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Histological Evaluation of Gingiva in Complete CrownRestorations

Meera Mahajan
Loyola University Chicago

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HISTOLOGICAL EVALUATION OF GINGIVA IN COMPLETE CROWN RESTORATIONS

by

Meera Mahajan, D.D.S.

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

June 1976
Meera Mahajan was born on January 31, 1946 in Sialkot, India.

She was graduated from Queen Victoria High School in Agra, India in May, 1961. From 1961 to 1963, she attended St. John's College, Agra, India.

In July, 1963, she began studies at Lucknow University, School of Dentistry, and received Bachelor of Dental Surgery in 1967.

Upon completion of dental school, she served one year of internship in All India Institute of Medical Sciences. She immigrated to U.S.A. in 1969 and completed two years of residency programme at University of Chicago.

In September, 1972, she entered graduate school at Loyola University, School of Dentistry, to pursue a specialty in Fixed Prosthodontics and M.S. degree in Oral Biology.
ACKNOWLEDGMENTS

I wish to express my thanks for the help and suggestions extended to me by the members of my board.

To Dr. Patrick Toto my deepest gratitude for his assistance and always having time for me.

To Dr. William Malone my appreciation for his inspiration and help in carrying out my research.

To Dr. Robert Pollock my thanks for his constructive criticism.

To my family: to my devoted husband, Jogindar, and to my son, Rohit, for their understanding patience during my graduate training; and finally to my parents who taught me the value of education.
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HISTOLOGICAL EVALUATION OF GINGIVA IN COMPLETE CROWN RESTORATIONS

CHAPTER I

INTRODUCTION AND STATEMENT OF THE PROBLEM

Introductory Remarks

The purpose of this investigation is to determine the histological response of the interproximal papillary gingiva to complete crown restorations. Tissue adjacent to extensively restored teeth were reviewed histologically to evaluate dental therapy. There is presently sparse literature which reviews the histological architecture of restorative teeth.

Keratinization, density of connective tissue, inflammatory changes and histochemical reaction of the tissues surrounding extensively restored teeth in patients were studied qualitatively. Quantitative study of Mitotic index and cell density was performed on the gingival epithelium.

This study is an effort to establish guidelines for histological evaluation of gingiva in extensive restorative dental therapy.
Statement of the Problem

It was the intent of this study to review histologically the tissue tolerance of extensive dental restorative treatment. A total of fifteen patients whose restorative work has been completed for two to nine years were reviewed. The collected data on mitotic index and cell density was analyzed statistically in order to ascertain the level of significance of the findings.
CHAPTER II

REVIEW OF THE LITERATURE

IN COMPLETE CROWN RESTORATIONS
HUMAN STUDIES

HERLANDS, LUCCA & MORRIS (1962) in their clinical study placed greater emphasis on forms, contours and extension of full coverage. They felt that in normal periodontal situation, the gingival margins of a cast crown should be placed slightly below the free gingival cuff. Axial contours, buccal and lingual contours were crucial in preventing food impaction, causing inflammatory reaction.

MILLER, FEINBERG (1962) reported in their paper on full coverage restorations placed great emphasis on preparation of the tooth prior to coverage. They stated that gingival inflammation and perio disease resulted from traditional techniques which were used by the clinician.

ERICSSON, MARKEN (1968) reported in their clinical study on patients, effects of fixed partial dentures on surrounding tissues. After measuring the pocket
depths, they found that depth of gingival pocket was shown to be greater around an abutment or around a tooth opposing a fixed partial denture than around the respective untreated control tooth. Also the gingiva more frequently showed retraction around the abutment when the occlusal load was greater on the fixed partial denture than when the occlusion was harmonious, however the difference was not significant.

MOUNT (1970) In his paper stressed that in the mature mouth, following crown placement, early change of the position of the gingival margin is often the result of trauma to the soft tissues or mishandling of materials during construction.

REDTENBACHER (1970) in his paper placed greater emphasis in preparation of teeth, casting, inadequate immediate replacement, laboratory failures for the limited success of oral rehabilitation.

MANNERBERG, (1971) In his clinical study on gingival changes following porcelain crown therapy found that porcelain crown whose cervical border is placed down to "half the depth of the gingival pocket" implies a
risk for permanent inflammatory irritation of the gingiva.

