

Several investigators have evaluated the response of the oral mucous membrane to dentures. No consensus of opinion exists yet.

Pendleton (1951) reported on biopsy material from 126 patients who underwent preprosthetic surgery. The epithelial surface was consistently parakeratotic, yet presented zones of cornification at the ridge area on occasion. The germinative layer was commonly thicker, rete pegs were elongated and subepithelial inflammation prevailed. Pendleton advised the factor of tissue tolerance must be taken into account in consideration of tissue change.

Van Scotter and Boucher (1965) discussed the nature of supporting tissues for complete dentures. Evaluation of tissue changes in denture-wearing
individuals may be compounded by the general increase in age of most prosthetic patients. A gradual retardation of cell division, a decreased capacity for cell growth, a reduced capacity to heal, and connective tissue changes occur in aging. The chemical nature of cells may vary during aging with or without concomitant morphologic change. The role aging plays is important when attempting to correlate physiologic changes with pathology.

The epithelial covering of the hard palate is described as masticatory mucosa. Healthy tissues are pale pink in color. The keratinized stratified squamous epithelium is immovable and firmly attached to the periosteum of the underlying bone by heavy dense bundles of collagen fiber bundles extending down from the lamina propria. Irregular elevations or rugae extend anterolaterally from the palatine raphe. According to Lund (1924) the rugae are not simply elevations of the mucous membrane, but contain a connective tissue nucleus called "ruga nucleus" as their base.

Van Scotter and Boucher (1965) divided the edentulous maxillary arch into various denture-supporting areas. The primary stress bearing area, the residual ridges, are grayish-pink as a result of their dense character and minimal vascularity. The tissue bounded laterally by the residual ridges and medially by the median raphe is the secondary stress bearing area. Its color is deeper pink, apparently due to increased vascularity.

Regions of the hard palate differ in the varying structure of the submucous layer. The submucous layer is filled with adipose tissue in the anterolateral portions and glands in the posterolateral areas. The submucous layer is absent in the midline of the palate, the palatine raphe.

Ostlund (1953) studied biopsy specimens of healthy palatal tissues from 22
subjects, aged 25 to 69. His observations were:

1. Normal mucosa (not supporting a denture) showed a horny layer with an average thickness of 13 microns. Remains of tonofibrils were seen among the epithelial cells. Desquamated epithelial cells in the saliva were nucleated and had short processes which could have been interpreted as remains of tonofibrils.

2. The mucosa showed no stratum lucidum.

3. The stratum granulosum was thicker than that generally accepted as normal for skin and usually contained 8 to 9 layers. It was tempting to assume the fairly thick stratum granulosum compensated for a lack of stratum lucidum. The arrangement of keratohyalin granules in the cytoplasm varied widely and bore no topographical relationship to the nuclei of cells.

291 biopsy specimens of palatal mucosa were collected from non-denture and denture wearers. The biopsies were taken near the midline of the vibrating line area. In comparing clinical with histological data, Ostlund (1958) made the following observations:

Within 6 months of denture wear, the epithelium of most patients showed a pronounced change despite the clinically normal appearance of the mucosa. The changes progressed in a typical fashion:

1. The stratum corneum decreased in thickness with a simultaneous decrease in the number of all layers in the
stratum granulosum.

2. The surface of the epithelium became parakeratotic.

3. As keratin disappeared from the surface, the cells of the stratum spinosum changed in appearance. The cell cytoplasm swelled in the outer zone. Edema (intercellular) increased and the cells “were separated” from one another. An increase in cell nuclei and cytoplasm volume occurred in the central portion.

4. During initial stages of tissue changes the number of mitoses increased. Mitosis decreased as tissue injury progressed.

5. The total thickness of the epithelium was greater due to an increase in the volume of individual cells and edema of the tissues.

Ostlund also reported an insignificant difference existed between the average thickness of inflamed, 0.28 mm, and uninflamed mucosa, 0.18 mm. Wearing a denture did not seem to influence mucosal thickness.

Kapur and Shklar (1962) investigated the effects of stimulation with an automatic toothbrush on the edentulous ridges and gingiva of 13 patients. An investigator applied stimulation 6 days a week for 4 weeks to experimental quadrants. Initially 7 of 16 areas developed areas of erythema. This occurred more frequently in the mandibular anterior region. No areas of erythema were present after 8 to 12 treatments. Post-treatment biopsy specimens revealed the maxillary experimental tissues had increased keratinization, acanthosis, a more obvious prekeratin layer, and greater activity in the connective tissue. The mandibular experimental tissues only showed a slight to moderate difference or keratinization as compared to controls. Kapur and Shklar
hypothesized these differences were due to the greater width, flat surface area and greater tissue thickness on the maxillary arch. Perhaps it was easier to massage. They stated stimulation of alveolar mucosa by a well adapted denture had a similar and possibly even greater keratinization effect.

Kapur and Shklar (1963) objected to Ostlund's (1958) choice of ventral to the vibrating line site for biopsy. They argued the vibrating line is an area normally subjected to displacement by a denture. They studied biopsies from the crest of edentulous ridges. Biopsies were taken at different time intervals before and after use of dentures from 9 patients. Total elapsed time from initial denture delivery to last observation was 12 weeks. One side of the arch also received stimulation from an electric toothbrush. The histologic study revealed an increase in width of the stratum corneum layer in the stimulated mucosa and after wearing a denture.

Porter and Flanagan (1963) conducted a histologic examination of 19 biopsy specimens from 15 patients who had inflammatory papillary hyperplasia. They found glycogen associated with parakeratotic areas; absent in hyperkeratotic areas; present in the prickle cell layer, in “fissures,” around epithelial pearls, and rarely in basal cells. Vacuolated epithelial cells contained heavy deposits of glycogen. Acanthosis and inflammation were also noted.

Al-Ani, Shklar and Yurkstas (1966) used exfoliative oral cytology smears as a means of evaluating tissue changes in response to dentures. Smears were collected from the hard palate of 84 denture wearers and 116 non-denture wearers. A mixture of keratinized and non-keratinized cells was most common in smears from denture bearing areas. Keratinized cells were predominant in the non-denture wearing group. Smears from patients with “denture
hyperplasia" or irritation had mixed to no cornification and a large number of nucleated cells.

Flanagan and Porter (1968) conducted a histochemical study of 40 specimens of inflammatory papillary hyperplasia and 13 specimens of clinically normal palatal mucosa. Dyskeratosis was usually found in specimens of inflammatory papillary hyperplasia. Mast cells were twice as numerous in inflammatory papillary hyperplasia. They were mainly concentrated around blood vessels and rarely present in the epithelium. Glycogen, not observed in the epithelium of normal controls was seen in all of the diseased sections. Very little acid mucopolysaccharide was seen in normal controls, but was found to be concentrated in the submucous layers rather than the lamina propria of diseased sections.

Nedelman, Gamer and Bernick (1970) took biopsies from the edentulous ridge mucosa of 62 complete denture and non-denture wearing patients. 42 patients had no previous removable denture experience. 20 had worn removable partial dentures. All were being prepared for complete or immediate dentures. The non-denture supporting ridge mucosa showed glycogen in the epithelial layer, a thickened stratum corneum and a heavy chronic inflammatory infiltrate in the underlying submucosa. The ridge mucosa of denture wearers consisted of a thin stratum corneum, loss of demonstrable glycogen, absence of inflammatory cells in the submucosa, and a relative increase in collagenous fibers in the lamina propria. Though some authors have reported small deposits of glycogen in clinically normal gingiva, Nedelman, Gamer and Bernick agreed with those who view the presence of glycogen in the epithelial cells as implying a disturbance in cellular
metabolism. Non-specific esterase was localized throughout the epithelial layer, with the heaviest concentration in the stratum granulosum and keratinizing layers. Localized enzymatic activity, presumed to be cholinesterase associated with nerve fibers and terminals, was found in the connective tissue papillae. Denture wearers exhibited an overall decreased activity throughout the various layers. Acid phosphatase activity was localized in the epithelial layer and most prominent in the strata corneum and granulosum in the non-denture bearing mucosa. A decrease in demonstrable acid phosphatase activity occurred in denture wearers. Both acid phosphatase and non-specific esterase are hydrolytic enzymes and associated with keratinization. Alkaline phosphatase was mainly localized in the connective tissue of non-denture bearing mucosa. The investigators associated its presence with chronic inflammation. A marked decrease of activity was seen in denture bearing mucosa. Both groups exhibited alkaline phosphatase activity around blood vessels.

Jani and Bhargava (1976) demonstrated a general increase in the thickness of the palatal epithelium, more marked in the rete peg region than in the inter-rete peg region. An increase in the thickness of the keratin layer occurred in most patients. These results were observed after 3 months of wearing well-fitted dentures. No signs of inflammatory changes were noticed in the underlying connective tissue.

Razek and Shaaban (1978) demonstrated an initial increase in keratinization during the first three years of denture wearing followed by thinning and parakeratosis in the fourth to sixth years. The lamina propria showed scattered mononuclear cell infiltration in the fourth to sixth years.
Succinodehydrogenase, acid phosphatase and alkaline phosphatase activity increased in the first three years and after remaking the dentures while a reduction in enzyme activity was associated with wearing dentures for more than 3 years. They postulated their observations may be the result of loosening of the dentures which usually occurs after 3 years. This lack of adaptation leads to scraping of the keratinized layer and inflammatory reactions.

Pronounced histopathologic and histochemical changes occur in denture-bearing oral mucosa following the insertion of a prosthesis despite the normal clinical appearance of the mucosa.
C. HISTOLOGIC AND IMMUNOLOGIC STUDIES RELATED TO GINGIVITIS

Zachinsky (1954) studied the range of histologic variation of biopsy specimens of clinical normal gingiva. The histologic criteria he set for healthy gingiva were:

- intact epithelial covering
- presence of papillary projections
- a free gingival groove
- presence of gingival fibers extending into free gingiva
- an intact crevicular epithelium
- an intact epithelial attachment
- a variable accumulation of inflammatory elements in the submucosa
- embedding of fibers in an optically homogeneous ground substance

Most epithelial surfaces were parakeratotic. He considered parakeratinization as the normal end phase of the gingival maturation cycle. According to Zachinsky, the demarcation between normal and pathologic gingiva was not clearly defined histologically.

Many investigators have studied the histologic features of inflamed gingival tissues.

Zachrisson and Schultz-Haudt (1968) described histologically normal gingiva as having a rather even pocket epithelium, mere indications of rete pegs and local areas of slight cellular infiltration in the connective tissues. Moderately inflamed gingiva was characterized with strongly acanthotic rete
pegs, polymorphonuclear leukocytes within the epithelium, and a dense infiltration of inflammatory cells, predominantly plasma cells and lymphocytes, in the connective tissue. Markedly inflamed gingiva exhibited intercellular edema in the epithelium. The connective tissue contained more numerous capillaries, dilated blood vessels. Inflammatory cells had replaced the normal fiber structures.

After local application in vivo of hyaluronidase, intercellular enzymes from leukocytes and in combination, Thilander (1963) did a light and electron microscope study of structural differences in gingival pocket epithelium. The observed widening of intercellular space was most marked after the application of combination of enzymes. This was probably due to the breakdown of intercellular polysaccharides and proteins. Modification of primary and secondary forces responsible for adhesion between cells may result in an increase in uptake of water to maintain a constant epithelial tonicity. Passage of irritant substances through the epithelium into the underlying connective tissue may be promoted. No widening of intracellular space was found in most desmosomes.

Thilander (1968) studied changes due to inflammation in the epithelial structure with the electron microscope. The material comprised biopsies from inflamed gingivae of 12 adults. The first change noticed was a widening of intercellular spaces. When the widening was of moderate degree, intermediate junctions, tight junctions and desmosomes were still observed. With increased widening, intermediate junctions disappeared first, followed by a loss of tight junctions and a concomitant reduction in the number of desmosomes.
Polymorphonuclear leukocytes were seen between epithelial cells. The basement membrane was diffuse or absent in some areas. Large glycogen granules and swollen mitochondria with reduced numbers of cristae were seen in the cytoplasm of epithelial cells. More advanced changes exhibited a further reduction in contact areas between cells and localized disintegration of cytoplasm, changes preceding cell death. This study illustrated morphological changes which may give rise to increased epithelial permeability in inflamed areas. Resistance varied among the types of junctional complexes. The presence of polymorphonuclear leukocytes in the epithelium suggested local breaches in the continuity of the basement membrane.

Wittwer, Dickler and Toto (1969) obtained gingival biopsies from 50 patients with a clinical diagnosis of chronic gingivitis. 96% showed the presence of plasma cells. More than 80% of the connective tissue cellular infiltrate consisted of plasma cells in 39 cases (78%). This strongly supported the concept of plasma cell preponderance in gingivitis. 2 cases (4%) showed a preponderance of lymphocytes while an approximately equal distribution of plasma cells and lymphocytes was present in 9 cases (18%).

Toto and Gargiulo (1970) found a loss of intercellular acid mucopolysaccharides in the stratified squamous epithelium in gingival tissue specimens collected from 25 patients with gingivitis and 4 patients with periodontitis. An accompanying loss of “intercellular bridges” was noted along with edema. A polymorphonuclear leukocytic infiltrate was seen intercellularly. The basement membrane was poorly defined or absent and also showed a loss of acid mucopolysaccharides. The connective tissue was largely replaced by plasma cells which arose from perivascular mesenchymal cells.
They suggested the polysaccharides participated in the function of biological cementation between cells, a function lost in chronic gingivitis. This loss of cementation compromised the integrity of the epithelium, and could lead to small microulcers in the sulcular epithelium. An imbition of fluid could follow with a water medium replacing the gel-like medium between cells.

