THE INTERRELATIONSHIP OF EPITHELIAL RESTS OF MALASSEZ WITH ORTHODONTIC ROOT RESORPTION AND REPAIR IN MAN

A project report submitted in partial fulfilment
of the requirements for the degree of
Master of Dental Surgery

by

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ADDENDA

Page xii; Add "IgG Immunoglobulin G" to the list of abbreviations under TEXT

Page xii; Add "dd Double Distilled" to list of abbreviations under TEXT

Page xiii, line 6. "orthdontic" should read "orthodontic"

Page 31, Paragraph 2, Line 3; Delete"E" and "F"

Page 31, Paragraph 2, Line 5; Delete "E" and insert "epithelial-like"

Page 70, Paragraph 1; Insert at the end "However some cervical regions were cut into 4 blocks."

Page 84, Last paragraph, Line 5; Should read "....in one of the lower premolars...."

Page 87; Insert at the bottom of page "Note: The numbers in the brackets after the specimen refer to the tooth extracted."

Page 119, Paragraph 3; First line should read 'One of the lower premolars...."

Page 143, Paragraph 2, Line 6; Correct the spelling of "discipate" to "dissipate"

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

FIGURES

AA	Adhesive Attachment
AR	Active Resorption
B	Basement Membrane
BV	Blood Vessel
C	Cellular Cementum
CA	Continuous Attachment
CB	Cementoblast
CC	Clear Cell
CE	Centriole
CF	Collagen Fibres
CI	Cilia
CM	Cementoid
CO	Companion Cell
CP	Cytoplasmic Process
D	Dentine
DJ	Desmosome-like Junction
DC	Dark Cell
E	Epithelial Cell
EC	Epithelial Cell Cluster
EE	Endocytosis/Exocytosis
ER	Epithelial Rest of Malassez
F	Fibroblast
G	Granular Substance
I	Invaginations of the Nuclear Membrane
L	Lipid Body
M	Mitochondria

MV	Microvilli
N	Nuclear Pore
O	Odontoclast
P	Phagocytosis
PF	Periodontal Fibres
R	Ribosomes
RB	"Ruffled" Border
RE	Rough Endoplasmic Reticulum
RL	Reversal Line
RR	Repairing Resorption
RS	Root Surface
T	Tonofilaments
TD	True Desmosome
TJ	Tight Junction
U	Undermining of the Root Surface
V	Vacuoles

TEXT

AIF	Anti-Invasion Factor
AEFC	Acellular Extrinsic Fibre Cementum
CMSC	Cellular Mixed Stratified Cementum
CIFC	Cellular Intrinsic Fibre Cementum
ECC	Epithelial Cell Cluster
EGF	Epithelial Growth Factor
ERM	Epithelial Rest of Malassez
HERS	Hertwig's Epithelial Root Sheath
RME	Rapid Maxillary Expansion
SEM	Scanning Electron Microscope
TEM	Transmission Electron Microscope

SUMMARY

A number of authors (LÖE and WAERHAUG, 1961; SPOUGE, 1980 and LINDSKOG et al.,1983,1988b) have suggested that epithelial rests of Malassez may contribute to the integrity of the periodontal ligament, prevent ankylosis and protect the root surface from resorption. However, based on previous light microscope studies, REITAN (1985) stated that epithelial rests are not present in the periodontal tissue adjacent to areas of orthdontic root resorption and subsequent repair. As the postulates of LÖE and WAERHAUG (1961) and the observations of REITAN (1985) are conflicting, the purpose of this study was to determine the interrelationship of epithelial rests of Malassez with orthodontic root resorption and repair in man.

Human, first premolars used as anchor teeth during rapid maxillary expansion were extracted for orthodontic purposes from five adolescent patients. Buccal slivers were removed and prepared for high power light microscope and transmission electron microscope evaluation.

Epithelial cell clusters were found in repairing root resorption bays of anchor premolars, in four patients. The epithelial clusters ranged in size from three to seven cells and were characterized by the presence of true desmosomes (macula adherens) and tonofilaments. Other common features included a discontinuous basement membrane, cilia, tight junctions, nuclear pores, numerous free ribosomes and vesicles. Similar ultrastructural features were found in epithelial rests of Malassez adjacent to active cementogenesis on non-resorbed surfaces. No epithelial clusters were observed in areas of active root resorption but collagen fibre bundles were seen attached along the actively resorbing surface.

It was concluded that regenerating epithelial cell clusters are present in repairing root resorption bays caused by orthodontic tooth movement in man and by their presence and ultrastructural features are implicated in the repair process.

SIGNED STATEMENT

This report contains no material which has been accepted for the award of any other degree or diploma in any other University. To the best of my knowledge and belief, it contains no material previously published except where due reference is made in the text.

Garth L. Brice.

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INTRODUCTION

Epithelial rests of Malassez are remnants of Hertwig's epithelial root sheath and the dental lamina which persist in the periodontal ligament (SCHROEDER, 1986). Although their presence can lead to the formation of dental cysts, their function within the ligament is still unknown (TEN CATE, 1985).

In a study of replanted teeth in dogs and monkeys, LÖE and WAERHAUG (1961) postulated that the epithelial rests may play a role in the maintenance of the periodontal ligament by limiting the degree of root resorption and preventing ankylosis. SPOUGE(1980) interpreted these findings to mean that the epithelial rests may limit the encroachment of bone within the periodontal ligament and protect the root surface from resorption.

Similarly, in a study of the repair of experimental cavities in the roots of replanted monkey incisors, LINDSKOG et al. (1983) found that epithelial cells were numerous in the connective tissue separating the reparative cementum from alveolar bone in the experimental cavities. They postulated that epithelial cells may contribute to the integrity of the periodontal membrane and prevent resorption of the dental root.

Studies of epithelial cultures grown in vitro from epithelial rests of Malassez , have demonstrated that epithelial cells are not quiescent in culture. They secrete several proteins (BRUNETTE et al., 1979b), secrete prostoglandins (BRUNETTE et al., 1979a; BIREK et al., 1980), secrete a potent bone resorbing factor (BIREK et al., 1983); are capable of phagocytosis of collagen (BIREK et al., 1980); interact with fibroblasts (BRUNETTE et al., 1977), secrete proteins capable of inhibiting collagenolytic enzymes (PETTIGREW et al., 1980) and respond to environmental stimuli through an alteration in their enzymatic

and protein synthesizing mechanism (NYLEN and GRUPE, 1969). These findings indicate that epithelial cells can proliferate, penetrate connective tissue, limit the encroachment of bone and perhaps the degree of root resorption, thus supporting the postulate of LÖE and WAERHAUG1961) and SPOUGE (1980).

More recently, LINDSKOG et al., (1988b) explanted enamel organ epithelium into experimental cavities in the root surfaces of extracted and subsequently replanted monkey incisors. Their results suggested that epithelial rests of Malassez may maintain the width of the periodontal space and prevent dento-alveolar ankylosis.

However, REITAN (1961) in an histological study of the behaviour of epithelial rests of Malassez during orthodontic movement of human teeth, noted that the rests were absent in the periodontal tissue adjacent to areas of root resorption and that repair of the root surface occurred in the absence of the epithelial rests of Malassez. This suggests that they are not involved in limiting root resorption and maintaining the integrity of the periodontal ligament, as suggested by LÖE and WAERHAUG (1961), SPOUGE (1980) and LINDSKOG et al., (1983,1988b).

Similarly, in the first and only transmission electron microscopic study of orthodontic root resorption in human teeth, RYGH (1977) found that root resorption takes place simultaneously with and after the elimination of hyalinized tissue. However, no epithelial rests were reported within the resorption lacunae.

In view of this apparent conflict in the literature and the importance of preventing root resorption during tooth movement, a light microscope and a transmission electron microscope study of the interrelationship between epithelial rests of Malassez and orthodontic root resorption is indicated. For

this purpose, the buccal surface of human premolars used as anchor teeth during rapid maxillary expansion was examined for the responses to root resorption.

Should the postulates of LÖE and WAERHAUG (1961) and LINDSKOG et al., (1988) prove to be correct, proliferating epithelial cells should be present in the vicinity of the repairing root surface and conversely, should not be present in areas of active resorption.

AIMS OF THE INVESTIGATION

1) To identify epithelial cells in the periodontal ligament of orthodontically moved teeth and by light microscope and transmission electron microscope techniques, determine the interrelationship of epithelial rests with orthodontic root resorption along the buccal root surface of premolars used as anchor teeth in rapid maxillary expansion.

2) Determine the morphology and distribution of epithlelial rests of Malassez in the following situations:

a. Active resorption

b. Repairing resorption

c. Active and repairing resorption

d. Non-resorbed surfaces

3) If ERM are present in areas of resorption, compare their morphology and ultrastructural features with ERM adjacent to non-resorbed surfaces.

4) Investigate the cellular components of the resorption bays, active and repairing, small and large, at an ultrastructural level, by use of the transmission electron microscope.

CHAPTER 1

LITERATURE REVIEW

1.1 EPITHELIAL RESTS OF MALASSEZ

A. EMBRYOLOGICAL ORIGIN OF ERM

Epithelial rests of Malassez (ERM) are remnants of Hertwig's epithelial root sheath (HERS) and the dental lamina persisting in the periodontal ligament (SCHROEDER, 1986).

Just prior to the start of root formation ameloblastic activity has essentially been completed, so that much of the anatomical crown is covered by reduced enamel epithelium. The cervical margin of the enamel organ is located at the future cementoenamel junction and consists mainly of an inner and outer layer of dental epithelium with no intervening cells, called the cervical loop (SPOUGE,1980).

The process of root formation is initiated by mitotic activity occurring within the cervical loop, to produce apical elongation of its double layer of epithelial cells, to form a diaphragm referred to as Hertwig's epithelial root sheath (HERS). This sheath directly induces formation of the specific tissues which comprise the root (SPOUGE, 1980).

During root formation the sheath consists of two parts, the diaphragm and the lateral sheath. The epithelial diaphragm is the short, horizontal, leading component, which is shaped like a solid disc with a central circular hole. This double sheet of cells is continuous on its outer perimeter with the remainder of the sheath, termed the lateral sheath, which runs coronally along the future dentino-cemental junction. Root elongation occurs

progressively as a result of cell proliferation within the sheath (SPOUGE,1980).

There is an equivalent proliferation in the adjacent connective tissue cells, both on its inner (pulpal) and outer (periodontal ligament) surface. Odontoblasts differentiate within the pulp under the influence of the root sheath and start to secrete dentine matrix between themselves and the root sheath. The preodontoblasts, along with an increasing amount of accompanying predentine, move progressively outwards until they reach the future dentine cemental junction. At this stage, the outer layer of dentine matrix starts to calcify. Immediately, as this happens, the epithelial cells of the root sheath separate from the dentine surface and breaks occur in the continuity of its previously continuous double layered sheets of cells. These remnants of the sheath then move out into the ligament to form a network referred to as the epithelial rests of Malassez (SPOUGE, 1980).

However, there is considerable conflict in the literature concerning the fate of the root sheath, particularly in the rat and mouse. It has been proposed that the epithelial remnants degenerate (DIAB and STALLARD, 1965; SHIBATA and STERN, 1967; LESTER, 1969a; FREEMAN and TEN CATE, 1971, MCLEAN, 1984), became encapsulated within the cellular cementum near the apex (DIAB and STALLARD, 1965; LESTER, 1969a; FREEMAN and TEN CATE, 1971; GURLING, 1982; MCLEAN, 1984) or migrate into the periodontal ligament and form well circumscribed ERM close to the root surface (WENTZ et al., 1950; BEERTSEN and EVERTS, 1979; GURLING, 1982; MCLEAN,1984; DAVEY, 1986).

KENNY and RAMFJORD (1969) studied histologically and autoradiographically the cellular dynamics in root formation of teeth in Rhesus monkeys. No discernable migration of labelled epithelial cells from Hertwig's root sheath into the periodontal ligament was detected, and no epithelial rests were present in the apical regions. They suggested that the rests which were

present in the upper portion of the developing periodontal ligament may have been derived from fragmentation of the root sheath at the initial stages of root development. However, at that period there was no lateral root sheath. There was no labelling of the coronal rests indicating that they were not actively dividing.

GURLING (1982), GURLING and SAMPSON (1985) proposed that the initial discontinuity between the enamel organ and the otherwise continuous bicellular lateral root sheath in 11 day old mouse material , results in isolated epithelial cells which probably form Type I rests. It was proposed that most of the epithelial cells could be carried occlusally within the periodontal ligament during tooth eruption. However, it was possible that some may have been retarded in their occlusal movement and thus contribute to more apically sited rests. Type II rests appeared to form from inner epithelial cells of the root sheath as they progressively separate from the coronal extremity of an otherwise continuous inner epithelial layer. (Figure 1.1)

DAVEY (1986) proposed that once the dentine formation was underway the continuous layer of Hertwig's epithelial root sheath disrupts through cellular contraction producing fenestrations, firstly in the outer layer and progressively in the inner layer. This resulted in an epithelial network, the continuity of which could not be demonstrated. (Figure 1.2) No autoradiographic labelling of DNA synthesizing cells was observed in the lateral sheath, suggesting that the cells were not dividing.

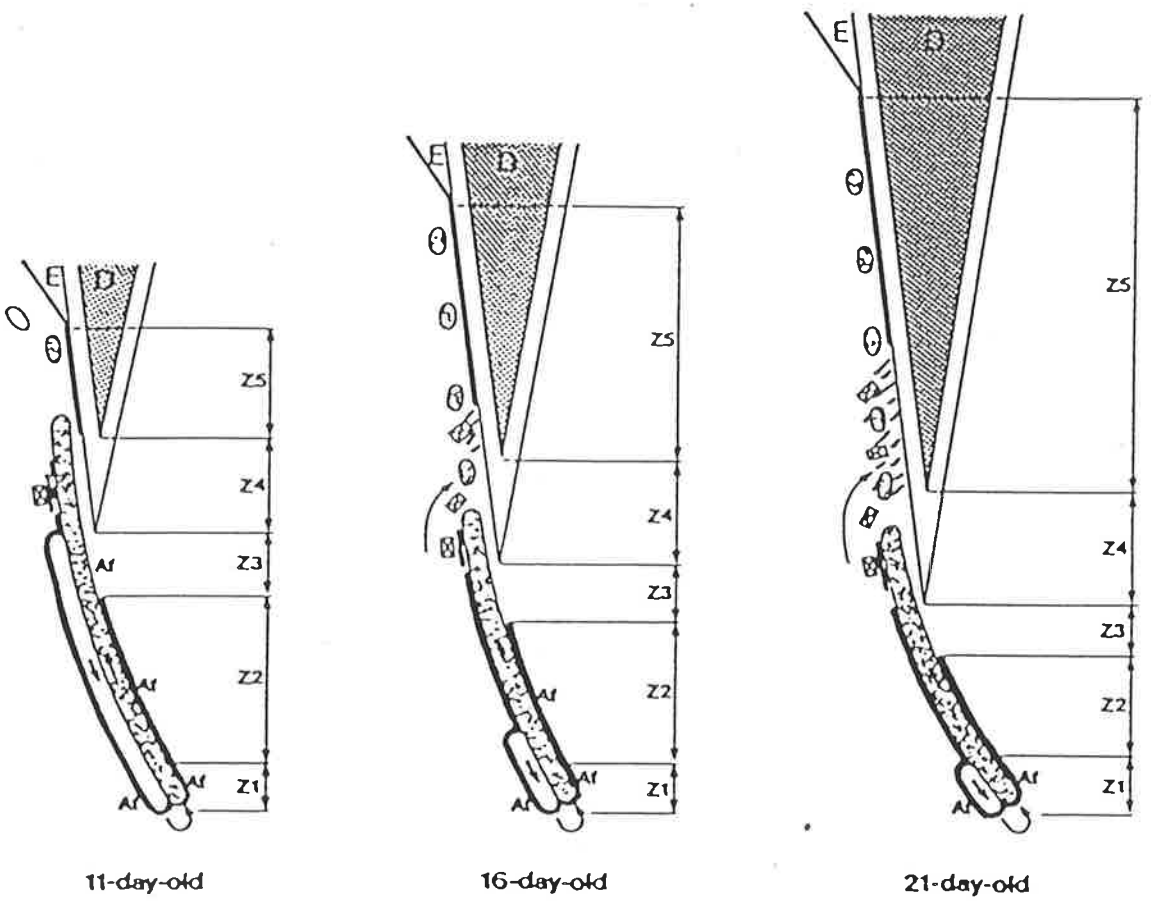

FIGURE 1.1 : Diagrammatic representation of root sheath changes from 11-21 days. The proposed direction of epithelial cell movement (arrows) favours maintenance of the inner layer of the sheath which subsequently disrupts to form ERM. (Adapted from GURLING, 1982)

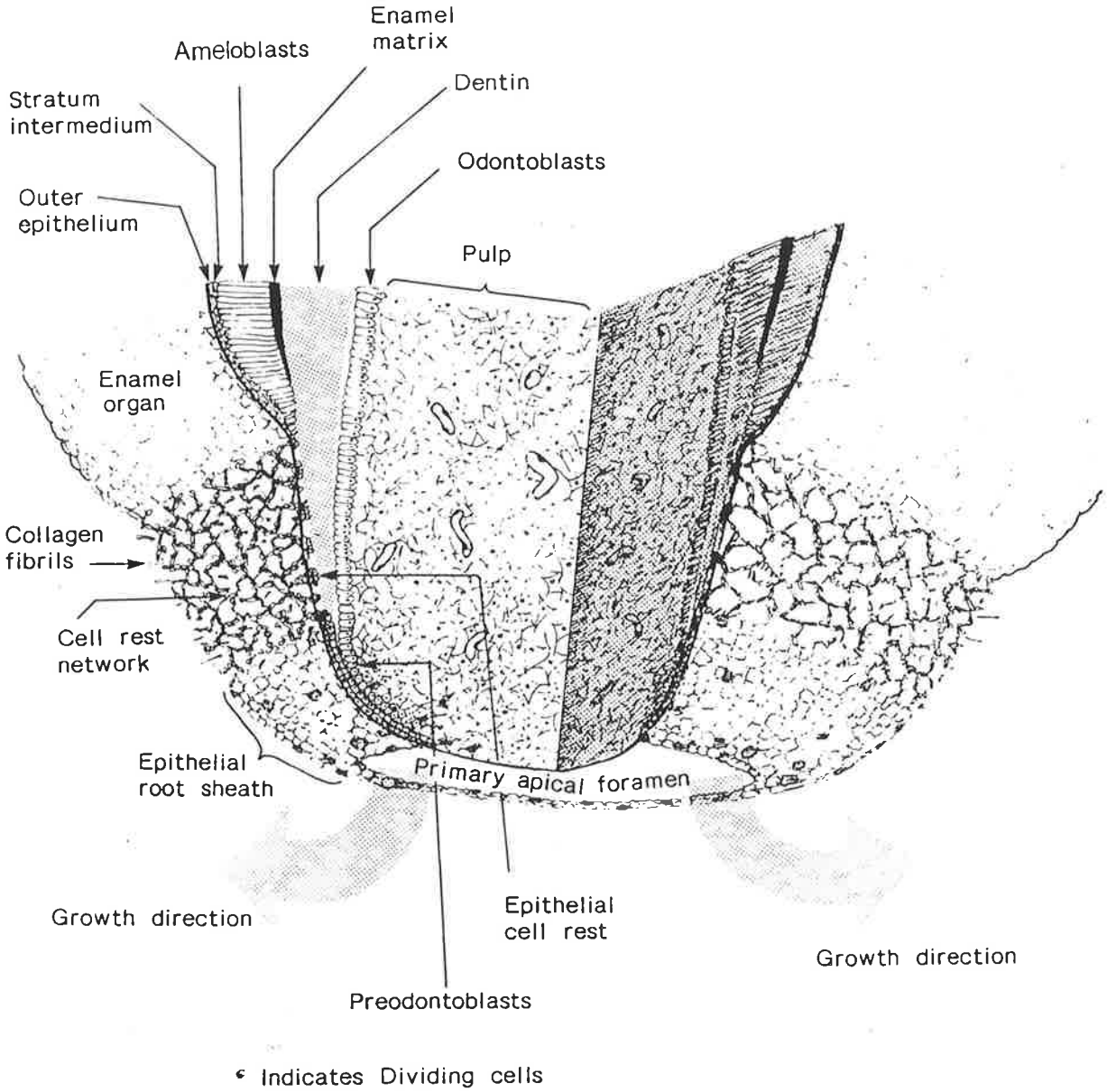

FIGURE 1.2 : Schematic diagram showing the formation of the epithelial network from the fenestration of Hertwig's epithelial root sheath. (Adapted from DAVEY, 1986)

B. MORPHOLOGY, DISTRIBUTION, PREVALENCE OF ERM

The distribution, morphology and prevalence of the epithelial rests of Malassez vary widely between species and with age, which may explain the apparent conflict in findings between various authors.

1) Human

SPOUGE(1980) in a review of the rests of Malassez, described their anatomy. They form an epithelial network which runs circumferentially within the periodontal ligament. Transverse sections of this network are most frequently seen as small, solid, circular or oval aggregates or strands, from approximately four to twenty epithelial cells. Individual clusters are dispersed at intervals down the ligament, like cross sections of the rungs of a ladder, running close to the cementum. Occasionally, the cords run further out into the ligament winding in and out of the fibre bundles. It was suggested that the divergence of opinion as to the relative density of the rests, may be due to the difficulty in distinguishing them under the light microscope. Similar descriptions were presented by TEN CATE (1985) and ORBAN (1986).

SPOUGE (1980) considered that it was likely that the rests of Malassez must be incorporated into the attachment in the cemental region which then contributed to the pathogenesis of pocket formation. The anatomical relationship resembled a basketball hoop with the network of rests suspended from, and in continuity with, the epithelial attachment.

VALDERHAUG and NYLEN (1966) in a study of fifty human teeth from patients aged 10 to 27 years, observed an extensive network of epithelial rests in almost all the periodontal ligaments. Strands, clusters and small islands of epithelial cells were observed, but usually all of these were found by examination to be interconnected. The strands and clusters contained

closely packed cells with large nuclei and a fairly narrow rim of cytoplasm. In the small cell islands, the nuclei were generally located peripherally while the larger portion of the cytoplasm was concentrated toward the centre. The rests were surrounded by an outer argyrophilic limiting membrane.

SIMPSON (1965), using the apoxestic (scrape off) technique, observed the degeneration of rests of Malassez with age in ninety-seven teeth from patients varying in age from 8.5 to 64 years. It was noted that in the youngest specimens the network of epithelial cells is well developed, resembling a perforated sheet rather than a net. With the passage of time, the amount of epithelium diminishes and the network becomes widely meshed. Later, the network breaks up to many isolated strands and islands. Finally, only scattered remnants of epithelium are present.

GRANT and BERNICK (1972) in a study of the periodontium of aging humans, found that epithelial rest aggregates were prominent by their size. The enlarged rests were variously situated in the periodontal ligament rather than being aggregated close to the cementum as in the young. The rests were encapsulated by a thick PAS positive basement membrane. Proliferated rests were found in inflamed tissue within marrow spaces indicating both tooth migration and cellular proliferation.

As rests degenerated there was a loss of the basement membrane, cells became widely spaced and their nuclei stained indistinctly. The periodontal fibres became hyalinized. Calcification appeared to accompany degenerative cell changes, resulting in calcified bodies within the periodontal ligament or in cemental exostosis when the calcified rest group approximated cementum.

VALDERHAUG and ZANDER (1967) studied the relationship of epithelial rests of Malassez to other periodontal structures in fifty human teeth, from patients

11 to 22 years of age. They utilized the apoxestic technique (SIMPSON, 1965) and 1µm sections cut from resin blocks and stained with toluidine blue. With toluidine blue, the nuclei of the epithelial cells stained dark blue while the cytoplasm appeared lighter blue. Fibroblasts had more lightly stained nuclei and cytoplasm whilst cementoblasts were larger, more cuboidal than fibroblasts and contained a large dark staining nucleus. Their cytoplasm stained more deeply than that of the fibroblasts but not quite as heavily as that of the epithelial cells.

The epithelial rests were less numerous apically than the mid root or cervical areas. Long strands parallel to the root surface were prevalent in the apical region. In contrast, round or oblong islands, each containing 4-10 cells dominated cervically and they were often orientated with their long axis nearly perpendicular to the cementum. In either area, orientation conformed to that of the adjacent collagen fibres.

The average distance from cementum to the epithelial cells varied: 27µm in the apical region, 33µm in the mid-root region and 41µm in the cervical region, suggesting that the distance between the rests and the cementum was not a constant factor. Rests were more numerous cervically than toward the apex of the tooth. No rests were observed in direct contact with cementum.

In silver impregnated sections, all the epithelial rests were surrounded by a dark brown argyrophilic zone 1-2µm thick. A dense PAS positive zone 1µm thick was also observed around the rests stained with periodic acid Schiff reagent (VALDERHAUG and ZANDER, 1967).

REEVE and WENTZ (1962) studied histologically the prevalence, morphology and distribution of epithelial rests in the human periodontal ligament of 280

permanent teeth obtained at necropsy, from persons ranging in age from 1 to 77 years. Three types of epithelial rests were observed:

1) RESTING TYPE: These rests varied in form from strands to oval groups with an average of ten polyhedral cells, depending on the line of section. They were observed close to the cementum and communicated with one another to form a netlike arrangement. They were present in all specimens from all age groups but decreased with age. Larger rest types were found in ovoid or spherical groups, composed of an average of twenty eight cells. These groups did not form strands and were surrounded by a loose concentric connective tissue. They appeared in the fourth and later decades. Occasionally they formed "ductlike" stuctures with a central amorphous mass and a thin basement membrane surrounding the rest.

2) DEGENERATED TYPE: This type was observed in all age groups but most frequently in persons in the first and second decades. They decreased with age and the majority were located in the apical and middle zones of the periodontal ligament in close proximity to the cementum. In cross section, these rests appeared oval shaped and contained an average of ten cells in each rest. The nuclei were dark and pyknotic and hydropic degeneration was apparent in individual cells. In the later decades, the rests calcified forming cementicles.

3) PROLIFERATING TYPE: Rests of this type, located close to the cementum, were observed in the later decades and increased with age. They were the largest rests, averaging eight to ten times the size of the small resting type and often surrounded by a well differentiated fibrous capsule.

The majority of the epithelial rests were located in the cervical area of the teeth in all ages except during the first and second decades, where they were greatest in the apical region. REEVE and WENTZ (1962) postulated that

constant chronic inflammation around the gingival sulcus may cause proliferation or at least, promote the persistence of the epithelial rests in this area of the tooth.

REEVE (1960) postulated that the three morphological types represent different stages in the life cycle of the epithelial rests. The distribution of the rests varies with age; the majority of the rests are in the cervical area in all age groups except those from eleven to twenty.

2) Rat and Mouse

There is considerable controversy in the literature over whether or not well defined rests persist in the periodontal ligament in rats and mice. DIAB and STALLARD (1965), SHIBATA and STERN (1967), FREEMAN and TEN CATE (1971) and LESTER (1969a) suggest that the remnants of HERS become encapsulated in the cellular cementum near the apex or degenerate and do not form ERM within the ligament.

However, numerous authors have suggested that ERM do persist within the periodontal ligament of rats and mice. (WENTZ et al., 1950; JOHANSEN, 1970; LISTGARTEN, 1975; GURLING, 1982; GURLING and SAMPSON, 1985). This divergence of opinion may be due to the difficulty in distinguishing them under the light microscope (SPOUGE, 1980). However, LESTER (1969a) used TEM but did not identify them.

WENTZ et al. (1950) classified the different histological forms of epithelial remnants within the periodontium of 105 rat molars into three main cell types:

1) RESTING TYPE: This form was arranged in either strands or small islands, the islands being cross sections of the strands. The islands consisted of an average of 7-10 cuboidal cells, arranged in spherical groups 30μm diameter and were found close to the cementum. The

strands contained an average of 2-5 cuboidal cells and appeared to communicate with other epithelial remnants. In most instances the connective tissue adjacent to the epithelial rests showed hyalinization. These rests decreased with increasing age and it was postulated that this may have occurred by a) disintegration and resorption b) degeneration and consequent calcification into cementicles or c) change into the proliferating type.

2) PROLIFERATING TYPE : This was the most frequent type and probably represents the stage in which the rests survive the longest. Two variations were observed:

a) Smaller form: consisting of 35-40 cells with small ovoid nuclei. Closely packed hyalinized fibres were distinguished in the capsule.

b) Larger form: consisting of 50 -100 cells with large, well formed nuclei. These were usually observed in the interdental space and the cervical third of the molars. It was postulated that these rests either degenerated or proliferated under the influence of some irritating factor and transformed into the differentiating type.

3) DIFFERENTIATING TYPE: These epithelial remnants increased with age and always occurred in either the bifurcation area or the supra-alveolar area and correlated to the resorption of the alveolar septum. They consisted of ovoid islands of cells averaging 390µm at the broadest diameter. The peripheral cells were cuboidal and arranged regularly on a basement membrane. Whereas the central cells were more loosely arranged and generally had smaller nuclei with oval to spherical shape. Numerous mitotic figures, a wide capsule surrounding the cells and epithelial pearl- like stuctures were also noted.

Rests were found in only 50% of their specimens. Of those found 47% were in the supra-alveolar area, only 8% in the apical region and 22-30% in the

mid root region. WENTZ et al. (1950) postulated that the three morphological types represented successive stages of the epithelial remnants which proliferated when stimulated by traumatic factors.

TROWBRIDGE and SHIBATA (1967) employing autoradiographic techniques, observed mitotic activity within epithelial rests of Malassez, in the bifurcation area of rat lower molars. Mitotic activity coupled with the apparent compression of adjacent connective tissue elements, suggested that these cells were actively proliferating. They appeared gland-like and a reticulum surrounding the rests indicated that they were contained within a basement membrane.

JOHANSEN (1970) observed that epithelial rests were in close relationship to the cementum in the marginal third of the periodontal ligament of the first maxillary and mandibular molars of Wistar rats. Rests were located less frequently in the apical two thirds of the periodontal ligament. All specimens showed the presence of the rests compared to 50% in the study of WENTZ et al. (1950).

LISTGARTEN (1975) used ultrastructural and high power light microscopic techniques to show that well defined cell rests persist in the periodontal ligament of rats and mice. Not all of Hertwig's epithelial root sheath became entrapped within cementum during root development. The rests were in close proximity to the cementum surface and were composed of clumped epithelial cells with typical ultrastructural features. There were three times the number of rests in the coronal half than in the apical half of the periodontal ligament. Cell rests appeared as more or less round cross sections of grouped epithelial cells.

GURLING (1982) and GURLING and SAMPSON (1985) in an ultrastructural study of epithelial root sheath changes during molar formation in the mouse, found

epithelial rests in the periodontal ligament just apical to the cemento-enamel junction (Type I rests). Epithelial cells also lined the cementum surface at reasonably regular spaced intervals (Type II rests). The Type I rests were equivalent in their locali to those described by WENTZ et al. (1950). Cell rests were present in the upper portion of the periodontal ligament but not in the apical region. Type II rests were more prevalent and easier to identify than Type I and were observed in small clusters or as single cells adjacent to the cementum.

3) Swine

GRANT and BERNICK (1969) investigated histologically the possible continuity between epithelial rests and epithelial attachment in miniature swine. They found that there may be a continuity of the root epithelial network with the reduced enamel epithelium before eruption and with the attachment epithelium after eruption. This continuum may be persistent or it may be transient. Their postulate was supported by the following observations:

1) Cords of epithelial cells arising from the reduced enamel epithelium of the unerupted tooth projected into the periodontal ligament to be continuous with the epithelial network.

2) In the erupted tooth, the epithelial rests swing outward and coronally toward an epithelial cord that projected from the attachment epithelium.

3) The root epithelium formed a lattice network encircling the tooth parallel to the cementum. At the apex, the network appeared to run in and out of the cementum.

The shape of the rests was influenced by the direction and tension of the periodontal fibre bundles. When the rests lay between tensely stretched fibre bundles, they were seen as elongated strands and in the looser connective tissue they appeared as ducts or follicles. The rests were encircled by a

membrane that was PAS and Alcian Blue positive. When the rests were about to be entrapped in cementum the membrane was not demonstrable.

4) Sheep

CUTRESS and CRIGGER (1974) studied histologically the distribution and appearance of epithelial cells rests in the periodontium of sheep. They described a distinct, well organised network of epithelium which commenced in the vicinity of the attachment epithelium and continued to the apical region. Rests were most prominent in the gingival third and least prominent in the apical third of the periodontal ligament. They appeared more consistently allied to cementum. In a three dimensional model, cell rests appeared as terminal appendages to the microvasculature in the gingival one third and one half region, whereas in the apical third no apparent relationship was observed.

C. EFFECT OF ORTHODONTIC FORCES ON ERM

REITAN (1961) in a study of the behaviour of the epithelial rests of Malassez during orthodontic tooth movement, used first upper premolars of twenty five patients aged 11-12 years, upper incisors of dogs and monkey material. He made a number of observations including the following:

1) Epithelial rests are more numerous in the periodontal membrane of 11-12 year old children than in that of 35-40 year old adults. An epithelial network exists around all roots studied.

2) In the dog, the epithelial rests were less numerous than in the 11-12 year old children and were observed as round or oval groups. They were normally located some distance from the root surface in the marginal area.

3) In the monkey, the rests were round or oval and slightly longer than those of the dog.

4) On the tension side, tooth movement may result in some compression of epithelial cells between stretched fibre bundles, moving the rests slightly away from the root surface. During direct bone resorption, epithelial rests on the pressure side move slightly toward the root surface.

5) Hyalinization of periodontal fibres causes atrophy of cellular elements in a circumscribed area. Connective tissue cells disappear rapidly and epithelial cells a few days later. Proliferation of fibroblasts and regeneration of capillaries start as soon as the subjacent bone area is removed by undermining resorption. The epithelial cells of the formerly hyalinized tissue do not reappear.

6) Hyalinization and loss of epithelial rests always lead to undermining bone resorption. Root resorption also occurred in a number of cases.

7) Epithelial rests were not observed in the periodontal membrane adjacent to resorption lacunae of the root surface.

8) In the animal structures, comparatively few epithelial remnants were lost during hyalinization. As a result, bone resorption was direct rather than by undermining. Similar observations were made in the adult material (species not stated), where the epithelial cells in the marginal region were comparatively few.

9) The absence of epithelial cells was considered to be characteristic of reorganised, formerly hyalinized fibrous tissue on the pressure side of the material from the 11-12 year old children.

GILHUUS-MOE and KVAM (1972) studied histologically and autoradiographically the prevalence, morphology and proliferation of the epithelial remnants of Malassez following orthodontic movement of rat molars. On the pressure side undergoing hyalinization, a marked reduction or a complete absence of epithelial remnants occurred. On the tension side,

there was an increased prevalence of rests which appeared to be in a proliferating state. A rapid reduction in the proliferating activity of the epithelial remnants occurred shortly after the cessation of the mechanical force.