YUODELIS, WEAVER, SAPKOS (1973) In their paper stated that final restoration should not follow the original anatomic crown and should recreate the original contours of the root portion. They said that the greater the facial and lingual bulge, the more plaque retained. For these reasons by flattening the facial and lingual contours of the restorations the gingival response is excellent, probably because the cervical region is made more accessible for routine home care.

RICHTER (1973) In his clinical study placed full crown restoration supragingivally and subgingivally. He did not get significant results by comparing two marginal locations; in the health of gingiva, in the change in sulcus depth, in gingival contour and in plaque accumulation. He came up to conclusion that fit and finish of full crown restorations may be more significant to gingival health than the location of the finish line.

WHEATCROFT, SCIANTARELLI (1974) In their study on dental students where they used water irrigation to
prevent gingival inflammation. They found that gingival inflammation increased in all students but was significantly less in those that used the water irrigation.

MARUYAMA, SIMOOSA, OJIMA (1976) In their clinical study on clinically normal gingiva on natural teeth and complete crowns. They found that clinically normal gingivae adjacent to natural teeth did not exhibit dilated capillaries, but more than one fourth of the capillary loops in the gingivae adjacent to the complete crowns showed dilation.

This study illustrated the degree of inflammation indigenous to complete crown restorations.
ANIMAL STUDIES

JENS WALERHAUG (1953, 1960) in his study on monkeys found histologically that subgingival restorations are among the major etiologic factors in periodontitis. He felt it was better to place the margins supragingivally from the beginning, but in cases such as caries rate, oral hygiene and esthetics, consideration must be given. He also used different materials to study the irritation exerted by the material per se. He implanted different pieces of materials subcutaneously or used as implants in tooth sockets and also studying the epithelium after extraction. He found gold, high and low fusing porcelain vitallium causing very slight irritation and inert. On the other hand self cure acrylic very irritating during and after polymerization, crown and bridge cement irritating and amalgam causing elective irritation.

MARCUM (1967) in his study on dogs found that crowns with margins located at or even with the gingival crest caused least inflammatory response; that crowns with margins located above and below the crest caused
the most severe inflammatory response. He also found that length of time a restoration was in place had little, if any, effect upon the severity or degree of inflammation.

PEREL (1971) carried his study on dogs for nine weeks, in which he studied the relationship between axial tooth contours and surrounding marginal gingiva. He produced undercontours and overcontours, on buccal and lingual crown surfaces. He made clinical and histological (microscopic) observations. He found that axial tooth surface undercontouring did not produce any significant changes in the gingival health. On the other hand overcontouring of similar surfaces produced inflammatory and hyperplastic changes in the marginal gingiva. He saw these changes clinically and microscopically after four weeks.
The early work of Minot (1908), concerning the variations of mitotic activity with different tissues, served as evidence of differential rates of growth in tissues.

Minot carried out his quantitative analysis of mitosis by using mitotic index (MI) which he described as "the number of cells to be found at any given moment in the active process of division out of a total of 1000 cells." The mitotic index has been used in many of the studies that followed.

In 1952, Katzberg found that the abdominal skin mitotic index varied with age, having a range of .23 to .76. He also noted a decrease in cell density with age.

In 1956, Marwah reported the first mitotic index of human oral mucous membrane. He studied the epithelium of attached gingiva and reported age differences in the mitotic index. The age ranged from 25 thru 34 years and from 50 thru 78 years with average mitotic indices of 0.98 and 1.56 respectively. The cell density was
also shown to increase with age going from 55 cells/(100u)$^2$ to 73 cells/(100u)$^2$. The older group was found to have 50% more mitotic activity and a 33% increase in cell density.

Thuringer, in 1959 reported on the effects of age on the human epidermis. There was an increase of MI from 0.245 to 0.482 and a change in location of the greatest number of mitotic figures. The mitotic activity of the basal layer showed a sustained increase with age. The mitotic activity of the spinous layer showed a decrease with age.

In 1960 Marwah reported on the effects of chronic inflammation on the epithelial mitotic index in human gingiva. Specimens were taken from men 25 to 34, and 50 to 78 years of age. In the non-inflamed tissue there was an increase in mitotic index with age from .55 to .84. There was also an increase in cell density with age from 49/(100u)$^2$ to 58/(100u)$^2$. In the inflamed specimens there was an increase in mitotic index from 1.5 to 3 times that on non-inflamed tissue. Cell size increased by 14-18%. The least increase was seen in specimens with high MI's.
Gargiulo in 1961 reported on the mitotic activity of human oral epithelium exposed to 30% Hydrogen Peroxide. Adult males ranging from 22 to 27 years and from 48 to 63 years were studied. The control biopsy specimens had an average mitotic index in the young age group of 0.79 and average mitotic index for the older group of 1.69. The treatment with 30% hydrogen peroxide resulted in an increase of the mitotic index by 8 times in the young and by 5 times in the older individuals. The average cell density for the younger group was 62 cells/(100u)² and 70 cells/(100u)² for the older group. Gargiulo also stated that the apparent increase in MI was due to a retarding of the mitotic process and therefore was not a true reflection of mitotic activity.

