ROOT RESORPTION AND REPAIR FOLLOWING RAPID MAXILLARY EXPANSION IN MAN

A SCANNING ELECTRON MICROSCOPIC AND HISTOLOGIC INVESTIGATION

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SUMMARY

This study was designed to investigate repair and possible reattachment of principal periodontal fibres in areas of resorption on anchor premolar root surfaces following rapid maxillary expansion in man. Upper first premolar teeth were obtained from patients requiring rapid maxillary expansion and extraction of these teeth after periods of retention varying between 14 and $53\frac{2}{7}$ weeks. The roots of the teeth were examined using scanning electron microscopy and light microscopy.

As a basis for comparison with root surface topography, human adult and fetal bone was examined in the scanning electron microscope. These tissues demonstrated features very similar to those seen on various root surfaces examined. Also, a number of apparently normal human premolar roots and trial tooth root specimens exhibiting resorption were examined in the scanning electron microscope and compared. Evidence of periodontal reattachment was found within defects on the surface of several of the trial specimens showing resorption.

Extensive root surface resorption characterized the roots of anchor premolars, especially on the buccal surfaces. Repair of the resorptive defects was found to occur exclusively with cellular cementum which seemed to be generally more advanced on anchor teeth retained for longer periods, up to the maximum of $53\frac{2}{7}$ weeks retention in this study. The surface areas involved by resorption did not show marked progression overall, from the shortest retention period of 14 weeks to the longest. This finding indicates that the advance of resorption is greatest during expansion and in the first three months of retention.

Periodontal fibre attachment was observed in the light microscope on repair cellular cementum and also on resorbed dentine surfaces. The exact nature of the attachments observed, whether adhesive or continuous, cannot be determined without ultrastructural study. Topographically, Sharpey fibre holes indicative of principal periodontal fibre insertion were found in repair cementum. However, these features were not numerous, or consistent in location and presence.

SIGNED STATEMENT

This project report is submitted in partial fulfilment of the requirements of the qualifying examination for the degree of Master of Dental Surgery in The University of Adelaide.

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

S. R. LANGFORD

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Dr M.R. Sims, Reader in Orthodontics, Department of Dental Health, suggested the topic investigated in this study and paved the way for the clinical aspects through his liaison with the Royal Adelaide Hospital Board of Management. In addition Dr Sims' continued guidance during the period of this project has enabled its successful completion.

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Mrs D. Crozier patiently and painstakingly typed the drafts and final copy of this report.

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Illustrations 9 to 65 inclusive are scanning electron micrographs except Figure 51 which is a light micrograph

Figures 67 to 76 inclusive are taken from the histological sections

SECTION 1

INTRODUCTION

Extensive root surface resorption of anchor premolar teeth has been shown by Barber (1978) and Harry (1977) to occur following rapid maxillary expansion. Barber (1978) could find no evidence of attachment of principal periodontal fibres to cellular cementum repairing the resorptive defects. He examined material from ten patients after fixed retention periods of up to nine months. The area involved by resorption on these teeth was quite extensive and consequent loss of periodontal attachment, if proven to be permanent, could seriously impair their functional support.

Rapid maxillary expansion is widely used by orthodontists to correct maxillary skeletal base width narrowness. Heavy forces (Isaacson and Ingram, 1964) are transmitted to the maxillary halves via an appliance connected to anchor teeth in the maxilla. Disruption and hyalinization of periodontal ligament have been described in association with orthodontic forces on teeth (Rygh, 1973; Storey, 1973; Rygh and Reitan, 1972). Such changes could be expected to occur in the periodontal ligaments of teeth used as anchor teeth for rapid maxillary expansion. If destruction of ligament cells was extensive, periodontal repair might be affected by progenitor cells from bone marrow as suggested by Ten Cate (1976) and Line, Polson and Zander (1974). The consequence of this would be ankylosis. Demonstration of root surface repair and periodontal fibre reattachment would rule out such a development.

Root resorption associated with orthodontic treatment apart from apical root loss has in the past been considered to be capable of complete restitution (Gianelly and Goldman, 1971). This cannot be assumed in the case of rapid maxillary expansion. Indeed, the few reports about the effects of this procedure on the tissues of the anchor teeth in man show damage and repair but not periodontal reattachment (Barber, 1978; Timms and Moss, 1971). In addition, the permanent loss of large areas of cervical acellular cementum with its marked concentration of inserting principal periodontal fibre bundles may significantly compromise the attachment and resistance to periodontal disease of these teeth.

The scanning electron microscope has been found useful in examining tooth root surfaces (Boyde and Lester, 1967; Jones and Boyde, 1972; Barber, 1978) and other hard tissue surfaces (Boyde and Jones, 1972). Specific topographical features have been associated with periodontal fibre attachment (Jones and Boyde, 1972). The use of this instrument to attempt to demonstrate periodontal fibre reattachment in areas of previous resorption on tooth roots could therefore prove beneficial. Conventional light microscopy may permit confirmation of the findings evaluated with the scanning electron microscope.

SECTION 2

AIMS OF THE INVESTIGATION

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1. To compare the mineralizing fronts of human adult and fetal bone with those of cementum, using the scanning electron microscope.

2. To examine the root surfaces of premolar teeth used as anchor teeth for rapid maxillary expansion in man, utilizing the scanning electron microscope and histologic techniques. Periods of fixed retention following expansion are to be varied up to a maximum of twelve months.

3. The repair of root resorption and any associated periodontal fibre reattachment are important features to be investigated on experimental teeth. The findings may then be related to the long term effect of rapid maxillary expansion on the functional status of anchor premolar teeth.

SECTION 3

REVIEW OF THE LITERATURE

Rapid Maxillary Expansion

The published history of rapid maxillary expansion dates back to 1860. Since that time much has been written and many investigations carried out particularly in relation to the macroscopic clinical effects of this procedure. In this review of the literature it is intended to give an overview outlining some of the historical and clinical features of this technique. Research related to the forces involved in rapid maxillary expansion and the tissue reactions of the teeth used as anchor units will be given more detailed attention.

Angell (1860) reported using a transpalatal screw device fixed to the premolars to expand the maxillary arch and thus increase arch length in one patient. Activation of the appliance produced a diastema between the upper central incisors which he felt indicated that separation of the maxillae had occurred. Other workers reported using similar devices clinically with comparable results (Goddard, 1893; Matteson, 1893).

Monson in 1898 seems to have been the first to suggest that mouth breathing could be associated with nasal stenosis, maxillary arch crowding and a constricted palatal vault. He thus advocated using rapid maxillary expansion to correct maxillary and nasal malformations. Such treatment was also recommended by Brown (1903), Ottolengui (1904), Willis (1911), Pullen (1912), Hawley (1912), Mesnard (1929) and others. However, Pfaff (1905) doubted the originality of Monson's (1898) proposal and stated that in Europe the efficacy of such treatment was not well accepted. Subsequent reports on the relief achieved, with rapid maxillary expansion, from rhinologic and respiratory ailments have been made by Griffin (1958), Gray and Brogan (1970), Timms (1974), Gray (1975) and Brogan (1977a, 1977b). Wertz (1968) has recorded increased capacity for nasal air volume during maximum effort following rapid maxillary expansion. This finding supported the work of Linder-Aronson and Aschan (1963) which showed a lowering to normal limits of the nasal resistance to breathing which was unchanged one year following rapid maxillary expansion in a group of ten patients.

Brown (1909) reported using rapid maxillary expansion as an adjunct to the treatment of cleft palates. More recently Grossman (1963), Isaacson and Murphy (1964) and Townend (1980) have described the use of rapid maxillary expansion in cleft palate patients.

Early investigations undertaken to demonstrate the effects of rapid maxillary expansion included the use of "green" skulls (Dean, 1909), human subjects (Dean, 1911) and dry skulls (Wright, 1912). Ketcham (1912) reported failure to open the midpalatal suture of a fresh cadaver and Federspiel (1913) claimed to find no evidence of suture opening on retracting palatal soft tissues following expansion. Dewey (1913) stated that the evidence supporting midpalatal suture opening was questionable but in 1914 he reported experiments on dogs and acknowledged the possibility of opening the suture. However, he still felt that rapid maxillary expansion was undesirable, supporting previously published statements by Angle (1910) that the great force and rapid movement associated with the procedure was not physiological.

As the Angle orthodontic philosophies and treatment methods rose to prominence in the United States, interest in rapid maxillary expansion waned. However, as detailed by Thörne (1956), the rhinological and respiratory aspects of the treatment continued to receive attention in Europe. It was not until the early 1950's that the technique again attracted interest outside Europe.

Quantitative studies of the effects of rapid maxillary expansion in man have now been undertaken by many workers. Thörne (1956, 1960), Haas (1961), Krebs (1964), and Wertz (1970) are among many whose published work now gives a well documented view of the skeletal and dental changes wrought by this procedure. Studies in animals (Posen, Bayne and Cleall, 1963; Starnbach, Bayne, Cleall and Subtelny, 1966) have also provided valuable information about the reactions to rapid maxillary expansion at more distant sutures and the bone and connective tissue responses (Murray and Cleall, 1971).

Investigations of the forces generated by the appliances are notably limited. A series of reports by Isaacson, Wood and Ingram (1964); Isaacson and Ingram (1964) and Zimring and Isaacson (1965) detail a unique study carried out on four human patients designed to measure the forces created during expansion and retention. These measurements were made electronically, using a dynamometer incorporated into each expansion appliance. The forces recorded during appliance activation ranged between several pounds and 22.5 pounds. Such forces are well in excess of accepted orthodontic forces between 50 and 400 grammes used to move teeth through alveolar bone (Gianelly and Goldman, 1971). Indeed, rapid maxillary expansion is considered orthopaedic rather than orthodontic and generally aims to correct a maxillary skeletal base discrepancy (Isaacson and Murphy, 1964; Wertz, 1970; Sassouni, 1972; Haas, 1973). Zimring and Isaacson (1965) reported that the residual loads acting on the appliances at the end of expansion were dissipated within five to seven weeks. They felt that this occurred predominantly by skeletal repositioning and later in retention when lighter forces were acting, by some independent tooth movement. These workers recommended the use of rigid retention with the appliance fixed *in situ*, over-expansion to compensate for relapse and an activation schedule reduced after the first few days of activation from twice-daily, producing 0.4mm opening, to once every day or every other day.