D. EFFECT OF INFLAMMATION ON ERM

VALDERHAUG (1974) studied histologically, the effect of periapical inflammation on the morphology, prevalence and distribution of epithelial rests of Malassez in primary and permanent teeth of monkeys. Epithelial cells in the shape of round elongated islands were seen in all sections but no net-like arrangement was observed. In the control teeth, the average distance from cementum to epithelial islands was largest in the marginal third and varied far more than in the apical region. The number of larger rests was also greater in the cervical region. Whereas, in the tooth with periapical inflammation, the average distance from the cementum to the rests and the number of epithelial islands were largest in the apical third of the periodontal membrane. Their findings suggested that periapical inflammation may have influenced the proliferative rate of epithelial cells in the periodontal membrane. About 50% of all the epithelial islands were located in the marginal third of the periodontal membrane in both control and experimental teeth.

SPOUGE (1984) utilised a three-dimensional reconstruction to study the gross morphology of epithelial residues in the periodontal ligament of a pig. The epithelial residues formed a continuous network, the coronal border of which was in intermittent direct continuity with the junctional epithelium.

E. ULTRASTRUCTURE OF ERM

Despite the differences reported in size, distribution and prevalence of ERM between species, they generally have very similar ultrastructural features.

1) Human

VALDERHAUG and NYLEN (1966) studied the ultrastructure of epithelial rests of Malassez of fifty human teeth from patients aged 10 to 27 years. They made the following observations:

1) Epithelial rests were separated from the connective tissue by a basement lamina.

2) The cells were irregular in outline, with many small interdigitating microvilli.

3) All cells appeared to have one surface abutting against the connective tissue and this was usually even, conforming to the smooth contour of the basement lamina.

4) Hemidesmosomes were observed regularly.

5) Collagen and thin non-striated fibrils were present in the immediate vicinity of the epithelial rests.

6) Desmosomes and tight junctions were seen frequently between the epithelial cells. The tight junctions followed straight or slightly wavy courses over variable distances, with the largest one seen measuring 1.5 μm.

7) The cytoplasm was usually rather dense.

8) The mitochondria averaged 0.2-0.3 microns in length and contained fairly indistinct cristae.

9) Rough-surfaced endoplasmic reticulum were seen occasionally and the Golgi apparatus was not identified positively.

10) A large number of fine filaments gather together into tonofibrils, some of which inserted into the attachment plaques of the desmosomes and hemidesmosomes.

11) Accumulation of dense glycogen granules with a diameter of about 250Å were frequent in the cytoplasm.

12) Large dense lipoid bodies, measuring up to one micron in diameter were present.

TEN CATE (1967) observed the ultrastructure of human Hertwig's epithelial root sheath. The presence of desmosomes and tonofilaments identified the root sheath with certainty. The cells contained very little endoplasmic reticulum. Free ribosomes, usually associated with endogenous protein synthesis, were plentiful. No secretory granules could be found, but in the region of the Golgi apparatus, electron-dense structures resembled lysosomes. The overall impression gained of their cytoplasmic ultrastructure was that the cells were inactive. He postulated that the ultrastructure of epithelial rests would be similar to the root sheath because their histochemistry was the same. Whether this is a valid postulate is debatable.

2) Human Explants / Proliferating

GRUPE et al. (1967) studied histochemically and radiobiologically human epithelial cell rest proliferation in vitro and in vivo . Epithelial cell rests from human periodontal ligament were cultured in vitro whilst epithelial trabeculae in apical granulomata and epithelial lining of dental cysts were studied in vivo . A number of features were observed which were characteristic of proliferating epithelial rests:

1) DNA synthesis and subsequent mitosis as confirmed in vitro by autoradiographic technique.

2) Greatly increased amount of cytoplasm.

3) Exhibited little succinic dehydrogenase activity and contained no glycogen.

4) Lipid accumulation increased.

5) Increased number of mitochondria.

From these findings, they suggested that in response to local environmental change, probably due to change in oxygen/carbon dioxide tension, epithelial cells proliferate because of their ability to undertake anaerobic glycolysis. Endogenous protein synthesis required for cell multiplication was brought about partly by pentose shunt activity, which is in turn linked to lipid synthesis. They noted that the histochemical features of proliferating cell rests were similar to epithelial proliferation associated with a disturbed supporting connective tissue in wound healing.

NYLEN and GRUPE (1969) described the ultrastructure of epithelial rests of Malassez from explants of human periodontal membrane. They differed from resting cells in vivo (VALDERHAUG and NYLEN,1966) as follows:

1) An apparent outgrowth of epithelial cells produced a disruption of basement lamina.

2) Hemi-desmosomes were infrequent and poorly structured.

3) Cytoplasm was more abundant.

4) Intercellular spaces were smaller but increased in number.

5) Junctional complexes were rare between interdigitating cell processes. Tight junctions and desmosomes were observed in areas where the membranes of adjoining cells were apposed more evenly. The desmosomes often lacked the intercellular contact layer.

6) Cells had a single nucleus with occasional infoldings of the nuclear membranes. The nucleoli were prominent. Nuclear pores were abundant and regularly spaced.

7) Organelles and inclusions were much more abundant. The mitochondria were much larger and contained many well developed internal cisternae with distinct outer membranes.

8) The endoplasmic reticulum was increased and partly covered by ribosomes.

9) Large numbers of ribosomes were seen throughout the cytoplasm, both singly and as polyribosomal aggregates.

10) The Golgi complex was well ordered, prominent and located toward the centre of the islands, indicating the existence of a pinocytotic or reverse pinocytotic mechanism.

11) Lipid droplets were frequent. They were bounded by dense membrane-like structures or bordered by small dense granules.

12) Several small dense membrane bound lysosome-like bodies were seen.

13) Explants seemed to be devoid of the dense aggregates of glycogen- like material.

14) Tonofilaments were less numerous and formed much thinner bundles.

15) Fine non-striated fibrils were observed close to the basement lamina on the connective tissue side. Interspersed with these and continuing deeper into the connective tissue, were fibrils of larger diameter which featured regular 640Å banding of collagen.

16) Numerous extracellular deposits of hydroxyapatite were found in the connective tissue, partly surrounded by epithelial cells. The basement lamina of these cells was usually absent.

These findings suggest that the cell rests may remain viable upon completion of root formation and are able to respond to environmental stimuli through an alteration in the enzymatic and protein synthesizing machinery. The arrangement of the organelles suggests protein utilization by the cells themselves and possibly also a contribution to continuing alterations of their surrounding basement membrane (NYLEN and GRUPE, 1969).

3) Monkey Explants / Proliferating

TEN CATE (1972) observed that the ultrastructural features of proliferating epithelium in the apical granuloma of the monkey, were similar to those of activated epithelium cultured in vitro . They had an extensive cytoplasm with a great deal of rough endoplasmic reticulum, contained no glycogen and had few lipid bodies. The change from a resting epithelial cell of Malassez to an activated proliferating cell, either in culture or in vivo , involves significent and consistent changes in both morphology and chemistry. These may be summarized as:

1) a decrease in the nuclear cytoplasm (increased cytoplasm).

2) the utilization of glycogen (no glycogen).

3) the synthesis of neutral lipid (increased lipid content).

4) the synthesis of ribonucleic acid (increased rough endoplasmic reticulum).

5) an increase in glucose-6-phosphate dehydrogenase activity and the depression of succinic dehydrogenase activity, indicating that the epithelial rest cells preferentially utilize the hexose monophosphate shunt (Pentose Shunt).

BRUNETTE et al. (1977) observed the ultrastructural features of explants of adult monkey periodontal ligament. Epithelial-like, E cells were sandwiched between fibroblast-like, F cells. Tonofilaments and desmosomes were observed in the epithelial-like cells. Although the membranes of E and F cells were sometimes closely apposed, no special junctional structures could be detected between them.

4) Rat and Mouse

LISTGARTEN(1975) described the typical ultrastructural features of epithelial rests found within the periodontal ligament of rats and mice. They included:

intracytoplasmic tonofibrils, intercellular desmosomal connections and a limiting basal lamina showing frequent interruptions in its continuity. Hemidesmosomes and occasional anchoring fibrils were associated with the basal lamina.

BEERTSEN and EVERTS (1979) found that the cytoplasm of epithelial rests of Malassez in mouse incisors and molars contained few tonofilaments and the cells were interconnected by desmosomes. The rests were surrounded by a basal lamina associated with hemidesmosomes and the basal lamina was interrupted in many places. They also observed autodesmosomes formed between opposing plasma membranes of surface invaginations of the same cell. The autodesmosomes were predominantly along the cell surface in areas where the basal lamina was absent. The basal lamina of the molar cell rests appeared to be interrupted less frequently than the incisors. They postulated that the presence of autodesmosomes is related to the small number of cells found within the rests and that they were not associated with any form of pathology.

GURLING (1982), GURLING and SAMPSON (1985) studied ultrastructurally the epithelial root sheath changes during molar formation in the mouse. Epithelial cells just apical to the cemento-enamel junction (Type I) displayed presumably a reconstituted basal lamina without aperiodic fibres. The mitochondria were large but showed indistinct cristae. Some endoplasmic reticulum was present and its cristae contained electron -dense amorphous material. Tonofilaments were not distinct and no Golgi apparatus was seen. The reconstitution of the basal lamina was suggested to be of mesenchymal origin.

Epithelial cells lining the cementum surface along the root had a high nuclear/cytoplasmic ratio with invaginating nuclear membranes. The cytoplasm contained rough endoplasmic reticulum, many free ribosomes,

mitochondria, dense bodies, a Golgi apparatus and tonofilaments. Where there was more than one cell, desmosome junctions were noted. No basal lamina or aperiodic fibres were found directly associated with the rests. All these ultrastructural features indicate that the cells are actively synthesizing and proliferating rather than resting (GURLING and SAMPSON, 1985).

MCLEAN (1984) observed the ultrastructural features of epithelial rests in mouse molars. Epithelial cells were identified which showed features characteristic of cell degeneration. These included swollen oedematous organelles, an increase in vacuoles containing lipid-type material, nuclear chromatin clumping, decrease in nuclear size and a loss of plasma and nuclear membranes. The presence of large tonofilament bundles however, remained as a distinguishing characteristic of these cells. The degenerating epithelial cells existed as either isolated cells within the periodontal ligament or as single degenerating epithelial cells amongst a cluster of healthier cells in an epithelial rest of Malassez. It was hypothesized that controlled cell degeneration is normally associated with tooth root formation.

5) Swine

BIRECK et al. (1980) noted that cells from porcine epithelial rests of Malassez cultered in vitro, had many interdigitating microvilli. Desmosomes associated with numerous tonofilaments were frequently seen between adjacent cells. However, basal lamina was not found at either the interface between the collagen in the culture or the plastic substrate of the container. Wholly intracellular collagen was located in a number of different vesicles, some of which communicated with the extracellular space, supporting the postulate that the collagen had been phagocytosed from the culture by the epithelial cells.

F. POSTULATED FUNCTIONS OF ERM

1) Protect the Root from Resorption

From his study on C-avitaminosis, WAERHAUG (1958) inferred that the epithelial rests may exert some protective activity against root resorption.

LÖE and WAERHAUG (1961) replanted 58 teeth in four monkeys and six dogs. In teeth replanted with vital periodontal membranes, ankylosis or a tendency of bone to bridge the periodontal space was not observed, although almost all the teeth showed active or arrested root resorption, particularly in the gingival third. As normal periodontal membrane was only found in places where epithelial rests were present, it was postulated that the epithelial remnants of Malassez play a role in the maintenance of the periodontal space by limiting root resorption. Three years after replantation, the periodontal space was normal width; the fibres were functionally orientated; epithelial rests were scattered throughtout the periodontal membrane and resorption lacunae were filled with secondary cementum.

VALDERHAUG and ZANDER (1967) found that very little or 100-300 μm of soft tissue remained on the root surface following extraction. Epithelial rests were found only in areas where sufficient amount of periodontal ligament adhered to the extracted tooth. Contrary to the postulates of LÖE and WAERHAUG (1961), they suggested that the success of replantation depends more on the presence of a certain amount of periodontal tissue than on the epithelial rests per se.

GRANT and BERNICK (1969) found that rests in miniature swine were numerous along the entire surface except in root resorption bays during the shedding of primary teeth. They were rarely present near the resorption lacunae. The occasional epithelial aggregate that could be identified was usually seen further away from the root surface, near bone and even in

marrow spaces. They postulated that this may indicate that the rests were "left behind" as progressive root resorption occurred, suggesting that the rests do not protect the root from resorption.

LINDSKOG and HAMMARSTRÖM (1980) using a casein-agar method for the detection of protease inhibitors, showed that human periodontal membranes or cementum contain a potent collagenase inhibitor which they postulated may prevent the roots of teeth from being resorbed.

LINDSKOG et al. (1983) suggested that protease inhibitors or "anti-invasion factors" produced by endothelial or epithelial cells in the periodontal ligament, may protect the root surface from osteoclastic resorption and contribute to the integrity of the periodontal ligament.

2) Prevent Ankylosis

ROBINSON (1926, cited in VALDERHAUG and NYLEN, 1966) suggested that rests had an endocrine function and produced a hormone which prevented the fusion of cementum and alveolar bone.

SPOUGE (1980) reviewed the functions of epithelial rests of Malassez. He concluded, both from the works of LÖE and WAERHAUG (1961) and the fact that nowhere in the body is epithelium in direct contact with bone, that in the absence of any special organizational properties, the role of the interposed epithelial rests could well be to act as an ankylosis inhibitor within the periodontal ligament. Similarly, the success of bone grafting would depend upon the ability of the epithelial network already in situ, adjacent to the operated area to repair or regenerate into the region of the graft.

3) Initiate Cytodifferentiation

GURLING (1982), GURLING and SAMPSON (1985) postulated that if the acellular cementum surface was damaged, cell rests derived from the inner

epithelial layer of Hertwig's root sheath could then come into contact with the exposed dentine. The rests could then influence cytodifferentiation of follicular cells to produce cementoblasts and effect cementum repair. The epithelial cells and cementoblasts may then become incorporated in the repair cementum matrix.

This postulate was based on an ultrastructural study of Hertwig's root sheath changes during molar formation in the mouse. They observed that pre-cementoblasts differentiated from follicular cells after the outer epithelial cell layer of the sheath shortened and cytoplasmic intercellular contact from inner epithelial cells to follicular cells was achieved. Pre-cementoblastic differentiation always occurred opposite where the premarginal dentine spicule existed and the inner basal lamina was either discontinuous or lost.

MERRILEES et al. (1983) studied in vitro the effect of explants of epithelial rests of Malassez and endothelial cells on the synthesis of glycosaminoglycans by the periodontal ligament fibroblasts. Their results supported the hypothesis that loose connective tissues are formed and maintained under the influence of epithelial, including endothelial, cells. However in vivo, epithelial rests are embedded directly in the dense fibrous ligament without intervening loose connective tissue.

DAVEY (1986) postulated that Hertwig's epithelial root sheath may be responsible for determining the site of root dentine formation. It was further suggested that the epithelial rests may be active in the orientation and attachment of collagen fibrils to root dentine and may play a role in maintaining periodontal width and integrity.

4) Produce Bone Resorbing Factors

HARRIS and GOLDHABER (1973) maintained vital explants of human dental cyst in tissue culture with mouse calvaria for seven days. Marked bone

resorption was seen when the explant remained vital and no resorption was evident with devitalized explants. It was proposed that dental cyst and tumour growth within bone is dependent on the synthesis and release of a potent bone resorbing factor, presumably by the proliferating epithelial cells within the cyst.

BRUNETTE et al. (1977) studied the interactions between epithelial and fibroblast-like cells in culture derived from monkey periodontal ligament. The epithelial-like E cell layer was sandwiched between two fibroblast-like F cell layers. This unusual relationship may be the result of cells mimicking in vitro their relationship in vivo , since E cells are derived from epithelial rests of Malassez which produce enamel-like proteins and Type IV collagen found in the basal lamina. Epithelial cells in vitro also secreted prostaglandins. It was postulated that epithelial cells in cysts which originated from epithelial rests contribute at least partially to the production of bone resorbing factor.

BIRECK et al. (1983) demonstrated that cells cultured from porcine ERM cells produce a factor or factors which stimulate bone resorption in vitro . The production of these factors was not affected by indomethacin, indicating that factors other than prostaglandins were responsible for the bone resorption stimulating effect of ERM cells. They postulated that one possible mechanism may involve collagenolytic breakdown of the bone matrix. However, ERM cells also produce inhibitors of collagenolytic enzymes. In order for a breakdown of bone matrix to occur, collagenolytic enzymes must be produced in excess of inhibiting activity. From their results BIRECK et al. (1983) suggested that the epithelial cells may contribute directly to bone resorption associated with cysts derived from rests of Malassez.

LINDSKOG et al. (1988b) placed explants of enamel organ, from which ERM are derived subsequent to root formation, into experimental cavities in the

root surfaces of monkey incisors. Alveolar bone was resorbed around the epithelial islands to a distance corresponding to the periodontal space.

5) Synthesize Protein

NYLEN and GRUPE (1969) in an ultrastructural study of the epithelial rests of Malassez from explants of human periodontal membrane, showed that the epithelial islands in the explants had assumed a more active state. This was supported by an increase in the number and differentiation of various organelles, accumulation of lipid and the absence of glycogen. The presence of fairly well developed endoplasmic reticulum and Golgi complex, together with a large number of nuclear pores was suggested to be indicative of enhanced protein synthesis. However, the lack of well ordered arrays of endoplasmic reticulum and the general absence of ribosomes along the perinuclear membrane, suggested that the cells are most active in the synthesis of proteins for retention by the cell. Probably all epithelial cells secrete their own basement membrane (PIERCE, 1966). Small vesicles in various stages of fusion with the plasma membrane are secretory rather than pinocytotic. The advanced internal development of the mitochondria suggested greater energy requirements, anoxia or efforts to compensate for metabolic shift.

6) Regulate Collagenolysis

PETTIGREW et al. (1980) reported that epithelial cells cultured in vitro from porcine and macaque periodontal tissues synthesized protein capable of inhibiting collagenalytic enzymes. These proteins are therefore important in the regulation of collagenolysis, maintaining the balance between synthesis and degradation during collagen turnover and could provide defense against pathological destruction of tissue.

7) Phagocytosis

KERR et al. (1972) proposed that epithelial cells when suitably stimulated, can display marked phagocytotic activity. They also suggested that epithelial cells are capable of taking up large structures such as apoptotic bodies.

BIRECK et al. (1980a) cultured cells in vitro from porcine epithelial rests of Malassez with collagen from rat tail tendons and examined serial sections of the material from the culture using an electron microscope. They found that the cultured epithelial cells phagocytose collagen in vitro. The phagocytosed collagen was incorporated into secondary lysosomes where it was actively digested. From their findings they postulated that the ERM that have been stimulated to divide and migrate during cyst formation, could destroy the extracellular substance of contiguous connective tissue by phagocytosis.

8) Formation of Periodontal Cysts

REEVE and WENTZ (1962) suggested that the epithelial rests are vestigial structures persisting within the periodontal ligament with a potential role in dental and periodontal cysts.

VALDERHAUG (1972) in a histological study of experimentally induced radicular cysts in monkeys, found that epithelial rests of Malassez were one of the sources for epithelium in periapical cysts. Serial sections revealed that proliferating epithelium in the granulomas was in the form of isolated islands and strands, which did not communicate with the oral surface epithelium. There was always a close connection between the root surface and the epithelium of the cyst wall.

TEN CATE (1972) noted that epithelial cell rests of Malassez behave in an identical manner to other epithelial cells when their local connective tissue

environment is altered. The fact that epithelial rests are surrounded by connective tissue rather than any distinctive morphologic or chemical properties, gives them their potential for cyst formation. Activation of the cell rests both in vivo and in vitro involves a switch in their metabolism and use of the hexose-monophosphate shunt. It was further suggested that the intraepithelial cavitation of trabeculae of activated cell rests is the mechanism for initial cyst formation.

HARRIS and TOLLER (1975) in a review of the pathogenesis of dental cysts stated that cell rests of Malassez appear to have a protective and reparative role when provoked by periapical sepsis. Epithelium, together with its inflammatory infiltrate appear to be a barrier, isolating pulpal irritants from the surrounding tissues and when complete, showed a distinct union with the margins of the apical foramen, similar to the attachment of gingiva to tooth. The existence of periodontal cysts is associated with continuous irritant stimulus. When the irritant is removed, the cyst reduces and the epithelial lining disintegrates. It was postulated that active proliferation of cyst lining epithelium induces the synthesis of prostaglandins which resorbs the surrounding bone, causing extensive symptomless enlargement.

9) Formation of Periodontal Pockets

ORBAN and WEINMANN (1942, cited in GRANT and BERNICK, 1969) postulated that the rests might play a part in the sudden appearance of deep pockets in periodontitis. They postulated that the rests might proliferate and become confluent with the attachment epithelium, which could then separate from the tooth to form a pocket.

GRANT and BERNICK (1969) suggested that the proliferating cords from the attachment epithelium in miniature swine seemed almost confluent with the

root epithelium and could be a response to inflammation. This confluence may play a part in pocket formation.

SPOUGE (1980) postulated that apical migration of the epithelial attachment during chronic marginal periodontitis is facilitated or may even be made possible in the first place by the presence and proliferation of epithelial cells already established within the substrate of the ligament.

10) Unknown Function

TEN CATE (1985) stated that many different functions have been proposed for the epithelial cell rests but since all are speculative, there is no known function for these cells at the present time. Epithelial rests proliferate in response to inflammatory changes within the periodontal ligament. If cavitation occurs within the resulting mass of epithelium, a dental cyst is formed.

ORBAN (1986) stated that the physiologic role, if any, of the epithelial rests in the functioning periodontal ligament, is unknown. Epithelial rests can undergo rapid proliferation and can produce a variety of cysts.

G. STATE OF ACTIVITY OF ERM

1) Proliferating

RAMJFORD et al. (1966) reported uptake of ^{3}H-thymidine in epithelial rests five days after experimental gingivectomy in monkeys, indicating that rests proliferate when stimulated.

JOHANSEN (1970) observed epithelial rests of Malassez using autoradiographic techniques in the first molars of Wistar rats. No uptake of tritiated thymidine was found in the rests on the control side. Following tooth fractures resulting from unsuccessful extraction attempts, labelled epithelial

cells were observed indicating that trauma and subsequent inflammation are stimuli for proliferation of the rests. It was postulated that the rests may take part in the formation of the epithelium of the pocket walls in periodontitis and epithelial lining of radicular cysts.

KVAM and GILHUUS-MOE (1970) using autoradiographic techniques, observed DNA synthesis and mitosis in a marginal epithelial rest of the periodontal membrane of the first maxillary molar of a rat.

2) Migrating

NYLEN and GRUPE (1969) presented the following as evidence of epithelial cell migration:

1) Desmosomes lacked an intercellular contact layer.
2) Reduction in number and organisation of the tonofilaments.
3) Breaks and voids in the basement lamina with extrusions of the cell processes from the epithelial cells into the connective tissue.

The irregular surfaces of the epithelial cells both laterally and basally were thought to reflect their accelerated activity and quest for adequate nutrients to survive. Hydroxyapatite deposits were randomly secreted by the explants (NYLEN and GRUPE, 1969).

3) Resting

TEN CATE (1965) established the presence of glycogen in the epithelial rest using histochemical techniques. He suggested that the presence of glycogen in the epithelial cells, together with the presence of a functioning pentose shunt and the presence of an anaerobic glycolytic pathway, is indicative of a metabolism requiring little energy. He believed that this interpretation accorded well with the inactivity of this epithelium and also suggested that the possibility of a functional role for the epithelial cell rests in

the adult periodontal ligament could be discounted. The species of the tissue source was not specified.

TEN CATE (1967) from a histochemical and ultrastructural study of human root sheath proposed that epithelial cell rests are capable of metabolizing anaerobically and via the pentose shunt. There was no evidence to show that they are actively secreting and have minimal activity.

Based on ultrastructural and histochemical study of fifty human teeth from patients aged 10 to 27 years, VALDERHAUG and NYLEN (1966) suggested that the epithelial rests of Malassez were truly resting cells.

The poorly developed endoplasmic reticulum, the morphology of the mitochondria and the scarcity of the Golgi apparatus, indicated that the epithelial rests are not glandular in nature and that very little protein is synthesized in the cells. They observed granules in the cytoplasm which strongly resembled glycogen and agreed with TEN CATE (1965) that this was indicative of a metabolism requiring little energy. However, they pointed out that this does not preclude that the cells could return to a more active state if properly stimulated. Large dense bodies were also observed and thought to be lipid in nature. They suggested the lipid may serve as a ready source of energy during increased metabolic activity or may reflect a certain degree of cell degeneration.

1.2 TYPES OF EXTERNAL ROOT RESORPTION

ANDREASEN (1985) reviewed the etiology and pathogenesis of external root resorption and classified three main types: 1. surface resorption 2. inflammatory resorption 3. replacement.

A. SURFACE RESORPTION

Surface resorption of the root surface is caused by injury to the periodontal ligament and possibly also to the root surface where the traumatized area is removed, and is mediated by cell proliferation from the adjacent periodontal ligament. If the injury is not repeated (eg. single trauma, arrest of the orthodontic treatment or removal of a displaced impacted tooth exerting pressure on a root surface), then the healing takes place with formation of new cementum and periodontal ligament fibres.

The histological description of repaired resorption cavities, associated with orthodontic tooth movement, appears identical to that of surface resorption found after acute traumatic dental injury. The resorption process takes place by uni- or multi-nucleated cells, but without significant inflammatory changes in the soft tissue.

B. INFLAMMATORY RESORPTION

This resorption process takes place by uni- or multi-nucleated cells present in inflamed granulation tissue. It is commonly associated with replantation of teeth and its development appears to be dependent on

1) Initial injury to the periodontal ligament during extraction and/or extended drying of the root surface prior to replantation.
2) The extension of the surface resorption into dentine.
3) Necrosis of the pulp and its communication through the dentinal tubules to the area of surface resorption.

It may also be caused by an inflammatory process other than infected pulpal tissue eg. periodontal inflammation.

C. REPLACEMENT RESORPTION

If the damage to the root surface is extensive and the alveolar bone is close, the osteogenic healing processes may overwhelm the periodontal ligament repair process and ankylosis occurs, with fusion between bone and root surface. A continuous replacement of tooth substance with bone will result (replacement resorption) because of the inherent nature of bone to remodel. Whether this fusion between the root surface and bone becomes permanent and progressive or transient, is apparently determined by the initial size of this ankylosis and possibly also the presence or absence of functional stimuli in the immediate period of healing after trauma.

HAMMARSTRÖM et al. (1986a) reviewed the pathology responsible for tooth loss following avulsion and recent replantation and attachment studies. They concluded that the management of the avulsed tooth should aim at the prevention of both ankylosis and inflammatory root resorption. Consequently, the vitality of the periodontal membrane must be maintained and bacterial invasion of the pulp prevented. Root resorption which follows an ankylotic fusion between the dental root and alveolar bone, progresses more slowly, if the cementum layer is intact.

1.3 ORTHODONTIC ROOT RESORPTION

A. AETIOLOGY

1) Hyalinization

RYGH (1977) found that orthodontic root resorption takes place simultaneously with and after the elimination of hyalinized tissue in man. It was postulated that the more mature periodontal collagen fibres adjacent to the cementum are possible barriers preventing root resorption. Elimination

of the hyalinized tissues leads to the removal of the cementoid and mature collagen, thus leaving a raw cemental surface without a barrier and open to attack by the odontoclasts. Once resorption lacunae were established, the cementum was resorbed from the rear as an undermining process. By continued orthodontic force application, the resorption process proceeded even after all hyalinized tissue was eliminated. If the orthodontic force was discontinued or fell under a certain level, the resorption lacunae repaired. Root resorption occurred around the hyalinized tissue and was mediated by cells from the adjacent periodontal ligament thus explaining how root resorption seemingly occurs behind compressed acellular parts of the ligament.

Active hyperaemia in the undamaged periodontal tissues adjacent to the hyalinization, establishes the circulatory conditions suited for the development of hard tissue resorbing cells. It was postulated that biologically active substances are released by destruction of tissue within the hyalinized zone. These may affect the development of various cells around the hyalinized zone including multinucleated giant cells with the ability to resorb cementum (RYGH, 1977).

2) Resorption Potential

MASSLER and MALONE (1954) conducted a roentgenographic study of root resorption in human permanent teeth, untreated and following orthodontic tooth movement. A definite resorption potential was present which varied in different persons and also in different teeth of the same person. The resorptive potential was found to be inherently high in approximately 10% of the sample. This figure correlated well with the approximately 14% of the teeth that showed severe degrees of resorption after orthodontic treatment. There were no striking differences between males and females but the

resorptive pattern was strongly bilateral. The severity of resorption was markedly increased by orthodontic procedures.

NEWMAN (1975) attempted to determine possible etiological factors in external root resorption using radiographic analysis of human teeth and determining the degree of apical deficiency. Persons with "idiopathic" root shortness (i.e. a high "resorption potential"), frequently had severe postorthodontic resorption. Orthodontic treatment increased the incidence and degree of root resorption. Systemic factors could not be implicated in root resorption. They postulated that the abnormal position of the tongue in anterior open bite cases may contribute to the increased root resorption found in these patients.

HARRY and SIMS (1982) found little variation in the susceptibility of different patients to root resorption.

LEVANDER and MALMGREN (1988) evaluated radiographically the risk of apical root resorption in human upper incisors, during orthodontic treatment. They noted that the degree of root resorption was significantly higher in incisors with bent or pipette shaped roots. The level of root resorption after treatment was significantly related to the resorption present after the initial 6-9 months of treatment. They found no correlation between root resorption and the type of mechanics employed.

3) Force, Magnitude and Duration

REITAN (1974) noted that increased force may increase the tendency to apical root resorption. Hyalinized areas observed as a result of relapse do not cause root resorption. It was suggested that root resorption is more likely to occur in cases where the compression is fairly strong and of some duration.

KING and FISCHLSCHWEIGER (1982) studied the effect of force magnitude on extractable bone resorptive activity and cemental cratering in orthodontic tooth movement in rats. Both bone resorptive activity and root resorption were found to be significantly increased with moderate and high forces. Light forces produce more rapid tooth movement with insignificant cemental cratering whereas intermediate heavy forces result in slower tooth movement and substantial cratering.

HARRY and SIMS (1982) completed a scanning electron microscope study of human root resorption under continuous intrusive orthodontic loadings, varying in magnitude and duration. Loss of root length occurred within 35 days with forces as light as 50 gms. After 70 days with activations ranging from 50 to 200 gms, progressive apical resorption was accompanied by regions of cellular cementum repair. There was a striking increase in root resorption compared with control teeth. The amount of resorption increased markedly with the duration of force and to a lesser extent with the magnitude of the force applied. These findings are similar to that of REITAN(1974) and STENVIK and MJÖR (1970).

COPELAND and GREEN (1986) studied radiographically the degree of root resorption in maxillary central incisors following active orthodontic treatment. The mean root resorption during treatment was 2.93 mms. Root resorption ceased with termination of active treatment. When post-treatment root resorption does occur, it is more likely associated with other factors such as traumatic occlusion and active force delivering retainers.

Root resorptions are affected by such factors as the duration, magnitude, type of force used, the distance of the tooth movement and the age of the patient (REITAN, 1967; DeSHEILDS, 1969; HOLLENDER et al.,1980).

REITAN (1985) stated several findings from his many years of research. The density and hardness of cementum and dentine may cause retardation of root resorption. If parts of the periodontal ligament have been transformed into granulation tissue, there may be formation of odontoclasts with resorption of the root surface. Like osteoid, cementoid tends to decrease in thickness on the side of compression. If orthodontic pressure continues for a longer period, root resorption may start even if the root was initially protected by uncalcified tissue.

REITAN (1985) proposed that the following types of root movement may lead to apical root resorption:

1. Prolonged tipping, notably of anterior teeth.
2. Distal tipping of molars causes resorption particularly of the distal roots. (Gradual tipping of these teeth by light interrupted forces may reduce the tendency to root resorption.)
3. Prolonged continuous bodily movement of small teeth such as upper lateral incisors. (Interrupted movement, or continous movement with rest periods, may diminish the occurrence of this type of root resorption.)
4. Intrusion. (It is important to initiate the intruding movement by forces as light as 25gms. Frequent rest periods will prevent any extensive resorption.)
5. Extensive edgewise torque of anterior teeth in the more mature young and adult patients.

Moving teeth bodily by light continuous forces may cause less root resorption than a tipping movement. Once an apical resorption is started, the process may be rapidly increased as the tooth is constantly tipped. Fairly painless and rapid tipping movement constitutes the type of tooth displacement that may cause a considerable degree of root resorption.

Apical root resorption is the only injury that can imperil the stability and normal function of the tooth. If the cementoid and predentine layers are fairly thick, there will be no apical root resorption. If the root surface is well calcified and the predentine layer is thin, tipping movement may lead to root resorption (REITAN, 1985).

There will be generally more root resorption after treatment in adults. However, there are few osteoblasts along the bone surface and the root exhibits a thick layer of cementum with some cementoblasts which in many respects make the adult root surface more resistant to root resorption than that of children. . Light wire torque may lead to root resorption if the force is acting over too long a period. To avoid this root resorption, the best technical solution would be to apply a light torquing force that acted interruptedly over a short distance. Less vacuolization occurred and less marked pulp reaction were observed after tooth movement with interrupted forces (REITAN,1985).

SHARPE et al. (1987) found a relationship between orthodontic relapse, increased root resorption and decreased alveolar bone. Subjects with relapse had undergone longer periods of treatment, exhibited a greater prevalence of root resorption and greater loss of alveolar bone support. Smaller amounts of tooth translation were seemingly more prone to demonstrate tissue loss i.e. increased root resorption and decreased alveolar height.

4) Increased Vascularity

HARRY and SIMS (1982) stated that resorption areas were often located near openings which appeared to be accessary canals at various locations on the root surface. It was suggested that these resorption zones could be associated with blood vessels passing through these openings.

BROWN (1982) also suggested that the blood borne origin of osteoclasts, their mobility and versatile morphology enable them to make an immediate resorption response. The proximity of blood supply plays an important role in root resorption. He infers that osteoclasts resorb both bone and hard dental tissues i.e. that odontoclasts, cementoclasts and osteoclasts are in fact the same cell type resorbing slightly different mineralized structures. As the capillary complex within the periodontal ligament is positioned closer to bone than to cementum, blood borne osteoclasts will resorb bone before cementum.

5) Bone Density and Age of Patient

GOLDIE and KING (1984) found that lactation coupled with calcium deficiency in rats, will produce decreased bone density which is consistent with increased parathyroid hormone secretion. Moreover, the decreased area of root surface resorption and enhanced tooth movement, suggest that increased bone resorption and decreased bone density, facilitate remodelling of alveolar bone in preference to root. A greater prevalence of root resorption has been reported in adults.

LEVANDER and MALMGREN (1988) could not find any correlation with age and the degree of root resorption.