The temporal sequence of histopathologic alterations in human gingival tissue following the beginning of plaque accumulation was observed by Payne, Page, Ogilvie and Hall (1975). The earliest change noted was a vasculitis of blood vessels immediately subjacent to the junctional epithelium. Edema, alteration of perivascular collagen fibers and a large increase in the number of neutrophils migrating from gingival vessels through the junctional epithelium into the gingival sulcus and oral cavity were seen within 2 to 4 days. This initial lesion exhibited no clinical manifestation and was thought to be a consequence of the elaboration and release of various chemotactic substances by plaque microorganisms. The early lesion occurred within 4 to 7 days. It was characterized by an infiltration of mononuclear cells, mainly small lymphocytes, a loss of approximately 70% of collagen immediately subjacent to the junctional epithelium and a pathologic alteration of fibroblasts. A fully developed established lesion was seen within 2 to 4 weeks. The lesion was confined to the gingiva, characterized by a predominance of plasma cells. At advanced stages, plasma cells continue to predominate and resorption of alveolar bone occurs.

Regarding the etiology of periodontal disease, many studies have revealed that bacteria are definite factors causing and maintaining the disease process. Although no single organism has been found to be the responsible agent, there
is strong evidence for a causal relationship between the microorganism and inflammatory periodontal disease.

Løe, Theilade and Jensen (1965) demonstrated withdrawal of all measures of oral hygiene resulted in gingivitis within 15 to 21 days. They concluded plaque was essential in the production of gingival inflammation.

Løe and Holm-Pedersen (1965) demonstrated crevices of normal human gingiva do not exhibit flow of fluid. Inflamed gingiva showed a presence of fluid flow which varied according to the severity of inflammation, began before structural changes could be ascertained clinically and persisted after signs of clinical inflammation were no longer present.

Holm-Pedersen, Agerbaek and Theilade (1975) studied the development of experimental gingivitis in 10 young and 11 elderly subjects. The development of gingivitis was considerably more rapid and severe in elderly individuals (within 7 days) than in younger ones (15 days). Plaque accumulation was greater in the elderly. Differential microscopic counts of gram stained smears of plaque failed to reveal any difference in the development of the complex plaque microflora. The values for the amount of gingival crevicular fluid remained higher in the elderly group once oral hygiene was reinstated, indicating a slower tissue recovery. The observed differences suggested an altered host response to plaque microorganisms.

The presence of bacteria within intact epithelial tissue was not observed in any of 50 gingival and 30 col specimens obtained from 39 patients by Sussman, Bartels and Stahl (1969). However, in areas of ulceration, bacteria were seen within the lamina propria. Since chronic inflammation was present in all tissue specimens without actual evidence of bacterial penetration, they concluded
gingival inflammation may well be the response to bacterial products rather than microbial penetration.

It is generally agreed most forms of periodontal disease are of microbial etiology. The mechanism by which microorganisms induce destruction of the periodontal tissues are unknown.

Two general possibilities exist:

1. Direct initiation of the inflammatory response by injurious microbial metabolites
2. Initiation of periodontal inflammation by antigens of oral organisms setting immunopathologic process into action

The immune system in man can be divided into four classes:
recognition component: lymphocytes
effector component: T & B lymphokines, immunoglobulins
complement system
monocytes, polymorphonuclear leukocytes, basophils, eosinophils

The thymus produces small lymphocytes, T cells, which are necessary for cellular hypersensitivity and cooperative effects in the induction of circulating antibody. B cells are plasma cell precursors, necessary for antibody production. Five major classes of immunoglobulins are recognized in man: IgG, IgA, IgM, IgD and IgE.

Gell, Coombs and Lachmann (1975) suggested a simple classification of allergic or immunopathologic reactions which are capable of inducing tissue damage:

TYPE I — Anaphylactic or Reagin-Dependent Reactions

This reaction is characterized by the production of reagin or
immunoglobulin IgE by plasma cells which fixes to or sensitizes mast cells, 
basophils, or membrane receptors. The reaction between an allergen or antigen with sensitized tissue components leads to the release of pharmacologically active substances causing a smooth muscle contraction, edema due to increased capillary permeability, construction of small venules, platelet aggregation, and mobilization of phagocytes.

TYPE II — Cytotoxic Reactions

These reactions are initiated by immunoglobulin IgG or IgM reacting with cell or tissue antigens. Tissue damage may then occur in the presence of complement or of certain kinds of mononuclear cells.

TYPE III — Antigen-Antibody Complexes or Arthus Reaction

Microprecipitation of antigen and IgG or IgM complexes occurs in and around small blood vessels, in membranes, or in the blood serum. Tissue damage is due to local inflammation or massive complement activation and its sequelae.

TYPE IV — Cell-Mediated or Delayed Hypersensitivity Reactions

This reaction is initiated by the reaction of actively allergized lymphocytes responding to the antigen by the release of lymphokines and/or the development of cytotoxicity.

This pattern seen in any one human disease is often complex, involving not just one but several of the above pathways or responses.
According to Muller-Eberhard (1968):

During the past decade, developments in immunology have led to an increased awareness of the biological importance of antibody. Concomitantly, it has become apparent antibody is biologically ineffective unless aided by effector systems. Complement constitutes the principal immunologically relevant effector system present in blood serum. It consists of nine components or eleven distinct serum proteins. Membranes are the primary target of complement. They may be irreversibly damaged, sustaining distinct ultrastructural lesions, by direct attack which requires participation of all nine complement components, or they may be otherwise affected by interaction with only certain components or split products thereof. Depending on the cell type involved, such noncytolytic reactions of complement may result in histamine release, directed cellular migration, or increased susceptibility to phagocytosis.

The classical mechanism of complement activation involves the interaction of C1 with immunoglobulin aggregates.

The role of antibody in immune cytolysis may be understood in terms of its ability to select specifically the target of complement action. Not all antibodies are able to bind C1. IgA appears to lack the ability to interact with C1. IgM and IgG do not. Only one molecule of IgM is required to bind C1 while two of IgG are necessary to form a potentially cytolytic site.

The following is a schematic of complement activation:

\[
\begin{align*}
\text{AgAb} + \text{Ca}^{++} & \longrightarrow \text{AgAbClq} \\
\text{Clq} & \longrightarrow \text{Clr} \longrightarrow \text{Cls} \\
\text{Cls} + \text{C4} & \longrightarrow \text{C4a} + \text{C4b} \\
\text{Cls} + \text{C2} & \longrightarrow \text{C2a} + \text{C2b} \\
\text{C4b2a} (\text{C3 convertase}) + \text{C3} & \longrightarrow \text{C3a} + \text{C3b} \\
\text{C4b2a3b} (\text{C5 convertase}) + \text{C5} & \longrightarrow \text{C5a} + \text{C5b} \\
\text{C5b} + \text{C6, C7, C8, C9} & \longrightarrow \text{C5b-9}
\end{align*}
\]

:active form
Ward (1972) reviewed the biologic activities of complement. They are listed as follows:

1. Inflammatory mediators
   A. Vascular permeability factors
      - C3a: the anaphylatoxins: contract smooth muscle
      - C5a: direct effect on blood vessels
            and/or release of histamine
            from mast cells
   B. Leukoattractants
      - C3a: attract polymorphonuclear leukocytes, eosinophils,
      - C5a: monocytes

2. Promotion of Phagocytosis — C3, C5

3. Coagulation Modifying Activity
   A. Promotion of blood coagulation — C6
   B. Promotion of clot lysis — C3, C4

4. Viral Neutralization — C1, C4, and C1,4,2,3

5. Cytotoxic Activity — C1-9

6. Inactivation of Bacterial Lipopolysaccharide — C5, C6

In general, gram-negative organisms are susceptible to the bactericidal effects of complement while gram-positive organisms are resistant. This may be due to the greater thickness and lower lipid content of the cell wall.

The alternate pathway of complement activation bypasses the need for antigen-antibody complex activation. C3 is cleaved by a serum protein or a system of proteins known as properdin. Properdin may be activated by
endotoxins and other microbial products. The properidin system is a non-immune alternative mechanism of defense (Anderson and Kissane, 1977).

A variety of antigens have been shown to induce inflammation upon repeated application to the periodontium of several animal species.

Rizzo and Mergenhagen (1960) elicited a local Schwartzman reaction in rabbit oral mucosa and skin with endotoxin prepared by a phenol-water extraction procedure from a strain of oral Veilonella. Extensive polymorphonuclear leukocytic infiltration, leukocytic thrombi and hemorrhage was seen.

Thonard and Dalbow (1965) challenged gingival tissues of conventionally reared rats and guinea pigs with whole sheep erythrocytes. The animals received biweekly injections for 3 weeks in the papilla between the maxillary incisors. Antibody forming cells were found in approximately 50% of the inoculated gingivae. Circulating hemolysins were found in the sera of all rats and over 80% of the guinea pigs. They concluded antibody production associated with indigenous cells can take place in suitably stimulated oral mucosa.

Rizzo and Mitchell (1966) exposed rabbits to protein antigen by triweekly insertion of egg albumin pellets into labial gingival pockets of mandibular incisors. Egg albumin treated rabbits developed serum antibody titers against egg albumin while saline treated control rabbits did not. The gingival lamina propria exhibited a moderately severe chronic inflammatory reaction consisting mainly of plasma cells and lymphocytes. Their findings supported the concept that gingival plasmacytosis of chronic periodontal disease was brought about by local absorption of bacterial antigens. The results indicated
antigen was absorbed into the gingiva and caused both a local and systemic immunologic response.

Dick and Trott (1969) investigated the synergistic action of inflammation and immunity in disease production using 90 albino mice. Inflamed tissues reacted more violently to presence of horse ferritin anti-horse ferritin complexes than do noninflamed tissues. The challenge reaction in noninflamed tissues incited an acute inflammatory lesion while in inflamed tissues it induced an Arthus reaction. An enhanced polymorphonuclear leukocytic response appeared to be related to altered vascular permeability. The traumatic inflammatory lesion appeared to enhance the immune reaction by maintaining a tissue environment characterized by increased vascularity and vascular permeability. The immune reaction was overactivated and tissue injury resulted.

Auer (1920) presented evidence that a mild inflammation can be severely aggravated by a local accumulation of serum antibodies combined with a systemical or topical supply of the corresponding antigen. Brandtzaeg (1973) surmised the initial gingivitis lesion should be an ideal situation for the development of an "Auer phenomenon."

Ranney and Zander (1970) created periodontal lesions in squirrel monkeys via hypersensitivity phenomena. The monkeys were sensitized by subcutaneous injections of ovalbumin emulsified with Freund's complete adjuvant. Challenge procedures were performed in several time sequences by placing ovalbumin soaked thread into gingival crevices. Resulting acute destructive periodontal lesions included chronic inflammation with vascular dilation, infiltration of gingival connective tissue by lymphocytes and plasma
cells, proliferation of crevicular epithelium into the underlying connective tissue and microulceration of pocket epithelium.

The chronic allergic response was shown to be immunologically specific by Ranney (1977). Immunofluorescent techniques revealed cells containing antibody to ovalbumin in the gingival connective tissues of animals challenged 3 times per week for 3 months. The cells appeared to be plasma cells. Such cells were not found in the gingiva from similarly treated unsensitized animals or from sensitized monkeys challenged for a shorter period.

McDougall (1974) found injection of an antigen, peroxidase, into the palate or skin of immunized rabbits reproduced a typical Arthus reaction. Topical application achieved by placing soaked paper points in the sulcus induced a severe, nonspecific inflammation.

In nonimmunized control animals, peroxidase had spread evenly through the intercellular spaces of the epithelium and nearby connective tissue by 15 minutes. By 45 minutes it was no longer detectable.

In immunized animals, peroxidase was found mainly as discrete, coarse clumps in the widened intercellular spaces in the outer half of the crevicular epithelium by 15 minutes. Little gained access to the connective tissue. It appeared antibody reacting with antigen in the intercellular spaces of the crevicular epithelium was largely successful in preventing access of antigen into the connective tissue. Numerous neutrophils migrated into the region of antigen-antibody precipitate reaching a maximum by 4 hours, and then declined. Most of the neutrophils were partially degranulated.

Nisengard, Beutner, Neugeboren, Neiders and Asaro (1977) induced Arthus reactions in the gingiva of 4 monkeys sensitized to bovine serum
albumin or crystalline egg albumin. Challenge by placing antigen soaked threads into the gingival sulcus or by gingival injection of the sensitizing antigen elicited inflammatory infiltrates composed largely of mononuclear cells: plasma cells, lymphocytes and foamy macrophages. Increased deposits of IgG and C4 were observed particularly in periovascular locations. No IgM and only trace deposits of IgA were observed. Injections of heterologous antigens and other control injections failed to induce such changes.