The design of appliances varies as exemplified by those of Haas (1961; 1970), Timms (1968; 1976), Biederman (1973) and Barber (1978). These appliances are not intended to be removed by the patient as they are cemented to teeth on either side of the maxillary dental arch by the operator. Each appliance is capable of producing maxillary expansion when activated correctly. Few direct comparisons of the results obtained with the different designs of appliance have been reported. Hershey, Stewart and Warren (1976) comparing two fixed appliance designs found statistically similar changes in first molar expansion, initial and final nasal resistance, and change in nasal width in a group of seventeen patients. Six of these patients were treated with wire and acrylic appliances and eleven with all-wire appliances.

Recommendations for fixed retention of the expansion obtained vary from two months (Krebs, 1964) to one to two years (Ketcham, 1912). Such retention is generally obtained by leaving the appliance in place. Thörne (1956) suggested that relapse might occur following fixed retention periods of less than three months. If removable retention devices alone are relied on, Stockfisch (1969) claimed that relapse would occur. Generally periods of retention with removable appliances for up to two years following fixed retention have been recommended (Haas, 1961; Krebs, 1964; Timms, 1968; 1976).

Root resorption following rapid maxillary expansion

Despite the long history of use and investigation of rapid maxillary expansion, there seems to be a paucity of studies examining the effects of this procedure on the teeth and their surrounding tissues at a histological level. Watson (1980) sounded a cautionary note against indiscriminate dental arch expansion by orthodontic treatment, including rapid maxillary expansion. Alveolar dehiscence, fenestration and root resorption were cited as possible deleterious effects resulting from such injudicious treatment. Graber (1969) warned that unfavourable changes such as buccally tipped teeth, open bites and devitalized teeth could result from misappropriate use of the palate-splitting technique as he called it. He claimed that relatively few cases of maxillary basal deficiency exist and that most lateral malocclusions are purely dental and therefore not suitable for rapid maxillary expansion. However, Graber (1969) did not elaborate any diagnostic criteria or present specific examples to support these claims. In a report of an investigation of the dental and skeletal changes associated with rapid maxillary expansion in 23 patients, Linder-Aronson and Lindgren (1979) found no unfavourable skeletal and dental side effects. This statement was true for the measurements they made and analysed but took no account of any possible damage at a histological level.

Watson (1979) stated that orthodontic tooth movement is always associated with a degree of resorption of cementum, the dependant factors being the amount and direction of root movement, the magnitude and duration of the force, the age of the patient and a factor he called the tissue stress potential. In rapid maxillary expansion the forces generated are high and continuous (Isaacson and Ingram, 1964, recorded some as high as 22.5 pounds) and directed laterally via anchor teeth.

It would seem therefore that some local tissue damage could be expected with rapid maxillary expansion.

One of the few workers to examine human tissue microscopically following palatal suture opening was Derichsweiler. According to Wertz (1970), Derichsweiler (1953, 1956) found no degenerative changes in the periodontal ligament in his histological examination.

In 1958 Debbane reported a study of midpalatal suture expansion in cats. Two of the seven cats used in the expansion experiment were subjected to continuous expansive pressure via a spring delivering force to bands on the maxillary canine teeth. Histologically, Debbane found no evidence of root resorption in one of these two animals. In the other, ground sections revealed active resorption on the pressure side of the canine roots associated with apparently normal alveolar bone. Intermittent expansive forces, produced by a periodically reactivated round gold wire, in two other cats were associated with a histological picture of extensive alveolar bone and cementum resorption on the pressure side of the anchor canine teeth. The remaining three cats served as controls. Debbane stated that light microscopic indications were that the force was greatest at the cemento-enamel junction. This correlated with the fact that, in general, tooth movement was seen to be tipping.

Starnbach, Bayne, Cleall and Subtelny (1966) investigated some of the effects of rapid maxillary expansion with a split acrylic plate having a central expansion screw. They used five Macaca rhesus monkeys, one serving as a control. In one animal sacrificed after two weeks expansion measured as 4mm across the molars, histological examination showed stretching of the periodontal membrane on the palatal aspect of the teeth involved. The periodontal membranes were disorganized and contained cell-free zones on the buccal side. The alveolar bone was irregular with many areas of resorption. These changes were uniform along the full length of the roots. A second animal, sacrificed after a 6mm expansion achieved in three months, showed similar but more extensive changes of the buccal bone with new bone deposition on the palatal side. The periodontal ligament elements were less severely disrupted. Small areas of cementum resorption were seen on some teeth. The third animal was sacrificed after a three month expansion period followed by a three month retention period, and the fourth after an additional three months without retention. These latter two monkeys showed an increasing return to normality of the alveolar bone and periodontal membrane compared with the other two animals. However, periodontal architecture was not completely re-established. No mention was made of changes in the tooth root surfaces apart from those described for the second monkey. The expansion schedules of three months do not correlate with those used for rapid maxillary expansion in humans which are usually of the order of three weeks. Thus, the apparent lack of tooth root damage cannot be directly extrapolated to humans.

The effects of palatal expansion in humans was investigated by Rinderer (1966), using records and material obtained from twelve patients. The appliances he used consisted of bands on molars and premolars or primary molars. Wire loops anchored the bands into an acrylic plate which was constructed to avoid resting on the roof of the palate. Expansion was obtained via a central screw given one quarter turn twice a day. The total opening produced was not stated. After a fixed retention period of at least three months, six unspecified anchor teeth from four cases were surgically removed with surrounding bone and examined histologically.

The light microscopic examination of the anchor teeth revealed resorption of the root surfaces on all specimens. This resorption penetrated variable distances into dentine and was found corresponding to pressure zones. Deposition of tissue, which Rinderer called secondary cementum or osteo-cement, in areas of resorption was interpreted by him as a reparative process.

From these observations Rinderer stated that "...with a sufficiently long retention period that results in fixation, a favourable situation is created for the re-establishment of normal paradontal conditions." The evidence presented for this conclusion was a number of photomicrographs of cellular cementum partially refilling resorption areas together with new alveolar bone on the opposite side of the periodontal ligament. However, there was no indication that reattachment of principal periodontal fibres had occurred or was occurring in such areas of resorption, nor that these areas would be fully repaired. Moreover, Rinderer did not detail the amount of expansion, the period of retention, or indeed which teeth were examined histologically. Further, he did not specify the length of time that he considered to be "a sufficiently long retention period."

At the end of the second part of a two part article, Moss (1968a, 1968b) reported the histological examination of teeth and adjacent alveolar bone removed from human patients after they had been treated by rapid maxillary expansion. He found microscopic areas of resorption, partially or totally repaired with so-called osteodentine. Little cellular activity was seen except at repair sites and according to Moss the periodontal ligament and alveolar bone appeared normal. No evidence of root resorption could be seen radiographically.

The period of expansion and retention of the specimens was not reported nor were the extracted teeth designated. Periodontal reattachment in repairing resorption areas was not mentioned and no indication of the extent of the observed resorption and repair was given.

Timms (1968) as part of a larger report, described the histological examination of upper first molars removed with a portion of buccal alveolar plate from two patients who had undergone rapid maxillary expansion and required these extractions, presumably to complete orthodontic treatment. This was done four months after active expansion. Details of the mode of retention for these cases were not specified. The expansion appliance employed by Timms utilized silver cap splints cemented to the maxillary teeth from the lateral incisors to the last erupted tooth and connected to a midpalatal screw.

The teeth examined showed areas of 'root decalcification' as Timms called it. Signs of repair were also observed but not described in detail. Only one photomicrograph was presented and this demonstrated loss of cementum and dentine on the pressure side of one root. No evidence of repair with reattachment of principal periodontal fibres was reported.

Timms and Moss (1971) used material obtained from eight patients who had undergone rapid maxillary expansion with an appliance similar to that used by Timms (1968). They examined histologically the effects of the expansion procedure on the teeth and their supporting tissues. The rate of expansion was from 0.15mm to 0.30mm per day and two to four months fixed retention was followed by varying periods with a removable retainer. The teeth for examination, either a first molar or second premolar, were removed surgically with adjacent alveolar bone and prepared for histological examination. The periods between expansion and tooth removal ranged from 6 to 23 months.

Histologically the teeth showed resorption of cementum and dentine on the distobuccal and mesiobuccal aspects of the roots, including the palatal root of first molars. Teeth removed six to seven months following expansion showed resorption mainly in the coronal third and trifurcation areas of the root. All areas of resorption of dentine and cementum showed repair taking place with so-called osteoid tissue.

In the teeth extracted one year after expansion evidence of resorption of dentine and cementum, which was repaired with osteoid tissue, was seen in the coronal and apical thirds. However, the relationship of the periodontal ligament to this repair cementum was not discussed or illustrated. Teeth removed 21, 22 and 23 months following expansion showed cementum exostoses and areas of resorption and repair in the apical third.

Alveolar bone resorption and deposition was evidenced by reversal lines. Bone deposition buccally was noted particularly in cases one year after expansion. The pulp showed deposition of secondary dentine not attributable to any apparent pathological agency and in some cases developing pulp stones were seen.

Timms and Moss (1971) concluded that relapse forces act on the teeth for some time, presumably 23 months, after the initial expansion. This was shown by reversal lines in buccal bone and resorption and repair at the root apices indicating lingual tooth tilting, at periods beyond 6 to 7 months and up to 23 months post-expansion in the retention phase. Moss (1976) commented on the results of this study (Timms and Moss, 1971), saying that the effect of rapid expansion on the roots of teeth was no more severe than that with orthodontic movements. He did not cite the basis for this comparison which therefore was probably only a subjective appraisal.