Silberkweit in 1963 described the pattern of mitotic activity and cell densities in the epithelium of children's inflamed gingiva. His subjects were divided equally with regard to sex, and ranged in age from 5 to 13 years. The average mitotic index for males was .514 and for females was .575. The mitotic index increased slightly as the age increased. The cell densities ranged from 26.30 to 73.23/cm² in males and from 21.53 to 72.00/cm² in females.
Silberkweit also noted that as mitotic activity increased, cell density decreased.

Hayes and Silberkweit in 1964 published the results of similar work. However, they studied normal gingival epithelium. The subjects ranged from 5 to 12 years of age. The average mitotic index for males was .454 and for females was .401. There was little variation in mitotic index with age. The average cell density of males was 60.32/cm² ranging from 38.05 to 91.39. The average cell density of females was 61.82/cm² ranging from 30.88 to 96.57. There was an inverse relation noted between cell density and mitotic index. The average mitotic index decreased with an increase in cell density.

In 1964 Krajewski and Gargiulo studied the mitotic activity in the oral epithelium of women. Twenty-four females between the ages of 20 and 35 years were studied, beginning 15 days after the onset of menstruation. The mitotic indices ranged from 0.12 to 1.48 and averaged 0.74. The cell density ranged from 31 to 89 and averaged 54 cells/(100u)².

Soni and Silberkweit in 1965 published their findings on mitotic activity and cell density in human gingival epithelium. They studied 65 men and
65 women between 14 and 24 years of age. They found that in non-keratinized epithelium the mitotic index was one and one half times higher than in keratinized epithelium. They found that in men with normal or inflamed tissue, cell density decreased with age. They also showed that in males the mitotic index increases with age in normal tissue and decreases with age in inflamed tissue. In females the cell density decreased with age in normal or inflamed tissue. The mitotic index increased with age in normal and inflamed tissue. The average cell density was 35.5 for normal males, 33.8 for male inflamed tissue, 35.7 for normal females and 35.7 for female inflamed tissue. The average mitotic index was .699 for normal males and .875 for male inflamed tissue, .656 for normal females and .798 for female inflamed tissue.

In 1967 Silberkweit described the mitotic activity in human gingival epithelium associated with Dilantin (Diphenylhydantoin sodium) therapy. One Hundred-forty four patients from 8 to 22 years of age were studied. Thirty were being treated with Dilantin. Males of all ages treated with Dilantin showed a lower mitotic index as compared to non-treated subjects. Young females
treated with Dilantin showed a higher mitotic index than the non-treated patient. But the older females treated with Dilantin showed a lower mitotic index than the non-treated patient. Cell density decreased in treated males and females. Mitotic index decreased in treated males but increased in treated females. The average cell density was 49.3 for normal males, 29.2 for treated males, 48.1 for normal females and 31.6 for treated females. The average mitotic index was .485 for normal males, .294 for treated males, .492 for normal females and .550 for treated females.

RYAN (1970) studied the mitotic index in the epithelium and cell density of epithelium and connective tissue of the attached gingiva in young and old men. Young group ranged from 18–22 years of age while older group ranged from 58–64 years. The average epithelial cell density was 144.2 cells/100u² in the young and 191.2 cells/100u² in aged men. The average mitotic index was 1.01 for young and 1.14 for aged men.
Table 1

Epithelial Mitotic Index and Cell Density
in Human Oral Mucosa

<table>
<thead>
<tr>
<th>Author and Year</th>
<th>Subjects</th>
<th>Age</th>
<th>Average MI</th>
<th>Average Cell Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marwah 1956</td>
<td>Normal males</td>
<td>25-34</td>
<td>.98</td>
<td>55/(100u)²</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50-78</td>
<td>1.56</td>
<td>73/(100u)²</td>
</tr>
<tr>
<td>Marwah 1960</td>
<td>Normal males</td>
<td>25-34</td>
<td>.55</td>
<td>49/(100u)²</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50-79</td>
<td>.84</td>
<td>58/(100u)²</td>
</tr>
</tbody>
</table>