Brown, Gargiulo, Toto and Suzuki (1978) applied an Actinomyces viscosus homogenate to the labial sulcus of New Zealand rabbits for 30 days. Clinical observations of chronic periodontal inflammation were supported by hematoxylin-eosin stained sections showing infiltration of lymphocytes, plasma cells and polymorphonuclear leukocytes. Direct immunofluorescence techniques demonstrated the presence of local and systemic immunoglobulins in the lamina propria and epithelium. Electron micrographs indicated discontinuities in the epithelial basal lamina. Evidence of antibody presence implied the penetration of antigen through the epithelium. Initial penetration of antigen was facilitated by mild inflammation from mechanical irritation of sulcular epithelium. Penetration of antigen lead to an increased density of inflammatory cells in the connective tissue and to structural alterations similar to those developing in gingival tissues in response to plaque accumulation. In vitro measurements of complement chemotaxis of polymorphonuclear leukocytes generated by the alternate pathway was equivalent to that generated by the classic and alternate pathways combined. Thus, endotoxin may activate the alternate pathway of complement activation, which in turn could mediate an inflammatory response and precede classic complement
activation of an immune response.

Fagraeus (1948) demonstrated the immature plasma cell not yet fully morphodifferentiated was the cell most active in the synthesis of immunoglobulins. An increase in the number of these cells coincided with steepest use in antibody production after intravenous injection of antigen of sensitized animals.

The development of a transitional cell into mature plasma cell showed the following characteristics:

1. diminution in the volume of the cell and a change in its shape from round to oval
2. the nucleus diminished, grew oval, increased in stainability and assumed an eccentric position
3. the nucleoli, big in immature elements, decreased in size
4. cytoplasm increased in volume and stainability, became more accentuated.

In case of an intense antibody formation, a differentiation of these cells into plasma cells took place. The mature plasma cell was regarded as the final link in a chain of development and a cell which has already passed the stage of greatest functional intensity.

Leduc, Avrameas and Bouteille (1968) used electron microscopy to localize antibody within differentiating and mature plasma cells in the spleens of hyperimmunized rabbits.

Antibody was localized by electron microscopy within differentiating and mature plasma cells in the spleens of hyperimmunized rabbits. Horseradish peroxidase was used as the antigen. Intracellular antibody to peroxidase was revealed in glutaraldehyde-fixed tissue by coupling it with its antigen and then
revealing the sites of peroxidase activity cytochemically.

Antibody first appeared in the perinuclear space of hemocytoblasts where it persisted through differentiation into immature plasma cells, but disappeared from this site in mature plasma cells. Antibody was present in the lamellar portion of the Golgi apparatus in all phases of plasmacytic differentiations. Mature plasma cells exhibited two types of antibody distribution: a concentration into large spherical intracisternal granules or an overflowing into all parts of the cytoplasm.

Many investigators have used immunohistochemical techniques in studying human inflammatory periodontal disease.

Brandtzaeg and Krause (1965) exposed sections of alcohol-fixed gingival biopsies to specific labeled antisera. The specimens were obtained from 8 subjects with a clinically healthy periodontium and 21 subjects with varying degrees of periodontal inflammation.

Using the direct immunofluorescence technique, the most striking difference demonstrated between clinically healthy and inflamed gingiva was the frequent marked increase in the number of plasma cells containing IgA in diseased tissues. An intense fluorescence was noted in severely inflamed specimens. Fibrous tissue, which fluoresced the weakest of all tissue components, was reduced. The number of blood vessels, which stained brightly, was increased. Intercellular staining was more common in acanthotic epithelium. The staining increased towards the epithelial surface and appeared to be associated with an increase in cellular separation. A band of staining occurred beneath the parakeratotic layer indicating a decrease of permeability.

Brandtzaeg and Krause postulated mucosal transfer of plasma proteins probably was a physiologic phenomenon, not brought about by pathologic
Plasma proteins may be carried to the oral surface by edema fluid. The staining pattern indicated transfer from the connective tissue to the epithelium could take place only in a few circumscribed areas. The parakeratotic or keratinized layer rarely exhibited cytoplasmic staining for plasma proteins, except in parakeratotic layers in severe inflammation. Cytoplasmic staining was due to antigen-antibody reactions. A transformation or exhaustion of proteins in the process of keratinization could account for the lack of cytoplasmic staining. IgA and IgG, with glycoproteins, may coat cell membranes contributing to cellular and epithelial adhesiveness.

Immunofluorescence tracings by Brandtzaeg (1966) indicate IgG and IgA permeate the connective tissue ground substance and further diffuse through both the oral epithelium and pocket epithelium of the gingiva. They pass the epithelium by with an intra and intercellular route, especially intercellular when inflammation is marked. The extravascular distribution of serum proteins is highly dependent upon concentration and molecular size: the majority of IgM (70%) is located in the intravascular space while the majority of IgG and IgA (52%-58%) is distributed extravascularly. Immunofluorescence revealed large concentrations of IgG, much less IgA and very little IgM in the gingival connective tissue.

Schneider, Toto, Gargiulo and Pollack (1966) demonstrated a local defense mechanism of the gingival tissue to bacteria present in the adjacent sulcus or pocket by identifying antigen-antibody reactions with fluorescent antibodies. Gingival biopsies and bacterial samples were taken from 18 adult males whose periodontal conditions ranged from marginal gingivitis to periodontitis. Frozen sections were reacted with specific fluorescein conjugated antibody to show the presence of globulins in the tissue. Bacteria, stained with
a contrasting fluorescent dye, were incubated with serial sections and the sections were reacted with fluorescein labeled antibody. An attraction of bacteria to the gingival tissue was always shown in areas of globulin concentration: perivascular connective tissue, collagenous fiber bundles, basement membrane, intercellular spaces in the epithelium, and within the cytoplasm of plasma cells.

Brandtzaeg (1965) showed IgG, IgA, IgM, albumin and fibrinogen were present in gingival pocket fluid by immunoelectrophoresis in preparations and concentrations comparable to plasma. IgG, IgA, IgM were present in a ratio of 12:4:1. Gingival pocket fluid, serum, and saliva were collected from 4 subjects exhibiting various periodontal conditions. Only IgG, IgA and albumin were detected in whole saliva and their concentrations were lower than in serum. The ratio of IgG, IgA was 1:1 as compared to 8:1 in serum and gingival fluid. He suggested plasma was the principal source of the five proteins detected in gingival tissue and pocket fluid. However, IgA and IgG may originate in part from plasma cells in the gingiva.

Results of a study by Nisengard and Beutner (1970) showed sera of 9 humans had IgG directed against bacteria from the gingival sulcus. The sera of 70 humans had antibodies to Actinomyces. Many individuals from this group were allergic or hypersensitive to Actinomyces. The levels of antibody and percentage of allergic individuals increased with the severity of gingival inflammation.

Organisms are present in periodontal plaque in extremely high concentrations, approximately $10^{11}$ organisms per mg in direct contact with the tissues. In spite of this, they do not seem to invade the tissues to any appreciable degree. Rizzo (1970) found topically applied antigens did not seem
to be capable of penetrating an intact gingival sulcus barrier.

Simon, Goldman, Ruben and Baker (1970) showed the quantity of endotoxin found in the gingival exudate of 39 patients was correlated with the degree of clinical inflammation. The correlation exceeded the 1% level of confidence. Gram-negative organisms are capable of liberating endotoxin upon their destruction. The authors related their results to those of Loe, Theilade, and Jensen (1965) and Theilade, Wright, Jensen and Loe (1966). Clinical signs of gingival inflammation became manifest just after a shift from a predominance of gram-positive flora to a gram-negative one in bacterial plaque.

Toto, Gargiulo and Kwan (1970) conducted an immunofluorescence study of 25 biopsy specimens of chronic gingivitis. The intact epithelium contained intercellular and intracytoplasmic immunoglobulin. The epithelium could serve in defense against antigenic substances found in the gingiva.

Platt, Crosby, and Dalbow (1970) investigated gingival tissues from patients with varying stages of periodontal disease. An immunofluorescence study revealed all immunoglobulin components were present. Their relative concentrations appeared to be related to the stage of pathology. IgM producing plasma cells were predominant in acute gingivitis, though cells with IgG were abundant. Sections from severe periodontitis contained numerous IgM cells, many IgA cells and few IgG cells.

Fluorescing endothelial cells of blood vessels were seen. Intense fluorescence of residual plaque adhering to gingiva indicated immunoglobulins combined with bacteria in the gingival region.

Berglund (1971) demonstrated immune complexes were formed by the reaction of gingival immunoglobulins obtained from thin slices of gingival
tissues and from spent media of gingival organ cultures with antigens from microorganisms of dental plaque (*Fusobacterium*, *Veillonella*, *Escherichia coli*). Immunoglobulins from diseased human gingiva apparently originated from cells in the inflamed tissue as well as from serum since larger amounts of antibody activity were observed over some gingival specimens than was expected from the magnitude of serum titers.

Indirect immunofluorescence staining by Nisengard, Beutner and Garto (1971) revealed cells containing IgE in inflamed human gingival connective tissue. Morphologically the mononuclear cells resembled plasma cells. The number of IgE containing cells appeared to be related to the severity of inflammation. Microorganisms from subgingival debris but not supragingival debris were coated with IgE.

Mayron and Loiselle (1973) treated tissues from patients with periodontal disease with fluorescein conjugated *Streptococcus mitis*, *Streptococcus salivarius*, and *Neisseria catarrhalis*. Microscopic examination revealed the presence of fluorescence primarily in the epithelium, demonstrating the presence of antibodies to these microorganisms in the tissue. The opposite technique of using rabbit antiserum resulted in visualization of areas containing bacterial antigens. Antigen was located in the subkeratin layers of the epithelium.

Brandtzaeg (1973) considered the number of activated B cells (plasma cells) in inflammatory periodontal lesions is impressive in view of the great dominance of T cells in the pool of peripheral blood lymphocytes (90% T cells). He stated infiltrates of established periodontitis lesions in man are dominated by IgG cells. IgG has great potency for complement and activation. Consequences of the immune response may be determined by a balance in the
local synthesis of IgG and IgA antibodies. A shift in favor of complement fixing
IgG over low complement fixing IgA may disturb immune homeostasis with
aggravation and perpetuation of inflammatory process.

Using gel diffusion techniques, Genco, Mashimo, Krygiea and Ellison
(1974) found a large percentage of human sera contain precipitating antibodies
directed to antigens found in extracts of sonicated *Leptotrichia buccalis*.

Treating flame-fixed *L. buccalis* smears with series of dilutions of subjects’
sera and fluorescein labeled goat antihuman IgG, IgM, IgA or IgE showed anti-
leptotrichial antibodies of either IgG and/or IgM were detected in all of the
adult sera tested.

The levels of IgG and IgM antibodies to *L. buccalis* in 5 edentulous adults
were comparable to those found in 14 dentulous adults, suggesting persistence
of *L. buccalis* at the gingival sulcus is not necessary for maintenance of adult
levels of antibodies.

Immunofluorescent localization studies carried out on human gingiva
showed IgG, IgM, C3, albumin and transferrin in the connective tissues and in
tracts through the sulcular epithelium.

Marttala, Toto and Gargiulo (1974) demonstrated antibodies to specific
bacteria were extractable locally in the gingiva. Three pedigree strains of
lyophilized *Actinomyces (israelii, naeslundii* and *viscosus*) and one of
*Streptococcus mutans* were prepared as smears on slides and reacted with
gingival tissue extracts obtained from 12 patients with chronic periodontitis.
The treated smears were reacted with goat antihuman antiserum to IgG, IgA,
and IgM, conjugated to fluorescein isothiocyanate. Fluorescence was
predominantly positive to all test organisms in all subjects.

Byers, Toto and Gargiulo (1975) assayed resected inflamed gingival tissue
obtained from 16 periodontal patients and a pooled sample of noninflamed gingiva from 5 patients using low-level diffusion plates. Marked increases in levels of IgG, IgA, and IgM were seen in inflamed tissues. These were attributed to the greater antigenic stimulus present in gingival pockets. The IgA/IgG and IgM/IgG ratios in inflamed gingiva were substantially different than those in normal serum. This supported the concept of local antibody production in inflamed gingiva.

The ratio of IgA/IgG was similar in inflamed and normal gingivae. This may be the result of the body's homeostatic mechanism regulating levels of blood and tissue immunoglobulins. IgM was present in 6 of 16 inflamed specimens. IgM may not be a consistent feature of chronically inflamed gingiva.

Attström, Laurel, Lahsson and Sjöholm (1975) conducted an electroimmunoassay of supernatants from gingival crevicular material. Higher concentrations of C3 and C4 were demonstrated in samples from chronically inflamed gingiva when compared to those from healthy gingiva. The concentration of C4 in samples from inflamed gingiva was significantly lower when related to plasma levels. This might indicate this factor had been consumed or was present in an altered form. C5 could only be demonstrated in material from inflamed gingiva. C3 proactivator was present in material from inflamed gingiva in the converted form. Analysis of C3 in samples from inflamed gingiva using crossed immunoelectrophoresis showed C3 was converted in the samples. This indicates complement system may be activated in gingival crevice material from inflamed gingiva.

Immunofluorescent studies conducted by Nisengard and Jarrett (1976) revealed 2 of 3 patients with gingivitis and all patients with periodontitis (20)
had crevicular bacteria that were coated in vivo with immunoglobulins IgG, IgA, IgM and IgE and complement factor C3.

Courts, Boackle, Fundenberg and Silverman (1977) collected crevicular fluid from patients with periodontitis and tested it for the presence of a functional complement system. Functional C1 and whole complement activities were rapidly inactivated by dental plaque. Results suggested complement was responsible for the hemolytic activity of gingival crevicular fluid.