6) Acid Phosphatase Levels

MATTISON et al. (1983) reported on two cases of external apical root resorption that continued after active and retentive orthodontic treatment had been terminated. Calcium hydroxide was used to stop its progress. It was suggested that the alkalinity of calcium hydroxide changed the local environment from one of high acid phosphatase to one containing alkaline phosphatase which in turn terminated the inflammatory process.

LILJA et al. (1983 a,b) examined orthodontic tooth movement in rats using histochemical techniques for some enzymes associated with bone resorption and tissue damage. Orthodontic force caused a redistribution of acid phosphatase containing cells to the margins of the hyalinized areas, followed by an increased activity of acid phosphatase. It was suggested that the lack of lysosomal enzymes in the hyaline zone as shown by the lack of staining for acid phosophatase, might explain why elimination of the hyaline zone is a slow process and why it proceeds from its periphery. It was concluded that the magnitude of the orthodontic force seemed to be a determining factor for the vitality of the periodontal membrane, but not for tissue degradation activity.

7) Piezoelectricity and Chemotaxis

MATTISON et al. (1984) quantitatively compared external root resorption of endodontically treated teeth with vital teeth in cats, when both were subjected to orthodontic forces. No significant difference was found. The mechanism which directs osteoclastic resorptive activity and therefore root resorption to specific areas on the bone and tooth surface are not known. Piezoelectricity and chemotaxis due to local tissue damage have been proposed.

B. BARRIERS TO RESORPTION

A number of barriers to resorption have been proposed throughout the literature:

1) Epithelial Rests of Malassez

LÖE and WAERHAUG (1961) in a study of replanted teeth in monkeys and dogs, found that ERM were always present in replanted teeth , with vital periodontal membrane and no ankylosis. They suggested that ERM may be

a factor in the limitation of root resorption in teeth replanted with vital periodontal membrane.

LINDSKOG and HAMMARSTRÖM (1980) found that when newly extracted teeth with the attached parts of the periodontal membrane were placed in physiological saline, a protease inhibitor called an anti-invasion factor (AIF) was released which inhibited bone resorption. It was suggested that these anti-invasion factors in the dental tissues participate in the maintenance of the integrity of the dental root.

LINDSKOG et al. (1983) in a study of the repair of periodontal tissues, suggested that epithelial cells in the periodontal membrane may produce a protease inhibitor (anti-invasion factor, AIF) which inhibits osteoclastic bone resorption. In this way, epithelial cells may protect the root surface from osteoclastic resorption and contribute to the integrity of the periodontal membrane.

LINDSKOG et al. (1988b) suggested that ERM, which are derived from odontogenic epithelium, maintain the width of the periodontal space and prevent dento-alveolar ankylosis.

BOYD (1985) studied cervical root resorption associated with ligating impacted canines and subsequent orthodontic tooth movement. He suggested that there was a downgrowth of the epithelial tissues from the palate to cover the denuded root surface before cells of the palatal connective tissue or alveolar bone could contact the denuded root surface. This downgrowth of epithelial tissue protected the root surface from resorption and ankylosis.

ANDREASEN and KRISTERSON (1981) found that periodontal cells proliferate apically from the crevicular gingival tissues when the root surface has been denuded of vital periodontal ligament. They postulated that if alveolar bone

growth across the periodontal space and thereby ankylosis could be prevented, a new periodontal ligament could form as a result of continued downgrowth from the crevicular site.

KARRING et al. (1984) studied root resorption during periodontal wound healing in monkeys. They found that it was possible to prevent root resorption by permitting apical downgrowth of epithelium along the root surface during the initial phase of healing. They postulated that the reason why root resorption is a rare complication following new attachment procedures in periodontal therapy, may be that an epithelial (root protective) barrier is regularly formed by the downgrowth of the dentogingival epithelium along the root surface, before granulation tissue from the bone or the gingiva makes contact with the root.

2) Cementoid/ Precementum

Precementum, found in young individuals resists resorption more readily than cementum (REITAN,1951).

KVAM (1972 a,b) suggested that areas of root surface not covered by lining cementoblasts, may be subjected to root resorptions in the pressure areas.

REITAN (1974) postulated that existing cementoid on the root surface may delay the onset of root resorption. The fact that uncalcified predentine was not attacked by resorbing cells suggested that permanent destruction of the apical portion of the root may be avoided if tooth movement is achieved before the root is fully developed.

RYGH (1977) noted that:

1) The chemical composition of cementum is similar to that of bony tissue (apart from fluorine content, which is considerably higher than

in bone, particularly in the outer layers of cementum). This increased fluorine content provides greater resistance to resorption.

2) The bony tissue has ample blood supply whereas the cementum is completely void of vascular tissue.

3) Unmineralized precementum layer or cementoid covering the cementum, is resorption resistant.

By contrast, BROWN (1982) concluded from BOYDE (1972) that cementoid and osteoid were not highly resistant to resorption and that any exposed part of the tooth root is readily susceptible to resorption by osteoclasts.

HAMMARSTRÖM and LINDSKOG (1985) in a review of the literature on resorption of teeth, suggested intermediate cementum formed by the epithelial root sheath sealed off the dentinal tubules preventing inflammatory resorption. Odontoblasts in combination with the underlying predentine also provide a barrier against resorption. In cases of profound external resorption, most of the dentine may be resorbed with the exception of the most pulpal part close to predentine. It was also suggested that the intermediate cementum is immunogenic and induces a reaction which seems to be a prerequisite for the formation of normal cementum. The resistance to resorption of non-mineralized predentine may modify the location of the resorption process. It was further postulated that protecting cells (cementoblasts?) may become replaced by an osteoblastic type of cell that responds to those factors which normally induce bone resorption.

LINDSKOG et al. (1983) suggested that the intermediate cementum forming an effective barrier between dentine and the periodontal membrane is critical to the progress of root resorption. Furthermore, it cannot be regenerated once it has been damaged.

LINDSKOG et al. (1987a) showed that reparative cementum-forming cells covering an experimental cavity in the root surface of replanted monkey incisors were unaffected by parathyroid hormone, a potent mediator of bone resorption. In parallel experiments, endocranial osteoblasts exposed bone surface by widening their intercellular spaces. It was concluded that the layer of cells covering the root surface forms a protective barrier against resorption and serves to protect the integrity of the dental root.

3) Sharpey Fibres

BROWN (1982) postulated that:

1. The close-packed Sharpey fibres that arise from the cementum in contrast to the more loosely packed fibres that are inserted into the bundle bone of the socket, act under normal circumstances as a physical barrier against the osteoclasts and explain why cementum is less likely to be resorbed than bone.
2. The smaller number of Sharpey fibres that arise from the apical third of the root cementum provide a less effective barrier against the odontoclasts and explain why the apices of the teeth are so susceptible to resorption.
3. The unpredictable and sometimes very severe resorption that may take place in some children is related to the varying effectiveness of the Sharpey fibre barrier.

Cementum is surrounded by older and more mature collagen which is more resistent to actual chemical changes than bone. There is continuous deposition of cementum throughout life while the alveolar bone is constantly remodelled (RYGH ,1977).

RYGH (1973) postulated that advanced degradation of fibrils at the cementum surface might induce root resorption processes involving the cementum.

Non-degenerated collagenous structures in and on the root surface may act as an accelerating factor in the formation of new cementum. The degree of fibrillar breakdown may produce variations in the process of cementum repair.

C. MORPHOLOGY OF HYALINIZATION AND ROOT RESORPTION

HENRY and WEINMANN (1951) studied the pattern of resorption and repair of human cementum in teeth and supporting tissues obtained by autopsy. Resorption areas were determined by the presence of resorption or reversal lines. As over 90% of the teeth showed histologic evidence of resorption, it was considered normal but not physiologic for a tooth to incur resorption to some degree during its life. The number of resorption areas and the susceptibility to resorption increased with age. Resorption areas were usually small, shallow and readily repaired, with the apical third being the most common site of resorption. Trauma was thought to be the most important local factor in the production of resorption.

MJÖR and STENVIK (1969) studied root resorption, microradiographically and histologically, in young human premolars which had been subjected to intrusive forces. The number of odontoclasts observed was small in comparison to the number of Howship's lacunae. Older odontoclasts with densely stained nuclei and eosinophilic cytoplasm, conformed to cells which had relatively radiodense cytoplasm. The young odontoclasts with lightly stained nuclei, which had distinct nucleoli and slightly haematoxyphilic cytoplasm, were more radiolucent than the older type. No fundamental difference was considered to exist between odontoclasts and osteoclasts. It was suggested that the increased radiodensity of certain odontoclasts may be due to shrinkage. The number of young odontoclasts was much smaller than the number of old odontoclasts. The total number of odontoclasts was small as compared to the number of Howship's lacunae. The findings

indicated that the turnover was high and the differentiation of odontoclasts occurs rapidly, while their subsequent breakdown is slower. A typical "brush border" was seldom observed. Large multinucleated cells were also found some distance from the root surface. These cells had many nuclei and nucleoli could usually be discerned. Their cytoplasm stained lightly and was often slightly haematoxyphilic. Organic material was removed evenly, without a border of partially dissolved organic matrix, intervening between odontoclasts and the calcified matrix during resorption.

KVAM (1972b) studied the cellular dynamics on the pressure side of the rat periodontium, following experimental tooth movement. The hyalinized tissue appeared to be gradually removed with the ingrowth of connective tissue cells. The number of labelled osteoblasts and fibroblasts increased initially, whereas the number of labelled cementoblasts decreased. They postulated that this finding may explain the frequent occurrence of root resorption near hyalinized zones. The differentiation of progenitor cells to cementoblasts or osteoblasts appeared to be dependent upon contact with their respective mineralized surface.

KVAM (1973) studied the organic tissue characteristics on the pressure side of human premolars following tooth movement. The formation of hyalinized areas was initiated by loss of the regular arrangement of the fibre bundles, disappearence of the periodic cross striation subsequently followed by a unification of these fibrous elements into a homogeneous substance, where no structural arrangement remains. A plexus of thin fibres tended to cover the associated resorbed lacunae of the root surface preventing their observation.

RYGH (1973) studied the ultrastructural changes of the periodontal fibres and their attachment in rat molar periodontium incident to orthodontic tooth movement. The glass-like appearance of hyalinized zones is related to

compression of fibres and to changes of the surrounding elements, rather than to a degradation of the collagenous structure themselves. Only a limited number of the collagen fibrils within the hyalinized zone revealed signs of breakdown characterized by longitudinal splitting. More advanced collagen degradation with a break of continuity between periodontal fibrils and adjacent cementum and alveolar bone was rarely observed. Periodontal fibres in such cases were reattached by apposition of new cementum and bone into which newly formed collagen fibrils are embedded. However, the continuity between periodontal fibrils and cementum was generally preserved in the hyalinized zones.

REITAN (1974) observed the initial tissue behaviour during apical root resorption caused by orthodontic tooth movement in man. Experimental extrusion, intrusion and tipping movement with moderate forces produced root resorption in the majority of cases. The resorbed lacunae were usually superficial and small. The resorbed root substance, except for a definitely shortened apical portion, was reconstructed by cellular cementum. Apical root resorption did not prevent further development of roots in which there was a fairly thick predentine layer. Two types of apical root resorption were observed; external and internal. The internal root resorption was caused by fibrous tissue compression when the predentine layer was thin or absent. Root resorption may occur rapidly, not only around, but also in the middle of the hyalinized zone. Less apical root resorption occurred by moving teeth bodily.

RYGH (1974) observed that replacement of hyalinized tissue from its borders, seemed to catch up with the enzymatic process of degradation occurring within the hyalinized areas before complete breakdown of the hyalinized periodontal ligament had taken place.

ROBERTS et al. (1981) reviewed the cellular response to orthodontic force. Available evidence suggested that the initial wave of active osteoclasts which appears within hours of application of orthodontic force, is derived from the following:

1. activation of previously present but inactive osteoclasts
2. migration of osteoclasts from adjacent bone
3. formation of new osteoclasts by local macrophages of the periodontal ligament
4. influx of monocytes from vascular spaces

Active osteoclasts are recruited and are not dependent on local periodontal ligament cell proliferation/differention as are new osteoblasts.

LINDSKOG and LILJA (1983) demonstrated the presence of fibrinogen and IgG in the hyalin zone after orthodontic tooth movement in man. It was suggested that the presence of plasma proteins in the hyaline zone contributed to its hyaline appearance. Fibrin and IgG may furthermore attract phagocytes and form a network for the reorganisation of the necrotic tissue in the hyaline zone.

LILJA et al. (1983 a,b) found that macrophages in various stages of differentiation were responsible for the degradation of the hyaline zone and alveolar bone during orthodontic tooth movement. The enzymatic differences were probably due to the influence of the immediate cellular environment. Prostaglandin secretion was associated with the degradation of the hyaline zone in man. Macrophages moderate their expression of enzymes in response to the material which they phagocytose. Osteoclasts were formed by the fusion of these macrophages.

ANDREASEN (1985) noted that the histological description of repaired orthodontic resorption cavities, appears identical to that of surface resorption

found after acute traumatic dental injury. Surface resorption takes place by uni- or multi-nucleated cells but without significant inflammatory changes in the soft tissues. After some weeks, newly formed cementum is deposited in the resorption cavities, thereby partially or completely restoring the contour of the root surface. At the same time, insertion of new periodontal fibres is seen, thus restoring the integrity of the periodontal ligament (ANDREASEN, 1985). Similar surface resorption cavities were found in 90% of normal human teeth examined by HENRY and WEINMANN (1951). Most of these resorption cavities showed repair with new cementum and were most frequently located in the apical region. It was suggested that trauma was the most common factor eliciting surface root resorption.

HAMMARSTRÖM and LINDSKOG (1985) reviewed the general morphological aspects of resorption of teeth and alveolar bone. The dentine resorbing cells were usually smaller than osteoclasts (BOYDE and JONES,1979). The number of nuclei in odontoclasts were also fewer and they have very small, if any, clear zones. It was suggested that the composition of dental tissues in some way influenced the appearance of the resorbing cells.

FOLLIN et al. (1986) studied histologically the occurrence and distribution of root resorption in beagle dogs. Fifteen teeth were moved bodily for 100 days and nine teeth were tipped for 180 days. All teeth were moved mesially with the same force. Root resorptions were more severe in the teeth that were moved bodily, than those that were tipped. The distal root was much more affected than the mesial root. It was considered important to use very light forces.

D. ULTRASTRUCTURE OF RESORPTION

1) Human TEM

FURSETH (1968) studied the resorption processes of human deciduous teeth by light microscopy, microradiography and transmission electron microscopy. Odontoclasts (multinucleated cells) were not observed frequently, but were found both on the tooth surface and in its immediate surroundings. The most conspicuous features of these cells, were large numbers of mitochondria, vacuoles and free ribosomes, while endoplasmic reticulum was scarce. Where the odontoclast was in contact with the tooth surface, a system of canals extending 2-3μm into the cytoplasm was observed and these canals contained mineral crystals. This was seen as a brush or ruffled border at the light microscope level. The tooth zone, in this area, showed a decreased mineral content, approximately 1μm wide. From these findings, it was concluded that odontoclasts are similar to osteoclasts and that they are a highly metabolically active cell. Odontoclasts play an active role in tooth resorption just as osteoclasts are believed to do in bone resorption.

FURSETH (1968) regularly observed that repair tissue within resorption areas resembled cellular cementum in structure and was separated from the underlying tissue by a reversal line. Cementoblasts (hard tissue forming cells) were characterized by a rich, rough surfaced endoplasmic reticulum and a large number of mitochondria.

RYGH (1977) studied by transmission electron microscopy orthodontic root resorption in man. Projecting cell processes of the pioneer cells invading the hyalinized areas were surrounded by a light zone containing collagen fibrils and a flocculent material. On the cementum side the projecting cytoplasmic process of these cells seemed to cut off the hyalinized fibrils, leaving a

naked tooth surface. Odontoclasts were in intimate relationship with the capillaries on the non-resorbing cemental surface. Whereas the odontoclasts at the resorbing cemental surface showed numerous mitochondria and vacuoles. There was a complicated system of folds and deep penetrating clefts corresponding to the ruffled border of the osteoclasts. Resorption of cementum occurred as an undermining process, whereby odontoclasts attack cementum from behind in the resorption lacunae in dentine. This indicated that the surface of the cementum was more resistant to resorption than the dentine and that deeper parts of root cementum are more readily attacked than the periodontal surface.

2) Human SEM

BOYDE and JONES (1968) examined human root cementum using scanning electron microscopy. A high frequency of occurrence of small areas of resorption of the root surfaces in young teeth was observed. These areas of resorption were more common near the tips of roots and were often seen to be sites of commencement of cellular cementum deposition. Orthodontically moved teeth, showed particularly large and prominent areas of resorption on the pressure side, which extended into dentine. At the edge of resorption bays, intrinsic matrix was removed preferentially to the Sharpey fibres, which appeared to be "eaten round". Extrinsic (Sharpey) fibres were found to occupy almost 100% of the surface in acellular cementum; about 40% of the surface in cementum with intrinsic fibres but no cells, and 15-40% in cellular cementum. It was postulated that cellular cementum deposition in the root apex occurs as a response to the need to repair resorbed areas which were caused by minor (vertical) occlusal trauma.

KVAM (1972 a) studied, utilising scanning electron microscopy, tissue changes on the pressure surface of human premolars following tooth movement. Removal of hyalinized tissue started shortly after its formation

and during this soft tissue removal, the underlying hard tissue was resorbed. Observations of circumferentially removed, coalesced fibres, corresponded with the finding of extensively resorbed lacunae in the mineralized tissue beneath. Resorption in the cementum was characterized by numerous small, thin walled, round lacunae, which confluenced into extensive, shallow resorption bays. Dentine was distinguished by the presence of tubules and in sites of active resorption, removal of mineral crystals had occurred, exhibiting a lattice-fibre arrangement. Resorption of dentine attained a characteristic honeycomb appearance. All marginal root surfaces became resorbed following orthodontic movement. Coalesced, hyalinized fibres were frequently observed in hyalinized areas.

3) Rat TEM

RYGH (1972 a) studied the ultrastructural vascular changes in pressure zones of rat molar periodontium, incident to orthodontic movement. Retardation and stasis of the blood flow occurred within 30 minutes after force application. After 2 hours, the vessels in the pressure zone, appeared extended and packed with red cells. This was followed by fragmentation of the erythrocytes, occasionally transforming to a crystalline structure. Disintegration of the walls of the blood vessels and release of their contents into the surrounding fibrous tissue, was observed after 1-7 days. Blood vessels were severely damaged and degraded to a level from which a restitution to normal function was inconceivable.

RYGH (1972 b) studied the ultrastructural cellular reactions in pressure zones of rat molar periodontium, incident to orthodontic tooth movement. Cellular changes not involving the nucleus, such as dilation of the endoplasmic reticulum and moderate swelling of the mitochondria, occurred within 30 minutes of force application (5-25gms). After 2 hours, this cellular swelling was marked. Large vacuoles had formed in the cytoplasm and the

mitochondria frequently revealed considerable enlargement. More advanced degradation was characterized by the rupture of the cell membrane, leaving isolated nuclei in various stages of decomposition. It was suggested from these findings, that hyalinization involves severe injuries of fibroblasts and other connective tissue cells, resulting in cell death. However, necrosis was limited to circumscribed areas in the periodontal ligament.

RYGH (1974) conducted a transmission electron microscopic study of the elimination of hyalinized periodontal tissues associated with orthodontic tooth movement in rats. Hyalinized structures disappeared concomitantly with an invasion of cells and blood vessels from the neighbouring periodontal ligament. The removal of collagen, cell remnants and degraded vascular elements was apparently mediated by different forms of cellular activity. Breakdown of collagen fibrils was observed around cell processes as well as inclusions within the cytoplasm of invading pioneer cells. Penetration of new blood vessels seemed to be an important factor in the elimination of hyalinized tissue. Remnants of cells and erythrocytes were removed by phagocytic activity, while more extensive amorphous masses of vascular components were eliminated by foreign body giant cell activity.

Pioneer cells were mononuclear and varied in shape and size (15-30µm). While richer in mitochondria, the endoplasmic reticulum was scarce. They had a large number of cell processes, surrounded by a light zone. Large amounts of extracellular material were enclosed by cytoplasmic processes indicating phagocytosis. A mononuclear cell type was generally observed behind the advancing pioneer cells. They had even contour with few cytoplasmic processes. Rough-surfaced endoplasmic reticulum was extensively developed, indicating a role in the synthesis of new periodontal tissue. The foreign body giant cells consisted of an aggregation of individual

cells which were bordered by well-defined interlocking cell processes. The cytoplasm of these cells contained a network of vacuoles with content of low electron density (RYGH 1974).

4) Monkey SEM

In a study of dentine resorption in replanted monkey incisors, LINDSKOG et al. (1988a) proposed a 3-stage spreading model of dentinoclasts. In stage 1, dentinoclasts were characterised by an abundance of filapodia projecting towards the dentine surface. The cells appeared to be exploring the surface to find an area suitable for resorption. This was followed by an increase in cell size accompanied by a progressive disappearance of the peripheral filapod fringe (stage 2). Stage 3 apparently represented the final stage of adaption and was characterized by an active resorption of the dentine surface.

E. REPAIR OF ROOT RESORPTION

Root resorption is repaired initially by cellular intrinsic fibre cementum (CIFC) which is identical in structure to the intrinsic component of cellular mixed stratified cementum (CMSC) i.e. CMSC without Sharpey fibres. Subsequently, the CIFC may become coated with acellular extrinsic fibre cementum (AEFC) (SCHROEDER, 1986).

HENRY and WEINMANN (1951) observed that in large areas of resorption, there was a tendency for repair to begin with cellular cementum and to change to acellular cementum as the state of the anatomical repair was approached. Infrequently, however, the first-formed and only repair tissue may be AEFC deposited on dentine. (KRONFIELD, 1933).

Initially, a thin layer of CIFC smooths the scalloped bottom of the lacuna, while most of the latter is still filled with periodontal ligament tissue and

numerous cells, probably cementoblasts, at the CIFC surface. In the later stages, most of the dentinal lacunae become filled with CIFC up to the level of the AEFC. In the adjacent periodontal ligament, there is no preferential orientation of collagen fibres and the latter are not incorporated into CIFC. Eventually, with the fibres assuming proper alignment, a variably thick layer of AEFC may form on top of CIFC (JONES, 1981).

LINDSKOG et al. (1983) studied the repair of experimental cavities in dental roots and of periodontal wounds , both in vivo and in vitro . The reparative cementum did not fill the experimental cavities in the replanted teeth, but the alveolar bone extended into the experimental cavities, producing a normal width periodontal ligament. Epithelial and endothelial cells were numerous in the connective tissue separating the reparative cementum from alveolar bone in the experimental cavities. It was postulated that they may contribute to the integrity of the periodontal ligament and prevent resorption of the dental root.

The inorganic structural appearance of CIFC formed in resorption lacunae of teeth moved orthodontically is identical to that seen in deciduous teeth prior to their exfoliation. In regions remote from active resorption, the inorganic root surface of these teeth exhibits large, smooth areas covered by CIFC, with islands of residual CMSC in between. The structure of the CIFC is reminiscent of fibre bundles running parallel to the cementum surface, a reflection of the arrangement of the numerous microspherulites, similar in shape to grains of rice. The CIFC surface also reveals great numbers of half open cementoblast lacunae, but insertion sites of extrinsic fibres of Sharpey are lacking. Such areas of CIFC seem to fill former resorption lacunae but also spread over unresorbed CMSC surface regions (SCHROEDER, 1986).

Almost identical structures i.e. CIFC deposition with intrinsic fibre bundles orientated at random and parallel to the surface, are seen in the apical

region of erupted permanent teeth, mostly molars. New cementum formation is rarely limited to the confines of the resorption bays. Instead the bays fill up first, with small clusters of mineral particles which usually flow over on to the surrounding non-resorbed cementum surface (JONES and BOYDE, 1972). Eventually, reincorporation of Sharpey fibres may occur either directly into superficial CIFC or indirectly by superimposition of AEFC. Successive periods of alternating resorption and repair may follow each other at a particular region, producing reversal lines, which may be seen in histological section (HENRY and WEINMANN,1951; JONES, 1981).

LINDSKOG et al. (1987b) examined the cellular colonization of denuded root surfaces on replanted teeth of rhesus monkeys, using the scanning electron microscope. The dentine surface was rapidly colonized by a few macrophage-like cells, which resorbed dentine in limited areas. The resorption activity was gradually inhibited by the ingrowth of a monolayer of fibroblast-like cells from the periphery of the denuded area. After 6 weeks, the entire cavity was covered by a monolayer of these cells beneath which a cementum-like tissue was formed, indicating that the cells were cementoblasts. It was concluded that wound repair on the root surface is dependant upon the differentiation and proliferation of pre-cementoblasts in the periphery of the wound and that cells in the main body of the periodontal membrane are of little significance in the healing process on the root surface.

F. ORTHODONTIC ROOT RESORPTION ASSOCIATED WITH RME

TIMMS and MOSS (1971) investigated the histological effects of rapid maxillary expansion (R.M.E.) on the anchor teeth and their supporting tissues. In all cases of rapid expansion, there was root resorption in the coronal third of the mesio-buccal and disto-buccal aspects of the root. Two years after expansion, evidence of root resorption and repair were still

present. Resorption and repair of the buccal alveolar bone was indicated by reversal lines. Secondary dentine was found directly facing the trifurcation and pulp stones were present in several cases.

ANDREASEN (1980) demonstrated that damage to the root surface is maximal on those surfaces where physical contact occurs with the bone socket during rotatory movement, when extracting for transplantation. Similar circumstances most likely occur on the buccal root surface of anchor premolars for rapid maxillary expansion.

BARBER and SIMS (1981) studied external root resorption in human premolars used for anchor teeth during rapid maxillary expansion, utilising the scanning electron microscope. A number of significant observations were made:

1) The longer a tooth was held in an overcorrected position during retention, the more comprehensive the resorption.

2) Anchor premolars extracted after a short retention period, revealed small areas of active resorption and repair confined mainly to the cervical regions of the buccal roots.

3) Anchor premolars extracted after 20, 33 or 60 weeks of fixed retention showed large resorption bays, scattered along the entire buccal surfaces, but predominantly in the cervical and middle third regions.

4) Mesial and distal root aspects were also variably affected.

5) Small resorption bays found on the palatal root surfaces were mostly situated within the apical third zone.

6) Most resorption bays showed both resorption and repair occurring simultaneously. (Figure 1.3)

7) Active resorbing surfaces were characteristically smooth and multilocular in appearance and were delineated by a rim of relatively sheer and undermined cementum. (Figure 1.4)

8) During repair, the first Howship's lacunae to be filled in were those forming the floors of resorption bays. Some defects were overcontoured whilst others remained as shallow depressions.

It was established that iatrogenic resorption is sustained up to 9 months after termination of the active phase of the R.M.E. Relapse forces cause the alveolar bone to be compressed toward the buccal aspect of anchor teeth held rigidly by the expansion devices operating as retainers. Defects produced by R.M.E. while ultimately involving large areas of root surface, remained comparatively shallow and included only superficial layers of dentine. Cellular cementum repair usually began centrally on the floor of resorption bays and spread out in the wake of ongoing peripheral resorption (BARBER and SIMS, 1981).

Cementum rims of active resorption were routinely found to be relatively sheer, with the progress of resorption apparently unaffected by the presence of Sharpey fibres. As very few Sharpey fibre depressions were observed in the advancing mineral front of repair cellular cementum within the root defects, it was suggested that only very little principal periodontal fibre reattachment occurs. This constituted permanent damage to the ligament (BARBER and SIMS, 1981).

LANGFORD and SIMS (1982) investigated human repair and reattachment of principle periodontal fibres in areas of root resorption on anchor premolar root surfaces, following rapid maxillary expansion, using light and scanning electron microscopy. Extensive root resorption characterized the buccal surfaces of the anchor premolars. The resorption areas were always repaired with cellular cementum, which appeared to be identical with normal

apical cellular cementum. Contrary to BARBER and SIMS (1981), no direct relationship between the total area of resorption and the length of the retention period was observed. Repair however, was generally further advanced on specimens retained for longer periods.

Sharpey fibre depressions in repair cellular cementum were notably sparse. Their distribution was again similar to that seen in apical cellular cementum. Fibre bundles orientated predominantly at right angles were observed in resorbed and unrepaired dentine. It was suggested that as dentine is resorbed, part of its fibre matrix is retained and serves as an ongoing source of direct attachment with the periodontal fibres. Periodontal attachment to resorbed and repairing surfaces was observed. Periodontal fibres and fibre bundles inserted directly into repair cellular cementum matrix, irrespective of the site of the lesion on the root (LANGFORD and SIMS, 1982).

LANGFORD (1982) added further to observations made by LANGFORD and SIMS (1982). Areas of root resorption did not increase significantly from 14 to 52 weeks, but the proportion of repair tissue in the defects became greater with more prolonged retention periods. Topographic findings suggested that strong relapse forces were probably dissipated after 3 months of retention. There was no correlation between the period of R.M.E., the length of retention and the total area of resorption affecting the anchor teeth. ZIMRING and ISAACSON (1965) found that residual loads acting on their fixed appliances at the end of expansion were dissipated within 5 to 7 weeks. The resorption observed appeared to be laterally expansive rather than axially invasive, so that large areas were involved but pulpal integrity was not compromised. Forces capable of causing significant root resorption operate for up to 3 months. Any further root surface resorption may be maintained by light and diminishing medially directed forces.

SIMS and WEEKES (1985) observed the pattern of resorption and cementum deposition of human premolars used as anchors for rapid maxillary expansion , utilizing light and scanning electron microscopy. It was suggested, that there may be a relationship between vascular architecture and the processes of cementum deposition and root resorption. Unique vascular loops which exist at the apical portion of the ligament and at certain sites more coronally may play a role in the particular patterns of resorption of cementum and dentine. Apical vessels were thin walled, predominantly venular structures, characterized by the presence of myelinated and unmyelinated nerve groups, similar to baroreceptors in human periodontal ligament. Rapid fluid transfer could occur with the extravascular compartment during masticatory loading and orthodontic tooth movement which may contribute to root resorption.

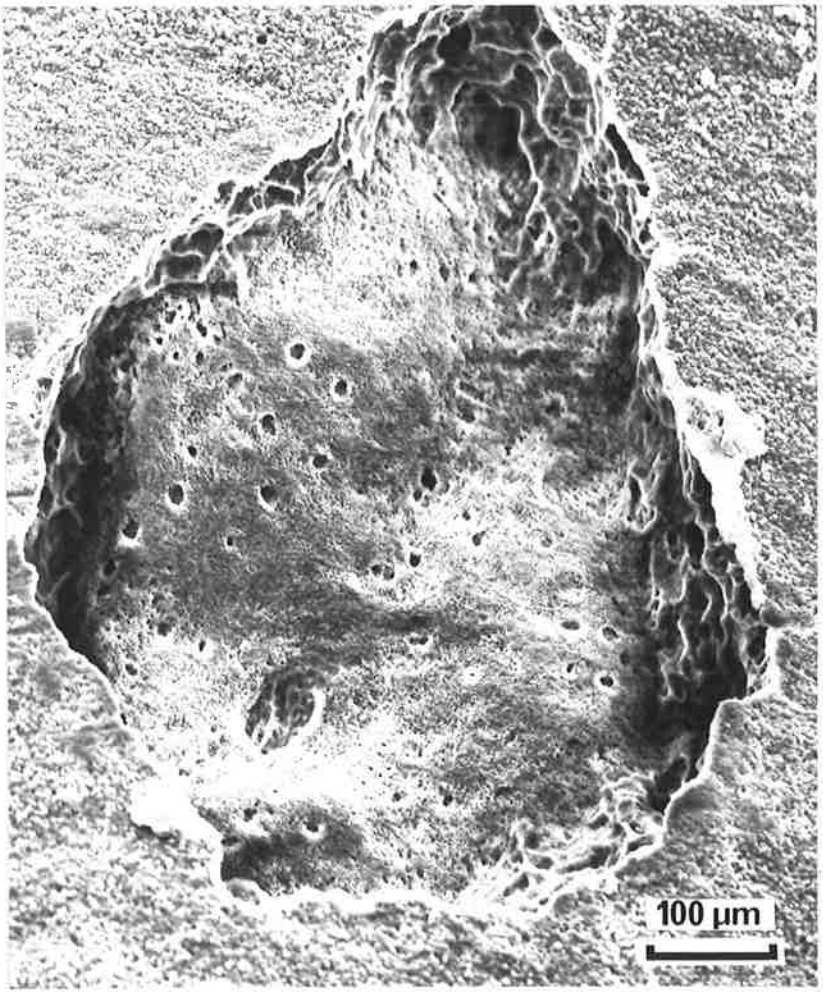

FIGURE 1.3 : Photomicrograph showing a repairing resorption bay with cellular cementum covering most of the Howship's lacunae in the floor of the bay. Distinct Howship's lacunae evident within the walls indicate sites of continuing resorption.

Magnification x 70 (Adapted from BARBER and SIMS, 1981)

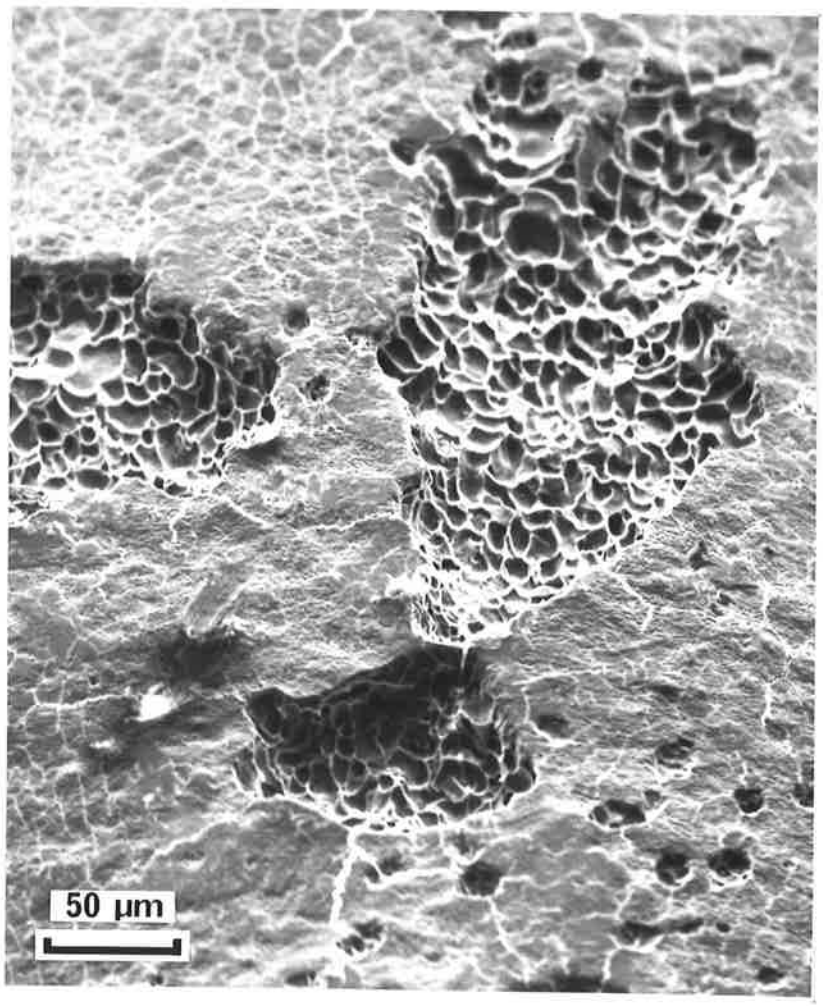

FIGURE 1.4 : Photomicrograph showing active resorption characterized by smooth multilocular surfaces. Typically, the resorption process has extended only into the more superficial layers of dentine.