Inflammation increased MI by $1\frac{1}{2}$ to 3 times.

| Gargiulo 1961   | Normal males  | 22-27 | .79        | 62/(100u)²           |
|                 |               | 48-63 | 1.69       | 70/(100u)²           |
|                 | Treated with  | 22-27 | 6.50       | 58/(100u)²           |
|                 | 30% Hydrogen  | 48-63 | 8.76       | 65/(100u)²           |
|                 | Peroxide      |       |            |                      |
| Silberkweit 1963| Inflamed      | 5-13  | .514       | 21.53-72.00          |
|                 | females       |       |            |                      |
|                 | Inflamed      | 5-13  | .575       | 26.30-73.23/cm²      |
|                 | males         |       |            |                      |
| Hayes 1964      | Normal females| 5-12  | .401       | 30.05-96.57/cm²      |
|                 | Normal males  | 5-12  | .454       | 38.05-91.39/cm²      |
| Krajewski 1964  | Normal females| 20-35 | 0.74       | 54/(100u)²           |
Table 1
(Continued)

Epithelial Mitotic Index and Cell Density
in Human Oral Mucosa

<table>
<thead>
<tr>
<th>Author and Year</th>
<th>Subjects</th>
<th>Age</th>
<th>Average MI</th>
<th>Average Cell Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soni 1965</td>
<td>Normal females</td>
<td>14-24</td>
<td>.656</td>
<td>35.7/cm²</td>
</tr>
<tr>
<td></td>
<td>Normal males</td>
<td>14-24</td>
<td>.669</td>
<td>35.5/cm²</td>
</tr>
<tr>
<td></td>
<td>Inflamed females</td>
<td>14-24</td>
<td>.656</td>
<td>35.7/cm²</td>
</tr>
<tr>
<td></td>
<td>Inflamed males</td>
<td>14-24</td>
<td>.875</td>
<td>33.8/cm²</td>
</tr>
<tr>
<td>Silberkweit 1967</td>
<td>Normal females</td>
<td>8-22</td>
<td>.492</td>
<td>48.1/cm²</td>
</tr>
<tr>
<td></td>
<td>Normal males</td>
<td>8-22</td>
<td>.485</td>
<td>49.3/cm²</td>
</tr>
<tr>
<td></td>
<td>Females treated with Dilantin</td>
<td>8-22</td>
<td>.550</td>
<td>31.6/cm²</td>
</tr>
<tr>
<td></td>
<td>Males treated with Dilantin</td>
<td>8-22</td>
<td>.294</td>
<td>29.2/cm²</td>
</tr>
<tr>
<td>Ryan 1970</td>
<td>Normal males</td>
<td>18-22</td>
<td>1.01</td>
<td>144.2/(100u)²</td>
</tr>
<tr>
<td></td>
<td>Males</td>
<td>58-64</td>
<td>1.14</td>
<td>191.2/(100u)²</td>
</tr>
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</table>
CHAPTER III

MATERIALS AND METHODS

Materials

A total of fifteen patients ranging in the age 30-68 years with extensive complete crown restorations were included. The following cases were utilized in this study:

a) Full upper reconstruction (teeth covered with complete crowns or replaced by fixed prosthodontics) and lower precision partial on one arch of full tooth supportive prosthodontics.

b) A selection of a subject requires at least the involvement of six teeth with complete crown restorations in one arch. The selected patients had all dental restorative work completed from 2-5 years. This would permit evaluation of long term effect of restorations upon the gingiva.

Methods

In this study three biopsies were taken from the
NORMAL GINGIVA

FIGURE 1
INFLAMED GINGIVA

FIGURE 2
RESTORATIONS

FIGURE 3

Anesthesia was provided.

Specimen Preparation

The biopsy specimens were fixed in formalin solution for 24 hours, after which they were washed in water for 24 hours. The tissue was then embedded in paraffin.
interproximal gingival papillary tissue (approximately 3x3 mm.) from each patient. The areas for biopsy were randomly selected from gingiva as follows:

a) Gingiva appearing clinically normal (pink, stippled form, sharp margins) adjacent to unrestored teeth.

b) Gingiva appearing clinically normal adjacent to restored teeth.

c) Gingiva adjacent to restored complete crown, which appeared clinically inflamed (red, edematous, unstippled).