Taichman, Tsai, Bachni, Stoller and McArthur (1977) demonstrated human peripheral blood polymorphonuclear leukocytes actively released lysosomal constituents upon in vitro exposure to either viable or irradiated, supragingival or subgingival dental plaque. Fresh sera amplified the release reactions. Modulation of polymorphonuclear leukocyte release may be modified by complement components and/or antibodies to plaque bacteria. Complement factor C5a can trigger lysosome release while antibodies facilitate phagocytosis by opsonization.

Schenkein and Genco (1977) determined concentrations of selected proteins of gingival crevicular fluid and serum by the single radial immunodiffusion method. Gingival crevicular fluid from severely inflamed periodontal tissue represented a 15% to 30% dilution of serum IgG, IgA, IgM. IgG showed a tendency towards higher gingival crevicular fluid concentration than IgA and IgM. They felt this was due in part to the production of IgG by 70% to 80% of plasma cells of chronically inflamed gingiva.

A marked decrease in C3 levels was found in most gingival crevicular fluid and a marked decrease of C4 levels was found in some fluids. This suggested complement may be activated during periodontal inflammation. C3 activation
products C3c and C3d indicated C3 had been degraded to its final products. Possible mechanisms suggested for its degradation were: antigen-antibody reaction promoting complement activation via classical pathway, nonspecific direct activation via the alternate pathway, proteolysis due to enzymes in the gingival sulcus. Quantitatively, C3d levels were comparable to levels attained consequent to immune complex and tryptic activation of human serum.

The alternate pathway of complement activation occurred in the periodontal pocket as demonstrated by conversion of C3 proactivator (factor B) to C3 activator Bb.

Mackler, Frostad, Robertson and Levy (1977) thought characterization of all associated immunoglobulins was important since investigations suggest lymphocytic infiltrates may be nonspecifically activated by immune complexes as well as specifically stimulated by bacterial antigens.

Altman, Chassy and Mackler (1975) found lymphocytes activated by immune complex binding via membrane complement (C3) receptors or Fc receptors with affinity for the Fc part of IgG produced pharmacologically active lymphokines equivalent to those induced by antigenic stimulation.

Mackler, Frostad, Robertson and Levy (1977) subjected gingival specimens from patients with varying degrees of periodontal disease to hematoxylin-eosin and immunofluorescence staining. Normal gingiva contained few lymphocytes and plasma cells. 94% of cellular infiltrate present at the sulcular epithelium-lamina propria junction of mild gingivitis were lymphocytes lacking membrane-associated immunoglobulins. Few plasma cells were seen. Tissues associated with periodontitis contained significant numbers of immunoglobulin bearing lymphocytes. Their distribution was 78% IgG, 9% IgM and 4% IgA. The plasma cells seen in these tissues had the
following distribution: 67% IgG, 24% IgM and 8% IgA. Distinctly different cellular infiltrates were seen associated with the two stages of inflammatory periodontal disease.

Toto, Lin and Gargiulo (1978) demonstrated the presence of immunoglobulin and C3 by means of the direct immunofluorescence technique in sections of frozen gingival specimens obtained from 20 patients with gingivitis and chronic periodontitis. The presence of IgG was seen in all cases, IgM in 10 and C3 in 7. Fluorescence was localized mainly in the lamina propria. Examination of the basement membrane at high magnification showed an irregular, diffuse fluorescence in all positively reacting sections. They concluded the presence of IgG, IgM and C3 suggested an antigen-antibody response binding and activating complement. Similar results were obtained by Suzuki, Gargiulo and Toto (1979).
CHAPTER III

METHODS AND MATERIALS

This retrospective study was conducted using formalin-fixed, paraffin-embedded tissue blocks of inflammatory papillary hyperplasia. These were selected from biopsy specimens received in the Department of Oral Pathology at Loyola University School of Dentistry. Hematoxylin and eosin stained sections were examined under the light microscope to confirm the diagnosis. A total of 36 tissue specimens of inflammatory papillary hyperplasia were used.

Hematoxylin and eosin stained sections were studied under the light microscope and scored for the presence or absence of the following parameters:

Epithelium:

- Keratinization
- Parakeratosis
- Hyperkeratosis
- Dyskeratosis
- Atrophy
- Vertical projections composed of stratified squamous etiology and central core of connective tissue
- Elongated rete pegs
- Flattened rete pegs
- Blunted or fused rete pegs
- Infiltration by polymorphonuclear leukocytes and lymphocytes
- Intercellular edema
- Acanthosis
- Ulceration
  - Surface etiology
  - In the depth of fissures
- Hydropic degeneration
- Vesiculation

52
Connective tissue:
  Infiltration by lymphocytes and plasma cells
  Lamina propria
  Deep connective tissue
  Edema
  Dilated capillaries
  Perivascular plasma cells
  Loss of collagen fibers

Sections from the tissue blocks were subjected to histochemical techniques similar to those described by Taylor and Burns (1974). They selectively stained for immunoglobulin IgG and C3 of the complement system.

3 to 4 micron sections were dehydrated in xylol and rehydrated through graded ethyl alcohols (100%, 95%, 75%) to distilled water.

The sections were placed in an alpha-naphthol solution for 5 minutes to block endogenous peroxidase activity to prevent staining when the antibody-conjugated peroxidase was subsequently developed. The alpha-naphthol solution was a combination of 100 ml of a solution containing 40 ml ethyl alcohol, 60 ml distilled water and 1 gm alpha-naphthol, and 0.2 ml of a solution containing 1 ml 30% hydrogen peroxide and 9 ml distilled water.

The sections were washed in running tap water for 15 minutes and placed in pyronin for 1 minute to lightly stain endogenous peroxidase. The pink pyronin stain was developed by combining 96 ml 40% ethyl alcohol, 0.1 gm pyronin and 4 ml aniline.

This procedure was followed by a running water wash and placing the sections in 3 changes of 0.01M phosphate buffered saline, pH 7.4-7.6 for 5 to 10 minutes. The phosphate buffered saline solution was made of 84.1 ml of 1.4 gm dibasic sodium phosphate (Na$_2$HPO$_4$) in 100 ml distilled water, 15.9 ml of 1.4 gm monobasic sodium phosphate (NaH$_2$PO$_4$H$_2$O) in 100 ml distilled
water, 8.5 gm sodium chloride and the addition of distilled water to 1000 ml.

Sections were then flooded with a 1:10 dilution of normal swine serum (Cappel Laboratories Inc., Cochranville, Pennsylvania) for 30 minutes. The swine serum nonspecifically binds and reduces extraneous immunoglobulin, further minimizing background staining.

Tissue sections were then washed in 3 changes of phosphate buffered saline, pH 7.4-7.6, 5 to 10 minutes each, to wash away excess swine serum and maintain a pH for maximum activation of horseradish peroxidase.

Sections were treated 60 minutes with either goat antihuman IgG conjugated with horseradish peroxidase or goat antihuman complement C3 conjugated with horseradish peroxidase. Both preparations were at a dilution of 1:10. The preparations were obtained from Cappel Laboratories Inc., Cochranville, Pennsylvania.

Following a thorough washing in phosphate buffered saline, pH 7.4-7.6, 3 changes of 5 to 10 minutes each, the tissue-bound peroxidase conjugated antibody was stained with a saturated solution of diaminobenzidine (5 mg of 3, 3' diaminobenzidine 4HCl in 10 ml phosphate buffered saline pH 7.4-7.6) to which was added 0.2 ml of a dilute solution of hydrogen peroxide (a 1:50 dilution of 30% H₂O₂ in phosphate buffered saline pH 7.4-7.6) for 1 to 3 minutes.

The sections were washed in a tap water rinse followed by 70% ethyl alcohol and counterstained 5 minutes with a 1% buffered methyl green solution pH 4.0. Originally only Mayer’s hematoxylin was used as a counterstain for the first series of 10 tissue specimens subjected to the above technique. However, better contrast was achieved by substituting methyl green, a nuclear (DNA)
stain.

Following the counterstain, the tissues were dehydrated through graded alcohols (70%, 95%, 100%), cleared with xylene and permanently mounted in Permount, a synthetic resin.

Sections were studied under the light microscope for the distribution of IgG and C3 deposits. The presence or absence of immunoglobulin IgG was noted in the epithelium — intercellularly

- as a band beneath surface layers
- on the basement membrane

and in the connective tissue —

- in plasma cells
- perivascularly

C3 deposits were scored in a similar manner with the exception of plasma cells. Deposits were generally noted around the periphery of plasma cells.

Eleven specimens of gingivitis were collected during periodontal surgery in the Department of Graduate Periodontics at Loyola University School of Dentistry. Ten specimens of clinically noninflamed attached gingiva were obtained during oral surgical procedures from the Department of Oral Surgery. Hematoxylin-eosin stained sections were scored on the following morphology:

**Epithelium:**

- Keratinization
- Parakeratosis
- Elongated rete pegs
- Infiltration by polymorphonuclear leukocytes and lymphocytes
- Intercellular edema
Connective tissue:

Infiltration by lymphocytes and plasma cells
   Lamina propria
   Deep connective tissue

Edema
Dilated capillaries
Perivascular plasma cells
Loss of collagen fibers

Sections from the tissue specimens were subjected to the immunohistochemical staining procedure described above. Light microscope observations were scored in the same manner as sections from inflammatory papillary hyperplasia.

In order to conduct a positive control of the immunohistochemical staining, sections of a tissue specimen of lupus erythematosus kidney were treated.
<table>
<thead>
<tr>
<th>Procedure</th>
<th>Steps</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>3 - 4 micron paraffin sections, xylol - alcohols - water</td>
</tr>
<tr>
<td>2.</td>
<td>alpha naphthol</td>
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<tr>
<td>3.</td>
<td>wash in running water</td>
</tr>
<tr>
<td>4.</td>
<td>pyronin</td>
</tr>
<tr>
<td>5.</td>
<td>running water</td>
</tr>
<tr>
<td>6.</td>
<td>phosphate buffered saline, pH 7.4-7.6, 3 changes</td>
</tr>
<tr>
<td>7.</td>
<td>normal swine serum 1:10 dilution</td>
</tr>
<tr>
<td>8.</td>
<td>phosphate buffered saline, pH 7.4-7.6, 3 changes</td>
</tr>
<tr>
<td>9.</td>
<td>goat antihuman IgG peroxidase conjugated, 1:10 dilution</td>
</tr>
<tr>
<td>or</td>
<td>goat antihuman C3 peroxidase conjugated, 1:10 dilution</td>
</tr>
<tr>
<td>10.</td>
<td>phosphate buffered saline, pH 7.4-7.6, 3 changes</td>
</tr>
<tr>
<td>11.</td>
<td>diaminobenzidine with $\text{H}_2\text{O}_2$</td>
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<tr>
<td>12.</td>
<td>tap water</td>
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<tr>
<td>13.</td>
<td>1% methyl green (as counterstain)</td>
</tr>
<tr>
<td>or</td>
<td>Mayer's hematoxylin (as counterstain)</td>
</tr>
<tr>
<td>(wash in tap water 10 minutes following hematoxylin stain)</td>
<td>5 minutes</td>
</tr>
<tr>
<td>14.</td>
<td>tap water</td>
</tr>
<tr>
<td>15.</td>
<td>dehydrate through graded alcohols to 100%</td>
</tr>
<tr>
<td>16.</td>
<td>clear with xylene</td>
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<tr>
<td>17.</td>
<td>mount with Permount</td>
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</table>

TABLE I
SUMMARY of METHOD for DEMONSTRATION of IMMUNOGLOBULIN IgG or COMPLEMENT FACTOR C3
in FORMALIN-FIXED, PARAFFIN-EMBEDDED TISSUES
CHAPTER IV

RESULTS

A. HEMATOXYLIN and EOSIN STAINED SECTIONS

The biopsy specimens of inflammatory papillary hyperplasia included in this retrospective study were received by the Department of Oral Pathology from 1966 to 1979. Nineteen were taken from female patients 29 to 60 years of age. Seventeen were taken from male patients ranging in age from 36 to 70. All specimens were from the hard palate.

All sections were examined using a light microscope and 40x-100x-450x magnifications. In all, 36 microscope slides with several serial sections from tissue specimens of inflammatory papillary hyperplasia were examined.

The epithelium of tissue specimens from inflammatory papillary hyperplasia was characterized by vertical projections composed of stratified squamous epithelium and a central core of connective tissue. In only one case was the epithelial surface relatively flat.

All sections examined exhibited a parakeratotic surface layer. 34 of 36 cases had elongated rete pegs. The morphology of the rete pegs varied: Some elongated rete pegs were fused and bifid, having a bulbous appearance. Others were fused and blunted. Many appeared single and elongated, especially at the bottom of crevices between papilliferous projections, hereafter referred to as papillae.

Atrophy, a reduction in the number of epithelial layers, was present in 7 of 36 cases. Acanthosis was seen in approximately one-third of the sections (13 of 36
cases). No evidence of keratinization, hyperkeratosis or dyskeratosis was found. Hydropic degeneration and vesiculation of epithelial cells was seen rarely, 2 in 36 cases. Ulceration was evident in 50% of the cases. Ulceration in the depth of the fissure or along the crevices was seen slightly more often than ulcerations along the surface of the papillae. Intercellular epithelial infiltration by polymorphonuclear leukocytes and mononuclear cells was observed in all cases. Edema, judged by widening of intercellular spaces, was noted in 26 of 36 cases.