Cynomolgus monkeys in early mixed dentition were used by Ohshima

(1972) to study the effects of slow and rapid maxillary expansion on the maxilla and its tissues. He used both all-wire framework and split-acrylic type appliances cemented in place for expansion and retention of various periods. At the end of each period, the experimental animal was sacrificed and radiographs and study models taken. Preparation for histological examination ensued.

The results of the histological examination of the midpalatal suture indicated some relapse after three months retention with rapid expansion but not with slow expansion. Root resorption of anchor deciduous molars was more extensive than of permanent first molars. This resorption was more marked in the slow expansion group. Repair of root resorption was also observed but periodontal reattachment was not demonstrated or described.

Harry (1977) used the scanning electron microscope to investigate root resorption in human premolar tooth roots which had been subjected to orthodontic forces prior to extraction. In addition, maxillary first premolar teeth which had been used as anchor teeth for rapid maxillary expansion and three months fixed retention were obtained from two patients. Root surface resorption extending into dentine involved large areas of the buccal aspects of these anchor teeth. Minor resorptions were also found on the tension side. The resorption was most extensive on the premolar roots of one of the patients whose palate did not appear to widen but exhibited arch expansion of dental origin only.

Further investigation of root resorption associated with rapid maxillary expansion was undertaken by Barber and reported in 1978. Ten patients were treated orthodontically, each undergoing rapid maxillary expansion and variable periods of fixed retention from 0 to 36 weeks prior to removal of maxillary first premolars in order to complete treatment with full banding. The extracted maxillary first premolars, which had acted as anchor teeth during expansion and retention, were examined with a scanning electron microscope. Comparison was made with untreated mandibular first premolars also extracted in six cases. In five patients one upper first premolar was deliberately not used as an anchor unit. In another two patients, periods of removable retention were employed and in one of these a period of six weeks with no retention occurred just prior to extraction of one premolar, the other having been removed earlier. These two patients came from a group of three, each of whom had one anchor premolar removed at two weeks following expansion. The remaining anchor premolars were extracted at 16, 24 and 32 weeks.

Barber found that resorption on teeth used as anchor units primarily involved the buccal root surfaces with the cervical one third most severely affected. The resorption tended to be peripherally expansive and relatively shallow, although often penetrating into dentine.

Increasing periods of retention up to nine months revealed not only more extensive resorptive areas but also increasingly greater repair by cellular cementum deposition centrally within these areas. Active resorption persisting after nine months retention was taken to indicate that relapse forces were still present. Importantly, no evidence of principal periodontal fibre insertion, namely Sharpey fibre depressions, was observed in any of the repair cellular cementum.

Some areas of resorption seen on 'non-buccal' aspects of anchor teeth were thought to be due to tension generated root damage. Limited resorption was found on only one of the mandibular first premolars examined. This finding supports reports by other workers of small areas of resorption on the roots of apparently normal teeth (Henry and Weinmann, 1951; Jones and Boyde, 1972).

Radiographic evidence of root resorption could be demonstrated in only one of the ten patients expanded by Barber. In this case apical root loss of 3 to 4 millimetres of an anchor premolar was diagnosed, based on a comparison of periapical radiographs of the anchor premolars taken prior to expansion and just before extraction. Directing the central x-ray beam mesially or distally as far as possible in the clinical situation did not reveal the surface root damage present. Harry (1977) had similarly failed to reveal root surface resorption radiographically and Black (1965) found radiographs unreliable in detecting root resorption.

Scanning electron microscopic appearance of cementum in man

It is the collected work of Boyde and Jones which is primarily responsible for the current state of knowledge concerning the topography of tooth root cementum. Forming and fractured surfaces of cementum and Sharpey fibre bone were examined in both the organic and anorganic states by Boyde and Jones in 1968. Their material was obtained from a wide variety of mammals, including man. They found it profitable to examine specimens which had been rendered anorganic to free the surface of adhering tissue. The deorganifying process also removed the unmineralized matrix.

In the anorganic specimens they found that Sharpey fibres presented either as projections above the general level of the mineralizing front or as depressions in the front. The surface texture of the anorganic preparations was found to be rougher in areas of Sharpey fibre depressions than projections. This roughness was due to prominent nodules which Boyde and Jones (1968) called microcalcospherites because of a similarity to the pattern seen in the mineralizing front of mammalian dentine but on a smaller scale. This mineral pattern in dentine presumably refers to the calcospherites ranging in diameter between one and 100 micrometres later described by Boyde and Jones (1972) and not to the mosaic pattern of closely packed particles approximately 250 Angstrom units in diameter found by Boyde and Lester (1967a) in peritubular dentine. The cement nodules illustrated by Boyde and Jones (1968) appeared to be spherical to fusiform in shape and probably about ½ to 1 micrometre in diameter although a size was not indicated.

Boyde and Jones (1968) described the Sharpey fibre projections in human permanent cementum as low, rounded mounds and as flat-topped or dish-shaped with slits or holes in human deciduous cementum. Projections and depressions did not intermingle but were found to occur in discrete patches of various size and distribution. In discussing the results of their study Boyde and Jones (1968) argued that Sharpey fibre projections represented areas of comparative inactivity where mineralization within inserting periodontal fibre bundles had outstripped surrounding matrix production. Depressions and the associated microcalcospheritic pattern indicated active mineralization but Boyde and Jones (1968) added that it was not possible to distinguish areas in which matrix production was continuing or had ceased and the mineral front had not caught up.

In Sharpey fibres with a central unmineralized core they sometimes observed dense mineralization and beading of the periphery indicating fusion of the microcalcospherites. Boyde and Jones (1968) postulated that this sequence of mineralization prevented ion diffusion to the central part, blocking mineralization of the core.

Developing cementoblast lacunae observed by Boyde and Jones (1968) showed canalicular openings and a fine surface pattern representing the collagen fibrils of the intrinsic matrix. Cementocyte lacunae exposed in fractured surfaces often presented a smooth homogeneous lining with canalicular openings still clear. The smooth lining was attributed to perilacunar mineralization of ground substance.

Boyde and Jones (1968) concluded from their observations of the mineralizing front that cementum formation is an uninterrupted process but subject to great local fluctuation in rate.

In 1972, Jones and Boyde reported a study of human cementum surfaces examined in the scanning electron microscope. They used the roots of 220 extracted human teeth, gaining most information from specimens with the periodontal membrane cut back and also from anorganic preparations. In the former specimens they observed that most of the principal fibre bundles entered the cementum obliquely without deviation within the field of view, often being aligned in rows.

In anorganic preparations Sharpey fibre projections in acellular cementum generally demonstrated a hexagonal packing pattern, being low mounds when close-packed and dish-topped when less densely packed. The plane of the mineralization front within each fibre was perpendicular to the long axis of the fibre.

Jones and Boyde (1972) divided the Sharpey fibre insertion areas into those showing 100% coverage and no intrinsic fibres this being acellular cementum, areas showing 40% coverage and having intrinsic fibres but with few cell lacunae, and areas with 15-40% coverage, intrinsic fibres and some cell lacunae. The latter two types were considered to represent cellular cementum. The peak of the Sharpey fibre insertion diameters for these three groups combined was 6 micrometres with a majority distribution between 4.5 and 9.5 micrometres. It was noted that there was a range of cementum from that which had no extrinsic fibres (sometimes seen near the root tip) to that which was purely mineralized Sharpey fibres with very little intrinsic matrix component.

In the coronal portions of newly formed roots the Sharpey fibre proportion of the acellular cementum allowed for no significant intrinsic matrix component. In older teeth further down the root this latter component increased to about 40% giving the topography characteristics similar to cellular cementum, although few cells were incorporated in it.

The gross pattern of mineral deposition studied showed 100% Sharpey fibre acellular cementum to have a smooth mineral front and that of resting 40% Sharpey fibre tissue to have an easily discernible textural pattern indicating spaces between the mineralized portions of the fibrils. The mineralization pattern of Sharpey fibres in cellular cementum was variable so that the mineral front appeared to be more irregular than in acellular cementum.

Intrinsic matrix exhibited a similar mineralization pattern to that described by Boyde and Hobdell (1969a) for adult lamellar bone, the mineralization occurring first in the collagen compartment of the matrix. The mineral clusters were oriented in the original fibre axis in anorganic specimens enabling study of the fibres' size and direction. Two groups of intrinsic fibres were distinguished. Firstly, smaller fibres close·to Sharpey fibre bundles and deflected to encircle them and secondly, larger bundles which followed straight courses for some distance.

Jones and Boyde (1972) pointed out that Sharpey fibre depressions in the mineralizing front in anorganic specimens were not seen in human acellular cementum, unlike some other mammals, probably because human acellular cementum never becomes very thick. The depressions were seen in actively mineralizing cellular cementum and tissue with a low density of cell lacunae, i.e. the 40% Sharpey fibre tissue.

Boyde and Jones (1972) stated that '50% Sharpey fibre cementum' mineralizes more rapidly than '100% Sharpey fibre cementum'. Mineralization spreads from new intrinsic matrix to the walls of the Sharpey fibres thus delaying mineralization in the centre of the fibres.

Importantly, Boyde and Jones (1972) reported that the cellular cementum found toward the root apex of completely formed teeth often contained very few cells. They stated that basically there is no difference between this tissue and frankly cellular cementum which exhibits intrinsic matrix fibres parallel to the surface in addition to Sharpey fibres which occupy 40-60% of the total volume.

Kvam (1973a) looked at demineralized tooth root sections in the scanning electron microscope and observed principal fibre bundles traversing the cementum in an orderly and parallel manner. The bundles were widest towards the root surface, dividing as they approached the cementodentinal junction. Fibres were observed connecting the principal fibres in a ladderlike or interlacing arrangement. These connecting fibres were derived from the principal fibres and extended from them at about 90°. The principal fibres were seen to be integrated with an extensive fibrous coat at a short distance from the root surface in the periodontal ligament. Such a fibre plexus was also described by Shackleford (1971a, 1971b) and Kvam (1972a). Shackleford believed that it might play a role in accommodation of the periodontal ligament to forces on the tooth. Kvam (1973a) made no mention of the location on the root where his observations were made stating only that principal fibres constituted the major part of acellular cementum.