The gingival interproximal tissue in the area to be biopsied was infiltrated with local anesthesia (xylocaine 1:100,000 epinephrine). The interdental papilla was excised by using a number 15 blade. The tissue was carefully removed from the teeth, washed in room temperature tap water to remove the blood. The specimens were then immediately placed in formalin for fixation. No patient discomfort was noted so no analgesics were prescribed.

**Specimen Preparation**

The biopsy specimens were fixed in formalin solution for 24 hours, after which they were washed in water for 24 hours. The tissue was then embedded in paraffin,
cut at six microns and stained with hemotixylin and eosin, Mallory and Periodic acid Schiff (PAS).

A slide of each specimen was selected at random and placed on the microscope and studied at 450x. 

In histopathology of gingiva, the sections were reviewed and the following characteristics noted:

1. Keratinization (Orthokeratinization) 
   (Parakeratinization) 
2. Histochemical reactions - Glycogen study 
3. Inflammatory changes - lymphocytes 
   - plasma cells 
   - polymorphonuclear leucocytes 
4. Density of collagen fibres 

**Mitotic Index**

Mitotic index is the number of counted mitotic figures in metaphase and anaphase per 1,000 cells.

An eyepiece reticular representing 100u² of surface area at 450x was used. Ten sample areas of epithelium were selected at random using basal cell layer as a baseline, for normal control gingiva associated with unrestored teeth and for epithelium of restored teeth with and

* AO Microstar, Rochester, N.Y.*
without clinical evidence of inflammation. It was very hard to see different stages of mitosis, so only metaphase and anaphase were taken into consideration. Metaphase is a stage where the chromosomes arrange themselves in an equatorial plane midway between the two centrioles forming the equatorial plate. In anaphase stage the chromatids diverge and move toward their respective centrosomes.

Mitotic index can be computed from this:

\[
\frac{\text{no. of mitotic figures}}{\text{Total no. of cells counted}} = \frac{\text{MI}}{1,000}
\]

Cell Density

Cell density was also taken into account while counting mitotic figures. The reticular eyepiece was placed along the basal cell layer and the total number of cells within the reticular boundaries were counted with hand mechanical counter. The reticular was moved along the basal cell layer to include ten \(100u^2\) fields. An average number of cells per \(100u^2\) was then computed for each area. This was repeated for all specimens to obtain a mean number of cells per \(100u^2\) for tissue appearing clinically normal without a crown and
clinically abnormal or inflamed with the crown.
CHAPTER IV

FINDINGS

Morphologic Characteristics of Gingiva Normal, Clinically Normal and Inflamed with Prosthesis.

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Keratinization</td>
<td>Parakeratosis</td>
<td>Parakeratosis</td>
<td>Parakeratosis</td>
</tr>
<tr>
<td>Glycogen</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Inflammatory infiltration</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Collagen density</td>
<td>Normal dense fibres</td>
<td>-</td>
<td>Loss of collagen fibres around blood vessels</td>
</tr>
</tbody>
</table>

Table 2
NORMAL GINGIVA ADJACENT TO UNRESTORED TOOTH

FIGURE 4
INFLAMED GINGIVA ADJACENT TO RESTORED TOOTH

FIGURE 5
 Histologic sections of three groups showed para-keratosis, which is characterized by remnants of epithelial nuclei in the keratin layer of epithelium.

b) **Glycogen study:**

Glycogen deposits were found in all three groups. Glycogen was found more near surface epithelium in the spinous layer rather than near the basal cell layer.

c) **Inflammation:**

Normal gingiva without prosthesis showed few inflammatory cells; lymphocytes, plasma cells, polymorphonuclear leucocytes. The gingiva which looked clinically normal adjacent to a crown showed more inflammatory cells than the control group. The blood vessels were dilated. Clinically inflamed gingiva adjacent to the crown showed the highest number of inflammatory cells. The blood vessels were dilated. In few sections the inflammatory cells were present in epithelium.

d) **Density of Collagen Fibres:**

The collagen density was studied only in Groups A and C. The specimens from clinically normal looking gingiva without prosthesis showed normal dense fibre
bundles and patent blood vessels. The gingiva adjacent to prosthesis, which appeared to be clinically inflamed showed a loss of collagen fibres around blood vessels in connective tissue.
NORMAL GINGIVA ADJACENT TO NATURAL TOOTH

FIGURE 6
CLINICALLY NORMAL GINGIVA

ADJACENT TO RESTORED TOOTH

FIGURE 7
INFLAMED GINGIVA ADJACENT TO RESTORED TOOTH

FIGURE 8
In the third group where the specimens were taken from clinically inflamed gingiva adjacent to a crown, mitotic indices ranged from 0.3 to 1.1 with an average mitotic index of 0.681.