Eleven biopsy specimens of clinically inflamed gingiva were collected during periodontal surgery in October to November 1979 in the Department of Graduate Periodontics. All patients had received preoperative scaling and curettage. The 5 female patients ranged in age from 25 to 53 while 6 male patients ranged in age from 13 to 54.

Eleven slides with several serial sections of gingivitis were examined. A light microscope with 40x - 100x - 450x magnification was used.

The oral epithelial surfaces were examined. Sulcular epithelium was not included in this part of the study. 7 cases exhibited a keratinized surface while 4 had a parakeratotic surface layer. The epithelium showed hyperplasia characterized by long epithelial ridges extending deep into the lamina propria in all cases.

The epithelium was accompanied by varying degrees of intercellular edema. Edema was noted in 5 of 11 cases. All sections showed an intercellular epithelial infiltration by polymorphonuclear leukocytes and mononuclear cells.

The connective tissue was generally edematous in 8 of 11 cases. All cases
showed a chronic inflammatory cell infiltrate which varied in location. 10 cases revealed foci of lymphocytes and plasma cells in the deeper submucosa while only 5 cases showed infiltration in the subepithelial lamina propria. Dilated blood vessels were seen in most specimens (10 of 11). Less than half, 5 of 11, had a loss of collagen fiber bundles.

Ten biopsy specimens from clinically noninflamed healthy appearing masticatory mucosa were collected during oral surgery procedures from October 1979 to January 1980. 3 specimens were taken during alveoplasty procedures while seven were distal wedges or tuberosity reductions performed after molar extractions. 9 female patients and 1 male patient ranged in age from 21 to 31 years.

Ten slides with several serial sections each were examined under 40x - 100x - 450x magnifications using a light microscope.

Ninety percent of the sections had a keratinized epithelial surface. Parakeratosis was evident in 1 case, or 10%. Elongated rete pegs were seen in 4 specimens. While no sections had evidence of intercellular edema, all showed intercellular infiltration by polymorphonuclear leukocytes and mononuclear cells.

Only 6 of 10 specimens had evidence of an inflammatory cell infiltrate. The foci of lymphocytes and plasma cells were located in the deeper connective tissue. No evidence of dilated blood vessels, edema, or loss of collagen fibers was found.

Table II summarizes the morphology observed in hematoxylin and eosin stained sections.
<table>
<thead>
<tr>
<th></th>
<th>Inflammatory</th>
<th>Papillary Hyperplasia</th>
<th>Gingivitis</th>
<th>Control</th>
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</thead>
<tbody>
<tr>
<td><strong>EPITHELIUM</strong></td>
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<tr>
<td>1. Keratinized</td>
<td>0</td>
<td></td>
<td>63.6</td>
<td>90</td>
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<tr>
<td>2. Parakeratosis</td>
<td>100</td>
<td></td>
<td>36.4</td>
<td>10</td>
</tr>
<tr>
<td>3. Hyperkeratosis</td>
<td>0</td>
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<tr>
<td>4. Dyskeratosis</td>
<td>0</td>
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<tr>
<td>5. Atrophy</td>
<td>19.4</td>
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<tr>
<td>6. Vertical projections composed of</td>
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<tr>
<td>stratified squamous epithelium and</td>
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<tr>
<td>central core of</td>
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<tr>
<td>connective tissue</td>
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<tr>
<td>7. Elongated rete pegs</td>
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<td>8. Flattened rete pegs</td>
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<tr>
<td>9. Blunted or fused rete pegs</td>
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<tr>
<td>10. Infiltrated by polymorphonuclear</td>
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<tr>
<td>leukocytes and lymphocytes</td>
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<tr>
<td>11. Intercellular edema</td>
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<tr>
<td>12. Acanthosis</td>
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<tr>
<td>13. Ulceration</td>
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<tr>
<td>Surface epithelium</td>
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<tr>
<td>In depth of fissures</td>
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<td></td>
</tr>
<tr>
<td>14. Hydropic degeneration</td>
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<tr>
<td>15. Vesiculation</td>
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<tr>
<td><strong>CONNECTIVE TISSUE</strong></td>
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<tr>
<td>1. Infiltrated by lymphocytes and plasma cells</td>
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</tr>
<tr>
<td>Lamina propria</td>
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<tr>
<td>Deep connective tissue</td>
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<tr>
<td>2. Edema</td>
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<td></td>
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<tr>
<td>Dilated capillaries</td>
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<tr>
<td>4. Perivascular plasma cells</td>
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<tr>
<td>5. Loss of collagen fibers</td>
<td></td>
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</tbody>
</table>
B. SECTIONS TREATED WITH ANTI-HUMAN IgG
HORSERADISH PEROXIDASE CONJUGATE

Table III summarizes the results of examining sections of inflammatory papillary hyperplasia, gingivitis and control tissues treated to localize the distribution of immunoglobulin IgG. All sections were examined under oil immersion, 1000x magnification, using the light microscope after scanning with lower power objectives.

By assigning a value of 1 to a positive identification of immunoglobulin IgG at a certain site and a value of 0 when no evidence was found, a chi-square analysis of the three groups and five tissue sites was done. Since some groups contained less than 21 specimens, a Fisher's corrected chi-square was used. The statistical data are presented in Tables V and VI.

Similar results were found between inflammatory papillary hyperplasia and inflammatory periodontal disease, i.e. there was no statistically significant difference was found in the distribution of immunoglobulin IgG deposits in the epithelium between inflammatory papillary hyperplasia, gingivitis and control tissues. Since all specimens demonstrated the presence of immunoglobulin IgG intercellularly, no chi-square values could be computed. Though a variable number of specimens in each group had a localization of immunoglobulin IgG as a band underneath the surface layer and along the basement membrane, no statistically significant difference at the .05 level of confidence could be demonstrated.

A statistically significant difference was evident in the connective tissues
between diseased and control tissues. A difference at the .01 level of confidence was demonstrated in the number of specimens infiltrated with plasma cells containing immunoglobulin IgG between control and diseased tissues. Perivascular deposition of immunoglobulin IgG was statistically different at the .05 level of confidence between gingivitis and control tissues. No statistically significant difference was found in the distribution of immunoglobulin deposits between inflammatory papillary hyperplasia and gingivitis.

Treatment of sections of tissue from a kidney with systemic lupus erythematosus served as a control of the method. Immunoglobulin IgG deposits were demonstrated particularly between the endothelium and glomerular basement membrane and in the mesangium.
### TABLE III

**DISTRIBUTION OF IgG DEPOSITS**

Number of Positive Specimens

<table>
<thead>
<tr>
<th></th>
<th>Inflammatory Papillary Hyperplasia (36)</th>
<th>Gingivitis (11)</th>
<th>Control (10)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EPITHELIUM</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercellularly</td>
<td>36</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Band beneath surface layer</td>
<td>19</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Basement membrane</td>
<td>31</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td><strong>CONNECTIVE TISSUE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma cells</td>
<td>35</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>Perivascularly</td>
<td>28</td>
<td>11</td>
<td>5</td>
</tr>
</tbody>
</table>
C. SECTIONS TREATED WITH ANTI-HUMAN COMPLEMENT C3 HORSERADISH PEROXIDASE CONJUGATE

Table IV summarizes the observations of the presence or absence of complement factor C3 deposits at certain sites in sections of inflammatory papillary hyperplasia, gingivitis and control tissues. All sections were examined under oil immersion, 1000x magnification, using the light microscope after scanning the slide with lower power objectives.

A chi-square analysis of the three groups, comparing the distribution of complement factor C3 deposits, was done. The statistical data are presented in Tables V and VI.

No significant difference was found in the distribution of complement factor C3 intercellularly or as a band underneath the surface layers of the epithelium between the three groups. All specimens of inflammatory papillary hyperplasia, gingivitis and control tissues demonstrated the presence of complement factor C3 on the basement membrane was statistically different at the .05 level of confidence between inflammatory papillary hyperplasia and control tissues.

A difference at the .01 level of confidence was demonstrated between diseased and control tissues in relation to perivascular deposition and distribution on plasma cells. No difference was found between inflammatory papillary hyperplasia and gingivitis.

Treatment of sections of tissue from a kidney involved by systemic lupus erythematosus served as a positive control of the method. Complement factor
C3 deposits were observed particularly between the endothelium and glomerular basement membrane and in the mesangium. The staining of complement factor C3 was heavier than that of immunoglobulin IgG.

The distribution of complement factor C3 deposits in relation to plasma cells appeared to vary. Often the deposits were localized around the periphery of these cells. At times, the deposits appeared to be deposited on the surface of the cell.
TABLE IV

DISTRIBUTION OF C3 DEPOSITS

<table>
<thead>
<tr>
<th></th>
<th>Inflammatory Papillary Hyperplasia(36)</th>
<th>Gingivitis(11)</th>
<th>Control(10)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EPITHELIUM</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercellularly</td>
<td>36</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Band beneath surface layer</td>
<td>11</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Basement membrane</td>
<td>30</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td><strong>CONNECTIVE TISSUE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma cells</td>
<td>33</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Perivascularly</td>
<td>30</td>
<td>10</td>
<td>1</td>
</tr>
</tbody>
</table>
## TABLE V

**CHI-SQUARE ANALYSIS OF DATE**

**COMPARISON OF INFLAMMATORY PAPILLARY HYPERPLASIA GINGIVITIS AND CONTROL SPECIMENS**

\[ df = 2 \]

* significant at .05
** significant at .01

<table>
<thead>
<tr>
<th></th>
<th>IgG</th>
<th>C3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EPITHELium</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercellular</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Band beneath surface layer</td>
<td>0.16</td>
<td>2.71</td>
</tr>
<tr>
<td>Basement membrane</td>
<td>3.44</td>
<td>7.74*</td>
</tr>
<tr>
<td><strong>CONNECTIVE TISSUE</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma cells</td>
<td>31.54**</td>
<td>37.25**</td>
</tr>
<tr>
<td>Perivascular</td>
<td>7.46*</td>
<td>23.28**</td>
</tr>
</tbody>
</table>
**TABLE VI**

**ANALYSIS OF DATA BY FISHER’S CORRECTED CHI-SQUARE**

\( \text{df} = 1 \)

* significant at .05

** significant at .01

<table>
<thead>
<tr>
<th></th>
<th>Inflammatory Papillary Hyperplasia/Control</th>
<th>Inflammatory Papillary Hyperplasia/Gingivitis</th>
<th>Gingivitis/Control</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IgG</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Intercellularly</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Band beneath</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface layer</td>
<td>0.002</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Basement membrane</td>
<td>1.934</td>
<td>0</td>
<td>0.387</td>
</tr>
<tr>
<td>Plasma cells</td>
<td>20.160 **</td>
<td>0</td>
<td>8.615 **</td>
</tr>
<tr>
<td>Perivascular</td>
<td>1.766</td>
<td>1.583</td>
<td>4.726 *</td>
</tr>
<tr>
<td><strong>C3</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Intercellularly</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Band beneath</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface layer</td>
<td>0.588</td>
<td>1.190</td>
<td>0</td>
</tr>
<tr>
<td>Basement membrane</td>
<td>5.510 *</td>
<td>0.953</td>
<td>0.417</td>
</tr>
<tr>
<td>Plasma cells</td>
<td>28.072 **</td>
<td>0</td>
<td>13.902 **</td>
</tr>
<tr>
<td>Perivascular</td>
<td>15.960 **</td>
<td>0.180</td>
<td>10.695 **</td>
</tr>
</tbody>
</table>
CHAPTER V

DISCUSSION

A striking similarity was seen in histologic morphology and in the distribution of immunoglobulin IgG and complement factor C3 in tissue samples of inflammatory papillary hyperplasia and gingivitis. The clinical and microscopic observations of tissues affected by either gingivitis or inflammatory papillary hyperplasia are similar. These features which represent a host response to toxic and antigenic substances present in intimate contact with the tissues for prolonged periods of time. Both inflammatory and immunologic processes are involved.
A. EVALUATION OF HEMATOXYLIN and EOSIN STAINED SECTIONS

Large numbers of microorganisms inhabit and colonize the gingival pockets in inflammatory periodontal disease. The periodontal pocket is lined by a nonkeratinized microulcerated sulcular epithelium. There appears to be a morphologically similar component in inflammatory papillary hyperplasia. The fissures separating papilliferous outgrowths are surrounded by a parakeratotic epithelium which is often ulcerated. Observations made from examining hematoxylin and eosin stained sections may be correlated to fundamental progressions of the inflammatory process.

Organisms found in dental plaque include Gram positive and negative cocci and rods, filamentous flora, vibrios, diphtheroids and spirochetes. These organisms produce exotoxins, endotoxins, enzymes and cell wall specific antigens. These products may stimulate the initial changes seen in epithelial structures as a result of inflammation. Enzymes, such as hyaluronidase, may directly affect epithelial structures, influencing their permeability to toxins and antigens.

A widening of intercellular spaces occurs as intercellular mucopolysaccharides and proteins are disaggregated and lost (Thilander, 1963; Toto and Gargiulo, 1970). As cellular junctions are altered, edema, an imbibition of fluid, occurs, altering the interstitial fluid. Tight junctions may act as a barrier impeding the flow of water, ions and small water soluble molecules along the intercellular spaces. As the cell junctions and their function are altered, intercellular spaces and epithelial permeability increase.