Magnification x 100 (Adapted from BARBER and SIMS, 1981)

CHAPTER 2

MATERIALS AND METHODS

2.1 THE EXPERIMENTAL MATERIAL

Ten human maxillary first premolars, utilized as anchor teeth during rapid maxillary expansion were extracted after varying periods of retention (3-6 months) from five patients, aged between 12 and 17 years. The lower premolars were also extracted for use as controls. The length of activation of the R.M.E. ranged from 2-3 weeks and the period of retention varied from 3-6 months.

2.2 FIXATION AND DEMINERALISATION

Immediately after their removal, the teeth were rinsed for 10 seconds in 0.06M Cacodylate buffer (Appendix 6.1) to remove excess blood. They were then fixed in a mixture of 10 mls of 25% gluteraldehyde and 90 mls of 0.06M cacodylate buffer for 12-36 hours. After fixation the teeth were washed in fresh 0.06M cacodylate buffer for 10 minutes.

The teeth were then demineralised for 21 days in .01M EDTA solution (Appendix 6.3) at 4°C. EDTA was changed daily and constant agitation was provided by a magnetic stirrer.

2.3 TISSUE SECTIONS

To facilitate demineralization, a mid-buccal sliver (Figure 2.1) was cut from each premolar to a depth of 2 mms using the finest jeweller's hacksaw blade. The tooth crown was held using a Spectog plier clamp. It was important to keep the specimen damp with EDTA in cacodylate buffer and to

handle it as little as possible to prevent damage. Once cut the slivers were placed immediately into beem capsules which were placed immediately into EDTA and jiggled to remove entrapped air and allow solution into the capsule. If done correctly, the capsule should sink. The beem capsule was then hooked onto the glass rod and placed on the rotator in the refrigerator at 4°C for further demineralization. Prior to embedding, the slivers were generally cut into 9 sections: 3 cervical, 3 mid-root and 3 apical. (Figure 2.1)

2.4 EMBEDDING

The embedding procedure was as follows:

a. Rinse in 0.06M cacodylate buffer pH 7.4 for 18 hours. (overnight)

b. Postfix in 4% OsO4 (Appendix 6.2) in cacodylate buffer for 1 hour.

c. Wash in 0.06M cacodylate buffer for 15 mins.

d. Wash in 70% alcohol for 15 mins

e. Block stain in 1% uranyl nitrate (Appendix 6.4) in 70% alcohol for 1 hour.

f. Wash in 70% ethanol for 15 minutes.

g. Dehydrate 70% ethanol for 15 minutes
2x 80% ethanol for 15 minutes
2x 90% ethanol for 15 minutes
2x 100% ethanol for 15 minutes
2x Propylene oxide for 15 minutes
2x Propylene oxide for 30 minutes

h. Infiltration; Agar resin was prepared as described in Appendix 6.5
1:1 propylene oxide : Agar for 15 hours
1:3 propylene oxide : Agar for 4 hours
100% Agar for 4 hours
100% Agar for 15 hours

i. Processed tissue samples were then transferred from the glass vials to silicon rubber moulds and embedded in freshly prepared Agar. Using a stereo dissecting microscope, the sections of the buccal slivers were then orientated so that the mesio-buccal edge of the section faced the block face.

j. The moulds were cured at 37°C for 48 hours then at 60°C for a further 48 hours. The blocks were then stored at room temperature in labelled plastic vials.

2.5 SECTIONING

Specimen blocks were secured in a Reichart specimen holder chuck and mounted on a Reichart - Jung Om U4 ultramicrotome (C. Reichart, Wien, Austria). Recording sheets were used to assist with subsequent block orientation. A mesa was prepared using single sided razor blades and 1 µm orientation sections were cut using glass knives prepared on an LKB knife maker Type 780 1B. These sections were transferred to clean glass slides and stained with millipored 1% borax (Appendix 6.9) and .05% toluidine blue solution (Appendix 6.6) at 90°C for 2 minutes. The stained sections were rinsed with millipored, double-distilled water, dried and viewed under an Olympus EHT light microscope, fitted with an Olympus polaroid camera containing black and white Polaroid type 107 land film. Polaroid photographs of orientation sections were used to assist the preparation of the final mesa . From the final mesa, 1 µm sections were collected onto clean glass slides for light microscope observation.

From the same mesa, sections in the silver interference range (approx. 750°A) were cut using a diamond knife (Diatome Ltd., Bienne, Switzerland) with a clearance angle of 6°. Ribbons of silver sections were collected on to clean 3 mms, 150 mesh, nickel grids for electron microscopic observation.

The grids were temporarily stored on Whatman grade 1 filter paper in plastic petri dishes.

2.6 LIGHT MICROSCOPY

lμm sections were flattened with chloroform vapour and dried on to clean glass slides on a 90°C thermostatically controlled hot plate. Staining was carried out as previously described for orientation sections. Slides were examined under a Zeiss Axiomat light microscope and relevant sections photographed using Ilford FP4 plate film (4" x 5"ASA 125) . The best negatives were obtained with the Axiomat set at 50 ASA, a Zeiss green filter and oil emersion for high power (> 800x).

2.7 ELECTRON MICROSCOPY

Grid mounted sections were stained by floating them, tissue side down, on a droplet of millipored 0.5 % uranyl acetate solution (Appendix 6.7) for 7 minutes at 37°C. Grids were then washed in four consecutive millipore filtered, double-distilled water rinses at 37°C. Lead staining was then carried out by floating the grids, tissue side down, on a millipored droplet of modified Reynolds' lead solution (Appendix 6.8) for 3 minutes in a covered petri dish containing CO_2 absorbing NaOH pellets. The grids were again washed in four consecutive rinses of millipored d.d. water at 37°C and stored on damp Whatman grade I filter paper in covered plastic petri dishes.

Specimen grids were mounted, tissue side up, in the vacuum column of a Jeol l00S transmission electron microscope (Jeol Ltd., Tokyo, Japan) set with an accelerating voltage of 60 kV, a beam current of 50 micro-amps, a gun bias setting of 5 and a spot size of 2. Appropriate sites were photographed using the inbuilt photographic unit, loaded with Ilford Electron Microscope Technical film (6.2 cm X l0 cm). Focusing was assisted by the inbuilt image wobbler.

Negatives were developed for 4 minutes at 20°C in Kodak D-l9 developer (agitating for 15 seconds out of every 30 seconds), rinsed with water and fixed in Hypam rapid fixer for 7 minutes (agitating intermittently). After fixing, films were washed in deionized water containing a few drops of Photo-Flo wetting agent. After drying, the negatives were stored in clear cellophane envelopes. Selected ultramicrographs were printed on Ilfospeed glossy photographic paper using a Durst Laborator 54 enlarger. Prints were developed in Ilfospeed paper developer for 1 minute, fixed in Ilfospeed fixer and dried in an air dryer (Model R.C.D. - 33, F.C. Manufacturing Co. Ltd., Japan).

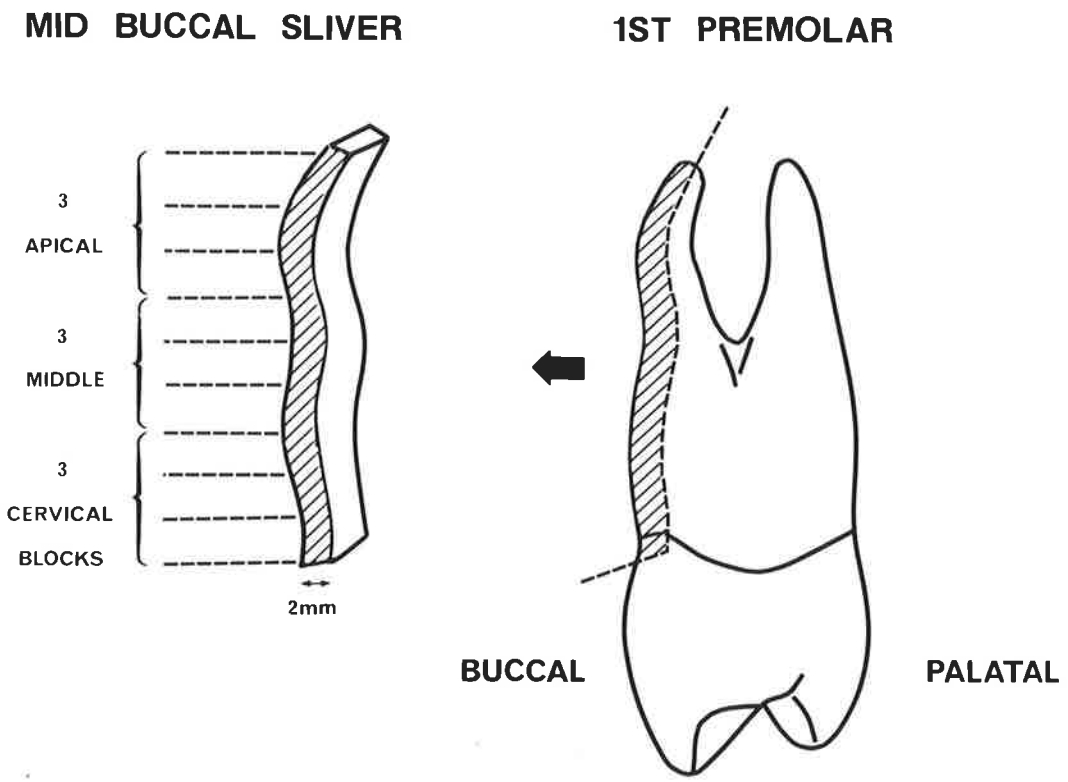

FIGURE 2.1: Diagram shows a mid-buccal sliver cut from each premolar to a depth of 2 mms. Each sliver was normally cut into 9 tissue blocks : 3 apical, 3 middle and 3 cervical.

CHAPTER 3

RESULTS

3.1 MATERIAL

Of the 92 experimental blocks of tissue, 42 blocks (46%) had less than 30μm of the periodontal ligament attached to the root surface. The majority of the ligament was lost during extraction of the teeth. However, the remaining 50 blocks of tissue (54%) had sufficient soft tissue covering for the purposes of this study.

3.2 EPITHELIAL CELL CLUSTERS IN REPAIRING RESORPTION BAYS

A. LIGHT MICROSCOPE

Twelve epithelial-like cell clusters were found in repairing root resorption bays of anchor premolars on the buccal surface of premolars used for anchor units during RME in five adolescent patients (Table 1) .

At the light microscope level, utilizing one micron sections stained with toluidine blue, the epithelial-like cell clusters appeared as darkly stained groups of cells relative to the adjacent repairing tissue (Figure 3.1). However, it was not possible to positively classify these cell clusters as epithelial at the light microscope level.

Subsequently, six of the twelve cell clusters listed in Table 1, from four separate patients, were positively identified as epithelial by their ultrastructural features, as detailed in later sections. The epithelial cell

clusters contained a mean of 5 cells, ranging from 2-8 cells. Their distance from the repairing cemental surface varied from 10-100 μm with a mean distance of 48 μm. Four of the six epithelial cell clusters were found in repairing resorption bays within the middle section of the root. The other two cell clusters were observed in the apical region within a predominantly repairing resorption bay which had a small area of active resorption at its edge (Figure 3.1). No epithelial cell clusters were observed in totally active resorption bays, but this may be due to the limited number studied in this project.

The presence of an epithelial network within the repairing resorption bays was not established.

B. TRANSMISSION ELECTRON MICROSCOPE

1) Low Power

The ultrastructural features apparent in all the epithelial cell clusters at magnification of x 6,000-8,000 included (Figure 3.2):

1) The cells were irregular in shape with single invaginated nuclei orientated toward the periphery of the cluster and abundant dark cytoplasm, containing many inclusions within the central area.

2) Each cell had one surface abutting the connective tissue. This irregular, pseudopodic outer surface was encircled by a discontinuous basement membrane.

3) Small cytoplasmic processes projected through the basement membrane toward adjacent undifferentiated mesenchymal cells.

4) The cell membrane adjoining other cells within the cluster contained numerous interdigitating microvilli.

2) High Power

At higher magnifications, epithelial cell clusters within repairing resorption bays were characterized by the presence of true desmosomes (macula adherens) and tonofilaments (Figures 3.3 and 3.4).

Other significant features more clearly seen at higher power included:

1) Numerous free ribosomes and polyribosomes were present throughout the cytoplasm, producing the dark appearance seen at lower power (Figures 3.3 and 3.4).

2) Golgi apparatus and endoplasmic reticulum were not consistently identified. However, a number of vesicle-like structures with numerous ribosomes at their periphery, resembling distended, rough endoplasmic reticulum, were observed throughout the cytoplasm (Figure 3.5). Profiles of what appeared to be Golgi apparatus were occasionally seen.

3) Nuclear pores were regularly observed (Figures 3.4 and 3.6).

4) The basement membrane was discontinuous (Figure 3.7).

5) Tight junctions were observed, which followed a wavy course over variable distances (Figure 3.6).

6) The mitochondria contained indistinct cristae and were not evenly distributed throughout the cytoplasm (Figure 3.7).

7) A small number of lipid bodies without an outer membrane were observed (Figure 3.5).

8) Non-striated collagen fibres were present immediately adjacent to the discontinuous basement membrane.

9) Solitary cilia were observed which had an outer arrangement of nine pairs of microtubules but no inner pair (Figures 3.3, 3.4 and 3.8).

10) There were no apparent dense aggregates of glycogen within the cytoplasm.

11) Numerous vacuoles were present throughout the cytoplasm.

3.3 EPITHELIAL RESTS OF MALASSEZ

A. LIGHT MICROSCOPE

Epithelial rests of Malassez were observed in the periodontal ligament adjacent to non-resorbed surfaces. Their size, shape, numbers and distribution along the root surface varied widely between patients, as did their distance from the root surface. A compilation of the results is presented in Tables 2 and 3.

There was less than 30μm of soft tissue attachment remaining on the root surface after extraction in 46% of the material collected. This prevented any valid statistical analysis of the distribution of the ERM adjacent to non-resorbed surfaces, as rests were found as far as 120 μm from the root surface.

The teeth with the least number of rests generally had tissue blocks with a limited amount of tissue attached to the root surface. The figures shown in Tables 2 and 3 may reflect the quality of the tissue collected rather than the true distribution.

Despite variations in attached soft tissue, some general observations concerning ERM can be made:

1) ERM were seen as either round, ovoid clusters containing a mean of 6 cells (Figures 3.10 and 3.11) or as long strands varying in size from 5 to 52 cells, mean 13 (Figure 3.9). The numbers of strands and clusters were approximately equal.

2) The majority of the ERM observed were in the middle and cervical regions. ERM were observed in the apical region in two patients.

3) Although the mean distance of the ERM from the root surface was 60μm, the range varied from 10μm to 120μm.

4) The cells were closely packed, with large nuclei and a narrow rim of cytoplasm. The nuclei in the clusters were generally located toward the periphery, with the larger portion of the cytoplasm concentrated toward the centre.

5) The clusters and strands were surrounded by a smooth, darkly stained basement membrane.

6) The ERM were not evenly distributed and no distinct epithelial network was observed.

7) The staining of the ERM varied from light to dark depending on their cytoplasmic inclusions, as was shown in subsequent TEM findings (Figure 3.9).

8) Epithelial rests of Malassez were observed in close approximation to active cementogenesis on non-resorbed surfaces (Figures 3.11 and 3.12).

9) ERM appeared to separate the surface from the adjacent blood vessels (Figure 3.11).

B TRANSMISSION ELECTRON MICROSCOPE

The epithelial rests of Malassez adjacent to non-resorbed root surfaces of the anchor premolars were characterized by the presence of true desmosomes (macula adherens), tonofilaments and a continuous basement membrane (Figure 3.16).

The epithelial rests normally contained two distinct cell types (Figures 3.13 , 3.14 and 3.15):

1) Clear cells whose cytoplasm contained very few ribosomes, tonofilaments or any other cytoplasmic inclusions. However, the

nuclei were not pyknotic and the nuclear and cell membranes were intact.

2) Dark cells which were characterized by an abundance of free ribosomes, polyribosomes, tonofilaments but no positively identifiable endoplasmic reticulum or Golgi apparutus.

The ratio of clear to dark cells varied from widely.

The cells within the rest (Figure 3.13) normally had one surface abutting the connective tissue which was usually even, conforming to the smooth contour of the encircling continuous basement membrane. The cell surfaces abutting each other within the rest were relatively even with very few interdigitating microvilli.

However, an ERM was observed adjacent to an area of cementogenesis on a non-resorbed root surface of an anchor premolar (Figure 3.17). The rest contained only dark cells and no clear cells were present. The irregular pseudopodic outer surface contained cytoplasmic processes projecting out through a discontinuous basement membrane. The inner surfaces contained many interdigitating microvilli (Figure 3.18).

It appears there may be a relationship between increased cementogenesis and the following changes within the ERM:

1) increased numbers of dark cells
2) increased outer surface irregularity
3) the appearance of cytoplasmic projections
4) increased number of interdigitating microvilli.

These are purely subjective morphological observations. Stereological point counting producing statistical correlations is required to verify these observations.

Further observations which support the concept that the ERM interact with the adjacent connective tissue were made:

1) The basement membrane was discontinuous in areas of exocytosis/endocytosis. Areas of possible phagocytosis were also observed (Figure 3.19).

2) Non-striated collagen fibres were found immediately adjacent to a discontinuous basement membrane (Figure 3.20).

3) ERM and mesenchymal cells were seen in close morphological relationship. In one section, a desmosome-like junction was seen between a fibroblast-like cell and ERM (Figure 3.21).

4) Structures resembling distended rough endoplasmic reticulum were observed in the dark cells (Figure 3.22).

Other significant ultrastructural features observed include:

1) Cilia were present in both dark and clear cells. They had an outer arrangement of 9 pairs of microtubules but no inner pair (9+0 axoneme) (Figure 3.23).

2) The mitochondria contained indistinct cristae and were not evenly distributed throughout the cytoplasm (Figure 3.19).

3) A small number of lipid bodies were observed in both clear and dark cells (Figure 3.14).

4) Numerous vacuoles were present throughout the cytoplasm.

3.4 COMPARISON OF ERM AND ECC

Many major ultrastructural features of the dark cells within the epithelial rests of Malassez were similar to those features found in the epithelial cell clusters

within repairing resorption bays. The common major features included:

1) An abundance of free ribosomes and polyribosomes throughout the cytoplasm.

2) An apparent lack of well developed Golgi apparatus and endoplasmic reticulum.

3) The presence of characteristic true desmosomes (macula adherens) and tonofilaments.

4) Invagination of the nuclear membrane and nuclear pores.

5) Mitochondria lacked distinct cristae.

The epithelial rests of Malassez adjacent to non-resorbed surfaces differed from epithelial cell clusters within repairing resorption bays in the following ways:

1) The basement membrane of ERM was continuous, whilst the ECC was discontinuous.

2) The cell surface of ERM abutting the connective tissue was usually even, conforming to the smooth contour of the basement membrane, whilst the pseudopodic outer surface of ECC contained cytoplasmic projections.

3) The internal cell surfaces within the ERM did not contain many interdigitating microvilli whilst they were numerous in the epithelial cell clusters.

4) ECC did not contain any clear cells observed with ERM.

The ultrastructural morphology of ECC in repairing resorption bays very closely resembled that of an ERM observed adjacent to an area of cementogenesis on a non-resorbed root. Common features included:

1) Only dark cells were present (Figures 3.17 and 3.18).

2) The irregular pseudopodic surface contained cytoplasmic processes projecting out through a discontinuous basement membrane.

3) The inner surface consisted of many interdigitating microvilli. However this was only a single observation. ERM similar to those described adjacent to non-resorbed surfaces were also observed adjacent to active cementogenesis (Figure 3.14).

3.5 RESORPTION BAYS

A. LIGHT MICROSCOPE

The number of resorption bays and their distribution along the root surface are shown in Table 4. Of the total number of resorption bays observed, the following percentages were of particular interest: 85% were undergoing repair ; 85% were located in the cervical and middle root region.

1) Active Resorption

Active resorbing surfaces (Figure 3.1) were characterized at the light microscope level by a sharp delineation between dentine and the adjacent soft tissue within the bays. There was no cellular cementum present on the surface of the resorbed dentine within the Howship's lacunae and very few collagen fibre insertions were observed. Numerous blood vessels were present within the active resorption bays. Classical multi-nucleated odontoclasts with ruffled borders were occasionally observed but more commonly, large mononuclear cells with vacuolated cytoplasm were seen in close relation to the resorbing surface. Active resorption was found in five blocks. Of these five, only two blocks contained adequate soft tissue for meaningful study (greater than 30 µm). In areas of active resorption, the

overlying soft tissue was often lost, leaving scalloped dentine with no tissue covering it.

2) Repairing Resorption

Repairing surfaces (Figure 3.1) were characterized by deposits of cellular, fibrillar cementum over the resorbed dentine, with a clear reversal line between them. Very few blood vessels were observed within the repairing resorption bay. Collagen fibres inserted into the repairing cementum and ran out into the adjacent soft tissues at right angles to the surface. Cementoblasts adjacent to the repairing cellular cementum had large round to ovoid nuclei with extensive light staining cytoplasm. Cellular cementum was deposited in all the repairing areas and was characterized by the presence of cementocytes and intrinsic fibres. The cementoid stained darker than cementum and dentine. Repairing resorption bays were found in 25 (27%) of the 92 tissue blocks studied.

3) Active and Repairing Resorption

Both active resorption and repair were observed within the same bay in 3 blocks (Figure 3.1). The small areas of active resorption were predominantly at the edges of the bay where it undermined the normal root surface. Repairing cementum was thicker toward the centre of the resorption bay.

All the resorption bays had penetrated the acellular cementum and involved dentine. No surface resorption of acellular cementum alone was observed. The bays varied in width greatly but the depth remained relatively shallow, such that the larger bays appeared wide but shallow. Large areas of root resorption and repair were also found in the lower premolars, extracted for control purposes. This effectively negated their use as controls.

B. TRANSMISSION ELECTRON MICROSCOPE

1) Active Resorption

Odontoclasts were not present in all the resorption lacunae in areas of active resorption. They had the following ultrastructural features (Figures 3.24, 3.25) :

1) The cells were large and irregular, with one to four nuclei. The chromatin was evenly dispersed and the nucleoli were prominent.

2) The "ruffled" border closely approximated the tooth surface and ran roughly parallel to the lacunal border. It consisted of a loose arrangement of small, irregular, microvilli projections which interdigitated with the adjacent hard tissue. The odontoclasts not adjacent to the root surface did not display a "ruffled border".

3) A "clear zone", that is, an area of organelle free cytoplasm encircling the ruffled border ,was not observed.

4) The cytoplasm contained a large number of distinct mitochondria with well-formed cristae. No signs of degeneration were evident.

5) The rough endoplasmic reticulum was numerous and evenly distributed throughout the cytoplasm. Golgi apparatus was observed in fine canals close to the nucleus.

6) The cytoplasm was highly vacuolated and contained abundant free ribosomes. The vacuoles varied in size and were separated from the cytoplasm by a single membrane. These vacuoles appeared empty or contained varying amounts of loose amorphous debris or membranous material or both.

7) Microtubules/filaments were abundant throughout the cytoplasm.

Collagen fibre bundles were attached directly to the resorbed dentine surface in areas of active root resorption in anchor premolars. Both

continuous type and adhesive type attachments (KURIHARA and ENLOW, 1980 a,b) were formed (Figure 3.26).

"Companion cells" were interposed between the odontoclasts and the resorbed dentine surface in areas of active resorption (Figures 3.24 and 3.25). These cells were similar in ultrastructure to cementoblasts and were thought to secrete the dark granular substance at the surface of the resorbed dentine adjacent to these cells (Figure 3.27). It is postulated that this darker granular substance "welds" the newly formed collagen fibres to the dentine, producing adhesive-type junctions.

2) Repairing Resorption

A reversal line between the resorbed dentine and the repairing cellular cementum could not be easily identified at the TEM level (Figure 3.28). Cementocytes were observed within the repairing cementum and were present at the repairing surface. The ultrastructure of these cells was not studied in detail.

Collagen fibres passed directly from the repairing cellular cementum surface into the adjacent tissue, providing attachment between the repairing root surface and the periodontal ligament (Figure 3.28).

Precementoblast-like cells were seen between the ECC and the repairing surface (Figure 3.29).

TABLE 1

MORPHOLOGY and DISTRIBUTION of EPITHELIAL CELL CLUSTERS (ECC) WITHIN REPAIRING ROOT RESORPTION BAYS

SPECIMEN	NUMBER of cells	DISTANCE from root	RESORPTION type	LOCATION on root
AA (14)	4	20 µm	RR/AR	C
AA (14)	3	10	RR	M
LB (24)	5	10	RR	C
LB (24)	4	10	RR	M
LB (24)	8*	10	RR	M
LB (24)	7*	10	RR	M
KL (14)	4*	40	RR	M
NM (14)	5*	10	RR	M
MR (24)	5	30	RR	A
MR (24)	3*	100	RR/AR	A
MR (24)	5	50	RR/AR	A
MR (24)	2*	80	RR/AR	A

RESORPTION - repairing resorption **(RR),** active resorption **(AR)**

LOCATION - cervical **(C),** middle **(M)**, apical **(A)**

* Epithelial cell clusters positively identified by their ultrastructure.

TABLE 2

MORPHOLOGY OF ERM NON-RESORBED SURFACE

SPECIMEN	TOTAL ERM OBSERVED	NUMBER OF CLUSTERS	NUMBER OF STRANDS
AA (24)	2	1(10)	1(16)
AA (14)	2	2 (8)	0
LB (24)	1	1 (5)	0
LB (14)	4	1 (5)	3 (7)
KL (14)	21	7 (6)	14 (10)
NM (14)	3	2 (5)	1 (7)
MR (14)	15	12 (5)	3 (14)
MR (24)	11	7 (6)	5 (26)
TOTAL	59	33 (6)	27 (13)

* The figures in brackets represent the mean number of cells within the ERM for each specimen.

TABLE 3.

DISTRIBUTION OF ERM / NON-RESORBED SURFACE

SPECIMEN	CERVICAL	MIDDLE	APICAL	TOTAL
AA (24)	2 (80)	0	0	2
AA(14)	1 (15)	1 (20)	0	2
LB (24)	1 (10)	0	0	1
LB (14)	4 (12)	0	0	4
KL(14)	0	16 (77)	5 (20)	21
NM(14)	2 (35)	1 (40)	0	3
MR (14)	6 (55)	4 (50)	5 (47)	15
MR (24)	1 (20)	11 (99)	0	12
TOTAL	17 (32)	33(57)	10 (33)	60

*The figures in brackets represent the mean distances of the ERM from the root surface (measured in microns).

TABLE 4

TYPE AND DISTRIBUTION OF ROOT RESORPTION

	CERVICAL	MIDDLE	APICAL	TOTAL
ACTIVE	3	0	1	4 (12%)
REPAIRING	8	17	4	29 (85%)
ACT/REP	1	0	0	1 (3%)
TOTAL	12 (35%)	17 (50%)	5 (15%)	34

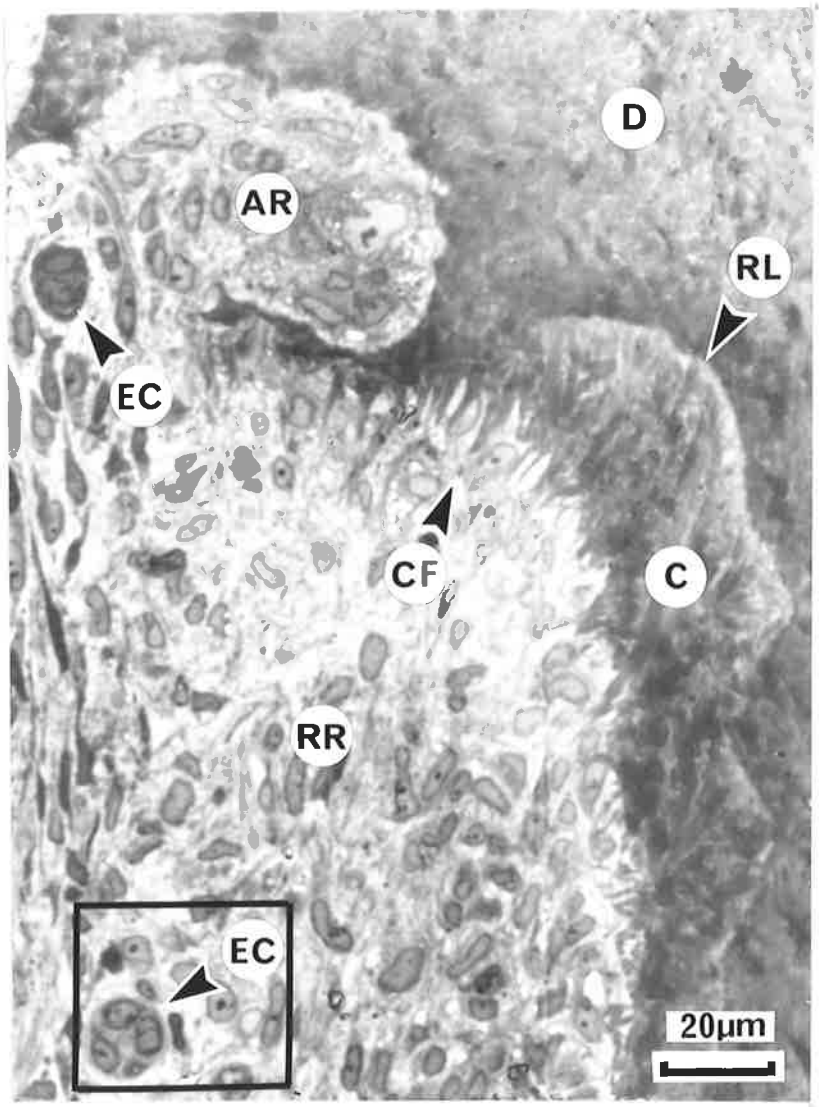

FIGURE 3.1 : Photomicrograph of a light microscope section stained with toluidine blue, showing two epithelial cell clusters (**EC**) within a large, predominantly repairing root resorption bay **(RR)** found in the apical third of the buccal surface of a human premolar used as an anchor unit during rapid maxillary expansion. The presence of a reversal line (**RL**) between the dentine (**D**) and the repairing cellular cementum (**C**) was indicative of repairing root resorption. Collagen fibres (**CF**) inserted into the cellular cementum. A small area of active resorption (**AR**) undermined the non-resorbed root surface and was characterized by the absence of repairing cellular cementum and a reversal line.

Magnification x 375 ; Enlargement x 1.8

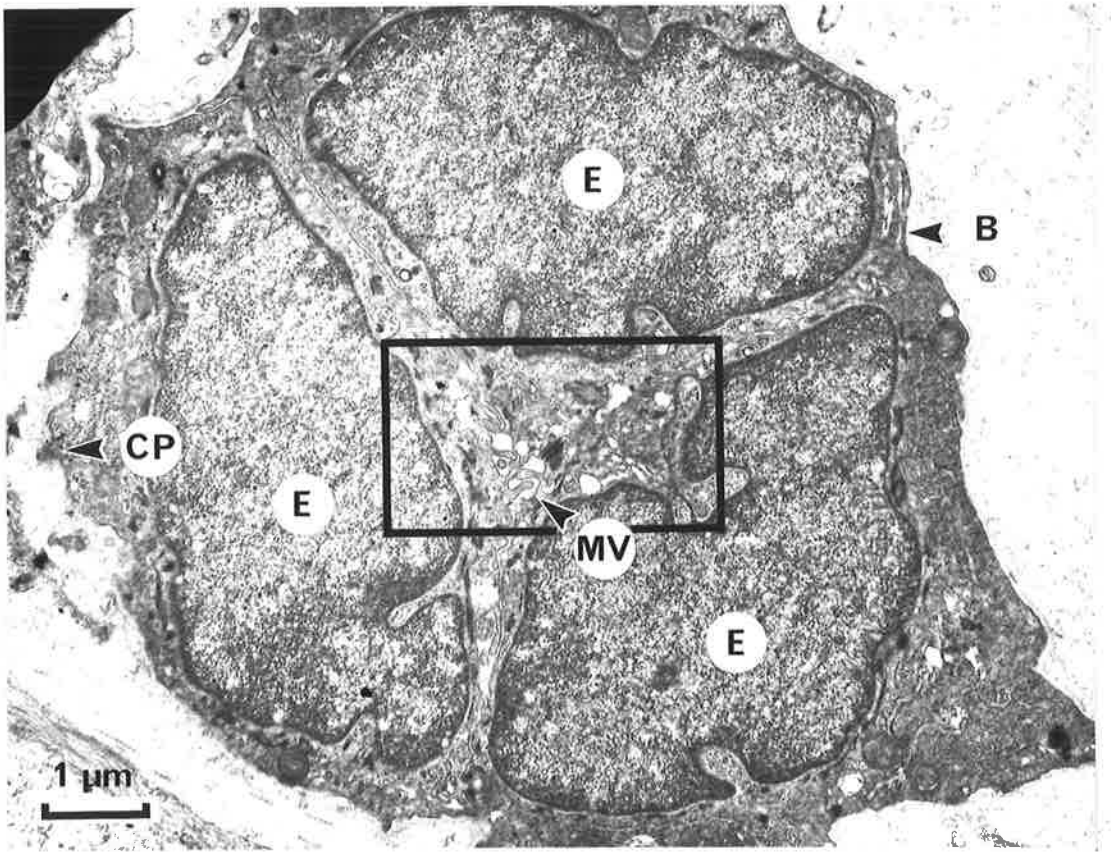

FIGURE 3.2 : Low power electron micrograph of the epithelial cell cluster outlined in Figure 3.1, within a repairing resorption bay. This cluster contained three epithelial cells **(E)** which were irregular in shape and contained single invaginated nuclei. The irregular, pseudopodic outer surface was encircled by a discontinuous basement membrane **(B)**. Cytoplasmic processes **(CP)** projected through the basement membrane. The inner surfaces contained many interdigitating microvilli **(MV).**

Magnification x 8000 ; Enlargement x 1.5

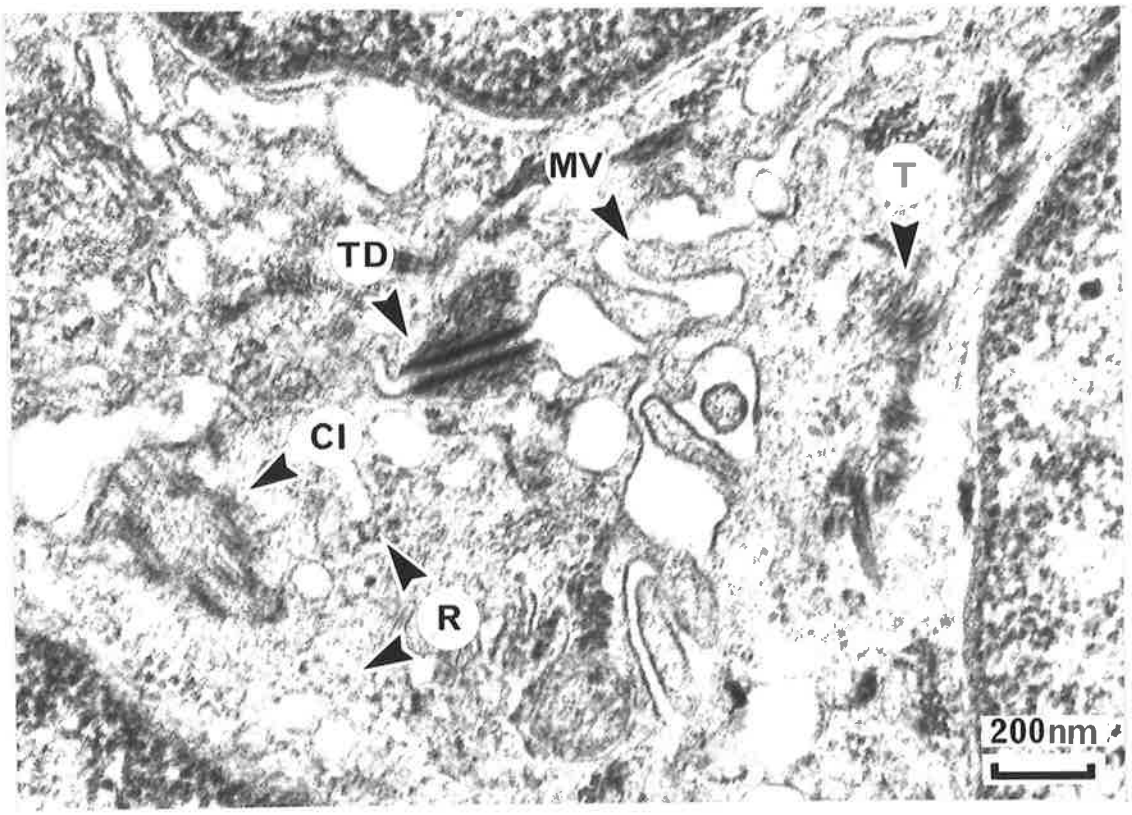

FIGURE 3.3 : High power electron micrograph of the central region outlined in the epithelial cell cluster in Figure 3.2. The epithelial clusters were characterized by the presence of true desmosomes **(TD)** and tonofilaments **(T)**. Other features include : numerous polyribosomes and ribosomes **(R)**, interdigitating microvilli **(MV)** and an oblique section through a cilium **(CI)**, with a 9+0 microtubular configuration.