By comparing Groups A and B, a t-value was 3.04, there was statistically significant difference at the...
Mitotic Index of Stratified Salivary Epithelium:

The mitotic index of the gingiva increases adjacent to teeth restored with complete crowns, that is whether or not the gingiva appear clinically normal or inflamed. For values, please see Table 3.

The mitotic index (number of cells in mitosis per 1,000 cells) was determined for each histological specimen. In the control group, clinically normal tissue without crown or prosthesis, the mitotic indices ranged from 0.2 to 0.5 with an average mitotic index of 0.340.

In the second group where clinically normal specimens were taken from gingiva adjacent to a crown, the mitotic indices ranged from 0.4 to 0.6 with an average mitotic index of 0.50.

In the third group where the specimens were taken from clinically inflamed gingiva adjacent to a crown, mitotic indices ranged from 0.3 to 1.1 with an average mitotic index of 0.681.

By comparing Groups A and B the t value was 5.87, there was statistically significant difference at the P less than .01 level. Between Groups A and C the t
value was 4.93, which is statistically significant difference at P less than .01 level.

Between Groups B and C the t value was 2.09, which is statistically significant at P less than .05.

A t comparison shows significant differences between Groups A and B (P less than .01). Significant differences were also shown when Group A was compared to Group C (P less than .01) and again when Group B was compared to Group C (.05 more than P less than .01).
Table 3

Mitotic Index

Epithelium

<table>
<thead>
<tr>
<th>Control Normal</th>
<th>Adjacent to Normal</th>
<th>Crown Inflamed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 0.3</td>
<td>0.4</td>
<td>1.0</td>
</tr>
<tr>
<td>2. 0.4</td>
<td>0.5</td>
<td>0.4</td>
</tr>
<tr>
<td>3. 0.2</td>
<td>0.4</td>
<td>0.7</td>
</tr>
<tr>
<td>4. 0.3</td>
<td>0.5</td>
<td>0.8</td>
</tr>
<tr>
<td>5. 0.4</td>
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<tr>
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<tr>
<td>7. 0.3</td>
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<tr>
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<td>0.6</td>
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</tr>
<tr>
<td>9. 0.3</td>
<td>0.4</td>
<td>0.7</td>
</tr>
<tr>
<td>10. 0.5</td>
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<table>
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<tr>
<th>Average</th>
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<tr>
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<td>Standard Deviation</td>
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<tr>
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</tr>
<tr>
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<tr>
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</table>
Cell Density:

The cell density in the epithelium along the basal cell layer was studied in two groups only, that is control Group A and clinically inflamed gingiva with crown Group C. The cell density was measured as the number of the cells per 100u² in the epithelium. The cell density in Group A ranged from 58.39/100u² to 87.69/100u² with an average of 69.7/100u².

In Group C the cell density ranged from 48.39/100u² to 92.3/100u² with an average of 66.2/100u². The cellular density did not differ significantly between control and experimental gingival epithelium. Please refer Table 5 for values.
Table 5

Cell Density (100u)$^2$

<table>
<thead>
<tr>
<th>Control gr. A (Normal Gingiva)</th>
<th>Inflamed Gingival (Adjacent to Crown)</th>
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</thead>
<tbody>
<tr>
<td>1. 86.7</td>
<td>71.3</td>
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<tr>
<td>2. 74.5</td>
<td>92.3</td>
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<td>59.5</td>
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<td>48.4</td>
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<tr>
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<td>11. 73.1</td>
<td>69.6</td>
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<td>12. 70.2</td>
<td>67.3</td>
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<tr>
<td>13. 63.8</td>
<td>61.2</td>
</tr>
<tr>
<td>14. 70.5</td>
<td>57.7</td>
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Average: 69.7, Standard deviation: 7.3

Average: 66.2, Standard deviation: 11.47
<table>
<thead>
<tr>
<th>Variable</th>
<th>Number of Cases</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>T-Value</th>
<th>Degrees of Freedom</th>
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<tbody>
<tr>
<td>A</td>
<td>14</td>
<td>69.7</td>
<td>11.47</td>
<td></td>
<td>0.98</td>
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<td>C</td>
<td>14</td>
<td>66.2</td>
<td>7.3</td>
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</table>
CHAPTER V

DISCUSSION

This study was undertaken to determine the histological response to complete crown restorations. Keratinization, histochemical reactions, inflammatory changes, collagen density of gingiva surrounding restored and unrestored teeth were taken into account to evaluate dental therapy.