Concomitantly, a vasculitis ensues in the vessels immediately subjacent
to the epithelium. Edema, alteration of perivascular collagen fibers and a large increase in the number of polymorphonuclear leukocytes migrating from gingival vessels through the epithelium occurs (Payne, Page, Ogilvie and Hall, 1975). The presence of polymorphonuclear leukocytes in the epithelium indicates a chemotactic response to plaque metabolites and suggests local breaches in the continuity of the basement membrane. The functions of the basement membrane are compromised. The basement membrane may maintain an electric potential influencing ionic exchange between the epithelium and connective tissue.

The acute inflammatory infiltrate of the connective tissue gradually changes to a chronic infiltrate if the lesion does not resolve. The cell population loses its polymorphonuclear predominance and becomes characterized by mononuclear inflammatory cells, evidenced by macrophages, lymphocytes and plasma cells in the connective tissue of gingivitis and inflammatory papillary hyperplasia. The “older” nature of inflammatory papillary hyperplasia demonstrated by the cellular infiltration of both layers of connective tissue, the subepithelial lamina propria and deeper submucosa. The deeper submucosa was predominantly involved in gingivitis.

The epithelium in sections of inflammatory papillary hyperplasia and gingivitis shows edema, characterized by widening of intercellular spaces, polymorphonuclear leukocytes and mononuclear cells throughout the entire thickness of the epithelium, and elongated rete pegs. Inflammatory papillary hyperplasia had a parakeratotic surface while the oral epithelium of gingivitis samples was predominantly keratinized. This difference is attributed to environmental changes brought about by wearing a denture prosthesis.
Numerous investigators have elucidated the many tissue changes occurring under maxillary dentures. A predominant change is the replacement of a keratinized surface by a parakeratotic one.

The lamina propria in sections from inflammatory papillary hyperplasia and gingivitis is characterized by edema, dilated blood vessels, loss of collagenous fibers and a chronic inflammatory cell infiltrate. The infiltrate consisting of plasma cells and lymphocytes was most marked in inflammatory papillary hyperplasia. It appears the fibrous base of the connective tissue disaggregates by depolymerization of the mucopolysaccharide ground substance and loss of collagen fibers to accommodate the increased cellular infiltrate.
B. EVALUATION of SECTIONS STAINED for IMMUNOGLOBULIN IgG and COMPLEMENT FACTOR C3

The similarity in the basic disease process underlying gingivitis and inflammatory papillary hyperplasia was further substantiated by a like distribution of immunoglobulin IgG and complement factor C3.

Globulin concentrations found in the perivascular connective tissue indicate a liberation of serum globulins. An abundance is expected in this area due to increased vascular permeability in the inflammatory reaction. Schneider et al (1970) attributed this increase to binding of the globulins by perivascular mucopolysaccharides in this area. However, the continual antigenic challenge produced by bacterial plaque in close proximity to the inflamed tissues suggests an Arthus reaction may be involved. This is in accord with results from animal studies by Dick and Trott (1969) and McDougall (1974). Antigen-antibody complexes fixing complement may be precipitated about the blood vessels, evidenced by positive immunoglobulin IgG and complement factor C3 staining in this area. The lack of fibrinoid necrosis in blood vessel walls suggests an insidious reaction.

Since the plasma cell actively synthesizes, stores, and liberates gamma globulin (Fagreus, 1948; Leduc et al, 1960), a concentration of immunoglobulin IgG is expected to be seen within these cells. It should be noted the simple demonstration of a substance within a cell is not necessarily evidence of its in situ synthesis. In the absence of other explanations for its presence, such as phagocytosis or absorption by specific receptors, in situ synthesis provides a tenable working hypothesis. As immunoglobulin IgG is
liberated, it may encounter antigens in the tissue. Ensuing antigen-antibody reactions fixing complement are suggested by the deposition of complement factor C3 around the plasma cells. It appears as if a defensive change in connective tissue structure and function occurs from one of masticatory mucosa to one adapted for an immunologic response.

Immunoglobulin IgG and complement factor C3 deposits were demonstrated on the basement membrane in gingivitis and inflammatory papillary hyperplasia. These deposits were mainly observed in localized areas. Occasionally, they appeared as a continuous line demarcating the epithelial-connective tissue interface. The presence of immunoglobulin IgG and complement factor C3 suggested binding of antigen-antibody complexes and complement activation on the basement membrane. Similar conclusions were reached by Toto et al (1978) and Suzuki et al (1979). The observation of IgG and C3 bound to the basement membrane suggests injury to the mucosa in inflammatory papillary hyperplasia may be a function of complement. Complement may serve as a chemotactic anaphylactic agent and destroy cell membranes by its phospho-lipidase activity. Schneider et al (1966) suggested the presence of globulin in the basement membrane may be due to binding to mucopolysaccharides on fibers.

The presence of immunoglobulin IgG and complement factor C3 in the intercellular spaces of inflamed epithelium of both gingivitis and inflammatory papillary hyperplasia indicates a possible pathway for their liberation. An alteration in all junctions, widened intercellular spaces and ulcerations of the epithelium may allow a greater escape of globulins and other tissue fluid elements from the connective tissue in the increased gingival crevicular fluid flow. Brandtzaeg (1965) demonstrated immunoglobulins were present in
gingival crevicular fluid in proportions and concentrations comparable to plasma. Since local antibody production by plasma cells has been demonstrated in inflamed gingiva, the serum proteins found in crevicular fluid most likely originate from blood serum and plasma cells. A local accumulation and exudation of antibodies in gingivitis is evident. It is tempting to postulate a certain amount of immunoglobulin was consumed by local antigen-antibody reactions. Thus local production of antibody would not result in an increased concentration of immunoglobulins in the gingival crevicular fluid. However, Schenkein and Genco (1977) demonstrated a tendency for higher gingival crevicular fluid concentrations of immunoglobulin IgG than IgA or IgM in severely inflamed tissue. They attribute this to local production, lack of integrity of the sulcular epithelium, and permeability of blood vessels. Greater amounts of macromolecules may be lost to the oral cavity with an augmented fluid flow as the severity of inflammation is aggravated. Since IgG and C3 are found in gingival crevicular fluid, it is likely these molecules migrate through the basement membrane and intercellular spaces or directly via gingival microulceration to reach the gingival sulcus. Nisengard and Jarrett (1976) demonstrated crevicular bacteria coated with immunoglobulins IgG, IgA, IgM and IgE, and complement factor C3.

Toto et al (1970) regarded the presence of intercellular and intracytoplasmic immunoglobulin in the epithelium as a defense mechanism against antigenic substances permeating the tissue.

Brandtzaeg and Krause (1965) conjectured intercellular globulins contributed to cellular adhesiveness. Though the exact function(s) of intercellular immunoglobulins and complement factors is not clear, it is evident they are localized in the epithelium of gingivitis and inflammatory papillary
hyperplasia.

Brandtzaeg and Krause (1965) attributed the band of immunoglobulin found in the epithelium to a decrease in permeability. It is interesting to note the band of immunoglobulin IgG and complement factor C3 concentrated in an area where antigenic challenge is prominent, even in clinically healthy specimens.

Loci of microscopic inflammation are commonly observed in clinically normal gingivae probably as a result of the continuous bacterial activities in the area (Zachinsky, 1954). All control specimens in this study showed epithelial infiltration by polymorphonuclear leukocytes and mononuclear cells while 60% exhibited localized accumulations of plasma cells and lymphocytes in the deep connective tissue. The presence of plasma cells indicates antibodies may be present in the tissue in response to ever present bacteria, maintaining a constant defense in the healthy tissue. Immunoglobulin IgG and complement factor C3 were found intercellularly in all sections examined. Brandtzaeg and Krause (1965) postulated mucosal transfer of plasma proteins was a physiologic phenomenon not brought about by pathologic change.

Chi-square analysis of the data revealed statistically significant differences in the frequency of localization of immunoglobulin IgG in the connective tissue. A Fisher's corrected chi-square analysis demonstrated a difference significant at the .01 level of confidence in the deposition of immunoglobulin IgG on the plasma cells found in inflammatory papillary hyperplasia, gingivitis when compared to control tissues. This is expected by the increased numbers of plasma cells in the diseased state. A significant difference at the .05 level of confidence was found in the deposition of immunoglobulin IgG perivascularly in gingivitis as compared to controls. The relative avascularity of palatal
mucosa in certain locations made it difficult to identify blood vessels in some sections of inflammatory papillary hyperplasia.

Statistical analysis revealed a significant difference in the localization of complement factor C3 in the connective tissue between diseased and noninflamed tissues. A difference significant at the .01 level of confidence was demonstrated in the deposition of complement factor C3 around plasma cells and perivascularly in inflammatory papillary hyperplasia and gingivitis when compared to controls. A difference in the deposition on the basement membrane, significant at the .05 level of confidence, was found between inflammatory papillary hyperplasia and controls.

No statistically significant difference was found in the distribution of immunoglobulin IgG and C3 between inflammatory papillary hyperplasia and gingivitis.
C. IMMUNOLOGICALLY MEDIATED TISSUE INJURY

During recent years it has become increasingly apparent there is hardly any pathologic process going on in the body that does not involve the immune apparatus.

Epithelial invasion of antigen into connective tissue must occur for contact with host immunocompetent cells. Initial access of antigens to the gingival tissues may be provided by the ability of bacterial enzymes to penetrate the epithelium or by a lytic effect on the epithelium by a toxic material produced by bacteria such as hydrogen sulfide. Penetration of plaque enzymes and nonenzymatic antigens leads to increased density of inflammatory cells in the connective tissue and to structural alterations in the epithelium.

Among cytological phenomena occurring is the chemotactic migration of leukocytes through the attached epithelium in response to specific factors released by the microbial plaque. As microorganisms elaborating such factors are almost continually present, this is a process which does not cease, as may the conventional inflammatory response. Once they are past the epithelial barrier, bacterial products or antigenic portions of bacteria could then stimulate the production of antibody. Histologic sections reveal a migratory accumulation and mitotic turnover of mononuclear cells, predominantly plasma cells, responding to immunologic factors also released by the microbial plaque or occurring with the general host response.

As plasma cells are associated with antigen-antibody reactions, it may be assumed an immunologic reaction is present in gingivitis. After antibody is induced, subsequent exposure of the connective tissue to the bacterial antigens could react with antibody now present resulting in tissue destruction and
inflammation. Ulceration of the overlying epithelium makes the connective tissue even more available to bacterial antigens leading to repetitious antigen-antibody interactions in the tissue, chronic inflammation, and epithelial proliferation characteristic of periodontal disease and inflammatory papillary hyperplasia.

The noninvasive character of the oral flora in general, the paucity of demonstrable microorganisms within the inflamed gingiva and the inability to indict a specific genus or group of genera as effectors of tissue destruction in human periodontal disease suggests that tissue damage is primarily mediated by the inflammatory process. If so, one role of the oral flora in tissue destruction would be to provoke and maintain inflammation. The chronic subepithelial inflammation which persists after modalities of nonsurgical treatment for inflammatory papillary hyperplasia may be due to the presence of bacteria harbored in crevices between the papilliferous outgrowths.

However, another possible mechanism for the production of periodontal disease and inflammatory papillary hyperplasia might involve sensitization and subsequent hypersensitivity reactions to antigens derived from bacteria in intimate contact with gingival and palatal tissues.

The possibility of Type I Anaphylactic reactions occurring in gingival tissues is raised by the presence of IgE-containing plasma cells as demonstrated by Nisengard and Beutner (1971). Their numbers were related to the severity of gingivitis present. Some researchers have reported an increased number of mast cells present in the connective tissue, directly related to the degree of gingival inflammation. Other investigators have demonstrated the opposite. The antigen-antibody reaction between antigen with IgE-sensitized mast cells releases histamine, a powerful acute inflammatory response
mediator (Charon, 1980). It is tempting to assume a relationship between these two cell lines.

The cellular response by plasma cells, lymphocytes and polymorphonuclear leukocytes as seen in the sections studied indicate a mixed type of host response. The findings suggest a hypersensitivity reaction of both Type II and IV. The possibility of Type III or Arthus reactions has already been alluded to.

It is reasonable to speculate that the source of antibodies may be derived locally from plasma cells, resident immunocompetent cells, or systemically through blood vessels. Regardless of the origin of antibodies, a normally protective mechanism of the host, antigen-antibody complexes can activate the complement system, a series of nine serum proteins which biologically amplifies the immune response. Tissue injury progressively worsens.

The complement system acts in part by enhancing phagocytosis of microorganisms by leukocytes. In diseases such as inflammatory periodontal disease and papillary hyperplasia, the possibility exists that phagocytic cells, amplified by complement activation not only immobilize microorganisms more quickly, but become overworked and eventually lyse. Lysis of phagocytic cells results in release of intracellular lysosomes containing a multitude of hydrolytic enzymes. These enzymes can damage host tissues if not contained intracellularly.

Complement chemotactic factors C3a and C5a result locally and mediate accumulation of polymorphonuclear leukocytes and monocytes with subsequent lysosomal release of hydrolytic enzymes in gingival and palatal tissues. Tissue injury is characterized by widened epithelial intercellular spaces, destruction of desmosomes and tight cellular junctions, and loss of
intercellular material and acid mucopolysaccharides. Thus, continued antigen penetration, exposure to immuno-competent cells, resultant antibody production and complement fixation become self-perpetuating.