Magnification x 25,000 ; Enlargement x 2.4

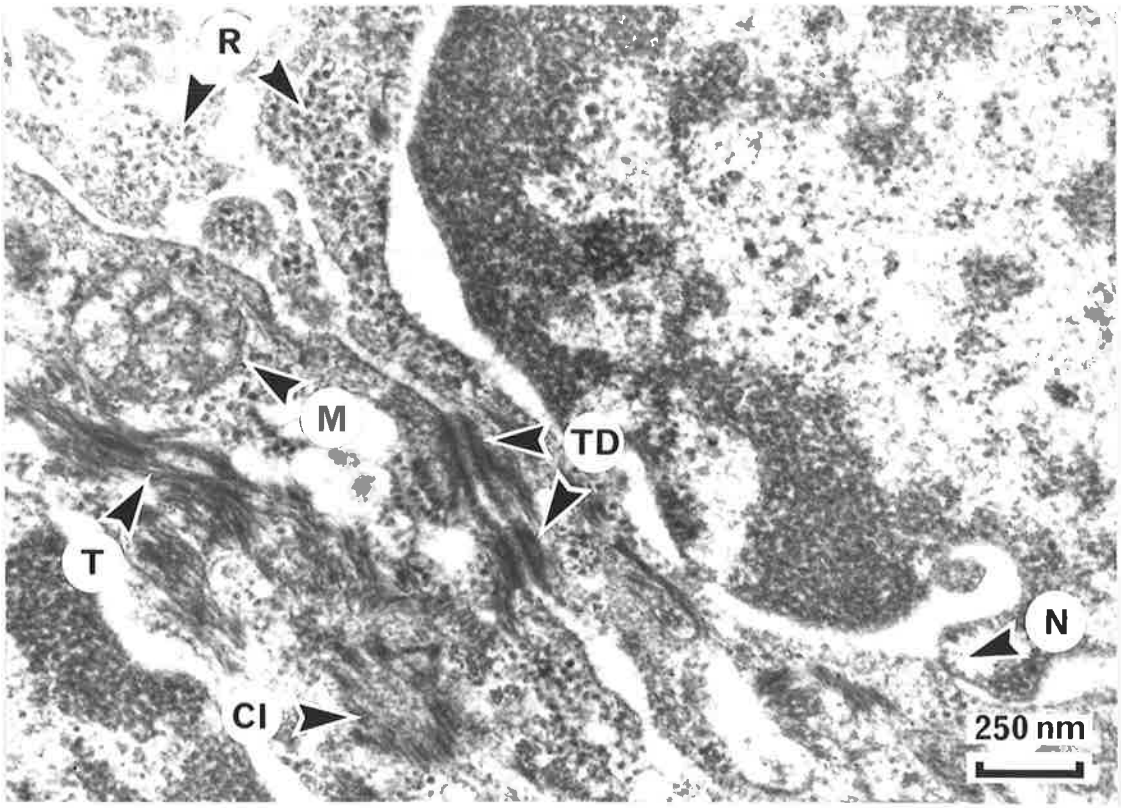

FIGURE 3.4 : High power electron micrograph of an epithelial cell cluster within a repairing resorption bay. The characteristic features were true desmosomes **(TD)** and tonofilaments **(T).** Other prominent features include: numerous polyribosomes and ribosomes **(R),** mitochondria **(M)** with indistinct cristae, nuclear pores **(N)** and cilia **(Cl)**, with a 9+0 configuration. Magnification x 30,000 ; Enlargement x 1.7

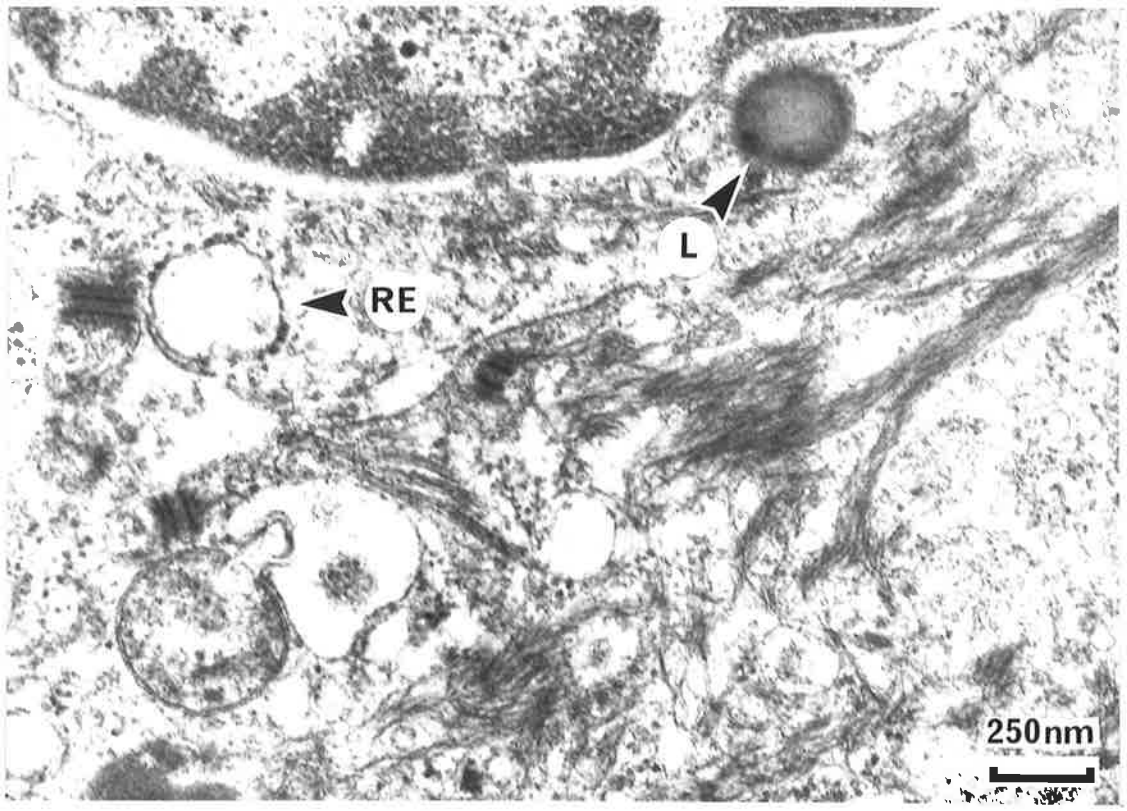

FIGURE 3.5 : High power electron micrograph of an epithelial cell cluster within a repairing resorption bay. Structures which resembled distended rough endoplasmic reticulum **(RE)** were observed. Lipid bodies **(L)** were seen occasionally.

Magnification x 30,000 : Enlargement x 1.6

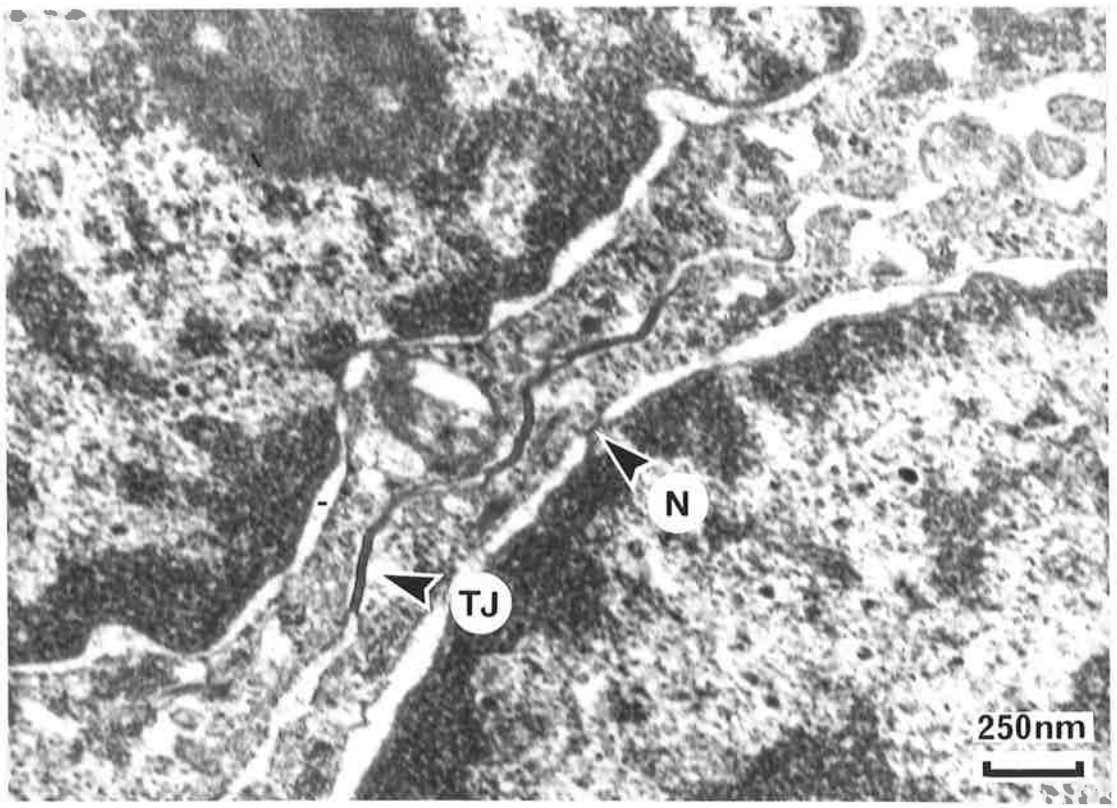

FIGURE 3.6 : High power electron micrograph of a tight junction **(TJ)** within an epithelial cell cluster in a repairing resorption bay. They followed a wavy course over variable distances. A number of nuclear pores **(N)** can also be seen.

Magnification x 30,000 ; Enlargement x 1.6

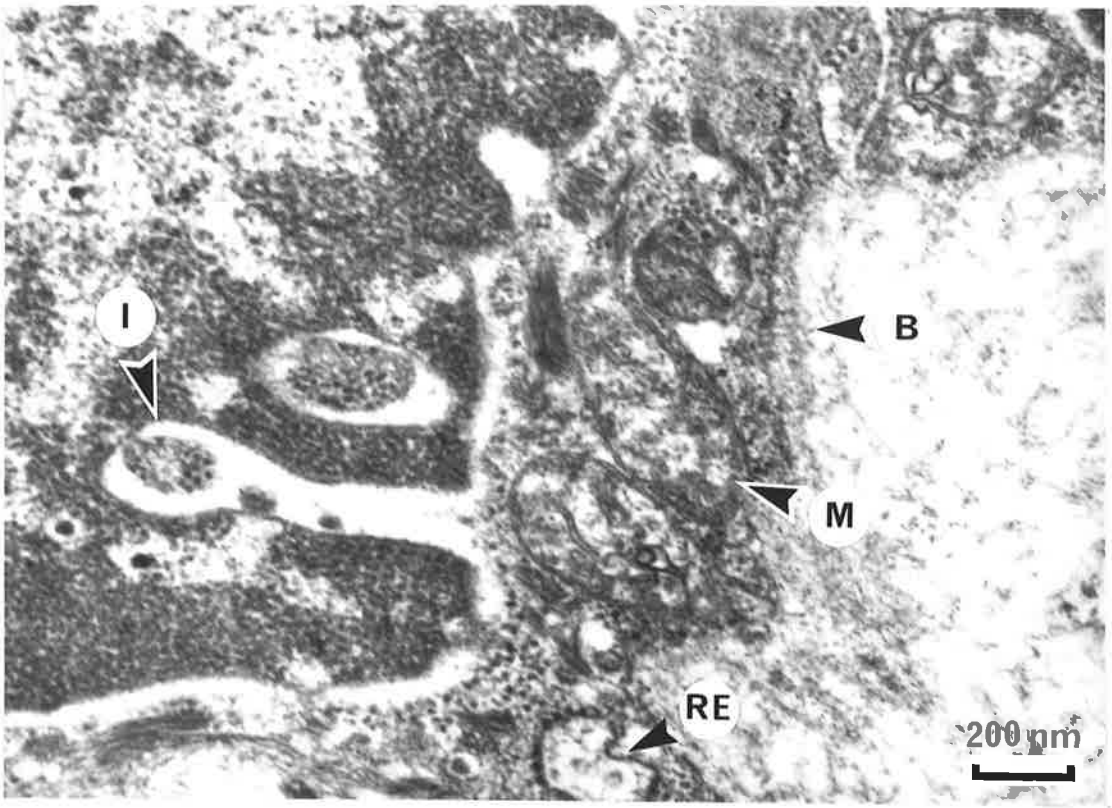

FIGURE 3.7 : High power electron micrograph of an epithelial cell cluster showing a discontinuous basement membrane **(B)**, mitochondria with indistinct cristae **(M)**, a vesicle with peripheral ribosomes, possibly a distended rough endoplasmic reticulum **(RE)** and an invagination of the nuclear membrane **(I)**.

Magnification x 30,000 ; Enlargement x 2.0

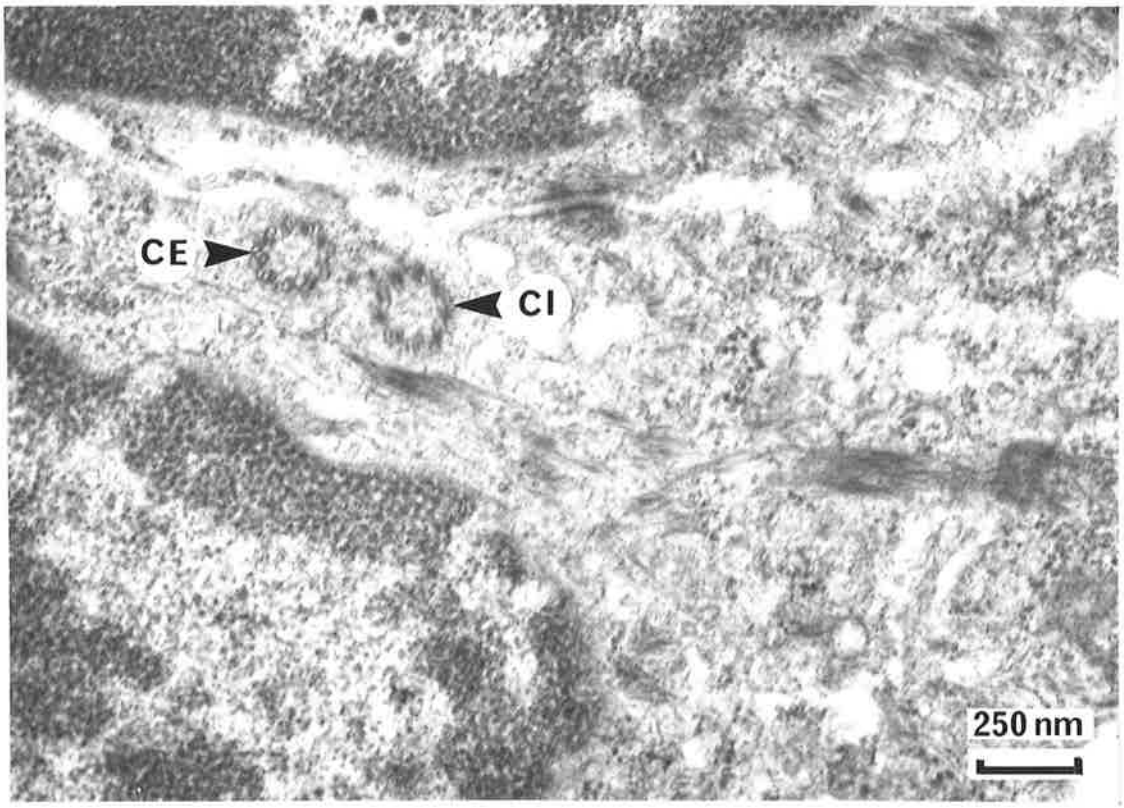

FIGURE 3.8 : High power electron micrograph showing an epithelial cell cluster within a repairing resorption bay. Cross sections through a centriole **(CE)** with its characteristic pattern of 9 triplets and a cilium **(CI)** with a 9+0 doublet arrangement of microtubules can be seen.
Magnification x 30,000 ; Enlargement x 1.6

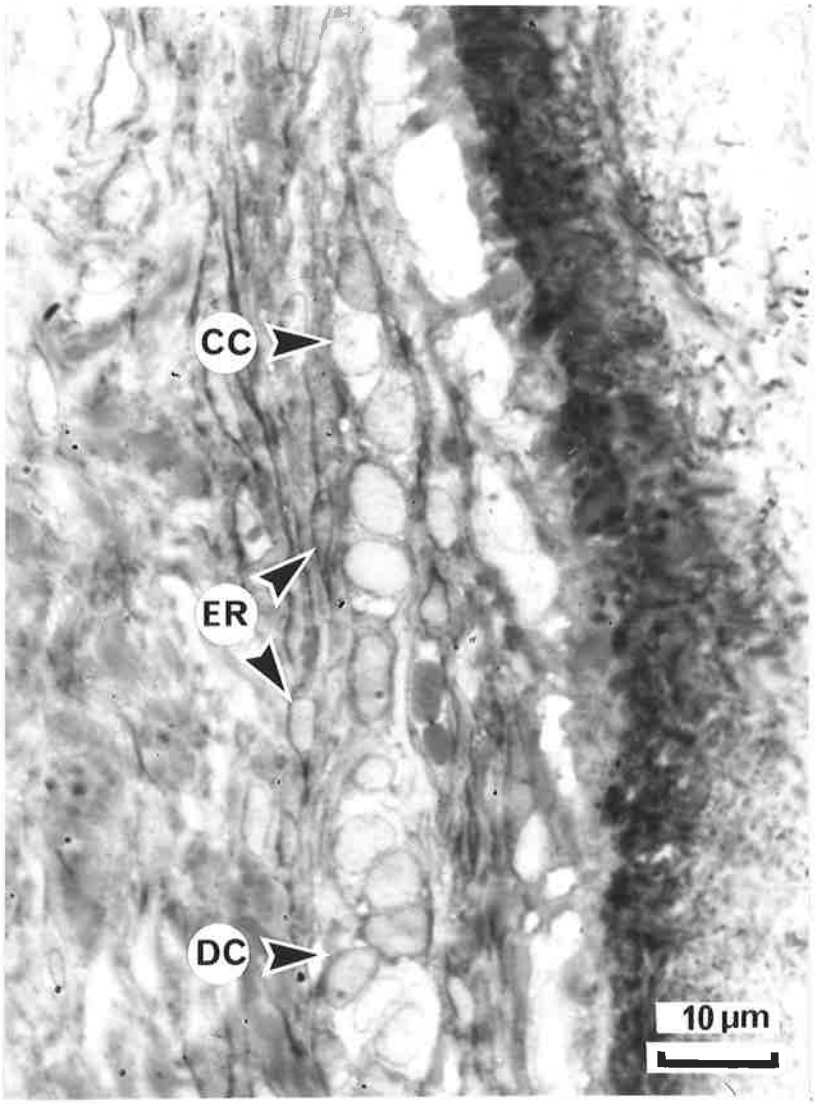

FIGURE 3.9 : Photomicrograph of an epithelial rest of Malassez **(ER)** forming a long strand close to a non-resorbing surface in the apical third of the root. Both clear cells **(CC)** and dark cells **(DC)** can be seen within the strand. Magnification x 1,000 ; Enlargement x 1.4

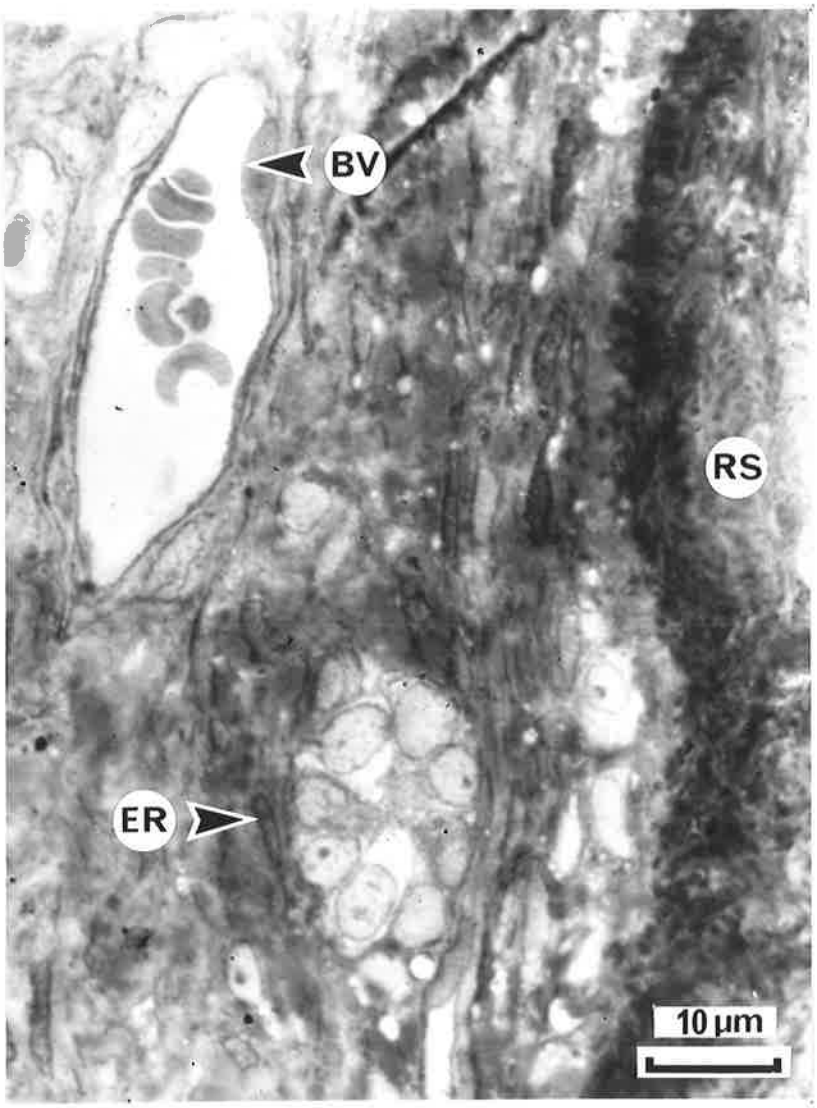

FIGURE 3.10 : High power photomicrograph showing a large ovoid ERM **(ER)** interposed between the non-resorbed root surface **(RS)** and an adjacent blood vessel **(BV)**, within the apical third of the root. The ERM contained both clear and dark cells.

Magnification x 1,000 ; Enlargement x 1.6

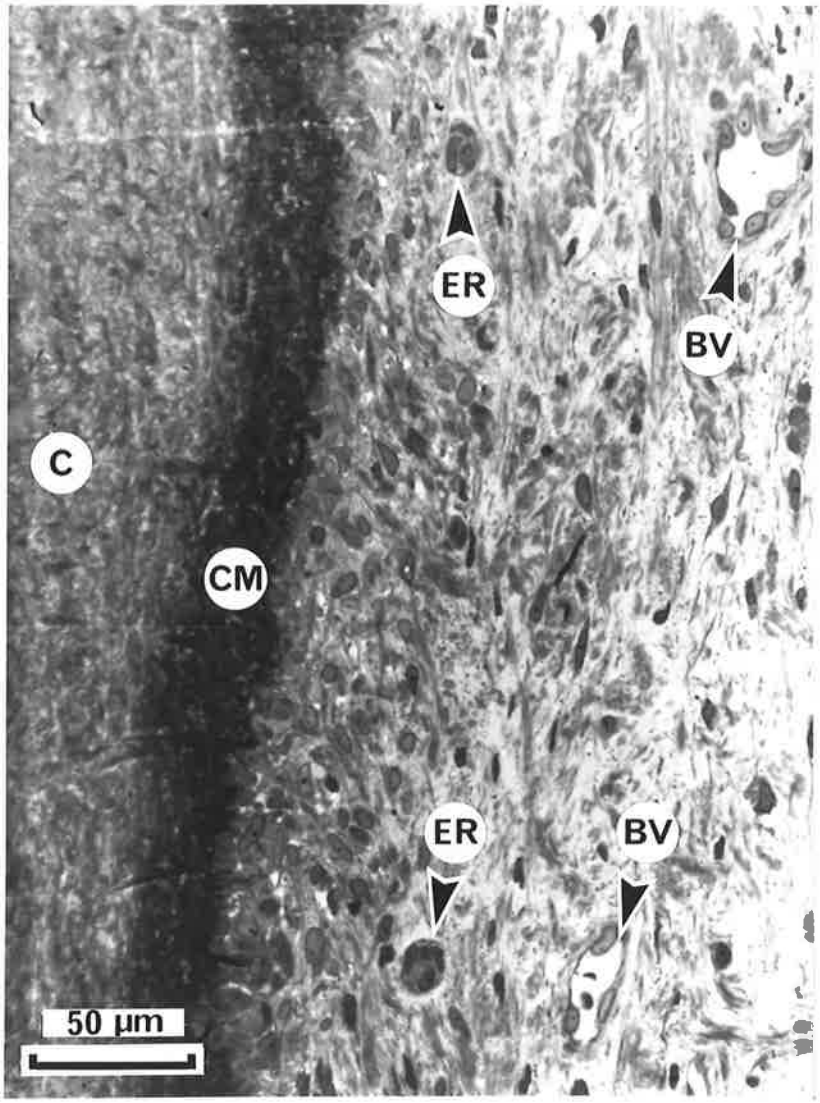

FIGURE 3.11 : Photomicrograph showing two ovoid epithelial rests of Malassez **(ER)**, adjacent to an area of cementogenesis within the apical third of the root. The cementoid **(CM)** stained darker than the cellular cementum **(C).** Note that the epithelial rests separate the root surface from the adjacent blood vessels **(BV)**.

Magnification x 300 ; Enlargement x 1.3

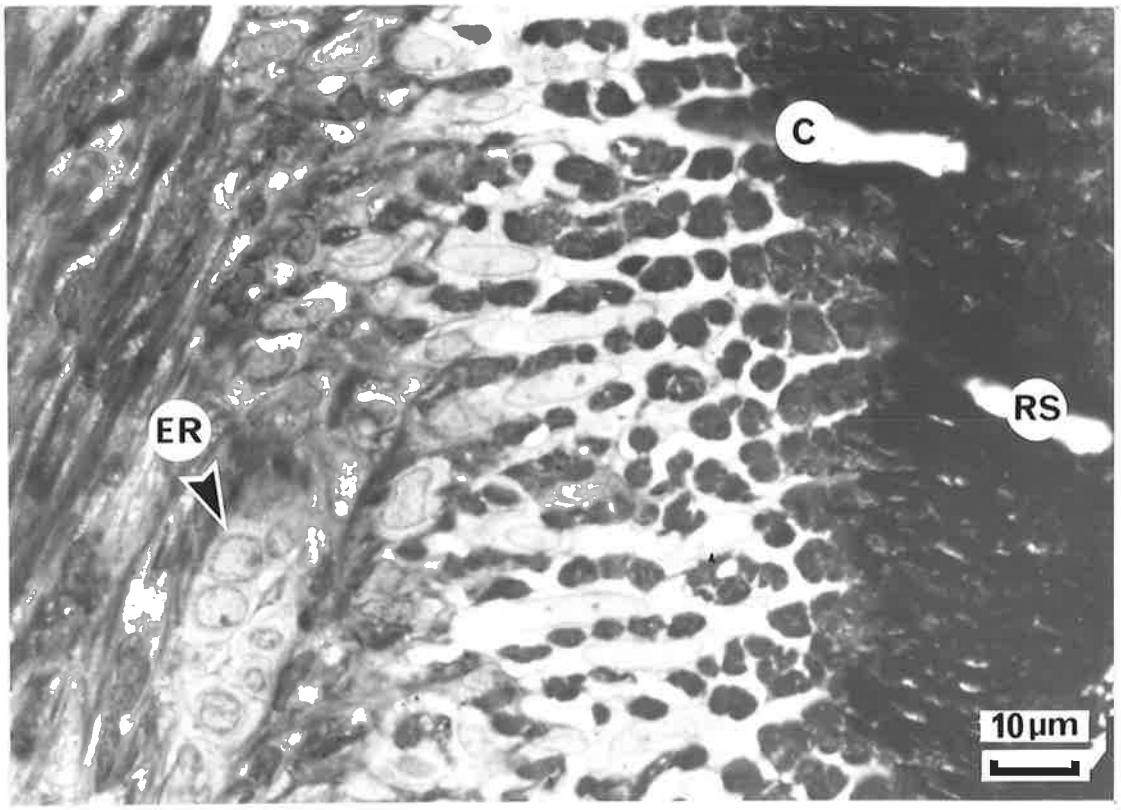

FIGURE 3.12 : A high power photomicrograph showing an ERM adjacent to an area of very active cementogenesis in the mid-root region of an anchor premolar. The cellular cementum **(C)** appears to "pallisade" from the ERM **(ER)** to the root surface **(RS)**.

Magnification x 800 ; Enlargement x 1.3

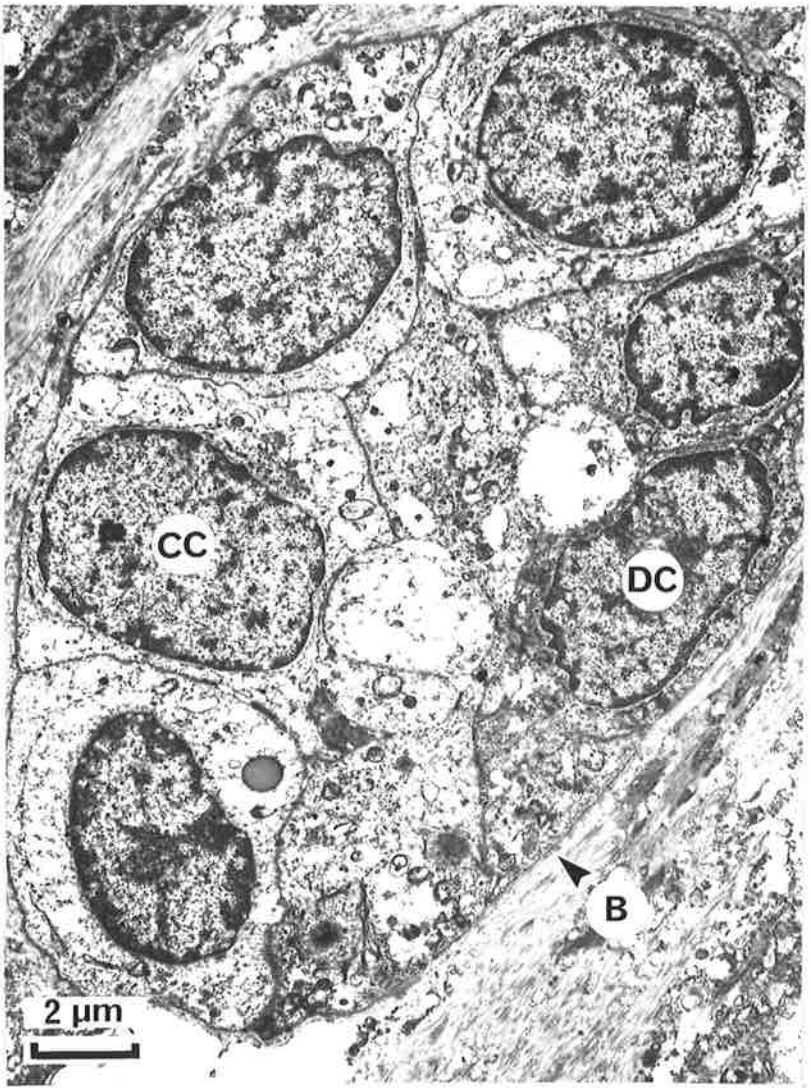

FIGURE 3.13 : A low power electron micrograph of an ERM adjacent to the non-resorbed surface of an upper premolar used as an anchor unit during RME. The ERM contained two distinct cell types. The lightly stained clear cells **(CC)** appeared vacuolated and contained very few cytoplasmic inclusions whilst the dark cells **(DC)** contained numerous ribosomes and tonofilaments. The cells appeared to have one surface abutting the connective tissue which was usually even, conforming to the smooth contour of the encircling, continuous basement membrane **(B)**. The inner surface did not contain many interdigitating microvilli.