Mitotic index (number of cells in mitosis per 1000 cells) was determined for epithelium near the basal cell layer. The cell density (number of cells per unit area (100u²) was also determined for epithelium using basal cell layer as a guide.

Qualitative Study:

Parakeratosis is found in the epithelium of clinically normal gingiva. However, it is seen also in the gingiva adjacent to crowns which appear clinically normal or inflamed. As parakeratosis is normal in attached masticatory mucosa, this study shows that complete crowns or prosthesis does not change the tissue keratinization.
Glycogen granules are found in the epithelium of all groups studied. These granules are found in the spinous layer cells towards the surface epithelium. The basal cell layer did not demonstrate any glycogen granules. As this is a normal finding for the keratinized attached gingiva, it shows that glycogen synthesis proceeds normally in gingiva adjacent to crown or prosthesis.

Inflammatory Response:

It is evident that a few inflammatory cells may be found in clinically normal gingiva. ORBAN has shown that few inflammatory cells are always found in normal gingiva. In teeth with prosthesis the gingiva show that inflammatory cells are present around dilated blood vessels. This indicates that clinically normal appearing gingiva is slightly inflamed when in contact with a crown. It suggests that prosthesis causes gingivitis. This is supported by the observation that clinically inflamed gingiva adjacent to prosthesis show heavy infilteration of polymorphonuclear leucocytes, lymphocytes and plasma cells. Also the capillaries and venules are dilated. Polymorphonuclear leucocytes were also found in the epithelium. These are expected and usually are seen in clinically inflamed gingiva.
WAERHAUG (53,60) in his study on monkeys, found that sub-gingival restorations cause inflammation as compared to supragingival restorations.

MARCUM (76) in his study on dogs confirmed the study of Waerhaug, that crown margins above the gingiva or at gingival margins caused least inflammation, while margins located below the gingival crest caused the most severe inflammatory response. But we feel that in cases where the caries rate, oral hygiene and esthetic is a problem, one has to consider the placement of the margins of the crown subgingivally. Then one must expect chronic gingivitis to arise in such cases.

RICHTER (73) in his clinical study placed complete crown restorations both supragingivally and subgingivally. He did not find any significant differences in gingival health by comparing the two locations or by plaque accumulation. His study differs from Waerhaug and Marcum.

WHEATCROFT, SCIANTARELLI (74) who stressed oral hygiene as the important factor in preventing gingival inflammation.

MARURAMA, SIMOOSA, OJIMA (76) found that the clinically normal gingiva adjacent to natural teeth did not exhibit dilated capillaries, while capillary loops in the gingiva adjacent to the complete crowns
showed dilatation. He also found capillary dilatation in gingival tissue adjacent to restored crown which appeared clinically normal. This experiment shows that few inflammatory cells always are seen in clinically normal gingiva, but the intensity is different. Normal gingiva adjacent to natural teeth showed few lymphocytes and plasma cells, while normal appearing gingiva adjacent to crowns show apparently more inflammatory cells and greater numbers of capillaries are dilated. Clinically inflamed gingiva adjacent to crowns show dense infiltration of inflammatory cells and dilated capillaries. It is believed that dilatation of capillaries in gingiva adjacent to complete crowns suggest that though the tissue looks clinically normal, there is an inflammatory process, following the placement of complete crown restorations. The density of collagen fibres is lost where inflammation is present. This finding is an expected finding in the pathogenesis of gingivitis accompanying inflammation.

**Mitotic Index:**

The mitotic index has been used as an experimental tool. Mitosis significantly increases in the epithelium of the interdental papillary tissue, when a crown is placed on a tooth. It is suggested that the induced
inflammation causes the increase in mitosis. The gingiva from subjects which appeared clinically normal shows essentially a normal morphology, histologically. The clinically normal gingiva adjacent to prosthesis shows both a slight increase in mitosis and inflammatory reaction greater than clinically normal gingiva. This indicates that prosthesis may cause low grade irritation with inflammation.