The complement system may be activated by the antibody dependent classic pathway. As C3 is a product of C3 esterase dependent upon pre-existing antigen-antibody C1,4,2, presence of IgG and C3 in the gingiva implicates antigen-antibody complexes form which bind and activate complement. A number of investigators have reported an increase in the number of IgG-containing plasma cells in gingivitis. A preponderance of IgG-containing plasma cells was seen in inflammatory papillary hyperplasia in this investigation. Immunoglobulin IgG has complement receptor sites and can bind complement. The presence of C3 fragment in the basement membrane suggests complement is activated by the classical route.

Alternatively, complement may be activated by polysaccharides, lipopolysaccharides and immunoglobulin aggregates to activate protein properidin, a beta glycoprotein in serum, which activates C3 causing C3-9 sequence.

As pointed out by Simon et al (1970) the quantity of endotoxin associated with gingival tissues was correleated with the degree of clinical inflammation. The signs of clinical inflammation became manifest as a shift occurred from Gram positive to Gram negative in plaque flora (Löe et al, 1965). Endotoxin may activate the alternate pathway, mediate the inflammatory response and precede classic complement activation.

In addition, Gram negative bacteria and spirochete coated with antibody may activate C1-7 causing bacteriolysis. Courts et al (1977) demonstrated functional complement activity in gingival crevicular fluid.
The picture presented of complement is by no means complete, but does emphasize its role as a local factor in the pathogenesis in inflammatory periodontal disease and papillary hyperplasia. Both pathways of complement activation may be involved just as inflammation and immunological processes may act synergistically together in producing the signs and symptoms of both gingivitis and inflammatory papillary hyperplasia.

The tissues are constantly exposed to massive amounts of pathogenic bacteria and their products. One might expect more severe infection and tissue damage than is seen. The pathologic process observed clinically is one of slow tissue damage. Individual variations in the severity of response may be due to individual differences in tissue resistance against bacteria aggressive factors, rather than to variations in the standard of oral hygiene. It is probable inflammatory papillary hyperplasia is an endogenous bacteria disease arising from a locally imbalanced host parasite relationship just as gingivitis and periodontitis are.
D. DIFFERENCES REPORTED IN THE PREDOMINANCE of VARIOUS CLASSES of IMMUNOGLOBULINS IN GINGIVAL TISSUES

The results of various investigators regarding levels of specific globulin fractions are not in agreement with one another.

Brandtzaeg and Krause (1965) reported an increase of immunoglobulin IgA-containing plasma cells in gingivitis. However, Brandtzaeg (1966) demonstrated an increase in immunoglobulin IgG in the connective tissue. Less immunoglobulin IgA and very little immunoglobulin IgG was found.

Platt et al (1970) reported IgM-producing plasma cells were predominant in acute gingivitis, though cells with IgG were abundant. Samples of severe periodontitis contained numerous IgM-containing plasma cells, many IgA cells and few IgG cells.

Nisengard et al (1971) demonstrated the numbers of immunoglobulin IgE-containing plasma cells were related to the severity of gingivitis.

Genco et al (1974) did not report relative concentrations, but did find immunoglobulin IgG, IgM, complement factor C3, albumin and transferrin in the connective tissue and sulcular epithelium of gingivitis samples.

The study by Mackler et al (1977) demonstrated plasma cells containing immunoglobulin IgG, IgM and IgA to be in an approximate ratio of 8:3:1 in sections from periodontitis.

These differences may be due to a number of factors:

An intrinsic variation in tissue areas examined may be present.

Since the predominance of certain globulin fractions appears to be related to the stage of pathology, the clinical criteria used to characterize the severity of involvement may have varied.
All the studies used immunofluorescence techniques with the exception of Byers et al (1975). Byers et al (1975) used low level diffusion plates. Variations in the specificity of technique could have resulted in the diversity of results. The use of unwashed specimens results in characterization of both cytophilic (plasma cell) and membrane bound (lymphocyte) immunoglobulins. Collagen fibers may give a positive fluorescence for immunoglobulin IgA and IgM.
E. REMARKS ON METHODOLOGY

As serum proteins, immunoglobulin IgG is predominantly found in the extravascular space while immunoglobulin IgM is located by and large intravascularly. IgG-containing plasma cells appeared to be predominant in combining results of previous studies. Therefore, characterization of the distribution of immunoglobulin IgG was chosen as one parameter in this pilot study.

The activation of complement results in the release of potent biological effector molecules. A discussion of the immune system seems incomplete without reference to complement. Complement factor C3 was chosen as the second parameter in the immunological portion of the study.

Prior to the development of immunoperoxidase methods, tissue or cellular localization of specific antigens or antibodies was studied with immunofluorescence methods. The development of enzyme-labeled antibodies, analogous to fluorescein-labeled antibodies, and their subsequent application to sections from formalin-fixed, paraffin-embedded tissues resolved many of the problems of immunofluorescence methods (Taylor, 1978).

Fresh or specially processed tissue is required for immunofluorescence methods. This constitutes a severe limitation, for often the pathologist has only fixed, embedded material available when the need for special diagnostic procedures becomes apparent. Immunoperoxidase methods make retrospective studies such as the present one possible.

Specialized ultraviolet microscopy is required for the examination of specimens labeled by immunofluorescence. The resulting resolution of morphological features is extremely poor. Reaction products are subject to
bleaching by light during microscopy and records are therefore impermanent.

A certain degree of nonspecific tissue autofluorescence occurs and may interfere with interpretation in studies of tissue sections.

The advantages of horseradish peroxidase procedures over immunofluorescence are:

1. They are applicable to routinely processed tissues;
2. A simple light microscope is required;
3. Preparations are permanently mounted;
4. Good morphological results are obtained;
5. The reaction product is not subject to change during examination;

The specificity and sensitivity of peroxidase-labeled antibody techniques have been favorably compared to immunofluorescence methods. Dorling et al (1971) showed a striking correlation of sensitivity between the two methods.

Formalin fixation apparently preserves the antigenicity of the immunoglobulin molecule to the degree necessary for its subsequent demonstration by immunoperoxidase methods. Taylor and Burns (1974) reported good results using formalin-fixed, paraffin-embedded tissues which had been routinely stored for five years or more. Good results were obtained in the present study using sections from tissue blocks which have been stored for thirteen years.

Nakane and Pierce (1967) attributed the sensitivity of immunoperoxidase methods to the amplifying effect of enzymatic activity. The enzyme and antibody are conjugated through stable covalent linkages, still retaining both enzymatic and immunologic activities. Enzyme is not consumed in the reaction with the substrate and each molecule of enzyme-labeled antibody bound to the antigenic site deposits many molecules of reaction product. Horseradish
peroxidase has a small molecular weight (40,000) and the conjugate penetrates tissue well. The reaction products of the histochemical reaction are insoluble in organic solvents.
F. THE MALIGNANT POTENTIAL of INFLAMMATORY PAPILLARY HYPERPLASIA

The etiology and clinical significance of inflammatory papillary hyperplasia has remained controversial in the dental literature since the 1940s.

Hobaek (1949) suggested palatal stomatitis was precancerous in some cases. However, in the same article, he stated cancer was certainly one of the less harmful effects of tobacco.

Thoma (1952) considered all papillomas as potential malignancies and advised their removal by wide excision.

Even though he encountered only occasional malignancies, Robinson (1957) considered inflammatory papillary hyperplasia premalignant.

However, Fisher and Rashid (1952) strongly emphasized the need to distinguish inflammatory papillary hyperplasia and true papillomas. The regression of the lesion with improved oral hygiene strongly refuted a neoplastic process. They argued the clinical course and histopathology of the lesion were compatible with inflammatory disease.

Sharp and coworkers (1951) reported mechanical trauma of negative pressure created by excessive relief on maxillary dentures had a remarkably low relationship to cancer. Trauma from ill-fitting dentures was often associated with oral malignancies, regardless of other numerous etiologic factors present.

The malignant potential of inflammatory papillary hyperplasia has been alluded to by other authors to this day, quoting Hobaek (1949) and Robinson (1957) among others.
Though the clinical behavior and histopathology of inflammatory papillary hyperplasia were compatible with inflammatory disease, Yrastorza (1963) felt the irreversibility of the lesion combined with occasional dyskeratosis suggested true neoplasia.

Guernsey (1965) found no dyskeratosis in his examination of 59 specimens. Dyskeratosis had been reported by Robinson (1957), Shafer et al (1963), Yrastorza (1963) and Flanagan and Porter (1968). Bhaskar et al (1970) reported no dyskeratosis was found in their study of 341 specimens. They concluded malignancy does not develop from papillary hyperplasia.

The histologic results of this study are in agreement with the morphology described by Yrastorza (1963), Shafer et al (1963) and Bhaskar (1970) with the exception of references to keratinization and dyskeratosis. No evidence of keratinization and dyskeratosis was found.

In conclusion, it may be stated studies substantiating the claim of malignant potential of inflammatory papillary hyperplasia are lacking.
G. PROSTHODONTIC CONSIDERATIONS

The role of the oral prosthesis as a causative factor in oral inflammation has been well established. Pronounced histopathologic and histochemical changes occur in the denture-bearing mucosa following the insertion of a prosthesis despite any normal clinical appearance of the mucosa.

Chacker (1973) described etiologic factors of inflammatory periodontal lesions. He included microbial irritants, mechanical irritants, inadequate restorative dentistry, chemical irritants, habits and mouth breathing. Several etiologic factors can lead to similar clinical manifestations. A multifactorial etiology may also exist for inflammatory papillary hyperplasia.

Many investigators have attributed inflammatory papillary hyperplasia as a response to trauma from ill-fitting dentures, occlusal problems associated with dentures, and movement of the denture base in function.

The very nature of a prosthesis precludes a supporting relationship to the maxillary mucosa. A denture is not a replacement for teeth; it is a replacement for no teeth (Malone, 1979). The diurnal changes noted in the palatal epithelium by Stephens et al (1966) supports the consideration that no removable denture can fit exactly. In tissues supporting an artificial replacement of missing body parts which move under function, the inciting cause of tissue change may be chiefly mechanical. Razek and Shaaben (1978) demonstrated an initial increase in keratinization during the first three years of denture wear followed by a thinning and parakeratinization of the epithelium in 4 to 6 years. The histologic results of the present study are in agreement with the parakeratosis noted by Pendleton
(1951), Ostlund (1958) and Razek and Shaaben (1978). The reports of increased keratinization by Kapur and Shklar (1962) and Jani and Bhargava (1976) were based on biopsy specimens taken after only three months of denture wear.

Tissue enzyme activity increased in the first three years of denture wear and after a replacement or new prosthesis was fabricated. A reduction in enzymatic activity was associated with wearing dentures for more than three years (Razek and Shaaben, 1978). The implication is to remake prostheses every three years. However, this is probably economically unfeasable for most patients.

The resultant parakeratinized epithelial surface may have a similar anatomic and physiologic structural weakness as the nonkeratinized sulcular epithelium. The lining of the gingival pocket does not seem to exhibit selectivity regarding diffusion of bacterial antigens inward and components of gingival crevicular fluid outward. The areas in question are continuously exposed to bacterial and leukocytic enzymes which may interfere with a potential barrier function.

Van Reenen (1973) demonstrated the number of bacteria associated with denture stomatitis. Lesions increased with years of denture wear and with increased severity of the lesion. No specific organism was associated with the lesions.

The close connection between Candida albicans and inflammatory papillary hyperplasia does not reveal anything regarding pathogenesis. A Candida infection may be secondary to the hyperplasia, the fissures between papillae affording a niche for candidal growth. Chronic traumatic injury to the oral mucous membrane may predispose it to candidal growth and infection.
Van Reenen (1973) found raised antibody titers to microorganisms isolated from denture stomatitis lesions. Genco et al (1974) indicated a gingival sulcus is not necessary for maintenance of adult plaque.

Though true allergy to denture base materials appears to be a rare phenomenon, poor tissue response to denture base materials has been alluded to.

Various base materials have been used in the fabrication of dentures. The materials that have been used include: vulcanite, cellulose acetate, cellulose nitrate, phenolic resins (Bakelite), acrylic resin, gold alloy, aluminum alloy, and chrome cobalt alloys. The most popular denture base material used today is polymethyl methacrylate (acrylic resin). However, scanning electron microscope studies have shown acrylic resin bases have a very irregular surface which may harbor bacterial plaque in the craters, pits, and porosities. Self-curing polymethyl methacrylate resins resulted in the least tissuephilic surface in a study currently being conducted by Scannichio (1979).

Budtz-Jorgensen and Bertran (1970) attributed poor denture cleanliness mainly to leukocytic emigration and continuous shedding of epithelial cells of inflamed mucosa rather than neglected hygiene. Several patients with severely inflamed mucosa brushed their dentures frequently and still had plaque-ridden dentures. The loci of organisms harbored in the crevices of the denture base probably were responsible for the relapses which occurred after treatment of denture stomatitis lesions. However, this should not preclude our responsibility as practitioners in emphasizing the need for a regimen of oral hygiene and tissue massage.