Magnification x 4,000 ; Enlargement x 1.6

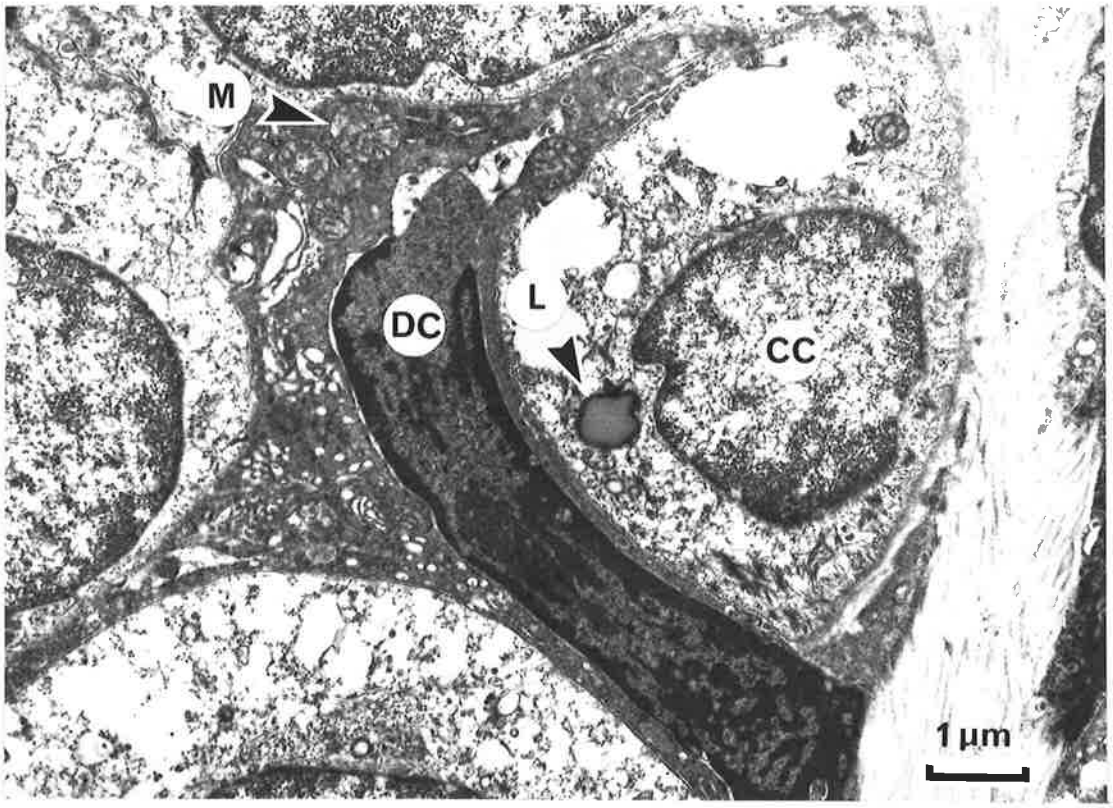

FIGURE 3.14 : An electron micrograph showing a dark cell **(DC)** surrounded by clear cells **(CC)** within an ERM, adjacent to the area of active cementogenesis shown in Figure 3.11b. Note the numerous mitochondria **(M)** within the dark cell, the highly vacuolated appearance of the clear cells and the lipid body **(L)**.

Magnification x 8000 ; Enlargement x 1.5

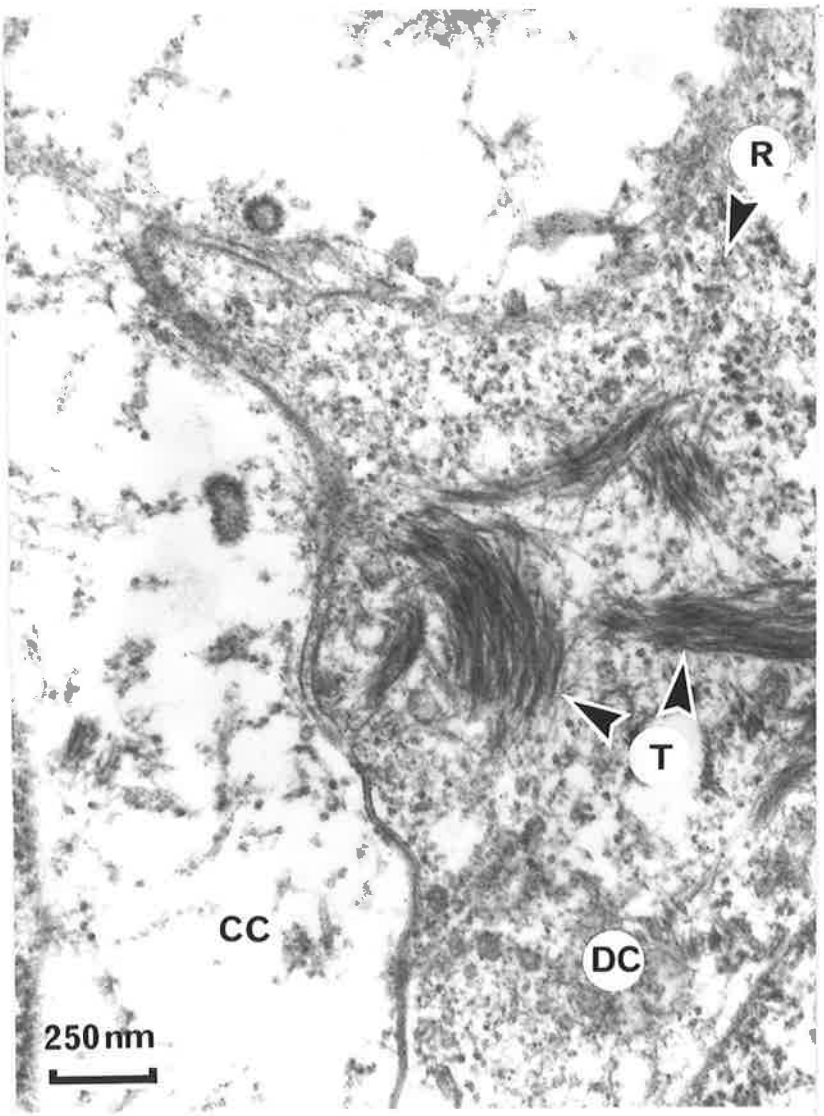

FIGURE 3.15 : A high power electron micrograph of a clear cell **(CC)** and a dark active cell **(DC)** within a ERM adjacent to a non-resorbed root surface. The highly vacuolated cytoplasm of the clear cell did not contain many ribosomes, tonofilaments or any other cytoplasmic inclusions. The cytoplasm of the dark cell contained many free polyribosomes and ribosomes **(R)** and tonofilaments **(T)** but no endoplasmic reticulum or Golgi apparatus were positively identified, indicating that they may be producing protein for their own renewal.

Magnification x 30,000 ; Enlargement x 1.6

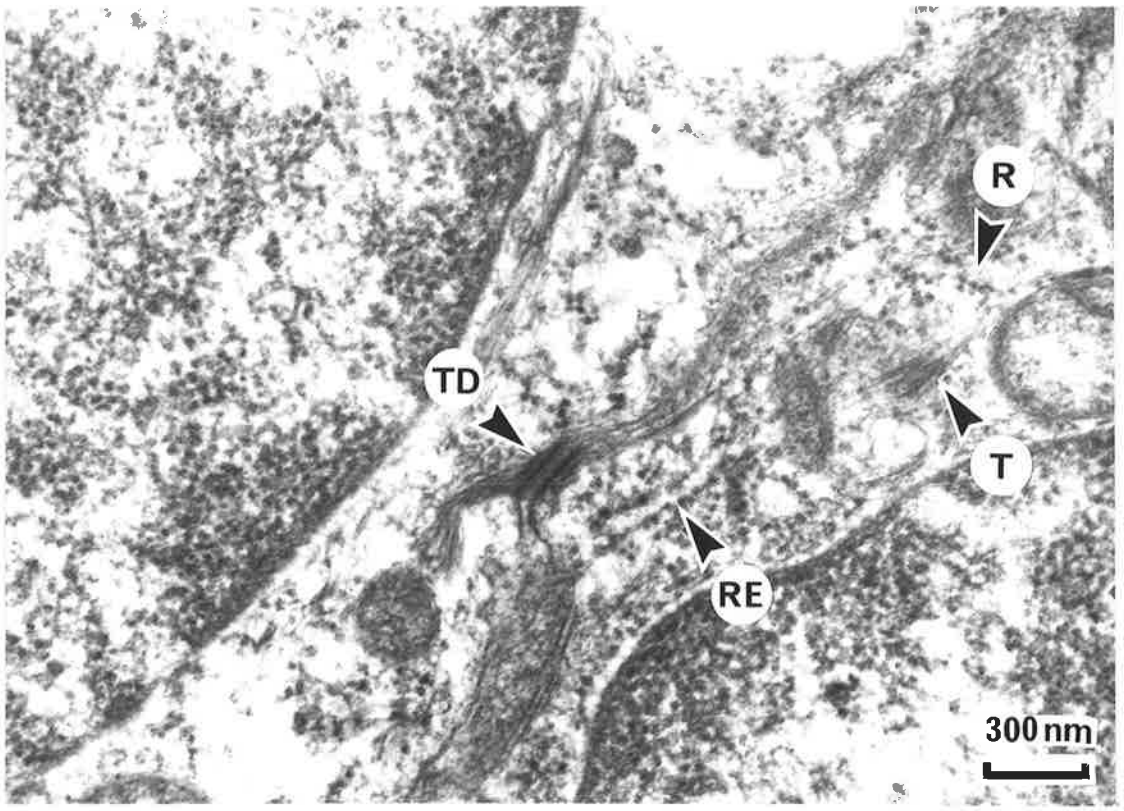

FIGURE 3.16 : A high power electron micrograph of an ERM adjacent to a non-resorbed root surface of an anchor premolar. The characteristic features were true desmosomes **(TD)**, and tonofilaments **(T)**. Numerous polyribosomes and ribosomes **(R)** were present throughout the cytoplasm. Structures similar to rough endoplasmic reticulum **(RE)** were observed. The same features were characteristic of epithelial cell clusters within repairing resorption bays. Compare with Figure 3.4.

Magnification x 30,000 ; Enlargement x 1.3

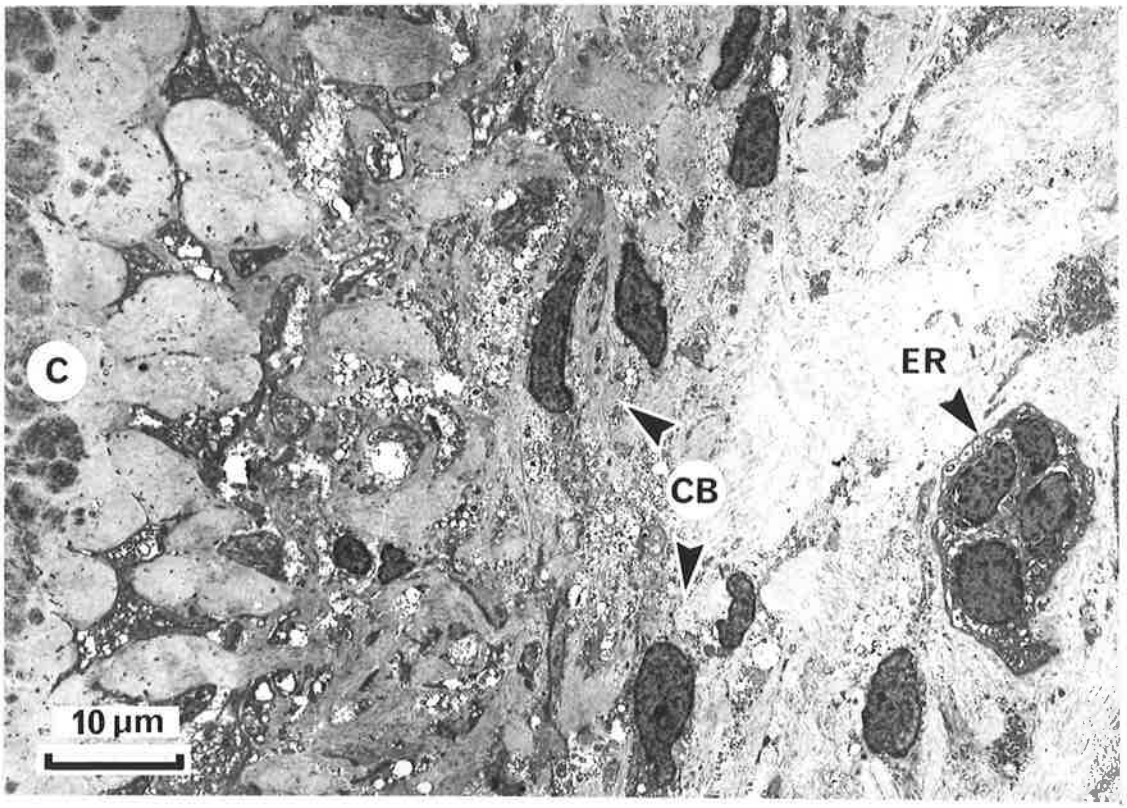

FIGURE 3.17 : A low power electron micrograph showing an epithelial rest of Malassez **(ER)** closely associated with an area of active cementogenesis on a non-resorbed surface of an anchor premolar. Cementoblasts **(CB)** were present on the surface of the globular repairing cellular cementum **(C).**

Magnification x 1,000 ; Enlargement x 1.6

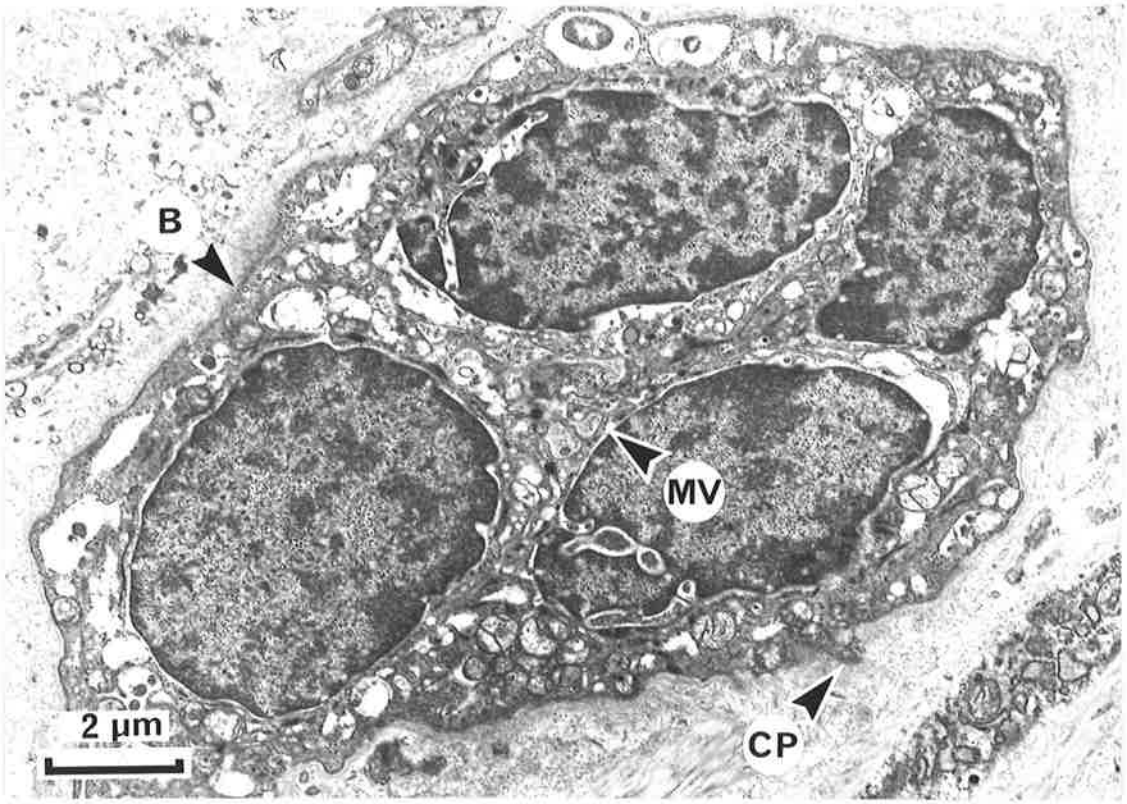

FIGURE 3.18 : A higher power electron micrograph of the ERM (shown in Figure 3.17), adjacent to an area of cementogenesis on a non-resorbed root surface of an anchor premolar. The irregular, pseudopodic outer surface was encircled by a discontinuous basement membrane **(B).** Small cytoplasmic processes **(CP)** projected through the basement membrane toward adjacent mesenchymal cells. The inner surfaces contained many interdigitating microvilli **(MV)**. The ERM contains only dark cells, suggesting an increased level of activity. The vacuolar nature of the cytoplasm may be due to poor fixation.

Magnification x 5,000 ; Enlargement x 1.6

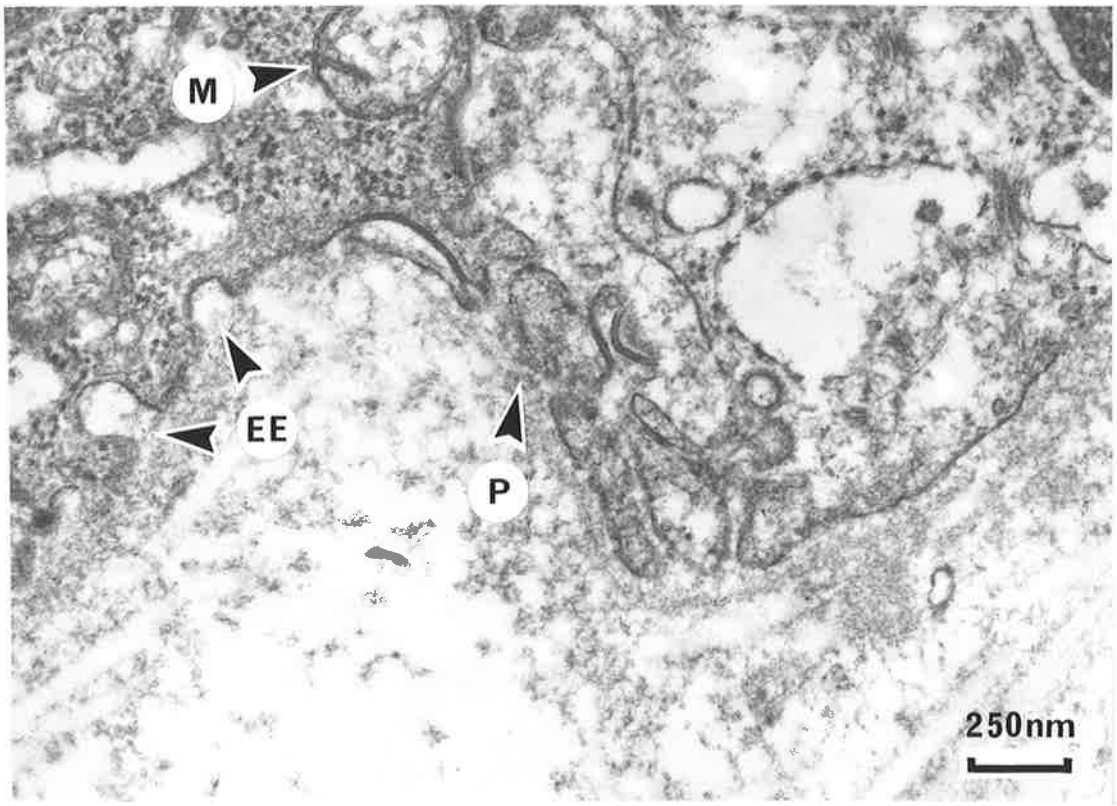

FIGURE 3.19 : A high power electron micrograph of an ERM adjacent to a non-resorbed surface of an anchor premolar. The basement membrane was discontinuous in areas of endocytosis/exocytosis **(EE)**. An area of possible phagocytosis **(P)** was also observed. The mitochondria **(M)** had indistinct cristae.

Magnification x 30,000 ; Enlargement x 1.6

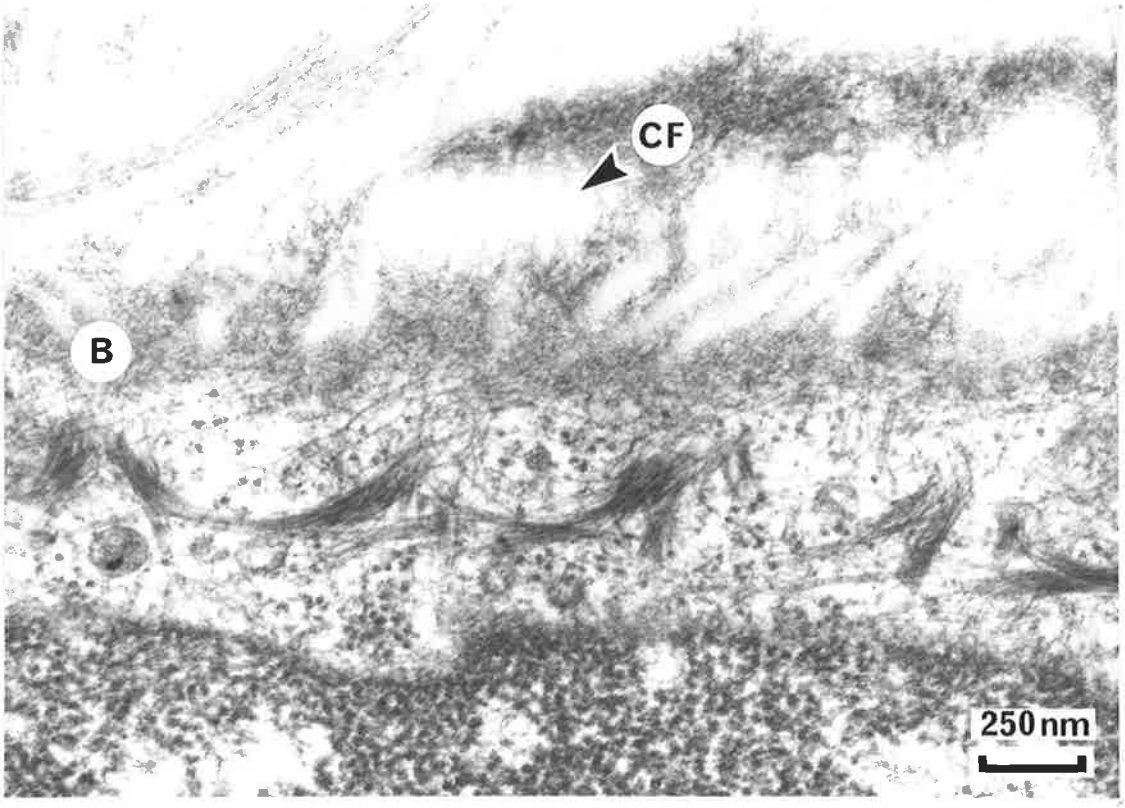

FIGURE 3.20 : A high power electron micrograph of an ERM adjacent to the non-resorbed surface of an anchor premolar. Non-striated collagen fibres **(CF)** were found immediately adjacent to the discontinuous basement membrane **(B)**.

Magnification x 30,000 ; Enlargement x 1.6

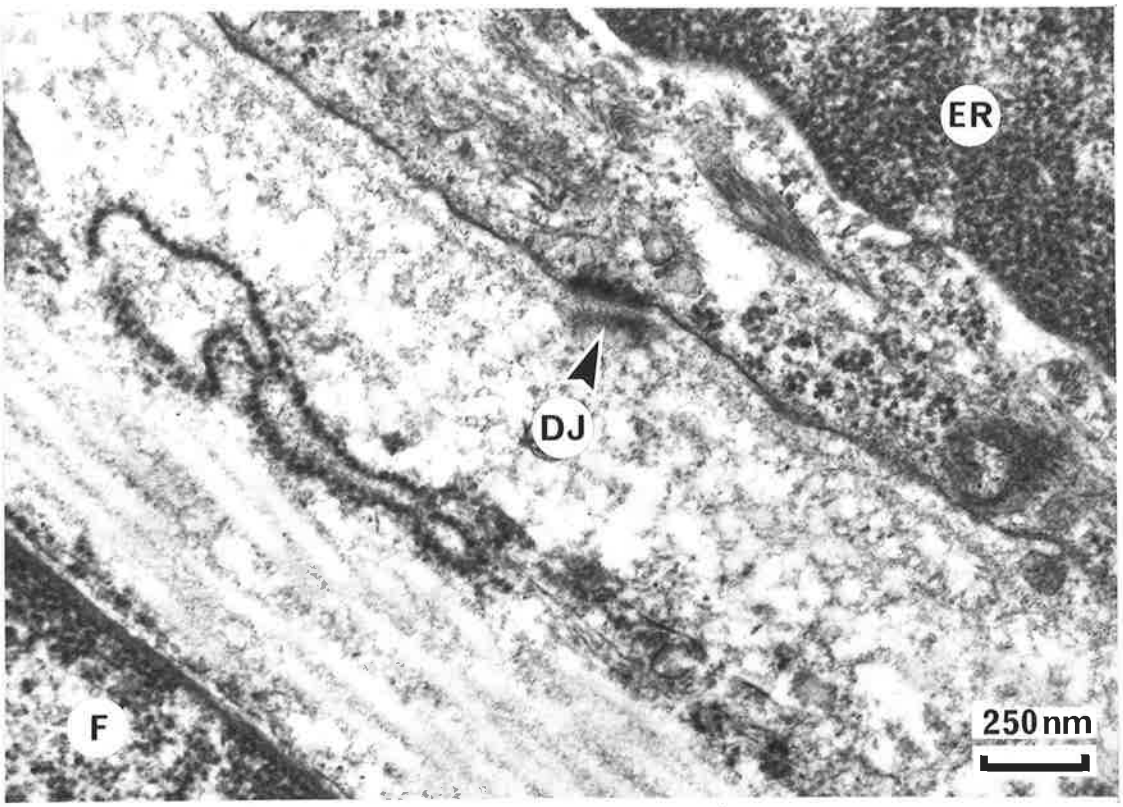

FIGURE 3.21 : A high power electron micrograph showing the close association of an epithelial rest of Malassez **(ER)** with a fibroblast **(F)** adjacent to a non-resorbed surface of an anchor premolar. A desmosome-like junction **(DJ)** has formed between the two cell membranes.
Magnification x 30,000 ; Enlargement x 1.6

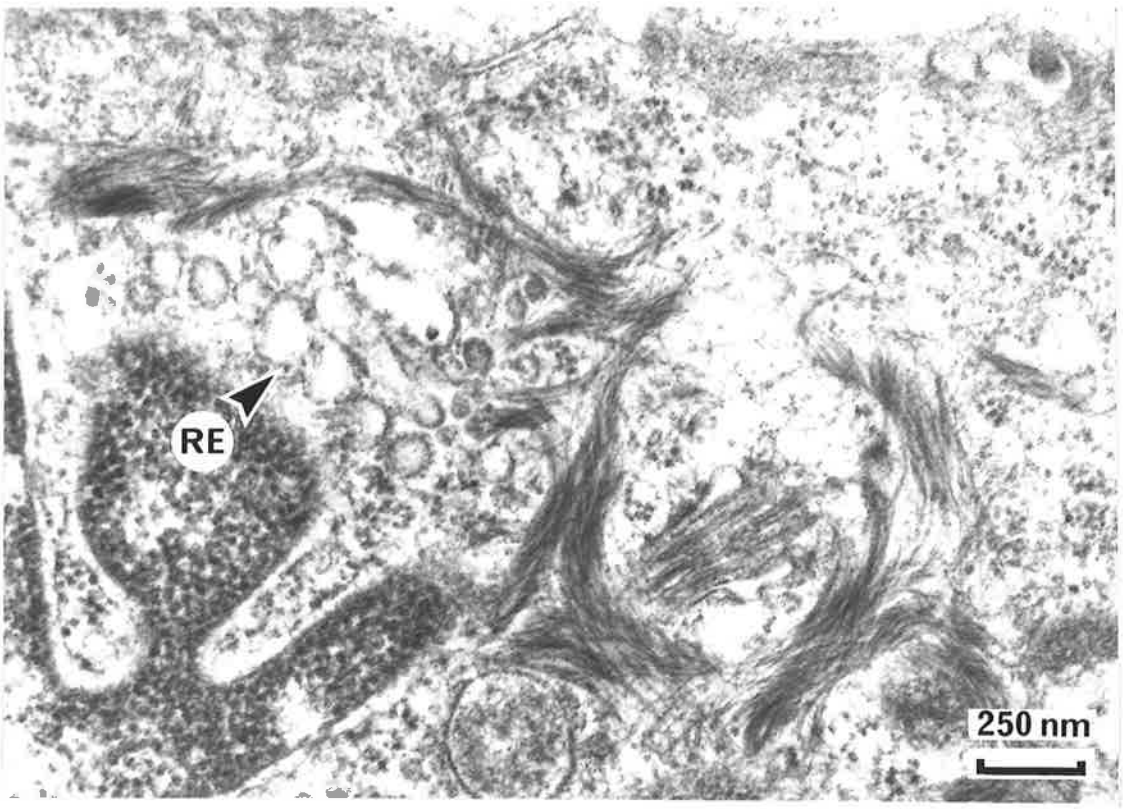

FIGURE 3.22 : A high power electron micrograph of an ERM adjacent to the non-resorbed surface of an anchor premolar. Numerous structures which resemble distended rough endoplasmic reticulum **(RE)** were observed. Magnification x 30,000 ; Enlargement x 1.6

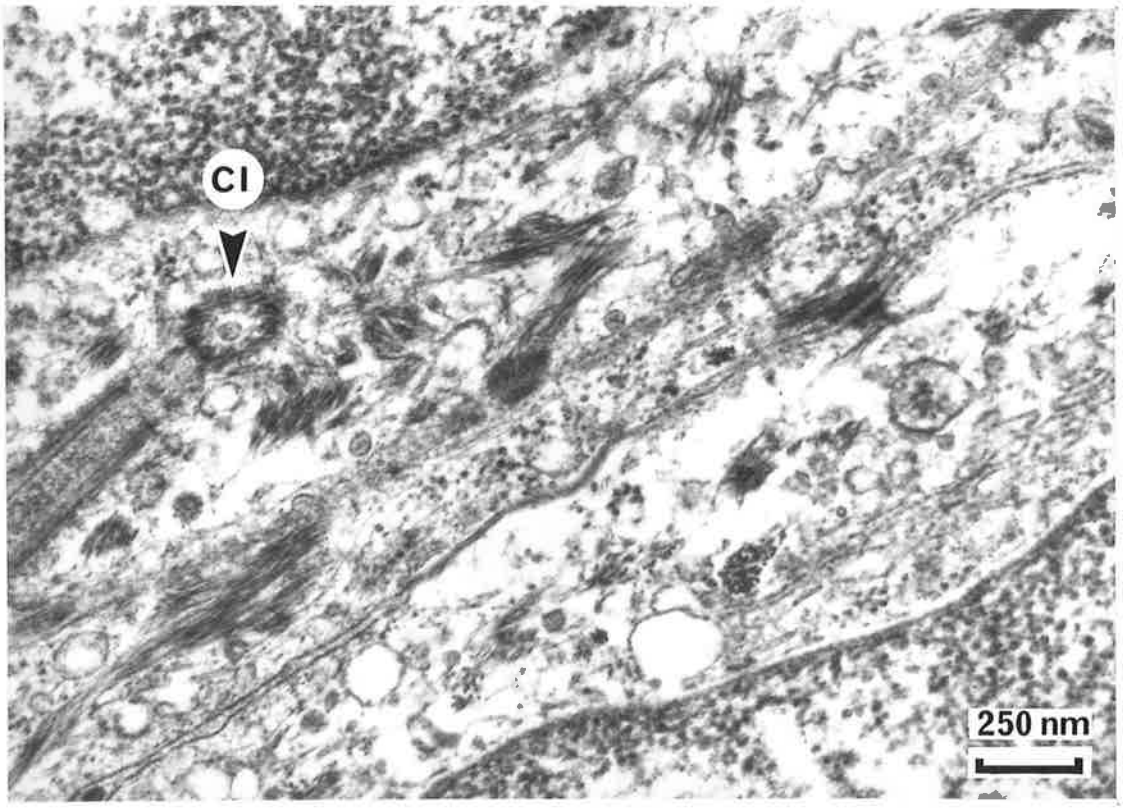

FIGURE 3.23 : A high power electron micrograph showing a section through a cilium **(CI)** in an ERM, adjacent to a non-resorbed root surface of an anchor premolar. The microtubules were arranged in 9 outer pairs but no central pair (9+0 axoneme).

Magnification x 30,000 ; Enlargement x 1.6

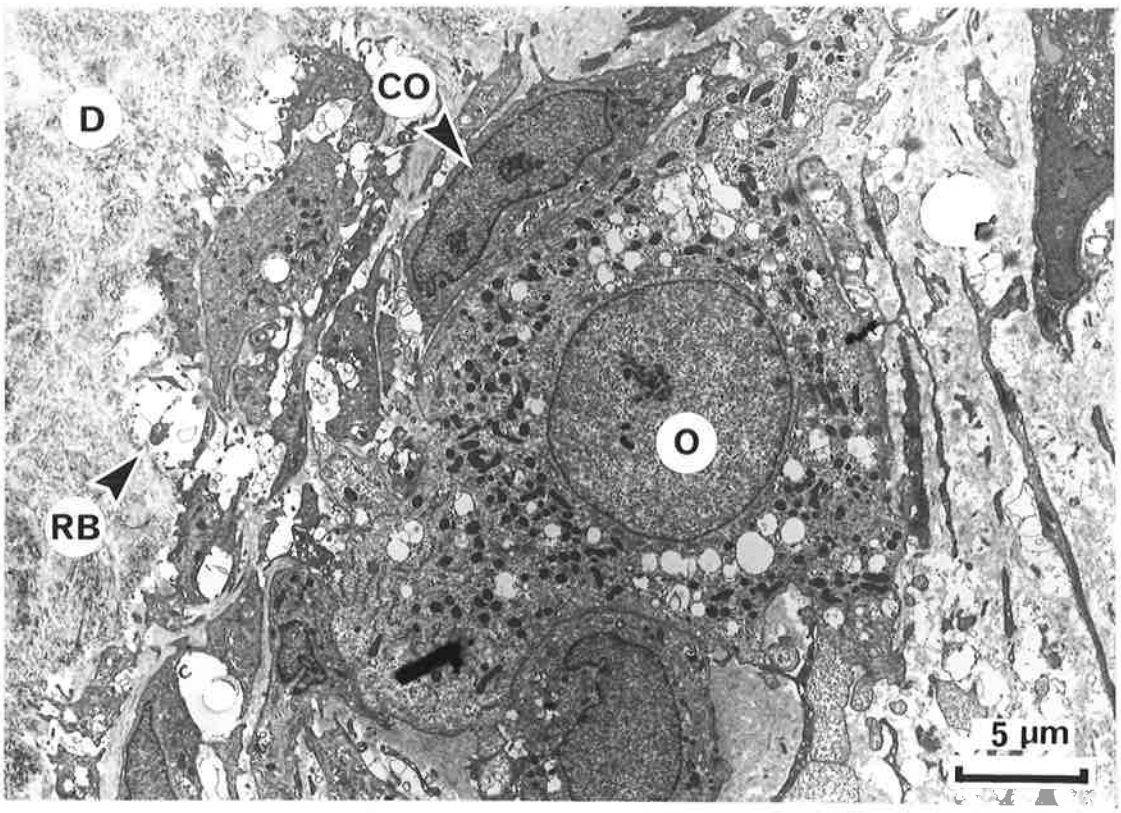

FIGURE 3.24 : A low power electron micrograph of an area of active resorption within an anchor premolar. The odontoclast **(O)** displayed a "ruffled" border **(RB)** which ran parallel to the resorbed dentine surface within the Howship's lacuna. The companion cell **(CO)** was interposed between the odontoclast and the dentine **(D)**.