Toda, Tojo, Takaki, Suzuki, Toda and Kasahara (1974) studying human cementum with the scanning electron microscope observed three types of cell lacunae. In type I the lacunar wall exhibited bundles of fibres of similar size to the intrinsic matrix fibres. These formed a network, with some individual fibres being independent of bundles. The bundles appeared to be arranged around the canalicular openings, with a slight deposition of homogeneous material. In type II the lacunar wall showed a granular or rough surface. Small irregular openings of various size were seen. In type III the wall had a smooth appearance with mild undulations and various sizes of canalicular openings. The material for 1 to 1.5 micrometres around these type III lacunae was seen to be homogeneous, probably due to the perilacunar mineralization described by Boyde and Jones (1968). The frequency distribution of observation was I:II:III = 8:1:1. This classification probably represents stages in the progressive perilacunar mineralization suggested by Boyde and Jones (1968).

Scanning electron microscopic appearance of root resorption

Boyde and Lester (1967b) examined the resorbing surfaces of extracted or exfoliated human deciduous molar teeth using the scanning electron microscope and transmission electron microscope. Significant undermining of dentine was found as it was resorbed and the peritubular dentine was seen to project above the level of the surrounding intertubular dentine. Completely sclerosed tubules were particularly prominent. Frequently a zone immediately around the peritubular dentine was eroded more deeply than the intertubular dentine. Boyde and Lester (1967b) attributed the resistance of peritubular dentine to a lower solubility of its matrix species and/or protection exerted by its mucopolysaccharide matrix component. Peritubular dentine they claimed to be highly mineralized, with a distinct morphology and a matrix notable by the predominance of sulphated acid mucopolysaccharides and the absence of collagen. Minute projections in the resorbing surface were taken to be sclerosed cementocyte canaliculi or if resorption extended into dentine, the peritubular dentine of sclerosed fine terminal branches of dentine tubules.

The transmission electron microscope showed the resorbing surface of dentine and cementum to consist of naked, unmasked collagen which indicated that demineralization of the collagen was the first step in resorption, a conclusion later supported by Boyde and Jones (1972) and Kvam (1972b).

In anorganic specimens, aggregations of large regularly shaped 'crystals' were found to accumulate at the periphery of Howship's lacunae this being the name given to individual resorptive depressions. These large 'crystals' were presumed to be formed by redeposition in a different form of the mineral component at the resorbing front. The 'crystals' did not appear to be limited in size and Boyde and Lester (1967b) assumed that they possessed no matrix.

The edges of the resorption bays in cementum were very sharp. A normal surface would suddenly give way to a large cavity formed by numerous Howship's lacunae. Often an impression of considerable undermining was present. The appearance of resorbing alveolar bone was similar to that of resorbing cementum according to Boyde and Lester (1967b).

Jones and Boyde (1972) looked at human tooth root surfaces in the scanning electron microscope and found a high frequency of small resorption areas in young teeth although no figures were given. They did not state whether deciduous or permanent teeth were involved. These resorption areas were common near the root tip and they believed that such areas were related to the normal establishment of occlusion of the teeth. Jones and Boyde (1972) also found that resorption pits were often the sites of the start of cellular cementum deposition. Large resorption bays distant from the root apex were rare and were attributed to abnormal occlusion or related to the pressure side of orthodontically moved teeth. Resorption bays extending into thick layers of cementum revealed a pattern of intrinsic fibres at right angles to the Sharpey fibres a description which was not convincingly illustrated. At the edge of resorption bays Boyde and Jones (1972) found that intrinsic matrix removal occurred preferentially to Sharpey fibre removal. However, no illustration was presented to support this finding. Thus the conclusion reached by Jones and Boyde (1972) was that the resorption process alters the intrinsic matrix in some way to allow its removal before that of Sharpey fibres. This resistance of Sharpey fibres to resorption was not observed by Harry (1977) or Barber (1978).

Large resorption bays were seen at times to have a slightly raised rim or a series of bumps formed of new, acellular cementum. Jones and Boyde (1972) supposed this to be a compensatory hyperplasia due to increased functional demands caused by the loss of periodontal attachment in resorbed areas. Barber (1978) attributed a similar feature, which he observed at the rim of a resorption area, to cementum having been undermined by resorption and pulled back by the attached periodontal fibres.

Jones and Boyde (1972) found that actively resorbing areas were smooth. Areas where repair was occurring showed a series of spherical mineral particle clusters at their free surface similar to those seen in primary bone formation. Usually these mineral clusters were evident on the surrounding unresorbed cementum surface also. Boyde and Hobdell (1969) described the mineralizing front at periosteal surfaces of primary bone as characterized by a collection of irregular mineral particles, each of about $\frac{1}{3}$ micrometre diameter, unrelated to collagen fibres in a specific fashion.

Boyde and Jones (1972) stated that in resorbing bone surfaces the osteocytes were unaffected by the presence of osteoclasts and protected the adjacent lacunar wall surface until released by removal of the surrounding bone. Seen in the scanning electron microscope, the cell lacunae showed a normal fine collagen fibre pattern or smooth perilacunar surface. Actively resorbing areas in bone demonstrated Howship's lacunae with sharp edges and those in which resorption had stopped showed shallow, poorly defined lacunae. Boyde (1972) pointed out that where resorption in bone had stopped the surface tended to be smooth whereas in areas of active resorption individual collagen fibres were evident. This topographical difference was attributed by Boyde (1972) to remineralization occurring within the superficial layer of chondroitin sulphate ground substance deposited at the cessation of resorption. Boyde (1972) also surmised that the pattern of initial repair in a resorbed area in bone is similar to primary bone mineralization. Such activity is characterized by mineral clusters with limited indication of collagen fibre presence due to the high mucopolysaccharide content of the first repair matrix. These observations concerning resorption in bone may be relevant to cementum because of its similarity to bone.

Barber (1978) found that repair of resorption initially produced a granular appearance on Howship's lacunae. Later stages of repair occurred with cellular cementum. Barber (1978) designated 3 types of mineral front in repair tissue. Type I exhibited mineral particles with a random orientation and was found in areas where repair was incomplete but progressing. Type II which was found less commonly, generally where repair had re-established root outline, demonstrated elongated mineral nodules united to reveal the fibre matrix pattern. Cell lacunae in this Type II repair tissue were round and had a fibrillar pattern covering their floors. A relatively homogeneous mineral front distinguished Type III repair cementum. Occasionally, where repair was exuberant, Type I and III were found together.

Kvam (1972c) found areas of root surface resorption on the marginal pressure side of orthodontically moved teeth in man, particularly in relation to hyalinized areas of the periodontal ligament (Kvam, 1972b). Kvam (1972c) assumed that such root resorption would be 'functionally repaired' but presented no evidence for this other than citing Massler and Malone (1954) who in fact made no mention of such a phenomenon.

Cementum: Aspects of structure in man

Morphologically cementum is of two types, acellular and cellular, the term acellular referring to layers of cementum which have not incorporated cells as cementocytes within their structure. A thin layer of acellular cementum usually covers the cervical one third to two thirds of the root. Cellular cementum is found on the remainder of the root surface and may also be found on the surface of the acellular cementum. The two types may occur in alternating layers and may be seen together in repair (Armitage, 1976).

Both acellular and cellular cementum exhibit incremental lines at light microscopic magnifications, those of cellular cementum generally being more widely spaced. According to Furseth and Mjör (1973) and Armitage (1976), histochemical studies indicate that these lines are highly mineralized areas with less collagen and more ground substance than other areas of cementum. Cementum is deposited throughout life, its width being approximately tripled between 10 and 70 years of age (Lammie, 1966; Furseth and Mjör, 1973).

Cementum is the medium by which the collagen fibres of the periodontal ligament attach to the tooth, the embedded portions being called

Sharpey fibres (Osborn and Ten Cate, 1976). Each Sharpey fibre is composed of numerous collagen fibrils passing well into the cementum (Armitage, 1976). Cementum also has a role in maintaining the width of the periodontal ligament, the root area available for the support of the tooth (Melcher and Eastoe, 1969) and in repair of damaged root structure (Melcher, 1969).

Osborn and Ten Cate (1976) distinguished two types of cementum based on differences between their organic matrices. This classification parallels that of Jones and Boyde (1972). One type of cementum, termed 'predominant' Sharpey fibre cementum by Osborn and Ten Cate (1976), does not contain cells. The bulk of the collagen fibres in this cementum are derived from inserting periodontal ligament fibre bundles forming Sharpey fibres. The other type, 'partial' Sharpey fibre cementum, usually contains cells but can sometimes be acellular. A variable percentage (40-60%) of the collagen content of this type of cementum is derived from Sharpey fibres many of which have an unmineralized core. According to Osborn and Ten Cate (1976) the main function of the predominant Sharpey fibre cementum is most likely to anchor the tooth in the alveolus, thus explaining why it covers the coronal two thirds of the root. They also stated that growth of cementum is most rapid in the apical regions in order to compensate for active eruption of the tooth which may occur throughout life.

This concept of cementum classification based on matrix derivation supports earlier work reported by Selvig (1965) on the collagen fibres in human cementum. From an electron and light microscopic study of ground sections and corresponding microradiographs of human teeth he pointed out that no region of cementum intercellular matrix was free of collagen fibrils and that the fibrils were orientated in two ways. He suggested that there are two groups of fibrils of different origin: the Sharpey fibre group which originates in the periodontal ligament and runs at right angles to the surface of the cementum and another group of fibres which run mainly parallel to the surface of the cementum and are probably laid down by cementoblasts. Scott (1977) described the arrangement of collagen fibres in cementum similarly. Selvig (1965) found that most of the acellular cementum contained closely packed Sharpey fibres, from which the matrix was derived. This has been supported by a histological investigation using polarized light reported by Giansanti (1970). Selvig (1965) also observed fibrils irregularly arranged or parallel to the surface in some areas of acellular cementum. At high magnifications in the electron microscope Selvig (1965) found that the surface of the acellular cementum was often characterized by a serrated appearance. This was produced by projections of calcified tissue, each of which corresponded to the insertion of one collagen fibril. Armitage (1976) stated that the collagen fibrils of both cellular and acellular cementum were arranged in a very complex fashion with little discernible pattern. However, he noted that relatively discrete bundles of collagen fibrils could be seen in some areas, particularly in tangential sections and these were Sharpey fibres making up a substantial portion of cementum.