The clinically inflamed gingiva adjacent to prosthesis shows both increased mitotic activity as well as increased inflammatory exudate, furthermore it is accompanied by a decrease in collagen density. This indicates that prosthesis does indeed contribute or more frequently is associated with gingivitis. This may represent increase in the inflammatory reaction in cases of prosthesis, adjacent to apparently clinical normal gingiva.

It is evident that when compared to normal gingiva, there is an increase in gingivitis both histologically as well as clinically in patients with fixed restorations or crowns.

This study differs from the results of MARWAH (56) who showed higher mitotic index, however he also showed that mitosis increases with age. He also
concluded that there is possible relation between carcinoma in older age groups to the loss of mechanisms, which govern cell division.

MARWAH (60) reported that mitotic index increases with inflammation from 1.5 to 3 times. Our study also shows that mitosis increases with inflammation. GARGIULO (61) showed mitotic index increases also with age. SILBERKWEIT (63) in his study on mitotic index in the epithelium of male and female inflamed gingiva, the average mitotic index for males was .514 and .575 for females. Our results were very close to the Silberkweit study.

Cell Density:

In this study the cellular densities of normal gingiva and inflamed gingiva adjacent to a crown does not differ significantly. The epithelial morphology appears similar in clinically normal gingiva and this is reflected by the similarity in cell density, although apparently more inflammation of the gingiva adjacent to crown is seen, the cell density remains normal. Soni and Silberkweit (65) also studied the cell densities in normal and inflamed gingiva. They also did not find any significant difference between the cell densities of normal and inflamed gingiva.
This agrees with our findings. Silberkweit also noted that as mitotic activity increased, cell density decreased. This differs from our study, as in the inflamed gingival sections where mitosis increases the cell density does not show any decrease.
CHAPTER VI

SUMMARY AND CONCLUSION

In this study, eleven to fourteen patients were used, whose extensive dental work had been completed from two to five years. Three biopsies were taken from each patient. Interdental papillary tissue was taken adjacent to unrestored tooth which appeared clinically normal and gingiva adjacent to restored tooth which appeared normal and inflamed. All the subjects selected were systemically normal and ranging in ages 30-68 years.

Biopsies were taken during daytime and specimens were fixed in formalin, embedded in paraffin, sectioned at 6 microns and stained with hemotoxylin and eosin, periodic acid schiff (PAS) and mallory. A section of each specimen was selected at random and placed on the microscope and studied at 450x for morphologic characters. All the specimens showed parakeratosis and glycogen in the stratum spinosus near the surface. Inflammatory cells were found in all sections, though intensity differed. In normal gingiva adjacent to unrestored
tooth, few inflammatory cells were noted, while gingiva adjacent to restored tooth, which appeared clinically normal showed more inflammatory cells and blood vessels were dilated. The gingival sections adjacent to restored tooth which appeared clinically inflamed, showed heavy infiltration of inflammatory cells and blood vessels were dilated. The collagen density was normal in gingiva which appear normal adjacent to unrestored tooth, while there was loss of collagen fibres around blood vessels, where inflammation was present. The mitotic index and cell density were determined by using an eye piece reticular representing $(100\mu)^2$ at 450x. An average mitotic index of 0.350 was determined in normal gingiva without any restoration. The gingiva, which appeared clinically normal adjacent to crown, the average mitotic index was 0.50 and for inflamed gingiva adjacent to restored tooth, the average mitotic index was 0.681.

The epithelial cell density for clinically normal gingiva without complete crown was 69.7 cells per $(100\mu)^2$ and for inflamed gingival tissue, adjacent to a crown the average was 66.2 cells per $(100\mu)^2$. 
There is always low grade inflammation and some loss of collagen in the gingival tissue adjacent to complete crown restorations, even when gingival tissue appears clinically normal. The mitotic activity of squamous epithelium increases with the placement of crown and probably results from the inflammation. The cellular density does not differ significantly in the clinically normal gingiva adjacent to natural teeth and inflamed gingiva adjacent to a restored tooth with a crown.

There is an apparent normal morphology of the epithelium and a normal distribution of glycogen in squamous epithelial cells after crown restorations.
CHAPTER VII

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APPROVAL SHEET

The thesis submitted by Meera Mahajan has been read and approved by three members of the faculty of the Graduate School.

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

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

Date: May 14, 1976

Signature of Advisor
PATRICK D. TOTO, D.D.S., M.S.