Magnification x 2,000 ; Enlargement x 1.6

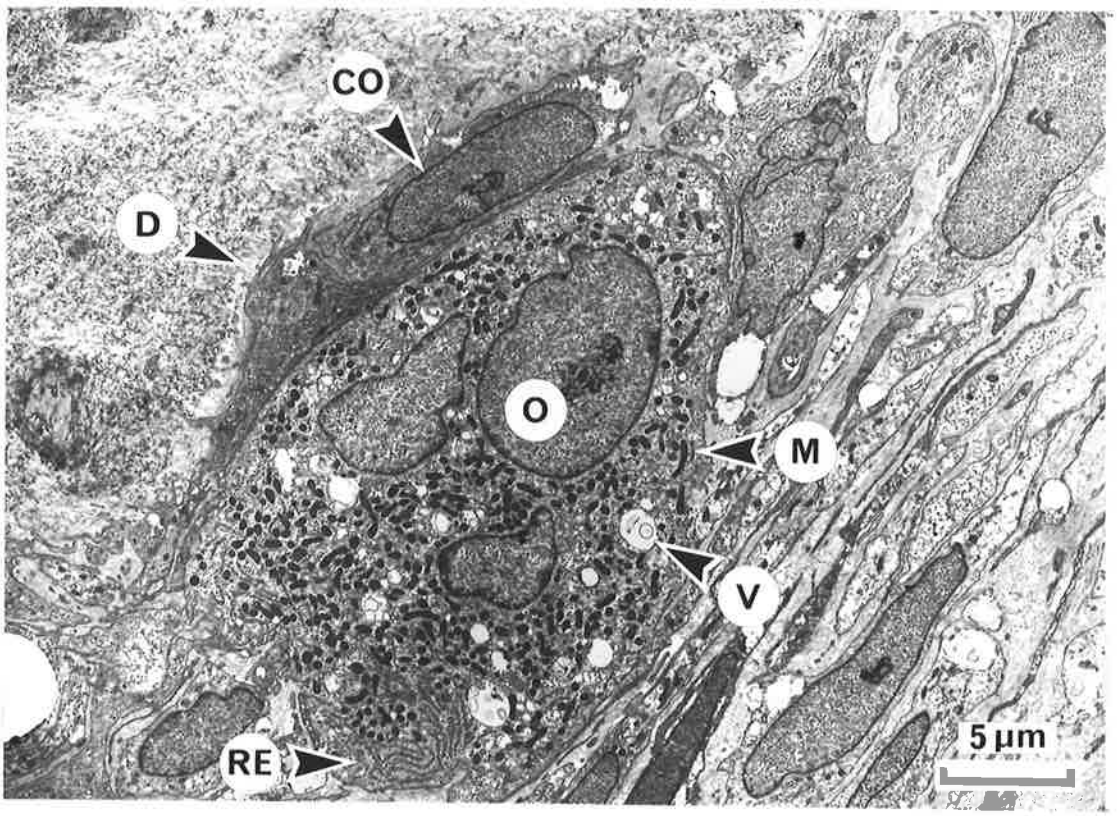

FIGURE 3.25 : A low power electron micrograph of an area of active resorption within an anchor premolar. A companion cell (**CO**) was interposed between the odontoclast (**O**) and the resorbed dentine surface (**D**). The multi-nucleated odontoclast did not display a brush border or a clear zone but was characterised by the presence of numerous dark mitochondria (**M**), vacuoles (**V**) and rough endoplasmic reticulum (**RE**).
Magnification x 2,000 ; Enlargement x 1.5

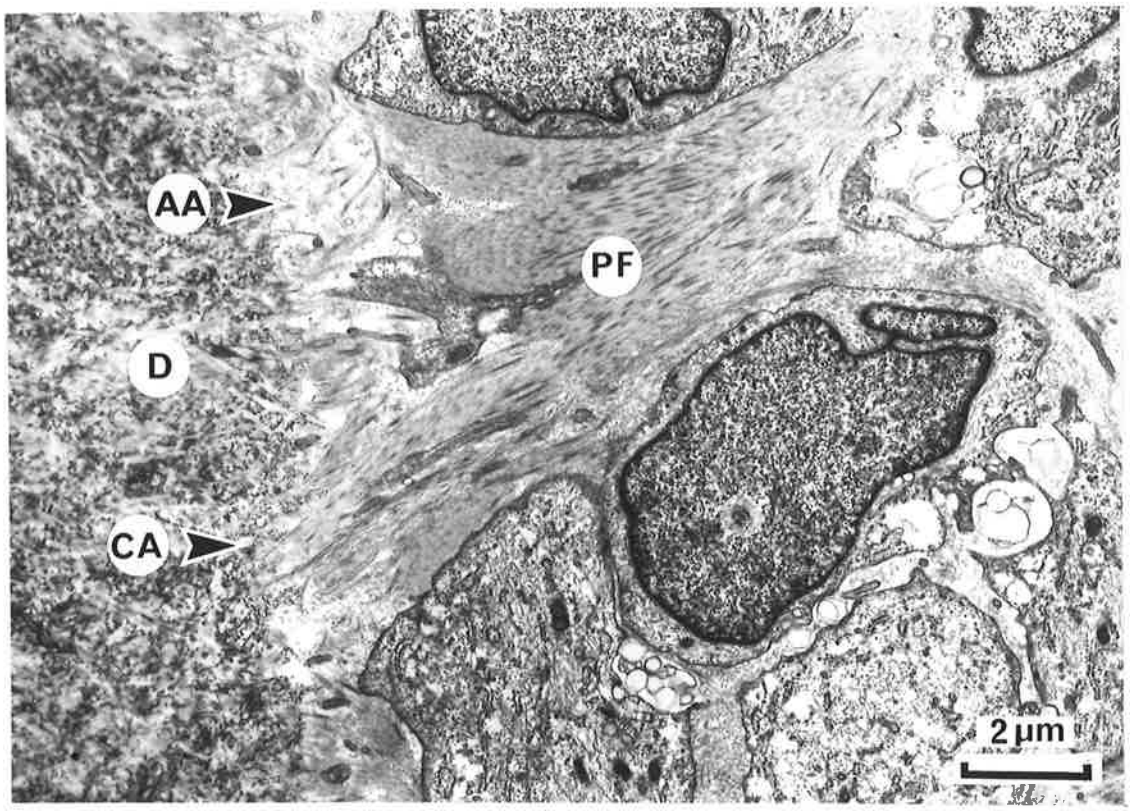

FIGURE 3.26 : A low power electron micrograph showing attachments between periodontal fibres **(PF)** and the resorbed dentine surface **(D)** during active root resorption of an anchor premolar. Both continuous **(CA)** and adhesive **(AA)** attachments were formed, similar to those shown on the bone side of the periodontal ligament by KURIHARA and ENLOW (1980 a,b).
Magnification x 5,000 ; Enlargement x 1.6

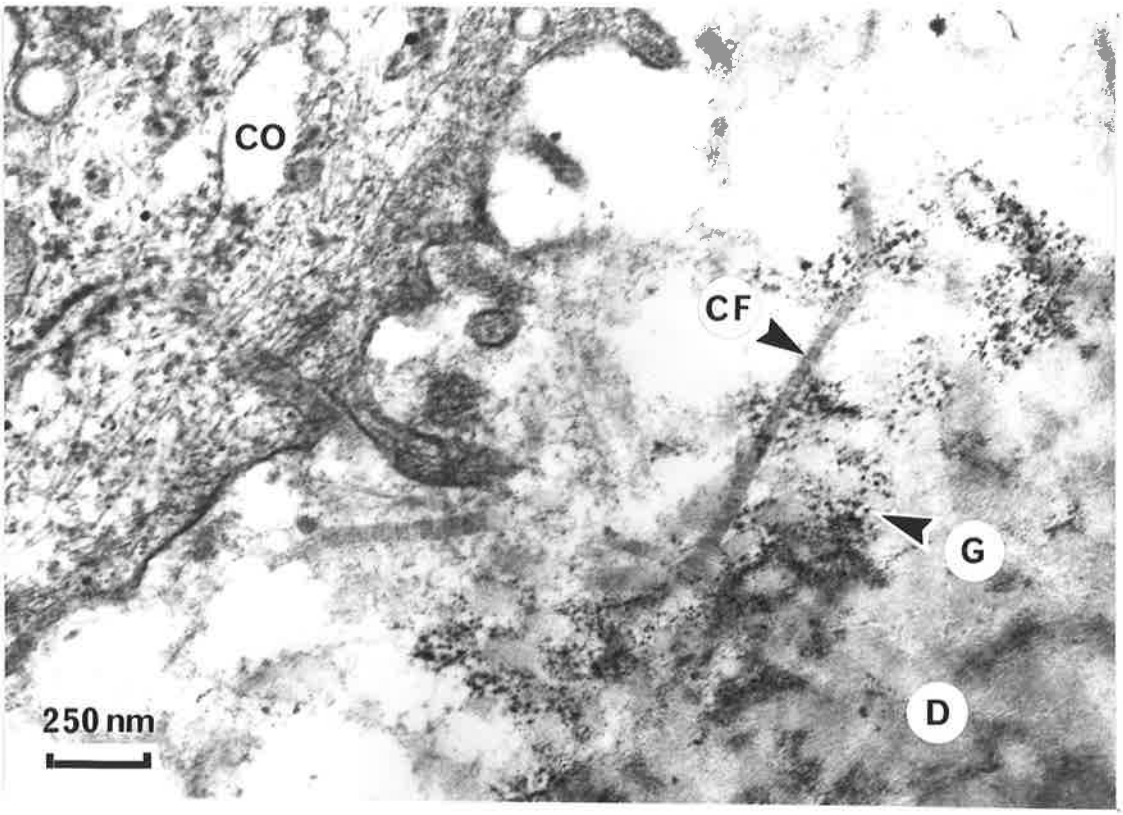

FIGURE 3.27 : A high power electron micrograph of the interface between cementoblast-like companion cells **(CO)** and the resorbed dentine surface **(D)** within a Howship's lacuna in an anchor premolar. It is postulated that the companion cells secrete the dark granular substance **(G)** which appears to "weld" the newly formed collagen fibres **(CF)** to the resorbed dentinal surface.

Magnification x 30,000 ; Enlargement x 1.6

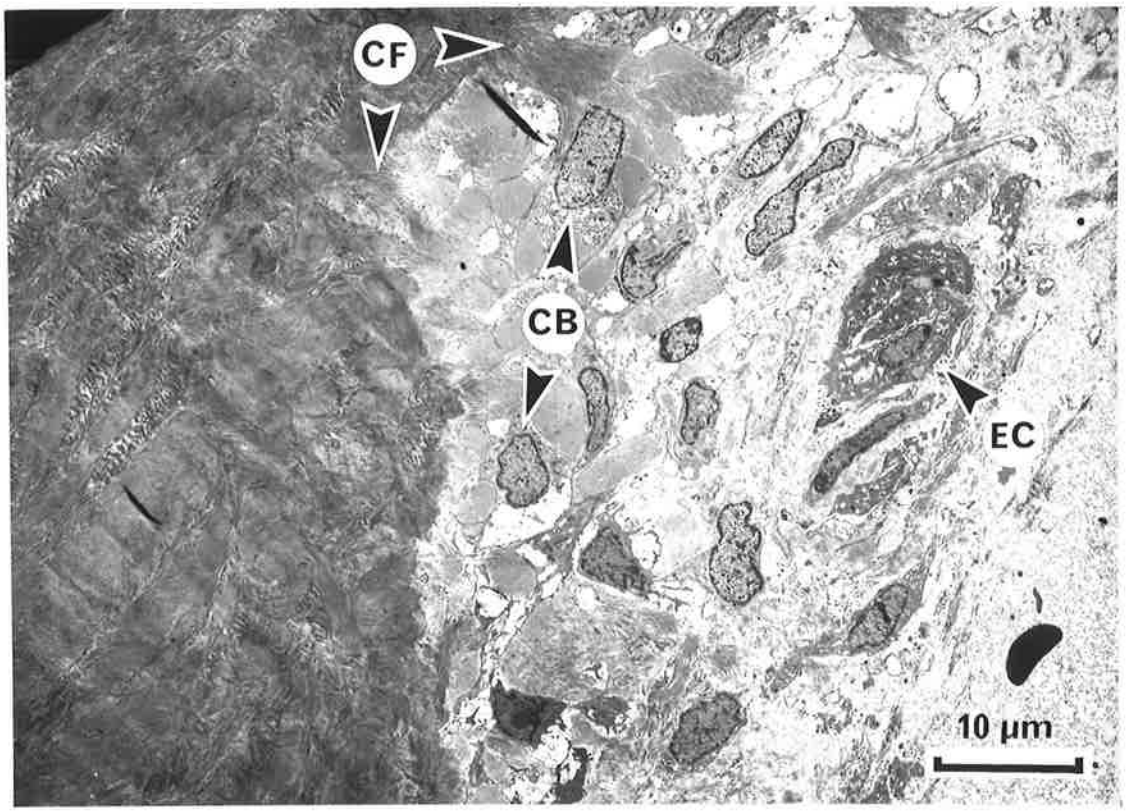

FIGURE 3.28 : Low power electron micrograph showing an epithelial cell cluster **(EC)** close to the centre of a large repairing root resorption bay in an anchor premolar. Collagen fibres **(CF)** passed directly from the repairing cellular cementum into the adjacent tissue, possibly providing attachment between the repairing root surface and the periodontal ligament. Cementoblasts **(CB)** were present along the surface of the repairing cellular cementum.

Magnification x 1,000 ; Enlargement x 1.8

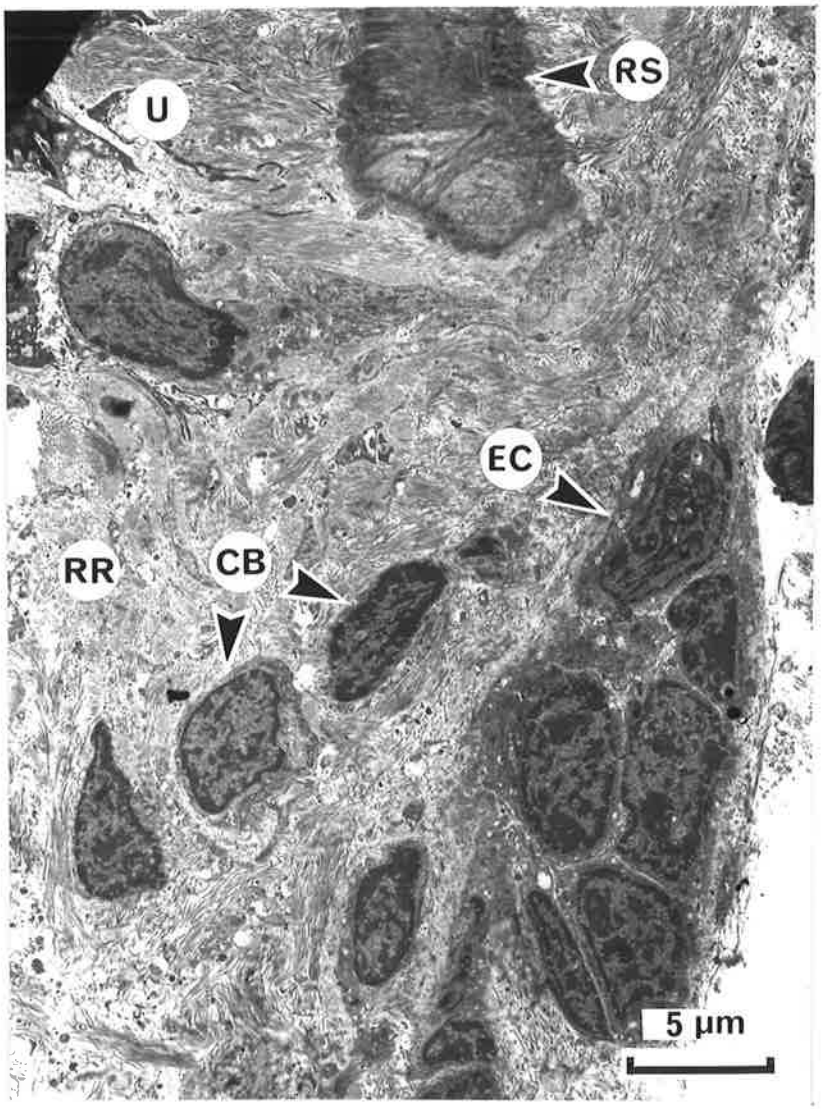

FIGURE 3.29 : Low power electron micrograph of an epithelial cell cluster **(EC)** within a repairing resorption bay **(RR)**. Precementoblast-like cells **(CB)** were seen between the ECC and the repairing surface, not in the picture. The non-resorbed root surface **(RS)** has been undermined **(U)** during active resorption.

Magnification x 2,000 ; Enlargement x 1.7

CHAPTER 4

DISCUSSION

4.1 HUMAN MATERIAL

As the premolars used in this study were extracted and then fixed by immersion, the quantity of periodontal ligament collected on the surface of the root and the quality of its fixation, varied widely. Of the 92 blocks studied at the light microscope level, 8 were selected for further detailed observation at the transmission electron microscope level. The selection of these blocks was based on the combination of root resorption and/or repair with a reasonable attachment of well-fixed tissue. The process of this reduction of the original sample restricted any statistical analysis of the distribution of ERM and ECC.

The material, although limited, was more than adequate to determine the presence or absence of epithelial cells within resorption bays, particularly those undergoing repair. The fact that the areas of active resorption were mainly denuded of soft tissue, restricted the study of these areas.

The lower premolars collected for control also had substantial amounts of root resorption and repair. This is in accordance with the observation of HAAS (1980) who reported that there is considerable dental expansion in the lower arch during rapid palatal expansion. Although this was an interesting finding in itself, it effectively negated the use of the lower premolars as controls.

In a SEM study of human anchor premolars, BARBER and SIMS (1981) reported that the opposing mandibular first premolars and the unattached

teeth, which moved laterally with the alveolar process during RME failed to show evidence of resorption.

However, VALDERHAUG and NYLEN (1966) studied the ultrastructure of epithelial rests of Malassez of 50 human premolars extracted for orthodontic reasons before any treatment had been undertaken. The results of their study were considered to be indicative of normal epithelial rests in man and were compared with the findings of this study, in lieu of appropriate controls.

Adequate controls may have been obtained if the lower premolars were extracted from each patient prior to rapid maxillary expansion. However, the orthodontic treatment plan for these patients would not allow early extraction of lower premolars as it would have compromised their treatment. Also, BROWN (1982) suggested that permanent root resorption is a normal process.

The material was collected on an opportunistic basis over a number of years. As the duration of retention after active expansion varied between 5-7 months, a predominance of repairing resorption bays would be expected (LANGFORD, 1982), as was indeed found to be the case.

4.2 METHODS

A. EXTRACTION

The extraction of upper and lower premolars was done under local anaesthetic with minimal trauma to the patient being the primary objective. The amount of soft tissue which was removed with the tooth varied widely and did not relate to the technique of extraction. No attempt was made to remove buccal bone with the premolar as this would not have been in the best interest of the patient.

B. FIXATION

The quality of fixation varied widely. This may have been caused by a lack of penetration of the fixative through the attached tissue during immersion. Surface contamination with blood may have contributed to the inconsistent fixation but rinsing in cacodylate buffer should have eliminated this problem. It has been suggested that Karnovsky's fixative may provide greater penetration (LINDSKOG,1988).

Anoxic changes and trauma before and during extraction may have also contributed to the variation in tissue quality. The local anaesthetic produces vasoconstriction which may have affected the mitochondria even before extraction.

C. DEMINERALIZATION

The technique used proved more than adequate. Buccal slivers were cut from partially demineralized teeth and reinserted into EDTA to reduce the overall time of demineralization.

D. TISSUE SECTIONS

Sectioning the mid-buccal sliver into approximately 9 blocks was necessary for successful embedding in resin. Resin will not always penetrate tissue blocks much greater than 2x2 mm. Larger blocks are also more difficult to section for slide preparation. The optimum size for a final mesa for silver sectioning was 1x1 mm. Larger sections may damage the diamond knife.

E. EMBEDDING

The procedure used was tried and proven within the laboratory. No problems were experienced so long as short cuts were not taken. A few blocks were moderately soft when sectioned but placing of them in a 45°C

oven overnight and subsequently storing them in a dessicator eliminated this problem.

F. SECTIONING

The technique utilized provided excellent sections for light and transmission electron microscopy. It was important to reposition the cutting edge of the glass knife after five sections had been cut. Attempts to remove more than five produced poor sections and damaged the block face.

G. STAINING

Toluidine blue did not differentially stain epithelial cells from the adjacent tissue which made it difficult and time consuming to locate them. Experiments with other stains proved unsuccessful as most stains cannot penetrate the resin. It was decided to stain with toluidine blue because its use was simple and predictable.

H. LIGHT MICROSCOPE

The light microscope techniques outlined produced good resolution up to magnification of 1000x, which enabled identification of epithelial-like cell clusters within repairing resorption bays. Selected sections were then taken to the TEM for positive identification utilizing ultrastructural features.

The light microscope was used to sort through a large amount of material, ensuring that the time spent at the TEM level was used most efficiently.

Even with the high resolution of the light microscope techniques utilized, it was often difficult and time consuming to find epithelial cell clusters. It is easy to see how epithelial clusters may have been overlooked by previous authors, such as REITAN (1961), who used low power light microscope

techniques with thick paraffin sections which produces relatively poor resolution.

I. TEM SECTIONING, STAINING and GRIDS

Sections cut at the silver interference level (70 nm) were found to give the optimum balance between resolution and ease of handling. Section thickness erred toward silver-gold (80nm) rather than silver-grey (60nm). The grey sections were extremely fragile and difficult to handle.

The double staining technique with Uranyl Acetate and Reynolds' lead was very sensitive to temperature variations. Ideally the temperature of the stain and the rinsing solutions should be kept at 37°C throughout the procedure. If the temperature went above 37°C, the Uranyl Acetate tended to wash out and numerous holes developed in the section. If the temperature was too low, the section was covered with lead precipitate.

Several grid patterns and sizes were tried to find the optimum for the sections cut. The hexagonal 150 mesh provided the best balance between grid size and tissue support. Considerable difficulty was encountered in gaining a clear unobstructed view of the epithelial cell clusters identified at the light microscope level. Routinely, 5-10 sections would have to be viewed before a clear view, free of the grid bars, was obtained. This problem could be reduced by the use of formvar or butvar coated grids which eliminate the grid bars. Unfortunately, there is a considerable reduction in resolution with this technique.

J. JOEL 100S

The Joel 100s is a relatively outdated transmission electron microscope with limited resolution. It functioned well up to magnifications of 30,000x, but magnifications greater than this were best achieved by enlarging

photographically. This limitation made it difficult to observe the intermediate dense line in the central region of the true desmosome (macula adherens), which can be best seen with a high resolution TEM at 80-100,000x magnification. Considerable care had to be taken not to burn or blow out the sections when moving to high power and then dropping back down to low power.

Time spent adjusting the condenser and objective apertures to correct focus and alignment, ensured that the Joel 100s was operating at its optimum level. Periodic checking for astigmatism using a holey carbon grid and correction when necessary, was also essential. The Joel 100s was very susceptible to contamination by sections of biological material, necessitating the stripping down of the entire column to remove the contaminant.

It was also important to condition the section at low power and beam strength for a couple of minutes to prevent it blowing apart at higher power.

K. PHOTOGRAPHY

Over 400 electron micrographs and 100 photomicrographs were taken throughout this study. At the TEM level, it was important to photograph at low power and progressively on up to the highest level. Reversing this procedure produced electron micrographs with dark shadows burnt into them by the electron beam at high power. However, it was often necessary to go to high power first to see if the structure was worth photographing. When this was required, the beam was kept dispersed and never over concentrated into the centre of the section.

The processing of the negatives was again critical. Old solution, inaccurate timing or inadequate agitation would produce disappointing results, considering the effort involved in arriving at this stage.

The photomicrographs produced at the light microscope required bracketing to determine the optimum ASA setting for the film. Again, it was important to ensure that the Axiomat, light microscope was operating at its optimum. The condensor and objective apertures were focused and aligned before using the microscope. Failure to do so resulted in poor resolution at high power and subsequently poor photomicrographs.

In conclusion, the materials and methods used in this study had many pitfalls. Despite this, they were more than adequate for the objectives sought. The findings have well and truly justified the expenditure in time and effort and have raised many more questions. Hopefully future researchers in this area will learn from some of the potential problems outlined.

4.3 EPITHELIAL CELL CLUSTERS IN RESORPTION BAYS

A. DIFFERENTIATION

The presence of epithelial cell clusters within areas of repairing orthodontic root resorption has not been previously reported in the literature and is contrary to the findings of REITAN (1961,1985). These cell clusters were initially located at the light microscope level and then taken to the transmission electron microscope for positive identification.

According to GHADIALLY (1982) , the presence of a "perfect,classic well differentiated" desmosome (macula adherens) in human tissue virtually guarantees that the tissue is epithelial. These true desmosomes have the following features:

1) A widened but not narrowed intercellular gap filled by a dense material which shows a line or pattern of lines.

2) Dense plaques on the cytoplasmic faces.

3) Intermediated filaments (tonofilaments) from the cytoplasm converging upon the plaques.

All of the above features were present in the desmosomes joining the epithelial cells within the repairing resorption bays, although the intercellular lines were often difficult to discern, due to the poor resolution of the Joel 100s and the obliqueness of some sections.

Another major feature which confirmed that these cells were epithelial was the presence of tonofilaments throughout the cytoplasm. The combination of both true desmosomes (macula adherens) and tonofilaments was considered to be characteristic of epithelial cells and was used to positively identify epithelial cell clusters within repairing resorption bays (Figures 3.3 and 3.4).

The morphological features of epithelial cell clusters as seen at the light microscope level were not sufficient to positively classify them as epithelial. This was only achieved at the ultrastructural level as described previously. There were a number of epithelial-like cell clusters observed at light microscope level which did not contain true desmosomes and tonofilaments and could not be unequivocably classified as epithelial.

A means of positively identifying epithelial cells at the light microscope level would greatly simplify future research into their origin and distribution throughout the repairing resorption bays. THESLEFF (1987) has used a radioactive label called epithelial growth factor (EGF) which specifically marks proliferating epithelial cells. BOYDE (1988) suggested that epithelial cells within freshly extracted teeth could be labelled with a specific fluorescent stain and studied using a confocal microscope and 3-dimensional computer reconstruction. This technique would remove the need for demineralization, embedding and sectioning.

Histochemical markers are available which differentially label the cytokeratin within the tonofilaments of epithelial cells. However, the tissue can only be embedded in paraffin, which would limit the study to thick 5μm sections at the light microscope level. Processing for TEM destroys the histochemical marker (WILSON, 1988).

B. ULTRASTRUCTURE/ FUNCTION

A number of functional interpretations can be derived from the ultrastructural morphology observed:

1) Protein Synthesis

The epithelial cell clusters contained numerous polyribosomes and ribosomes throughout their cytoplasm (Figure 3.4). No endoplasmic reticulum or Golgi apparatus were positively identified. According to FAWCETT (1981), these ultrastructural features suggest that the epithelial cells are synthesizing protein to renew their own cytoplasm. Similar interpretations were made by NYLEN and GRUPE (1969) concerning the ultrastructure of human ERM cultured in vitro.

However, small vesicle-like structures with ribosomes at their periphery were observed (Figure 3.5). It is possible that this was a rough endoplasmic reticulum which was distended during processing for TEM. Similarly, small vestiges of what could have been a Golgi apparatus were observed. If these were valid observations, it could be assumed that the cell clusters were secreting some of the protein produced.

A number of different interpretations can be placed on the presence of lipid bodies. The one that supports the previous findings is that put forward by TEN CATE (1972). In observations of proliferating epithelium in an apical granuloma of a monkey, TEN CATE (1972) implied that there is an increase in

the synthesis of lipid associated with increased protein synthesis. He also noted that there is an increase in glycogen utilization. The use of glycogen during protein synthesis would explain why no dense aggregates of glycogen were observed in epithelial cell clusters. The similarity of epithelial cell clusters to proliferating epithelial cells in a apical granuloma indicates that epithelial clusters may also be proliferating. However, dividing cells were not observed.

The general pattern of increased protein synthesis was supported by regularly spaced nuclear pores, relatively evenly distributed nuclear chromatin and invaginations of the nuclear membrane.

Nuclear pores allow the transfer of messenger RNA molecules from the nucleus to the ribosomes within the cytoplasm during protein synthesis. An increase in their number indicates an increase in activity (FAWCETT, 1981).

2) Proliferation

NYLEN and GRUPE (1969) described the ultrastructure of proliferating epithelial rests of Malassez cultured in vitro from explants of human periodontal tissue. Many of the features described by them were observed in the present study in the epithelial cell clusters. These included a disrupted basal lamina, abundant cytoplasm, cytoplasmic projections, desmosomes lacking a clear intercellular contact layer, infoldings of nuclear membranes, prominent nucleoli, regularly spaced nuclear pores, increased numbers of organelles and cytoplasmic inclusions, large numbers of free ribosomes and polyribosomes, lipid bodies and no discernable glycogen aggregates. Again, the similarities between epithelial cell clusters and epithelial explants actively proliferating in culture, suggest that ECC may also be proliferating. NYLEN and GRUPE (1969) suggest that the irregular surfaces of the epithelial

cells, both laterally and basally, may be an expression of accelerated activity or may reflect their quest for adequate nutrients to survive.

TEN CATE (1972) observed that the change from a resting cell of Malassez to an activated, proliferating cell either in culture or in vivo, involves significant and consistent changes in both morphology and chemistry including : a decrease in the nuclear cytoplasmic ratio, utilization of glycogen, synthesis of neutral lipid, synthesis of ribonucleic acid and an increase in glucose-6-phosphate dehydrogenase activity.

3) Epithelial / Mesenchymal Interaction

Each cell within the epithelial cell cluster had one surface abutting the connective tissue (Figure 3.2). This irregular pseudopodic outer surface was encircled by a discontinuous basement membrane. Cytoplasmic processes projected through the basement membrane into the adjacent connective tissue. LESTER (1969 a,b) postulated that similar cytoplasmic processes within Hertwig's epithelial root sheath (HERS) may be related to the differentiation of cementoblasts and cementum formation. He noted that cytodifferentiation of cementoblasts during root formation was related to the initial discontinuity of the outer basal lamina of HERS, with invasive cytoplasmic projections of the epithelial cells which establish a junction between epithelial cells and connective tissue cells. It was postulated that the cytoplasmic projections establish contact with the adjacent undifferentiated mesenchymal cells and initiate their cytodifferentiation into cementoblasts.

Similar observations were made by SLAVKIN and BRINGAS (1976) during the initiation of amelogenesis. Following the appearance of an epithelial cell process projecting through the basement membrane of the inner enamel epithelium, adjacent undifferentiated mesenchymal cell processes penetrate

clefts within the preameloblast forming heterotype cell contacts. Such contacts are necessary for the cytological and functional differentiation of odontoblasts and ameloblasts.

SLAVKIN (1972) noted that in vitro, neither epithelium nor mesenchyme differentiate in the absence of the adjacent dissimilar tissue. If this finding can be extrapolated to repairing resorption bays, it would suggest that epithelial cells must be present within the repairing tissue to initiate cytodifferentiation of undifferentiated mesenchymal cells into cementoblasts and fibroblasts.

Epithelial cell clusters observed in repairing resorption bays had very similar ultrastructural features to the inner layer of epithelial cells in Hertwig's epithelial root sheath in the mouse molar as described by GURLING (1982). Common features included: numerous ribosomes and polyribosomes, tonofilaments, desmosomes, cytoplasmic projections and a discontinuous basement membrane. GURLING (1982) proposed that these cells initiate the cytodifferentiation of odontoblasts and cementoblasts. It is postulated that epithelial cell clusters are derived from ERM, which according to SCHROEDER (1986) are remnants of Hertwig's epithelial root sheath, which persist in the periodontal ligament. It is therefore suggested that epithelial cell clusters, which are ultrastructurally very similar to the inner layer of HERS and originate indirectly from HERS, are also capable of initiating cementogenesis, which is found in repairing root resorption bays.

GURLING (1982) suggested that during a stage in advance of mineralization, HERS became more active and was characterized by; more numerous mitochondria, Golgi apparatus became more conspicuous, moderate numbers of rough endoplasmic reticulum, abundant ribosomes, increased numbers of desmosomes and abundant tonofilaments. It is suggested that

similar features seen in epithelial cell clusters indicate that they may also have an increased activity, associated with the initiation of cementogenesis.

Epithelial cell clusters within repairing resorption bays differed considerably from "normal" ERM as described by VALDERHAUG and NYLEN (1966).

1) In "normal" ERM, the outer surface of the rests is bound by a continuous basement membrane, producing an even, smooth contour. Whereas the irregular pseudopodic outer surface of epithelial cell clusters contained cytoplasmic processes projecting through a discontinuous basement membrane.

2) In epithelial cell clusters, numerous ribosomes are seen throughout the cytoplasm but no accumulations of dense glycogen granules. In "normal" ERM the reverse is true.

These differences suggest that epithelial cell clusters were more active than "normal" ERM and may be involved in cytodifferentiation of cementoblasts.

The presence of cilia in epithelial cell clusters (Figure 3.8) could indicate that the cells were either motile or were passing fluids across their surface (FAWCETT, 1981). However, the cilia, although regularly observed, were few in number and lacked the central pair of microtubules. FAWCETT (1981) suggests that these solitary aberrant cilia are probably immotile but may have a sensory function. However, it is more probable that they are merely anomalous rudimentary structures with no function (FAWCETT, 1981).

Similar solitary cilia with 9+0 pattern have been reported by GURLING (1982). He suggested that such cilia have potential for some oscillatory binding and their growth may be reponsive to Ca^{2+} environment.

LESTER (1969 a) recorded well developed cilia in Hertwig's epithelia at a site just apical to the mineralizing dentinal front during cementogenesis. This

suggests that cilia in ECC within resorption bays may have a role in cementogenesis.