Selvig (1965) found Sharpey fibres in cellular cementum to be mineralized only peripherally as opposed to intrinsic fibres which were totally mineralized. He related this finding to the rate of cementum mineralization and the origin of Sharpey fibres from generally uncalcifiable periodontal ligament. Armitage (1976) attributed unmineralized areas of one to five micrometres in diameter seen in cementum to be poorly mineralized cores of Sharpey fibres.

Listgarten (1966) found that in human cervical cementum from comparatively young people Sharpey fibres tended to run a straight course to the cemento-dentinal junction. Gustafson and Persson (1957) found that Sharpey fibres emerged from cementum and bone in a straight line. They suggested that changes in tooth position necessitated new layers of cementum and bone to readjust Sharpey fibre orientation at points of emergence. Thus, forces would be directed along the long axis of the fibres enabling maximum resistance to be achieved.

Armitage (1976) stated that although continuing apposition of cementum incorporates a larger part of the principal periodontal fibres, the attachment is confined to the most superficial or recently formed layer of cementum. Thus, increasing thickness of cementum would not enhance the strength of attachment. The size and number of principal periodontal fibres and corresponding Sharpey fibres in cementum depends on the functional demands placed on the teeth. Thus, these fibres are numerous and well developed in functioning teeth (Furseth and Mjör, 1973).

In 1963 Amazawa described cementocyte canaliculi seen in the transmission electron microscope as radiating and connecting with one another. He claimed that the walls of the lacunae were more highly mineralized than the rest of the tissue. Albright and Flanagan (1962) using the transmission electron microscope, described the presence of unmineralized collagen fibrils, in the space between cementocytes and the walls of their lacunae.

Cementum and bone are structurally similar but differ in that cementum is avascular and bone is vascularized. Mineralization of cementum occurs after matrix production, and apatite crystals are deposited within, on the surface of and between collagen fibrils (Furseth and Mjör, 1973). The crystals have been described as thin plate-like structures measured in hundreds of Angstrom units (Albright and Flanagan, 1962; Selvig, 1965).

Albright and Flanagan (1962) also described small cementum crystals with a needle-like shape.

An important and interesting feature of cementum is the cementogenesis of the cervical acellular cementum. It appears from the work of Paynter and Pudy (1958), Listgarten (1968), Listgarten and Kamin (1969), Lester (1969), Slavkin and Boyde (1974) and Owens (1978; 1980) that at least the first formed acellular cementum during tooth formation may be a product of Hertwig's epithelial root sheath. Ten Cate has shown that human cementum, including the first formed cementum, is an ectomesenchymal derivative, being laid down by cells differentiating from connective tissue between the inner layer of the dental follicle and the dentine surface (Ten Cate, 1969; 1972 and Freeman and Ten Cate, 1971). Stahl (1977) pointed out that both these proposed modes of cementogenesis may profoundly influence the repair of cementum and if the ectodermal origin was confirmed in man, the reformation of acellular cementum as a regeneration response following injury would be most unlikely. Histological observations by Stahl (1977) of repair cementum in rats have supported the contention that regeneration of acellular cementum is limited or absent.

Tooth Root Resorption and Repair

Henry and Weinmann (1951) attributed the first mention of resorption of permanent tooth roots to Bates (1856). Although the dental profession acknowledged resorption as a problem, it was not until the early part of this century that serious investigations of the phenomenon began to be reported. The introduction of radiography as a diagnostic tool in the early 1900's enabled workers to detect resorption *in situ*.

Ottolengui (1914a) reported on pathological root resorption due to lesions and impacted teeth, and physiological resorption of deciduous tooth roots, using radiographs of cases to illustrate his paper. In a second article, Ottolengui (1914b) discussed root resorption of reimplanted teeth, illustrated with radiographs of a case. At this time, orthodontic treatment was not cited as a direct cause of root resorption, but caution against moving roots of teeth into unerupted teeth was advised.

Early histologic studies illustrating the reparative potential of cementum include Sippy (1927) using dogs, Orban (1928a) using material of unstated origin and Zemsky (1929) utilizing human material. These reports claimed that normal functional periodontal attachment would occur with repair of root resorption. Indeed a number of photomicrographs reproduced by Sippy (1927) and Orban (1928a) demonstrate a type of periodontal reattachment associated with repair cementum. However, Sippy (1927) was dealing with experimental wounds through the full thickness of mucosa, bone, periodontal ligament and into the root structure of dogs. Such a situation is not necessarily analogous to lesions of purely root surface resorption in man. Orban (1928a) failed to define any of the details of his material including its origin, thus severely limiting its usefulness. The shortcomings of such early research meant that the pathogenesis of root resorption in man and its sequelae was not scientifically well established. Despite this, few investigations since have been concerned with the ultimate outcome of resorption of the surface of tooth roots.

Great attention has been focused on root resorption associated with orthodontic treatment, but this has been limited mainly to studies of the loss of apical root length. Such root loss is acknowledged to be permanent (Reitan, 1974). Root surface resorption on the other hand can be repaired with cementum as has been shown by many workers, including Reitan (1974) and Barber (1978). Unfortunately, repair in this context has been equated by many writers with regeneration of a normal functioning periodontal attachment. There is little published evidence of any reattachment following resorption, one of the few examples being a photomicrograph of apparent attachment to early repair cementum in tissue of unstated origin presented by Gottlieb in 1942.

An investigation of resorption and repair in human cementum was reported in 1951 by Henry and Weinmann. They studied 15 human dentitions (261 teeth) obtained at autopsy. Records included photographs, models and radiographs taken prior to histologic preparation. Some degree of root resorption was demonstrated histologically in 90.5% of the 261 teeth examined, although serial sections were not available for all. The number of resorption areas per tooth and per dentition tended to increase with age and showed a 'remarkable degree of bilaterality' per tooth in each arch. The majority of resorption areas (76.8%) were found in the apical one third of the root. Resorption areas were more numerous on the mesial and buccal surfaces compared with the distal and lingual surfaces respectively. There was little difference in the incidence of resorption in upper and lower teeth. Trauma and an inherent resorption potential were cited as the main factors responsible for the observed lesions.

Repair was reported by Henry and Weinmann (1951) to occur with a combination of acellular and cellular cementum, explained by a variation in the rate of formation. Large areas of resorption exhibited repair with relatively rapidly formed cementum initially, which changed to acellular ('homogeneous') as restoration of root outline was approached. In most areas repair was 'anatomically complete', restoring the former root surface outline. Periodontal reattachment to the repair tissue was not discussed. The authors also concluded that small areas of root surface resorption could be diagnosed from radiographs. This claim, however, has not been well supported (Andreasen and Hjørting-Hansen, 1966; Harry, 1977; Barber, 1978).

According to Armitage (1976) resorption of cementum may occur after trauma or with excessive occlusal forces. Scott (1977) stated that evidence of small areas of cementum resorption may be found in about 90% of permanent teeth, usually in the apical region, supporting earlier reports by Harvey and Zander (1959), Henry and Weinmann (1951) and Jones and Boyde (1972). Factors responsible for such resorption have been identified only broadly. For instance Lammie (1966) stated that toxic action, as in advancing periodontal disease, or pressure may cause resorption. He also stated that mild stimuli causing deposition of cementum, acting with increased intensity, may cause resorption.

One of the functions of cementum is protection of the root of the tooth (Jenkins, 1978). Repair is generally said to follow removal or cessation of factors initiating root surface resorption (Furseth and Mjör, 1973; Scott, 1977) and is accomplished by the formation of cellular or acellular cementum either separately or in alternating layers (Armitage, 1976). That repair can occur with cellular or acellular cementum or both has been stated by other writers including Henry and Weinmann (1951) and Kerr (1961). Photomicrographs reproduced in both these papers to illustrate acellular repair cementum are unconvincing since the layers of cementum in question were darkly stained and relatively thin. In addition, the apparent lack of cell lacunae in any cementum layer of limited area in a single histological section can be the result of the plane of section. Other workers have described only cellular cementum as the repair tissue of resorbed root substance (Helldén, 1972; Reitan, 1974; Stahl, 1977).

Armitage (1976) stated that repair generally tends to re-establish the former root outline (anatomic repair) but if a recess remains the alveolar bone profile will be altered to restore normal periodontal ligament width (functional repair), as suggested by others including

Orban (1928a) and Henry and Weinmann (1951). However, scant attention has been paid to the reattachment of principal periodontal fibres in repair cementum and indeed few comments have been offered. Kronfeld (1938) argued that following repair, the organism would attempt to establish a workable functional relationship between bone and tooth but presented no evidence for this. It seems that in the literature repair has been equated with reattachment (i.e. regeneration), despite the fact that most of the photomicrographs published to illustrate repair do not demonstrate such reattachment.

Kurihara and Enlow (1980a, 1980b) investigated the periodontal attachment to remodelling alveolar bone in rats. They distinguished three types of periodontal membrane-to-bone attachments, these being an adhesive type, a continuous type and a composite of these two. Interestingly, one photomicrograph in their first report (1980a) shows an adhesive periodontal attachment to a resorbed dentine surface from a tooth subjected to experimental orthodontic forces. This finding was not enlarged upon or discussed further in these reports. The continuous and intermediate types of attachment correlate well with the description by Gianelly and Goldman (1971) of the mechanism of periodontal attachment adaptations which occur during tooth movement.