Though no denture base material is ideal, it is pragmatic to use as
tissuephile a denture base as is currently available in making prostheses for patients who exhibit a propensity for soft tissue inflammation. Since the escalating price of gold makes its use prohibitive, metal bases of chrome cobalt alloys are recommended. Though not yet tested by scanning electron microscopy in any current studies, the vinyl denture bases may be more tissuephile than polymethyl methacrylate. The study by Scannichio (1979) raises questions regarding the “inviolate” denture intaglio. It may well be more tissuephile to reduce sharp ridges before insertion of the prosthesis. Many schools have taught the maxim of not altering the denture intaglio except for warranted post-insertion adjustments.

Though the oral mucosa can endure mechanical stress such as denture-induced stress, it is more favorable to have supporting tissues which will afford an optimum denture-bearing base due to their inherent physiologic characteristics. Lytle (1957, 1959, 1962) emphasized the need for adequate recovery of tissues that had been abused by ill-fitting dentures in order not to perpetuate tissue injury in new prostheses.

Physical health plays a dominant role in response of body tissues to mechanical stimulation. General resistance factors influence the status of the connective tissue ground substance and integrity of the epithelium. Patients suffering from diabetes and Sjögren’s syndrome often find it difficult to tolerate dentures well.

Tissue rest appears to be a mitigating factor. Continuous wearing of a denture prosthesis except for time spent for oral hygiene was correlated with the incidence of inflammatory papillary hyperplasia.

Deleterious habits such as bruxing place additional stress on denture-supporting mucosa. An occlusal splint described by Braun and Shotwell (1979)
was effective in improving inflamed maxillary supporting mucosa.

The retention of the maxillary denture effectively cuts off palatal tissues from the free flow of saliva. Dental plaque formation and bacterial growth may increase in this isolated environment. The reduced pressure may affect tissue permeability. Denture stomatitis and inflammatory papillary hyperplasia are rarely seen in the edentulous mandibular arch. Mandibular dentures rarely exhibit positive retention. Therefore, the tissues are constantly flushed with saliva and its component antibodies. Denture sore mouth is a term commonly used in the early literature describing burning sensations and/or erythema underneath dentures. This was often attributed to poor tissue response to denture base materials, e.g. excess unreacted monomer in acrylic base dentures. Denture stomatitis apparently replaced the term “denture sore mouth”. However, in their literature review Weaver and Goebel (1980) stated denture stomatitis is used specifically to describe Candida infection of the maxillary denture bearing area.

Tucker and Heget (1976) observed inflammatory papillary hyperplasia had been identified as

- papillomatosis
- denture sore mouth
- pseudopapillomatosis
- stomatitis protectia
- hypertrophic stomatitis
- denture stomatitis
- palatal granulations
- pseudoepithelomatosis hyperplasia
It may well be that inflammatory lesions described as denture stomatitis are incipient lesions in the progression of tissue changes leading to inflammatory papillary hyperplasia, analogous to the relationship between gingivitis and periodontitis.

While inflammation attendant to inflammatory papillary hyperplasia may be reduced, the hyperplastic response of the tissues appears to be irreversible. Fisher and Roshid (1952) noted a regression of inflammation with improved oral hygiene and removal of the plaque-ridden denture. Van Huysen et al (1954) noticed improvement when new dentures were made. Inflammation diminished with tissue rest [Yrastorza (1963), Miller (1977)]. Smith (1964) used medicaments in treating inflammatory papillary hyperplasia. An attenuation of inflammation resulted. However, resolution of the papilliferous outgrowths did not occur with any conservative treatment modality. Surgical removal appears to be the treatment of choice, as it does with periodontal pockets.

Just as many dentulous patients with poor oral hygiene exhibit no periodontal pathosis, many denture wearers with a similar lack of hygiene do not appear to develop inflammatory papillary hyperplasia. Predisposing causes, some of which have been identified, are important in the pathogenesis of inflammatory papillary hyperplasia.
H. FUTURE OF THIS STUDY

One of the shortcomings of the present study was its qualitative nature. Biopsy samples of gingivitis were obtained after scaling and curettage were done to reduce inflammation and tissue friability prior to periodontal surgery. The retrospective nature of “collecting” tissue samples of inflammatory papillary hyperplasia precluded scoring the degree of pathology present at the time of biopsy. Therefore, computing immunoglobulin IgG-containing plasma cells per unit area would have been invalid.

Though it was not scored, intracytoplasmic immunoglobulin staining was noted in specimens. Light microscope adsorption studies may yield quantitative data comparing inflamed and control tissues.

Staining comparative classes of sections for immunoglobulins IgM, IgA, and IgE would probably yield data facilitating comparison to similar studies conducted on tissue samples of gingivitis and periodontitis alone.

Investigation of Type IV cell mediated or delayed hypersensitivity reactions would complete the overview of immune system reactions in inflammatory papillary hyperplasia.

The relationship of inflammation papillary hyperplasia to gingivitis could be further corroborated by conducting studies on plaque maturation underneath dentures and characterization of tissue exudate from inflammatory papillary hyperplasia lesions. Perhaps inflammatory papillary hyperplasia is the manifestation of periodontal disease in the edentulous arch.

The health of the soft tissues is an index to the character and relative
permanence of the underlying bone. The preservation of denture-supporting (basal seat) tissues is of prime importance to the denture patient. Since prolonged inflammation of tissue in contact with bone usually results in loss of bone structure, inflammatory papillary hyperplasia may progressively reduce the quantity and quality of denture-supporting tissues. This decreases the ability of the patient to maintain masticatory function in later years. If lesions analogous to inflammatory papillary hyperplasia could be induced in laboratory animals, studies of its effects on underlying bone may yield information on as yet unknown clinical implications of the lesion. Postmortem studies may achieve a similar result. Just as periodontitis affects alveolar bone, inflammatory papillary hyperplasia may have adverse effects on the alveolar ridge and basal bone, affecting effective denture support.
CHAPTER VI

SUMMARY AND CONCLUSIONS

In health, host-parasite interactions are balanced to the extent no clinical symptoms or tissue lesions occur. When the balance shifts in favor of the parasite, disease results as expressed by clinical signs of inflammation.

The most obvious host response to local environmental change is inflammation. Responses to local factors and mechanisms of tissue repair may be modified by systemic factors. The immune response overlaps both local and systemic factors.

The presence of immunoglobulins in the inflamed tissues, elevated serum antibody titers to various oral microorganisms, plasma cells and lymphocytes in the inflamed connective tissues, and polymorphonuclear leukocytes confronting bacterial plaque and its products indicate the activation of the immune system and complement. The inflammatory reaction resulting from antigen-antibody reactions appears to possess additional features relating to platelet agglutination, mast cell degranulation, polymorphonuclear leukocyte chemotaxis and fixation of various elements of the complement system. Both the inflammatory mechanism and the immune reaction are essentially homeostatic mechanisms. Immune reactions sometimes lead to host injury as in allergic reactions. The immune mechanism as a host defense reaction is a general rather than a specific one.

While it is generally agreed that microorganisms of dental plaque comprise the primary etiologic agent responsible for chronic periodontal
disease, the wide variation in host susceptibility leads to the concept host tissue factors may play an overriding role in the induction and progress of the disease state. Thus, in this regard, periodontal disease and inflammatory papillary hyperplasia may be similar chronic inflammatory diseases in which much of the tissue destruction appears to be a consequence of the immunopathologic response of the host to the presence of the etiologic agent rather than a direct toxic effect of the agent or its products upon the tissues.

A multifactorial etiology exists for inflammatory periodontal disease. Elements in the multifactorial etiology of inflammatory papillary hyperplasia include the intrinsic nature of prostheses, mechanical trauma resulting in tissue alterations and increased tissue susceptibility, bacterial plaque harbored in the intaglio of the denture base and in crevices between hyperplastic papillae, and tissue tolerance affected by systemic factors and pre-existing oral masticatory mucosa pathology.

The histology of specimens of inflammatory papillary hyperplasia, gingivitis, and clinically healthy masticatory mucosa was studied. From the observations made, it was noted that:

1. A similarity regarding elements of the chronic inflammatory process was seen between inflammatory papillary hyperplasia and inflammatory periodontal disease.

2. No significant difference in the distribution of immunoglobulin IgG and complement factor C3 was seen in between inflammatory papillary hyperplasia and gingivitis. The presence of immunoglobulin IgG and complement factor C3 were demonstrated intercellularly in the epithelium, on the basement membrane, associated with plasma cells and
perivascularly. At times, such deposits were present as a band beneath the surface layers of the epithelium.

3. The presence of immunoglobulin IgG and complement factor C3 in inflammatory papillary hyperplasia suggested antibody complexes form which have the potential to bind and activate complement.

4. The inflammatory process and immune system appear to be involved in the pathogenesis of inflammatory papillary hyperplasia. Reactions of the immune system may be an aggravating factor in the persistence of the lesion.

5. It is probable inflammatory papillary hyperplasia is an endogenous bacterial disease arising from a locally imbalance host-parasite relationship just as are gingivitis and periodontitis.
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Fig. 1. Inflammatory papillary hyperplasia. IgG. 100X magnification. Counterstain: Hematoxylin and eosin. Localized areas of IgG deposits are seen along the basement membrane. A cellular infiltrate of the epithelium is evident.
Fig. 2. Inflammatory papillary hyperplasia. IgG. 1000X magnification. Counterstain: Hematoxylin and eosin. IgG deposits may be seen intercellulary in the epithelium. Polymorphonuclear leukocytes and a monocyte are seen infiltrating the epithelium from the connective tissue.
Fig. 3. Inflammatory papillary hyperplasia. IgG. 1000X magnification. Counterstain: Hematoxylin and eosin. Plasma cells containing brown granular deposits of IgG in their cytoplasm are seen. Plasma cells negative for IgG are also evident in the connective tissue.
Fig. 4. Inflammatory papillary hyperplasia. C3. 1000X magnification. Counterstain: Hematoxylin and eosin. C3 deposits are present intercellularly in the epithelium. Perivascular deposits are noted surrounding the blood vessel in the lamina propria. Diffuse C3 deposits are seen in the connective tissue as well as associated with plasma cells.
Fig. 5. Inflammatory papillary hyperplasia. C3. 1000X magnification. Counterstain: Hematoxylin and eosin. Brown granular C3 deposits are evident intercellularly in the epithelium.
Fig. 6. Inflammatory papillary hyperplasia. C3, 250X magnification. Counterstain: Hematoxylin and eosin. C3 deposits are seen peripheral to a number of plasma cells in this section of connective tissue.
Fig. 7. Inflammatory papillary hyperplasia. IgG. 1000X magnification. Counterstain: Hematoxylin & eosin and methyl green. Intercellular and intracytoplasmic deposits of IgG are evident in the epithelium and along the basement membrane.
Fig. 8. Inflammatory papillary hyperplasia. IgG. 1000X magnification. Counterstain: Hematoxylin & eosin and methyl green. Perivascular deposits of IgG are seen upon examination of the connective tissue. Plasma cells staining positive for IgG are evident in the background cellular infiltrate.
Fig. 9. Inflammatory papillary hyperplasia. IgG. 250X magnification. Counterstain: Hematoxylin & eosin and methyl green. Deposits of IgG are seen in the cytoplasm of a number of plasma cells and perivascularly.
Fig. 10. Inflammatory papillary hyperplasia. C3. 250X magnification. Counterstain: Hematoxylin & eosin and methyl green. Intercellular C3 deposits in the epithelium, C3 deposits along the basement membrane and perivascularly are demonstrated.
Fig. 11. Inflammatory papillary hyperplasia. C3. 250X magnification. Counterstain: Hematoxylin & eosin and methyl green.
Deposits of C3 may be seen on the surface of some plasma cells and perivascularly.
Fig. 12. Gingivitis. IgG. 250X magnification. Counterstain: Hematoxylin & eosin and methyl green. Deposits of IgG are noted intercellularly, along the basement membrane and perivascularly.
Fig. 13. Gingivitis. IgG. 250X magnification. Counterstain: Hematoxylin & eosin and methyl green. Cytoplasmic deposits of IgG are seen in a number of plasma cells.
Fig. 14. Gingivitis. C3. 1000X magnification. Counterstain: Hematoxylin & eosin and methyl green. Perivascular deposits of C3 are evident in this section of connective tissue. Red blood cells are seen in the lumen of the blood vessels. Margination of white blood cells is seen in one vessel.
Fig. 15. Noninflamed attached gingiva. IgG. 40X magnification.
Counterstain: Hematoxylin & eosin and methyl green.
Deposits of IgG are evident intercellularly in the epithelium
and as a band underneath surface layers. Discrete areas of
cellular infiltrate are seen in the connective tissue.
Fig. 16. Noninflamed attached gingiva. C3. 100X magnification. Counterstain: Hematoxylin & eosin and methyl green. C3 deposits are seen intercellularly in the epithelium and within blood vessels in the connective tissue.
Fig. 17. Systemic lupus erythematosus kidney. IgG. 100X magnification. Counterstain: Hematoxylin & eosin and methyl green. Deposits of IgG are demonstrated between the endothelium and glomerular basement membrane. A sclerosed glomerulus is evident in the section.
Fig. 18. Systemic lupus erythematosus kidney. C3. 40X magnification. Counterstain: Hematoxylin & eosin and methyl green.
C3 deposits are evident between the endothelium and glomerular basement membrane.
APPROVAL SHEET

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

4/16/80
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