The overall picture that was formed both from the individual ultrastructural interpretations presented and comparisons made with findings from other studies, was that the epithelial cell clusters were active proliferative cells producing protein for their own renewal and perhaps for secretion. They were interacting with the adjacent tissue and may be initiating cytodifferentiation of cementoblasts and subsequent cementogenesis in repair.

C. ORIGIN

This study has shown beyond any doubt that epithelial cell clusters are present in areas of repairing root resorption in human premolars used as anchor units during RME. However, no epithelial cell clusters were found in areas of active resorption. It was concluded that the epithelial network was destroyed in the areas of hyalinization, induced by the forces of the RME. Similar observations were made by REITAN (1961,1985) and RYGH (1977).

It is postulated that epithelial cell clusters proliferate or migrate in from epithelial rests of Malassez at the periphery of the repairing resorption bay to re-establish a network of epithelial cells across the repairing root surface. However, with the limited number of epithelial cell clusters observed, it was not possible to establish how and when they moved into the resorption bay and whether or not they were part of a larger network.

It is also remotely possible that the epithelial cell clusters observed were remnants of the original epithelial rests of Malassez, which may have survived the process of hyalinization. REITAN (1961) noted in animal structures that comparatively few epithelial remnants are lost during hyalinization. However, REITAN (1985) also noted that the only cellular

elements that disappear permanently during hyalinization produced by orthodontic tooth movement were epithelial rests of Malassez. Future research using 3-dimensional computer reconstruction of serial sections through repairing resorption bays could possibly resolve these questions.

No epithelial cell clusters were found in purely active resorption bays. However, an epithelial cell cluster was present adjacent to a small area of active resorption at the edge of a predominantly repairing resorption bay (Figure 3.1). This finding may be due to the limited number of purely active resorption bays observed in this study. Most of the root resorption found was undergoing repair. At what stage the ECC move into the resorption bays has yet to be resolved.

D. CONTRARY OBSERVATIONS

REITAN (1961) in a study of the behaviour of epithelial rests of Malassez during orthodontic tooth movement in man, proposed that epithelial cells disappear during hyalinization induced by orthodontic forces and do NOT reappear during subsequent repair. He added that epithelial rests are not present in the periodontal ligament adjacent to resorption lacunae of the root surface. REITAN (1985) restated his earlier observations by noting that the only cellular elements that permanently disappear during hyalinization induced by orthodontic forces are epithelial rests of Malassez. ERM were not observed in active resorption bays in this study, in accordance with REITAN (1961). However, epithelial cell clusters closely resembling ERM were found in repairing resorption bays which is contrary to REITAN (1961, 1985).

An explanation for this, most probably lies in the methods used to look for and confirm the presence of these cells. REITAN (1961) used low power light microscope techniques with paraffin sections several microns thick. However, in the present study high power/ high resolution light microscope

techniques with 1µm resin sections were used to locate possible epithelial cell clusters. The ultrastructure of these cells was then studied using TEM techniques for positive identification. It was not possible in this study to positively identify epithelial cell clusters at the light microscope level, even with higher resolution and magnification. It is most probable that REITAN (1961) was faced with the same problem of positive identification at the light microscope, particularly with low power and low resolution techniques.

E. SUPPORTING LITERATURE

The findings of this study support earlier observations of LÖE and WAERHAUG (1961), SPOUGE (1980) and LINDSKOG et al. (1983, 1988 b). They suggested that epithelial rests of Malassez protect the root surface from resorption, prevent ankylosis and maintain the integrity of the periodontal ligament. The fact that epithelial cell clusters are present in human repairing root resorption bays lends weight to their postulates.

LINDSKOG et al. (1983) in a study of repair of experimental cavities in monkey incisors, found that epithelial cells are numerous in the connective tissue which separates the reparative cementum from alveolar bone in the experimental cavities. They stated that epithelial cells are close to the surface of the reparative cementum in the experimental cavities. These strands are 2-4 cell layers thick, with no distinct lumen between the cells. Identification was based on low power light microscope features and an elevated activity of glucose-6-phosphate dehydrogenase, which has been shown to be higher in epithelial cells (TEN CATE, 1965).

A collaborative study is planned with Dr. Lindskog to investigate the ultrastructure of these epithelial/endothelial cells and to compare them with ECC found within human resorption bays. If these experimental cavities in monkeys prove to be a suitable experimental model for orthodontic root

resorption in man, a number of experiments could be designed to investigate the role of epithelial cell clusters in the repair of damaged root surfaces.

4.4 EPITHELIAL RESTS OF MALASSEZ / NON-RESORBED SURFACES

A. MORPHOLOGY and DISTRIBUTION

The size and shape of the epithelial rests of Malassez adjacent to non-resorbed surfaces were similar to those described in other human studies by REEVE and WENTZ (1962); SIMPSON (1965); VALDERHAUG and NYLEN (1966), and VALDERHAUG and ZANDER (1967). However, no epithelial network was observed. The number and distribution of the ERM along the root surface varied widely from patient to patient and from block to block. This variation was caused by a number of possible factors:

1) Variation in soft tissue attachment on the root surface.

2) Destruction of the epithelial network during rapid maxillary expansion without subsequent root resorption.

3) Serial sectioning, which may have demonstrated the three dimensional distribution of these rests, was not performed.

Depite these limiting factors an increased number of rests were observed in the middle and cervical, regions which supports the findings of VALDERHAUG and ZANDER (1967). However, the variables listed prevent any valid statistical quantification of this observation.

The distance of ERM from adjacent non-resorbed surfaces also varied widely. The mean of 40 µm is in keeping with earlier observations by VALDERHAUG and ZANDER (1967) but the range of 10-120µm is greater than any previously reported.

VALDERHAUG and ZANDER (1967) noted that ERM stained with toluidine blue are darker than adjacent fibroblasts and cementoblasts. In this study both light and dark staining rests and light and dark cells within individual rests were observed which suggests that the ERM may have been affected by the rapid maxillary expansion.

BARBER and SIMS (1981) reported that after 20-36 weeks of fixed retention following RME, the anchor premolars show large resorption bays scattered along their entire buccal surfaces but predominantly in the middle and cervical regions. As ERM are destroyed during hyalinization of the periodontal ligament prior to orthodontic root resorption (REITAN, 1961,1985), it would be expected that a large portion of the epithelial network along the buccal surface of anchor premolars would be destroyed during RME. The ERM found adjacent to the non-resorbed buccal root surface in the study may be remnants of the network destroyed during hyalinization or could represent a network rebuilt subsequent to the hyalinization.

An association was observed between ERM along the non-resorbed root surfaces and the adjacent blood vessels. The ERM were interposed between the blood vessels and the non-resorbed surfaces. As dentinoclasts are thought to be blood borne (JEE and KIMMEL, 1976), the ERM may act as a protective mechanism against root resorption by preventing the movement of dentinoclasts to the root surface. Whereas on the bone side the odontoclasts are brought into close apposition to the bone by increased vascularity (BROWN, 1982).

A valid statistical comparison of the effects of RME on the normal morphology and distribution of epithelial rests of Malassez was prevented by lack of soft tissue attachment (i.e. less than 30μm) to the root surface in 46% of the material collected. The presence of large areas of repairing root

resorption in the control material indicated that it had also been affected by RME (HAAS, 1980) and precluded its use as representative of normal tissue.

B. ULTRASTRUCTURE

1) Clear and Dark Cells

The ERM adjacent to non-resorbed root surfaces subjected to RME contained two distinct cell types (Figures 3.13, 3.14 and 3.15):

1) clear cells, with highly vacuolated cytoplasm containing no tonofilaments and very few inclusions.

2) dark cells with dense cytoplasm containing many ribosomes, tonofilaments and other inclusions.

The ultrastructural features of the dark cells resembled the features of epithelial cells within normal human rests as described by VALDERHAUG and NYLEN (1966). The significant differences were that "normal" epithelial rests frequently contain accumulations of dense glycogen granules but very few ribosomes are present throughout the cytoplasm. This suggests that the dark cells, with their increased number of ribosomes and absence of glycogen, are in a more active phase than "normal" ERM.

In addition, the ultrastructural features of the dark cells found within ERM adjacent to non-resorbed root surfaces subjected to RME, were similar to those found in epithelial cell clusters adjacent to repairing orthodontic root resorption. Both contained many free ribosomes, polyribosomes and tonofilaments, with no apparent endoplasmic reticulum or Golgi apparatus. According to FAWCETT (1981), these features indicate that they are both actively synthesizing protein to renew their own cytoplasm.

In an ultrastructural study of epithelial rests of Malassez cultured in vitro from human explants NYLEN and GRUPE (1969) found features similar to those

present in the dark renewing cells observed within ERM in this study. These included the presence of large numbers of ribosomes and polyribosomes throughout the cytoplasm. However, they noted that the tonofilaments are less numerous and form much thinner bundles, the Golgi complex is well ordered and endoplasmic reticulum is increased and partly covered by ribosomes, compared to ERM in vivo . They postulated that this arrangement of organelles indicates protein utilization by the cells themselves and possibly also a contribution to continuing alteration of their surrounding basement membrane. This postulate is somewhat conservative, considering the presence of a well ordered Golgi complex and increased number of rough endoplasmic reticulum. These features are normally indicative of a secretory function (FAWCETT, 1981).

There appears to be no previous reference in the literature describing the presence of clear cells within epithelial rests of Malassez. However, clear cell rests have been reported in the dental lamina, the lateral periodontal cyst and the gingival cyst (WYSOCKI et al., 1980). They suggest that lateral periodontal cysts originate from the dental lamina because clear cells are not found in either epithelial rests of Malassez or the reduced enamel epithelium As their observations were based on histological studies of lateral periodontal cysts, it was not possible to make a direct comparison of ultrastructural features of clear cell rests in the cyst and clear cells in human ERM, adjacent to non-resorbed surfaces following RME. Further investigation is required to clarify these conflicting findings.

The presence of two distinct cell types within ERM has not previously been described in human tissue. However, in mouse molars, MCLEAN (1984) found degenerating epithelial cells exist as either isolated cells within the periodontal ligament or as single degenerating cells amongst a cluster of healthier cells in an epithelial rest of Malassez. Histologically, REEVE and

WENTZ (1962) also described degenerative, resting and proliferative types of epithelial rests in man. The classification was based on the number of cells within the rest and the appearance of the nuclei,i.e. whether or not they appeared pyknotic.

It is unlikely that the clear cells observed in this study were degenerative as there was no evidence of nuclear chromatin clumping or breakdown of the nuclear or cytoplasmic membranes.

2) Outer and Inner Surfaces

The formation of a desmosome-like junction between the outer surface of a dark cell in an ERM and a mesenchymal cell (fibroblast-like) adjacent to an area of cementogenesis (Figure 3.21), supports the concept of an epithelial-mesenchymal interraction in these areas. SLAVKIN (1972) reported that such junctions are rare.

BIREK et al. (1983) reported that ERM can secrete collagenase. It is suggested that the presence of non-striated collagen fibres found in the study, immediately adjacent to the discontinuous basement membrane in both ECC and ERM (Figure 3.20), may represent areas of secretion of collagenase and the breakdown of collagen.

However, PETTIGREW et al. (1980), reported that ERM synthesize protein capable of inhibiting collagenolytic enzymes. In order for a breakdown of collagen to occur, collagenases must be produced in excess of the inhibiting activity.

BIREK et al. (1980) reported that ERM phagocytose collagen in vitro . They postulated that they could destroy extracellular substances of contiguous connective tissue during their migration. A few areas of possible

phagocytosis were observed in this study, but none contained discrete fragments of collagen (Figure 3.19).

The outer surface of the epithelial rests adjacent to inactive non-resorbed surfaces was contained by a continuous basement membrane which produced an even, smooth contour, similar to "normal" ERM described by VALDERHAUG and NYLEN (1966). However, cytoplasmic processes projecting through a discontinuous membrane were observed in ECC within repairing resorption bays (Figure 3.2) and ERM adjacent to areas of cementogenesis on non-resorbed surfaces (Figure 3.18). Similar cytoplasmic processes have been described in ERM proliferating in vitro (NYLEN and GRUPE, 1969); in the inner layer of epithelial cells of Hertwig's epithelial root sheath during cementogenesis (GURLING, 1982; McLEAN, 1984) and in the inner enamel epithelium during amelogenesis (SLAVKIN and BRINGAS, 1976).

In a study of Hertwig's epithelial root sheath and root formation, LESTER (1969 a,b) suggested that epithelial cytoplasmic processes projecting through a discontinuous basement membrane of the sheath initiated the cytodifferentiation of cementoblasts and thereby cementogenesis during root formation. The lack of such projections in ERM adjacent to inactive non-resorbed surfaces may be associated with the minimal cementogenesis that was taking place.

It is postulated that the initiation of cementogenesis is associated with a change in the ultrastructural morphology of ERM as follows:

1) Increased number of dark cells.

2) The outer surface changes from a smooth, even contour contained by a continuous basement membrane to an irregular pseudopodic surface with cytoplasmic processes projecting through a discontinuous basement membrane.

3) The inner surfaces change from a relatively even contour to numerous interdigitating microvilli.

Active cementogenesis was observed adjacent to ERM with a predominance of clear cells. However, the outer surface of the dark cells within these ERM had the characteristic features of activity, previously described. Dark cells were also observed in ERM adjacent to relatively inactive surfaces. It is obvious that the relationship of ultrastructural morphological features and cementogenesis is not as straight forward as the previous postulate would suggest. It is however, a reasonable starting point for future research.

4.5 RESORPTION

A. DISTRIBUTION AND MORPHOLOGY

Of the ninety two blocks examined, 32 (34%) contained areas of resorption and/or repair. This closely compares with the observations of BARBER and SIMS (1981) during a scanning electron microscope study of external root resorption following RME. They found that as much as 36% of the buccal root surface can be involved in root resorption. However, LANGFORD and SIMS (1982) in a similar study, reported an average of 24%, ranging from 12%-45%. No attempt was made to accurately measure areas of resorption in this study as only one plane of section through the buccal sliver was observed.

The predominance of repairing resorption bays (85%) indicated that only limited active resorption occurs within anchor premolars after cessation of active expansion and 3-6 months of retention.

The distribution of resorption, active and/or repairing, along the buccal root of anchor premolars following RME was mainly in the cervical (35%) and mid-root regions (50%). Only a small amount of resorption was observed in the apical region (15%). This pattern of distribution was also observed by BARBER and SIMS (1981), who reported large resorption bays scattered along the entire buccal surface of the anchor premolars but predominantly in the cervical and middle regions.

It was difficult to discern active and repairing resorption in the apical area because of the large quantity of cellular cementum normally present and the uneven undulating surface. Repairing resorption bays were only scored if a clear reversal line could be seen and active resorption was scored when resorbed dentine with no covering cellular cementum, was observed. These criteria for selection may have eliminated several possible areas of resorption, which could not be positively identified. Thus, the distribution stated may reflect the difficulty of identifying resorption in the apical area.

BARBER and SIMS(1981) suggested that the distribution of resorption may be caused by tipping of the anchor premolars rather than bodily movement under the forces of the RME. This would produce greater forces in the cervical region, which would have reduced as the centre of rotation was approached. If the centre of rotation was at the apex then the apical third would have received the lightest forces. If the centre of rotation was one third of the root length from the apex, then the apical third would not have been compressed at all, but instead placed under tension, resulting in cementogenesis rather than resorption.

The large amount of cellular cementum observed and the lack of discernable resorption in the apical area would suggest that the latter was true. It is more than likely that the force/couple ratio varies throughout expansion and subsequent retention causing the centre of rotation to move

along the root length (SMITH and BURSTONE, 1984) . Whether there was compression or tension in the apical third would be dependent on the force /couple ratio at the time. Whilst the forces are heavy, during early expansion, the force/couple ratio favours excessive compression in the bucco-cervical region and tension in the bucco-apical region.

ZIMRING and ISAACSON (1965) found that the residual load acting on the fixed appliances at the end of expansion dissipated after 5-7 weeks. It is suggested that as the forces dissipate, the force/couple favours less cervical compression and increased compression of the apical region, depending on how much the appliance was flexed during active expansion. The more flexible appliances would take longer to discipate their forces than more rigid appliances. This may explain why more active resorption continues for up to 9 months after RME with the flexible Hyrax type of appliance (BARBER and SIMS, 1981).

Perhaps the use of a more rigid expansion appliance may reduce the degree of root resorption. A controlled experiment could be set up to test this concept. REITAN (1974) showed that once root resorption is started by strong continuous forces, much lighter pressures maintain or increase the resorptive process.

One block contained both active and repairing root resorption with a small area of active resorption at the side of a large shallow repairing resorption bay. This finding supports the observations of BARBER and SIMS (1981) and LANGFORD and SIMS (1982), that the resorption is laterally expansive rather than axially invasive.

One explanation for this self-limiting effect of root resorption was put forward by LINDSKOG and PIERCE (1988). In an in vitro study of dentinoclasts, they concluded that development of membrane structures specialized for

resorption will not proceed unless a mineralized collagenous matrix is available to a spreading clastic cell. The combination of the mineral and collagen components of hard tissues is essential for attachment and expression of specialized resorbing structures by dentinoclasts. The removal of hydroxyapatite exposes intrinsic collagen which limits the resorption by dentinoclasts (PIERCE, 1988).

Active resorption at the edges of larger repairing bays undermined the adjacent non-resorbed surface. This finding supports the postulate proposed by many authors , including RYGH (1977) and BROWN (1982) that the root surface is more resistent to resorption than the underlying dentine. This increased resistence is due to: unmineralized cementoid (REITAN,1974 ; RYGH, 1977); increased fluorine content of cementum (RYGH, 1977); presence of Sharpey fibres (BROWN, 1982); intermediate cementum (HAMMARSTRÖM et al.,1986a,b) and the layer of cementoblasts covering the root surface (LINDSKOG et al., 1987a).

In a scanning electron microscope study of cellular colonization of denuded root surfaces on replanted teeth, LINDSKOG et al. (1987b) noted that the resorption activity was gradually inhibited by the ingrowth of a monolayer of precementoblasts from the periphery of the denuded area. However, BARBER and SIMS (1981) noted that cellular cementum repair usually begins centrally on the floor of resorption bays and spreads out in the wake of ongoing peripheral resorption. Similar observations were made in this study. Repairing cellular cementum was always thickest toward the centre of the resorption bay and thinned out toward the periphery.

Nowhere in the literature is ankylosis reported to be a common sequel to RME. However, LINDSKOG et al. (1983) reported that permanent ankylosis can develop when experimental cavities greater than 4 mm^2 are placed into the root surface of monkey incisors. If this finding can be extrapolated to

man, it would suggest that most areas of root resorption in anchor premolars during RME must be less than $4mm^2$, otherwise ankylosis can occur. However there may be considerable species differences.

This is contrary to observations of BARBER and SIMS (1981), who showed that as much as 36% of the buccal root surface of anchor premolars was involved in resorption processes. Either ankylosis is a common occurrence and has not been reported or a much larger area of resorption can occur in man without resulting in ankylosis.

The lack of any long term follow up of periodontal health of anchor teeth following RME is apparent. It may be possible that the varying forces/couple ratio and contour of the alveolar bone results in small areas of resorption at any one time and that these areas coalesce to form larger areas reported by BARBER and SIMS (1981).

B. ULTRASTRUCTURE

As far as can be determined, the only previous TEM study of orthodontic root resorption in man was conducted by RYGH (1977). The human material consisted of eleven premolar teeth that were moved buccally by means of fixed appliances for periods between 2 and 50 days. Experimental forces of 20,100,120 and 200 gms were utilized. The resorption studied was therefore mainly active, whereas the resorption within the present study was predominantly repairing. No other TEM study of repairing orthodontic root resorption in man was found in the literature.

The presence of a large number of mitochondria, vacuoles and rough endoplasmic reticulum would suggest that the odontoclasts observed in areas of active resorption (Figures 3.24, 3.25) were metabolically highly active cells (FURSETH, 1968). However odontoclasts were not present in all the lacunae along the active resorbing surface.

Cementoblast-like cells were seen adjacent to the odontoclasts and sometimes between the odontoclast and the resorbed dentine (Figure 3.24). Similar "companion cells" have been described by KURIHARA and ENLOW (1980 a,b) adjacent to osteoclasts in areas of alveolar bone remodelling. They postulated that these osteoclast "companion cells" secrete a glycoprotein onto the resorbed bone surface, which provides a transient Type III adhesive attachment between bone and the periodontal ligament. This attachment subsequently undergoes resorption along with new formation of attachments as the bone surface continues to be resorbed. It is suggested that the cementoblast-like cells adjacent to odontoclasts in areas of active root resorption perform a similar function. A dark granular substance was observed along the resorbed dentine surface and within secretory vesicles adjacent to these "companion cells" (Figure 3.27). GREVSTAD (1987) has suggested that a similar dark, granular substance in rats "welds" collagen fibres to the surface of the dentine.

The ultrastructural features of cementoblasts at the surface of the repairing resorption bays were not studied in great detail. However, it was noted that they closely resembled the active cementoblasts found in human resorption lacunae (YAMASAKI et al., 1986). These cells were characterised by a plump cytoplasm with a well developed rough endoplasmic reticulum and Golgi complex. No large accumulations of glycogen were observed.

Cells appeared to be pallisading down from the ECC to the repairing surface, within resorption bays (Figure 3.29) and from ERM to areas of cementogenesis on non-resorbed surfaces (Figure 3.12). This observation may represent the movement of precementoblasts from the ERM/ECC to form cementoblasts at the root surface. The ultrastructure of these cells requires further study before any conclusions can be drawn.

Many other cells were observed within both repairing and active resorption bays which could not be positively identified. A great deal more study of the various cells within the repairing resorption bays is required to resolve this problem.

C. PERIODONTAL FIBRE ATTACHMENT

In areas of active resorption, soft tissue attachment was mostly denuded during the extraction of the tooth. In a few sections in which soft tissue was found adjacent to active resorption, only a small number of collagen fibre attachments could be seen at the light microscope level. These findings suggest that in areas of active resorption, the attachment of the periodontal ligament to the tooth is reduced. The attachment mechanism was studied closely at the TEM level and is described below.

In areas of repair, the collagen fibre attachment to the repairing cellular cementum was markedly increased in comparison to the areas of active resorption. These fibres were at right angles to the surface between the cementoblasts, indicating that the periodontal ligament was again firmly reattached to the root surface.

In histologic sections of human anchor premolars, following RME LANGFORD and SIMS (1982) reported that resorbed and unrepaired dentine often exhibits fibre bundles orientated at right angles to its surface. Similar fibres were observed in this study. Using TEM techniques it was observed that these fibres ran directly and predominantly continuously into the dentine but were occasionally separated from the dentine by a clear zone (Figure 3.26). The junctions were similar to Type I continuous attachments and Type III adhesive attachments between periodontal fibres and bone during alveolar remodelling in rats, as described by KURIHARA and ENLOW (1980).

Some fibres also appeared to be attached to the resorbed dentine by a dark, granular substance (Figure 3.27), similar to that described by GREVSTAD (1987). He postulated that this dark, granular substance "welds" the fibres to the exposed resorbed dentine. He offered no explanation as to where this "welding" substance is derived.

However, in this study, similar dark granular substance was observed in exocytotic vesicles released from cementoblast-like "companion" cells interposed between odontoclasts and the resorbed dentinal surface. The granular substance was deposited along the resorbed dentine surface adjacent to the fibroblast-like cell (Figure 3.27). It is proposed that this dark granular substance, which "welds" collagen to the dentine was secreted by these cementoblast-like cells.

On the bone side of the periodontal ligament in rat, KURIHARA and ENLOW (1980 a,b) describe similar fibroblast-like or osteoblast-like cells that always accompany active osteoclasts as "companion cells". They suggest that these cells secrete a network of strand-like material ("ground substance") which provide initial adhesion of the newly formed collagen. The frayed dissociated ends of the fibrils of the periodontal membrane become joined with the new collagen perhaps using the interfibrillar ground substance material just mentioned as a bonding agent. They have shown histologic evidence in the rat of a "clear zone" between periodontal fibres and resorbed dentine, which suggests that adhesion type attachments are formed. Similar adhesive junctions have been observed in this study at the surface of resorbed dentine in human anchor premolars subject to RME (Figure 3.26).

KURIHARA and ENLOW (1980 a,b) also describe the formation of a continuous type attachment of periodontal fibre to bone during alveolar remodelling. Some (not all) of the bone matrix fibrils survive the resorption process and

become incorporated into the periodontal membrane, forming a continuous band of collagen. Similar continuous attachment of fibre bundles were observed at the resorbed dentine surface in this study (Figure 3.26).

Fibre bundles were observed regularly along the surface of repairing cellular cementum, within the resorption bays. This is contrary to SEM observations of LANGFORD and SIMS(1982) and BARBER and SIMS (1981). They noted that there are very few Sharpey fibre depressions within the forming mineral front of repair cementum. However, LANGFORD (1982) demonstrated histologically that periodontal attachment was not lost in areas of resorption and repair.

D. ASSOCIATION WITH BLOOD VESSELS

Although blood vessels were a predominant feature within active resorption bays, none were observed between epithelial cell clusters and the root surface in repairing resorption bays. According to BROWN (1982), there is a close association between a rich blood supply and active resorption. JEE and KIMMEL (1976) have shown evidence that osteoclasts are of blood cell origin and are delivered to the resorption site through the blood stream. BROWN (1982) postulated that dentinoclasts and osteoclasts have similar origins and may be the same cell. The absence of blood vessels found within repairing resorption bays also supports this postulate.

The presence of the rests between the blood vessels and the cementum (Figure 3.10 and 3.11) may act to protect the root from resorption as suggested by LÖE and WAERHAUG (1961). Areas of active resorption, where blood vessels are in close association with the resorbing surface and do not have an intervening layer of E.R.M., adds credence to this postulate.

SIMS and WEEKES (1985) suggested there may be a relationship between vascular architecture and the processes of cementum deposition and root

resorption. Unique vascular loops which exist at the apical portion of the ligament and at certain sites more coronally, may play a role in the particular patterns of resorption of cementum and dentine.

CRIGGER et al. (1972) proposed that many of the epithelial rests are continuous with, or even part of, the microvasculature.

CHAPTER 5

CONCLUSIONS

1.) This study has shown that active renewing epithelial cell clusters are present in repairing root resorption bays subsequent to orthodontic tooth movement in man. This finding has not been previously reported and is contrary to earlier observations made by REITAN (1961,1985) .

2.) As epithelial cell clusters are present in repairing root resorption bays and have active, renewing, ultrastructural features similar to cells found in epithelial rests of Malassez and Hertwig's Epithelial Root Sheath, it is postulated that they may be involved with the repair of orthodontic root resorption and the re-establishment of the periodontal ligament.

3.) Epithelial cell clusters have ultrastructural features which suggest they may initiate cytodifferentiation of undifferentiated mesenchymal cells into cementoblast and are involved in the cementogenesis of repairing root resorption.

4.) It is also postulated that the epithelial cell clusters may originate from the epithelial rests of Malassez at the periphery of the resorption bays. As to how and when they move into the areas of resorption has yet to be established . Future research utilising three dimensional computer reconstructions of serial sections, through selected resorption bays, should resolve these questions.

5.) Active resorption, although reduced relative to the areas of repair, is still present on the buccal surface of premolars used as anchor premolars for R.M.E. after 6 months of retention.

6.) Periodontal fibres are attached by Type III adhesive junctions and Type I continuous junctions to the resorbed dentinal surface in areas of active resorption.

7.) Odontoclast companion cells which are similar to cementoblasts in ultrastructure, may secrete a dark granular substance which "welds" the periodontal fibres directly to the dentine surface.

This project has created more questions than it has answered. A great deal more research is required to gain a complete understanding of orthodontic root resorption and repair in man.

FUTURE RESEARCH

Future research is recommended in the following areas:

1.) Determine how and when epithelial cell clusters move into areas of active resorption. Denuded root surfaces in monkey incisors, as utilized by LINDSKOG et al. (1983), bracketed over a suitable period may provide a means of resolving these questions. The use of a histochemical marker, specific to the cytokeratin within epithelial cells, would greatly simplify the identification of epithelial cell clusters at the light microscope level and negate the need for time consuming TEM techniques. Alternatively, epithelial growth factor as described by THESLEFF (1987) may provide appropriate differentiation of epithelial cells.

2.) By utilizing 3-dimensional computer reconstructions of serial sections through selected repairing resorption bays, establish whether or not epithelial cell clusters in the resorption bays are continuous with the epithelial rests of Malassez adjacent to non-resorbed surfaces at the periphery of the area of resorption.

3.) Compare stereologically the ultrastructure of epithelial cell clusters (ECC) with ERM, adjacent to non-resorbed root surfaces of anchor premolars, Hertwig's epithelial root sheath, normal ERM from control material and clear cell rests in lateral periodontal cysts.

4.) Compare the ultrastructure of ECC with the epithelial cells found in repairing experimental cavities placed in monkey incisors (LINDSKOG et al., 1983), and establish whether or not these cavities are suitable experimental models for orthodontic root resorption.

5.) Closely examine the ultrastructure of the cells between the ECC and repairing root resorption bays and between ERM and areas of active cementogenesis. This may add to the understanding of the role of ERM/ECC in cementogenesis.

6.) Investigate the possibility of using implants of cultured epithelial rests of Malassez or odontogenic epithelium to initiate repair of invasive cervical root resorption.

CHAPTER 6

APPENDICES

6.1 0.06M CACODYLATE BUFFER

Solution:	0.06M cacodylate buffer (arsenic)	
Formula:	sodium cacodylate (E.M. grade)	25.68 gm
	double distilled H_2O	2000 ml
	pH the solution to 7.4 at 20°C.	
Shelf life:	7 days 4°C.	
Precautions:	High toxicity to humans, hence it is recommended that gloves, mask, and a fume cupboard are used.	

6.2 4% OSMIUM TETROXIDE SOLUTION

Solution: 4% osmium tetroxide in cacodylate.

Preparation: The osmium tetroxide solution should be made up the day before the experiment, to allow the crystals to completely disslove. To prepare the 4% osmium tetroxide, remove the label, and clean the outside of the 2 gm OsO_4 ampoule with ethyl alcohol. Warm the ampoule in a beaker of hot water and rotate it carefully to get an even film of OsO_4 on the inner walls, then drop the ampoule into a thick walled glass bottle containing 50 mls of cacodylate buffer and seal with a screw top lid. Shake the bottle to break the OsO_4 ampoule and cover with aluminium foil to exclude light. The 4% osmium

solution tetroxide should be kept in a fume cupboard at all times.

Shelf life: Approximately 10 days but discard if not clear. If the solution becomes straw coloured, or darker, its fixative should be discarded.

Precautions: Whenever solutions containing osmium tetroxide are being used always wear protective clothing (goggles, mask, gown, and gloves) and work in a fume cupboard, as osmium tetroxide has a high human toxiciy. All waste must be stored in sealed containers in a fume cupboard and later disposed of properly.

6.3 DECALCIFYING SOLUTION FOR ELECTRON MICROSCOPY

Solution: 0.1M E.D.T.A. in 2.5% glutaraldehyde (pH 6.0).

Formula: E.D.T.A. 74 gms 45 gms

0.06 M cacodylate buffer (pH 7.4) 1800 mls

25% glutaraldehyde (TAAB E.M. grade) 200 mls

Dissolve the E.D.T.A. in the cacodylate buffer by gently heating, and when cool add the glutaraldehyde. pH the solution to 6.0 at 4°C.

Shelf life: 7 days at 4°C.

Precautions: Human toxicity, therefore gloves and masks recommended.

6.4 1% URANYL NITRATE BLOCK STAIN

Solution: 1% uranyl nitrate in 70 % alcohol.

Formula:	uranyl nitrate	1gm
	absolute alcohol	70 mls
	double distilled water	30 mls
N.B. :	Exclude light from the solution by covering the jar with aluminium foil.	
Shelf life:	7 days at room temperature.	
Precautions:	Avoid skin contact.	

6.5 AGAR EMBEDDING RESIN

Agar embedding medium was obtained from Ladd Research Industries, Inc. The hardness of the resin blocks depends upon the ratio of mixture A (containing DDSA) to mixture B (containing NMA), and considering the type of tissue to be sectioned, it was decided to use a hard embedding medium. The two resin solutions were therefore mixed in the proportion of 1 part of A to 2 parts of B.

The weight per oxide equivalent (W.P.E.) for the Agar of the batch used was 147. Volumetric measurements were used for making the embedding resin. The measurements were based on the results published by Luft ('61).

Preparation	WPE = 147	Total volume approximating 30 mls.
Mixture A:	Agar	5 mls
	DDSA	6.3 mls
	(Shake vigorously for 10 minutes)	
Mixture B:	Agar	10 mls
	MNA	8.45 mls
	(Shake vigorously for 10 minutes)	
Mixture C:	Mix A + Mix B + 29.75 mls	
	(Shake vigorously for 10 minutes)	

Add DDMA 0.41 mls (Shake vigorously for 10 minutes)

Thorough mixing is essential, otherwise blocks with poor sectioning properties will be produced.

Precautions: Agar is a suspected carcinogen and hence should be handled with great care. Agar NMA, DDSA and BDMA are all toxic if swallowed, and can cause skin irritation. Work with the components must be conducted in a fume cupboard, avoiding exposure to vapours, and avoiding contact with skin, eyes and clothing. Gloves and gown should be worn.

6.6 0.05% TOLUIDINE BLUE STAIN FOR 1μm LIGHT MICROSCOPIC ORIENTATION SECTIONS

Solution: 0.05% toluidine blue in double distilled water.

Formula:		
	toluidine blue	0.05 gm
	double distilled H_2O	100 mls
	Dissolve by stirring.	

Shelf life: 6 months at room temperature.

Precautions: Avoid contact with skin.

6.7 0.5% URANYL ACETATE T.E.M. GRID STAIN

Solution 0.5% uranyl acetate in 30% ethyl alcohol

Formula:		
	uranyl acetate	0.125 gm
	absolute alcohol	7.5 ml
	millipored double-distilled H_2O	17.5 ml

Shelf life: Cover with alfoil to exclude light.

Will last 7 days at room temperature.

Precautions: Avoid skin contact.

6.8 LEAD CITRATE T.E.M. GRID STAIN

Solution: Modified Reynold's lead solution

Formula:

lead nitrate	1.33 gm
sodium citrate	1.76 gm
millipore double-distilled H_2O	30 ml
1M sodium hydroxide	8 ml

Mix lead nitrate, sodium citrate, and double-distilled water, shaking vigorously for 1 minute, then allow to stand for 30 minutes. Add the 8 mls sodium hydroxide solution and dilute the entire solution to 50 mls with double-distilled water, and mix by inversion. Final solution must be clear with pH no less than 11.

Shelf life: 30 days at 4°C. Discard if pH drops below 11.

Precautions: Recommend wearing gloves when using T.E.M. grid stains, to avoid skin contact.

6.9 1% BORAX FOR 1μm LIGHT MICROSCOPIC ORIENTATION SECTIONS

Solution: 1% borax in double distilled water.

Formula:

sodium thiosulphate (borax)	1gm
double distilled water	100mls

Dissolve by stirring.

Shelf life: 6 months at room temperature.

Precautions: Avoid contact with skin.

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