Ankylosis of a tooth may occur when the periodontal ligament is lost and repair takes place with bone deposition (Ten Cate, 1976). This replacement type of resorption may follow traumatic injury to a tooth (Andreasen, 1972). The origin of the cells repopulating or repairing areas of damaged periodontal ligament has been cited as a major determinant for either reconstitution of the ligament or ankylosis supervening (Ten Cate, 1976; Line, Polson and Zander, 1974).

Repair of the periodontal ligament and its reattachment to cementum has been shown to occur following replantation of exarticulated teeth in dogs and monkeys (Löe and Waerhaug, 1961) and man (Andreasen and Hjørting-Hansen, 1966). Areas of repaired surface resorption associated with a normally structured periodontal ligament have been found in histological studies of replanted, exarticulated teeth in dogs (Andreasen, 1972). In a histological study of cementum repair following apicoectomy in man, Andreasen (1973) found that reattachment of collagen fibres to form a functional periodontal ligament was associated with an eosinophilic repair cementum, often of the cellular type. The presence of periodontal fibrous scar tissue was followed, he noted, by a marked reduction in the amount of the cementum repair.

The possibility of periodontal reattachment to tooth roots has been of interest to periodontists for some time. Skillen and Lundquist (1937) found no connective tissue reattachment in artificially created pockets in human material, due to epithelial proliferation. Linghorne in 1954 stated that, at that time, there was doubt whether reattachment was possible. However, his work and that of many others (Beube, 1947; Linghorne and O'Connell, 1950; Morris, 1969; Listgarten, 1972; Frank, Fiore-Donno, Cimasoni and Ogilvie, 1972; Register, 1973; Canton and Zander, 1979; Cole, Crigger, Bogle, Egelberg and Selvig, 1980) would seem to indicate that reattachment is possible following various treatment procedures for periodontal disease. The literature relating to reattachment associated with the treatment of periodontal disease is extensive and has been reviewed by Burfield (1971) and Daryabegi, Pameijer, Ruben and Ricchetti (1980). In the main, consideration and experimentation has involved only the area of attachment apparatus coronal to the alveolar bone crest or in infra-bony pockets, often concentrating on the gingival epithelial attachment.

The exact nature of the reattachment observed in these studies is variable and apparently unpredictable. Stahl, Slavkin, Yamada and Levine (1972) and Stahl (1979) discussed three possible modes of connective tissue to tooth root attachment following healing, which summarize the observations of other studies in this field. Firstly, reattachment may occur by fibre union to residual Sharpey fibres on the tooth surface. Secondly, connective tissue fibres may adhere to the tooth surface like a modified periosteum or thirdly, cementum deposition with fibre insertion may occur on the root surface. However, Stahl (1977) stated that since cementum repair and reattachment had seldom been reported it must not occur frequently. Experimental models for the study of the repair and reattachment of periodontal tissues below the level of the alveolar bone crest have generally involved the initial destruction of overlying bone, for example Helldén (1972) and Nalbandian and Frank (1980). Experimentation of this kind is similar to that of Sippy (1927) and shows that repair and regeneration of an apparently functional tooth support apparatus is possible under these extreme conditions.

Even though cementum is structurally similar to bone in many respects, it is less readily resorbed under pressure than is bone (Scott, 1977). This feature of cementum allows teeth to be moved orthodontically. However, although the pressures used for orthodontic tooth movement are ideally not more than 20 grammes per square centimetre of root surface area, extensive resorption of cementum sometimes extending into dentine may occur (Scott, 1977). A number of factors have been cited by Gianelly and Goldman (1971) as responsible, at least in part, for the resistance of cementum to resorption. The fact that cementum is more dense than trabeculated alveolar bone may be involved. Gianelly and Goldman (1971) also suggested that the duration of applied forces might be important since cementum is not immune from resorption altogether. Another factor might be the cellularity of the cementum. Most root resorption is reported to be found in the apical region of the tooth (Gianelly and Goldman, 1971), where cellular cementum is predominant. They postulated that cementocytes could modulate into a resorbing type of cell. This situation would parallel that in bone, where osteocytes may resorb bone (Pritchard, 1972; Yaeger, 1971). Boyde and Jones (1972) however, stated that the walls of osteocyte lacunae exposed by resorption were essentially identical topographically to those exposed by fracturing bone tissue specimens. This indicated, they said, that osteocytes were unlikely to be involved in resorption. Reitan (1974) stated that there was no definite evidence to prove cementocytes' involvement in resorption. Gianelly and Goldman (1971) further stated that cellular cementum was less dense than acellular cementum, presumably due to a slightly lower overall mineral content although this was not specified. The implication made was that the lower density of cellular cementum rendered it more susceptible to resorption. Gianelly and Goldman (1971) pointed out that cementum is continuously, though slowly, laid down throughout life and thus cementoid or pre-cementum would always be present on the root surface. This cementoid, they contended, is resistant to resorption. Similar claims have been made previously by Sicher and Weinmann (1944) and Stenvik and Mjör (1970) and later by Reitan (1974) and Rygh (1977). Rygh (1977) proposed that as the turnover of unmineralized surface matrix on the bone side of the periodontal ligament was faster, the collagen in the cementoid would be more mature and thus better able to resist changes. Stenvik and Mjör (1970) found that odontoclasts were unable to resorb any unmineralized matrix, including predentine.

Another proposed reason for the different responses of bone and cementum to pressure has been the rich vascularity of bone (Kerr, 1961; Storey, 1973; Armitage, 1976). Bone resorption may be related to degenerative processes produced by interference with circulation in bone (Armitage, 1976) or to pressure being more readily transmitted to bone via the vascular system (Kerr, 1961). Bien (1967) felt that the smoother surface of cementum compared with alveolar bone accounted for the former's lack of resorption. This contention was based on his hypothesis that resorption is related to the formation of minute gas bubbles forming oxygen concentration cells in the periodontium. These gas bubbles lodge on the rougher alveolar bone more readily than the smoother cementum.

Root resorption of human permanent teeth related to orthodontics

Oppenheim (1911) was the first researcher to closely study the tissue reactions associated with orthodontic tooth movement. In an experimental histologic investigation of this subject, he used a baboon with a full deciduous dentition. Passing mention was made of tooth root resorption with the comment that firm conclusions could not be made as the resorption seen could have been physiological.

In 1927 Ketcham reported apical root loss associated with orthodontic treatment. At this time radiography had become widely used as an adjunct to diagnosis. Ketcham's report, based on radiographic diagnosis, created a great deal of interest and probably was the impetus to much research and reporting to follow. A further report by Ketcham in 1929 included additional cases from his practice and overall he found definite apical resorption of anterior teeth in 19% of these cases. In persons not subjected to orthodontic treatment, Ketcham put the proportion showing root resorption at 1%. It should be remembered when reviewing these early reports that radiographic technique and interpretation was not standardized nor as well developed as today. It is likely that some error was present, with only very obvious resorptions being discovered and reported.

In a series of articles published in 1935 and 1936, Oppenheim discussed the biological aspects of orthodontic tooth movement. With reference to cementum resorption, Oppenheim expressed the belief that this was related both to unphysiological forces which exceeded the permissible compression of the periodontal ligament and also to the constitution of the patient. He stated that the degree of resorption was related to the magnitude of the force, its duration and any change in its direction.

Repair or filling in of cementum resorptions with secondary cementum, after removal of the apparent cause of the resorption, was demonstrated histologically by Oppenheim. With reference to functional regeneration Oppenheim referred to a number of works published in German. Euler-Mayer (1927), he said, questioned whether the filling in of cementum resorptions and restoring of root surface contour was associated with a full '*restitutio ad integrum*', that is a return of functional attachment. Kronfeld (1933) however, was reported by Oppenheim to believe that the normal function of the root surface was restored.

In a histologic and radiographic study reported in 1930, Marshall used monkeys to investigate apical root resorption of permanent teeth related to orthodontic treatment. The results for the various tooth movements (including rotation, tipping, extrusion and intrusion) showed that root resorption occurred in most experimental teeth. Further, it was seen that as the pressure was released repair commenced, this being accomplished by tissue resembling either bone or cementum but never dentine. Often, the proximity of young blood vessels to eroded areas was associated with repair. Although Marshall stated that usually the contour of the root was rebuilt, he did not demonstrate in the published article reattachment of periodontal fibres to cementum, although such reattachment was assumed by Ketcham in the discussion following the paper. In 1934, Marshall again discussed root resorption and claimed that the state of general nutrition was an important factor in repair.

Becks (1936) looked at a number of patients with root resorption which had been demonstrated radiographically. He found an association between root resorption in patients with previous orthodontic treatment and systemic disturbances, particularly endocrine disturbances such as hypothyroidism. It should be pointed out that the radiographic technique and interpretation as well as the medical diagnosis employed by Becks (1936), while undoubtedly good for its day, was probably not as accurate as it would be today. The statistical construction of Becks' study was also open to bias.

Stuteville (1938) histologically examined extracted human premolar teeth which had been subjected to various orthodontic manipulations. From his results he concluded that root surface resorption would occur in virtually all cases of malocclusion corrected orthodontically. Factors cited by Stuteville (1938) as important in the production of this injury were, the amount and type of force, the distance through which the force is active and other forces, for example those from occlusion. His material also showed that repair by secondary cementum occurred. He stated that resorptions could only be seen radiographically as reduction in the length of the root, if this occurred.

In reply to a question at the end of his paper, Stuteville (1938) said that repair of periodontal membrane injuries was characterized initially by connective tissue with fibres running parallel to the root surface. He claimed that function would then demand arrangement as in the normal periodontal membrane. No material was presented, however, to show definite reattachment of periodontal fibres to repair cementum.

In 1938 Kronfeld published a paper about cementum in which he stated that root resorption resulting from orthodontic movement showed a marked reparative tendency on the cessation of pressure and that therefore it was of no practical significance. He claimed that repair cementum would cover resorptive defects in cementum, dentine or enamel. However, no evidence for reattachment of periodontal fibres to repair cementum was presented in the paper.

Skillen (1940) reported on tissue reactions to orthodontic tooth movement and trauma. He reproduced histological photomicrographs from human material to support his discussion and in summary said that tooth root resorptions, among other injuries, heal readily with no apparent functional defect. This was a subjective assessment and again no evidence to show periodontal fibre reattachment was presented. As the resorption areas shown were not extensive it is not unreasonable to suppose that no immediate loss of function would have been detected clinically even without periodontal reattachment in such areas.

An analysis of 76 patients' radiographs taken before, during and after orthodontic therapy led Massler and Malone (1954) to conclude that there existed a high degree of correlation between the amount of periapical root resorption seen before and after treatment. A radiographic study reported by Massler and Perreault (1954) compliments that of Massler and Malone (1954). Massler and Perreault (1954) concluded that the root ends of untreated, permanent teeth exhibited a definite resorptive potential, resulting in varying degrees of apparently idiopathic resorption. The individual resorptive potential evident from these two studies could perhaps be equated with Becks' (1936) 'systemic predisposition' to root resorption. Although later work, for example VonderAhe (1973), has argued against many of the specific systemic predisposing factors being generally important, the concept of individual resorption potential still remains (Newman, 1975).

Apical root resorption and its relation to orthodontic therapy was studied radiographically and reported on by Phillips (1955). He pointed out that apical root resorption permanently and irreparably shortened the tooth root, thus differing from cementum surface resorption which may occur on the sides of the root and can repair.

Importantly, Phillips demonstrated that for maxillary incisors a 2mm loss of apical root length decreased the periodontal attachment area by 5 to 10 per cent, a 4mm loss reduced it by 15 to 25 per cent and a 6mm loss resulted in a 30 to 40 per cent reduction of attachment area. This finding supports the contention of other workers that loss of crestal alveolar bone is more harmful to the retentive apparatus of the tooth than apical root loss (Jacobson, 1952; Sjølien and Zachrisson, 1973; Zachrisson, 1975, 1976). Such a suggestion has been based on the greater loss of attachment area per unit of root length cervically, compared to apically, and the fact that the periodontal retentive apparatus of the tooth is concentrated in the cervical two thirds of the root. In addition, Hemley (1941) stated that only loss of one third of the root or more was clinically significant as only then would the lever arms of crown and root alter relatively enough to cause concern.

Goldson and Henrikson (1975) conducted a longitudinal radiographic study of apical root resorption associated with Begg orthodontic treatment. Forty-two first premolar extraction cases were evaluated and the results showed no visible resorption in 23% of the teeth at the end of treatment, less than 2mm apical root loss in 48% and 2mm to one third root loss in 3%.

The remaining teeth exhibited either irregular root contour, oblique resorption or were unevaluable. Torque on upper central incisors tended to increase the incidence of resorption on these teeth. In addition, teeth exhibiting resorption prior to treatment showed more than the average amount during treatment.

Reitan has extensively studied the local tissue reactions to orthodontic movement both in animals and humans, utilizing histological techniques. In relation to root resorption a general conclusion evoked by a number of his reports (Reitan 1960, 1961, 1969, 1972, 1974) is that root resorption, both surface and apical, commonly occurs with orthodontic tooth movement generally in association with hyalinization of the periodontal ligament. Further, root surface resorption generally repairs with cellular cementum. Factors particularly important to the location and extent of root resorption would seem to be the amount, direction, duration and nature of the orthodontic force used.

Support for these conclusions has been afforded by the published work of other researchers including Mjör and Stenvik (1969) and Stenvik and Mjör (1970). Despite the wide-ranging investigations by Reitan, reattachment of principal periodontal fibres in areas of repaired resorption on tooth roots has not been given much attention. The impression conveyed by Reitan's publications is that repair of root surface resorption implies functional periodontal reattachment, but nowhere is this adequately demonstrated by him.

Root surface resorption on the pressure side of orthodontically moved teeth was investigated histologically by Wainwright (1973) in Macaque *speciosa* monkeys. One of his conclusions was that the stimulus for root resorption was more likely to be related to the stresses on the root and the time involved than to the density of the bone. This conclusion was based on the observation of a similar extent of resorption on the buccal and lingual sides of tooth roots moved out through the bony cortical plate and then back into the alveolus. Repair of resorptive defects with cellular cementum was a feature but reattachment of periodontal fibres was not demonstrated or discussed.

Apical root resorption of upper incisors occurring during stage 2 and stage 3 of Begg orthodontic treatment has been reported by Hall (1978) and Ten Hoeve and Mulie (1976) respectively. These workers attributed this resorption to movement of the root apices against the cortical plate of bone during tipping and torquing of the upper incisor teeth.

Rygh (1977) reported a transmission electron microscopic examination of teeth and surrounding tissues from Wistar rats and human subjects. The experimental teeth were moved orthodontically with different forces for various time periods prior to removal. In specimens from both sources large multinuclear cells were seen close to the cementum surface in the periodontal ligament at a distance from hyalinized tissues. Interestingly these cells appeared to be engaged in cementum and dentine resorption but were not directly in contact with the root surface. Cementum was seen to be resorbed peripherally by undermining from behind its periodontal surface, while the adjacent periodontal ligament demonstrated bundles of fibrils inserting almost at right angles to the root surface. Within resorption lacunae the blood vessels seemed to be in close contact with the odontoclasts. Under the electron microscope the odontoclast surface adjacent to resorbing cementum appeared as a series of folds and clefts. The striated pattern of the cementum collagen fibrils became less clear in these clefts. Similar features were reported by Furseth (1968) to be found during the resorption of deciduous teeth.

SECTION 4

MATERIALS AND METHODS

Clinical phase

The major part of the material for this study was derived from patients treated in the Orthodontic Clinic in the Dental Department of the Royal Adelaide Hospital. These patients have been treated by the author. Six patients who were expanded and retained as described did not require extraction of teeth to complete their orthodontic treatment and were thus lost to this study. Additional material was obtained from patients undergoing similar treatment by orthodontic specialists in private practice in Adelaide. The patients required treatment with a rapid maxillary expansion (RME) appliance to correct a maxillary base width discrepancy. Following this active treatment, a period of retention was necessary and finally removal of the upper first premolar teeth to correct a tooth-jaw size discrepancy and allow completion of the orthodontic treatment with full banded therapy.

The rapid maxillary expansion appliances used by the author were of a common design and utilized a mid-palatal expansion screw (type 600-010, Dentaurum, West Germany) set as high as practicable in the vault of the palate, centrally between the first premolar and first molar. The screw was attached to bands on the first premolar and first molar each side by stainless steel wire of 1.1 mm diameter passing through the two holes for this purpose on each side of the screw. Each wire to band or wire to screw contact was soldered. This provided a rigid appliance for separating the maxillae as desired (Figure 1). Patients not treated by the author were expanded using appliances and activation schedules similar to that described for the author's patients.

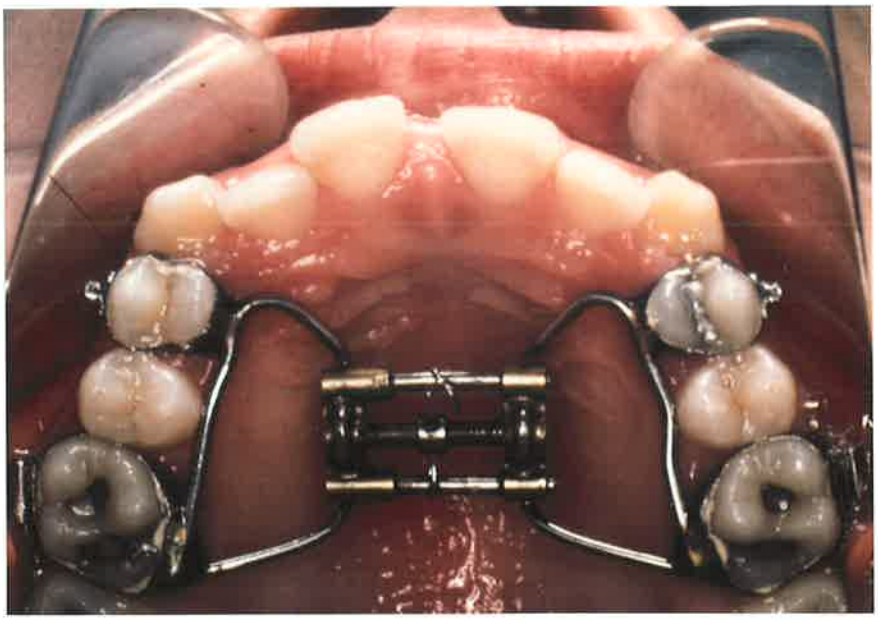


Figure 1

Occlusal view of the type of RME appliance used in this study.

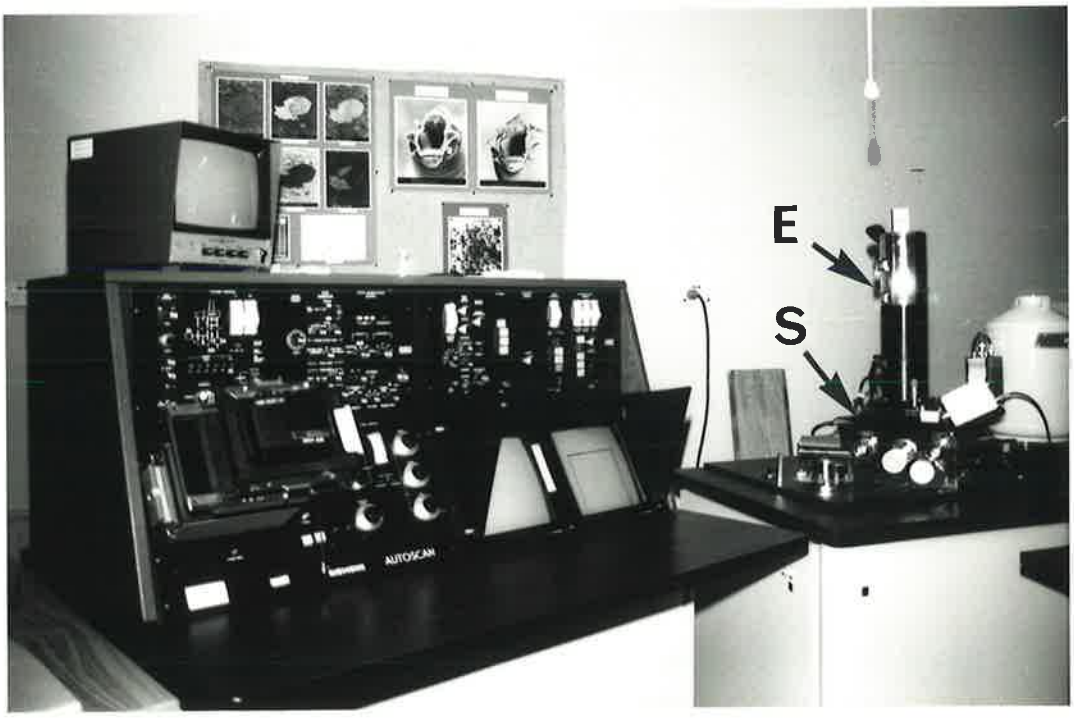


Figure 2

The scanning electron microscope used in this study, with its control panel and video displays on the left. The electron optical column, E, is on the right, with the specimen chamber, S, underneath.

Activation was undertaken by the patients, following instruction. The screw was given one quarter turn (0.2mm expansion) each morning and evening until the desired expansion was achieved. At this stage, the screw was ligated through the keyholes and around the guidepins with 0.012 inch ligature wire to prevent spontaneous unwinding. The appliance was then left *in situ* to provide rigid retention. The amount of the expansion was dictated by the degree of the skeletal base width discrepancy and accompanying malocclusion, the aim being to overcorrect the maxillary narrowness thus allowing for partial relapse expected following removal of retention.

A minimum of three months fixed retention was used as a lower limit by the author, to prevent relapse. The upper limits of retention time recommended for clinical practice vary considerably. For the group of patients treated by the author the length of the retention period was determined in each case by dental and orthodontic parameters. These included the extent of the original discrepancy and the skeletal maturity of the patient, the amount of expansion achieved, the eruption of permanent teeth and the timing of further treatment. Expansion and retention periods were therefore determined by patient variables and were consistent with good clinical practice.

At the end of each retention period the upper first premolars were removed to facilitate completion of the orthodontic treatment. Extraction was performed with forceps, under local anaesthesia, with all possible care to minimize damage to the root surface areas of the teeth. The teeth were placed immediately into 10% neutral buffered formosaline (Appendix I). The volume of the fixative used was 400 to 600 ml in order to ensure adequate fixation. After 24 hours the extracted premolars were transferred to 70% ethanol for holding, 50 ml being used for each tooth. This regime used for material from the author's patients was followed as closely as possible for the additional material. Further preparation of the specimens, a description of which begins on p.4.8, was carried out as expediently as possible.

In this report, material will be designated by the initials of the patient from whom it came. Table 1 lists the patients in the author's treatment group and gives details pertinent to this investigation. The expansion across the premolars was determined by subtracting the horizontal distance between the mesial developmental grooves of the upper first premolars on the pretreatment study models, taken just prior to commencing rapid maxillary expansion, from the same measurement made on study models taken at the end of the retention period. The inter-premolar expansion is not listed for the additional material shown in Table 2, as study models for these private patients were unavailable. Patients in the author's treatment group had not previously had orthodontic treatment. During expansion and retention none of the author's patients had additional orthodontic treatment except for M.C. In this patient correction of an upper lateral incisor lingual cross-bite was achieved in 16 weeks during retention using an .016 inch upper arch wire. As indicated in Table 2, a number of the private patients not treated by the author had additional orthodontic therapy prior to removal of retention and extraction of the upper first bicuspids.

The bar graphs in Figure 3 show the period of retention and the period of expansion for each patient in this study. A comparison of the two graphs shows a lack of correlation between the retention and expansion periods.

TABLE 1

Patients treated by the author

Patient Initials	Sex M: Male F: Female	Age at start of expansion (years; months)	Period of Active Expansion (weeks and days)	Period of fixed retention (weeks and days)	Amount of expansion across the premolars to nearest half mm.
M.D.	F	$16\frac{3}{12}$	$2\frac{2}{7}$	16	2.5
W.J.	M	$15\frac{6}{12}$	$3\frac{3}{7}$	$17\frac{5}{7}$	6.5
K.B. *	F	$12\frac{8}{12}$	4 + 1*	20	7.5
L.B.	F	$16\frac{2}{12}$	$2\frac{3}{7}$	$25\frac{4}{7}$	7.0
A.S.	F	$14\frac{3}{12}$	$3\frac{6}{7}$	29	8.0
M.C. **	M	$12\frac{1}{12}$	$3\frac{1}{7}$	$38\frac{6}{7}$	7.0
M.I. *	M	$13\frac{1}{12}$	$2\frac{5}{7} + 3\frac{3}{7}$*	$3 + 37\frac{6}{7}$*	11.0
E.C.	F	$10\frac{6}{12}$	3	52	6.5
L.H.	F	$11\frac{10}{12}$	$3\frac{2}{7}$	53	7.0
J.S.	M	$13\frac{4}{12}$	3	$53\frac{2}{7}$	7.5

* These patients required two appliances to achieve the desired expansion. The second appliance was inserted for M.I. three weeks after activation of the first was stopped (shown in retention column) and was activated only one quarter turn per day.

** For orthodontic reasons only the upper left first premolar of M.C. was extracted.

Patients from whom extracted RME anchor first premolar teeth were utilized in this project are listed by initials with details relevant to the investigation.

Patient records

Standard pretreatment orthodontic records for each patient in the author's group were obtained prior to the commencement of treatment. These were:

1. Complete history.
2. Study models.
3. Photographs - colour transparencies of profile, full face and dentition.
4. Extra-oral radiographs - lateral cephalogram
    - orthopantomogram
    - hand/wrist radiograph.
5. Intra-oral radiographs - left and right bitewings
    - long cone periapicals of incisors and canines.

In addition to these, long cone periapicals of first premolars with the central x-ray beam at right angles to the tooth and an occlusal view of the anterior maxilla were obtained. Periapical radiographs of the premolars were repeated just prior to their removal. A lateral cephalogram was also taken at the end of the retention period for each patient.

The pre- and post-expansion periapical radiographs of premolars were masked peripherally, illuminated by variable intensity transmitted light from a radiograph viewer and examined under 2 to 3 times magnification provided by a hand held magnifying glass. An assessment of any resorption of the roots of the premolars evident in these radiographs was made.

TABLE 2

Patients not treated by the author

Patient Initials	Sex Male M Female F	Age at start of expansion (years; months)	Period of expansion (weeks;days)	Period of Retention (weeks;days)
T.H.	F	$13\frac{9}{12}$	$3\frac{3}{7}$	14
B.B.	F	13	$4\frac{4}{7}$	$1\frac{3}{7} + 13$
G.B.	F	$15\frac{9}{12}$	$3\frac{3}{7}$	17
C.T.	M	$15\frac{6}{12}$	2	17
R.H.	M	14	$1 + 1\frac{2}{7}$	$3\frac{3}{7} + 17\frac{4}{7}$
R.E.	F	12	3	$17\frac{2}{7} + 4\frac{2}{7}$
S.W.	F	$12\frac{3}{12}$	2	$22\frac{3}{7}$
S.M.	F	13	$4\frac{4}{7}$	23 + 28

Patients from whom extracted RME anchor first premolar teeth were utilized in this project are listed by initials with details relevant to this investigation.

Features varying from the protocol used by the author were as follows.

B.B. : Retention with expansion appliance was changed after 10 days for a removable palatal acrylic appliance.

R.H. : Expansion and retention were carried out simultaneously with full banding (Begg lightwire) and the use of class II intermaxillary elastics. After 24 days retention with the expansion appliance, this was removed and the first molars and premolars rebanded to continue orthodontic treatment. The first premolars were extracted after a further 17 weeks and 4 days due to anchorage demands for the orthodontic treatment.

R.E. : The expansion appliance was removed 4 weeks and 2 days prior to the extraction of the upper first premolars and no other form of retention was used.

S.W. : Expansion and retention were carried out simultaneously with full banding (Begg lightwire) and the use of class II intermaxillary elastics.

S.M. : Fixed retention of 23 weeks was followed by a period of 28 weeks with no appliances used for retention prior to removal of the upper first premolars.

Records for the additional patients were not standardized and were generally unavailable.

Preparation of teeth for viewing in the scanning electron microscope (SEM)

Initially, trial human tooth root specimens were prepared and examined in the scanning electron microscope. Included in this group were two upper first molars which had been subjected to headgear therapy for 20 weeks, several untreated first premolar teeth from teenage patients, a retained upper deciduous canine and an impacted, unerupted second premolar from a 23 year old female patient. Several human bone specimens were prepared as a basis for comparison with cementum structure. A piece of lingual cortical bone was obtained at an operation to remove an impacted third molar. Fetal bone was obtained from an aborted 20 week human fetus.

These specimens were fixed and stored in 10% neutral buffered formosaline (Appendix I). The crowns of the tooth specimens were removed by sectioning prior to further preparation. The specimens were rendered anorganic in 5% sodium hypochlorite at room temperature for 24 hours, washed in distilled water and dehydrated in a series of graded alcohols before final air drying. Mounting and coating of the specimens was as described on p.4.12 except for a period of 12 hours under vacuum in the coating unit to further dry the specimens prior to coating.

Lower first premolars from experimental patients T.H., M.C., S.M., E.C. and J.S., were prepared in the same way as the trial specimens for viewing in the SEM. The roots were mounted vertically onto the SEM stubs by attaching them at the coronal transverse cut surface in a manner similar to the trial tooth root specimens.

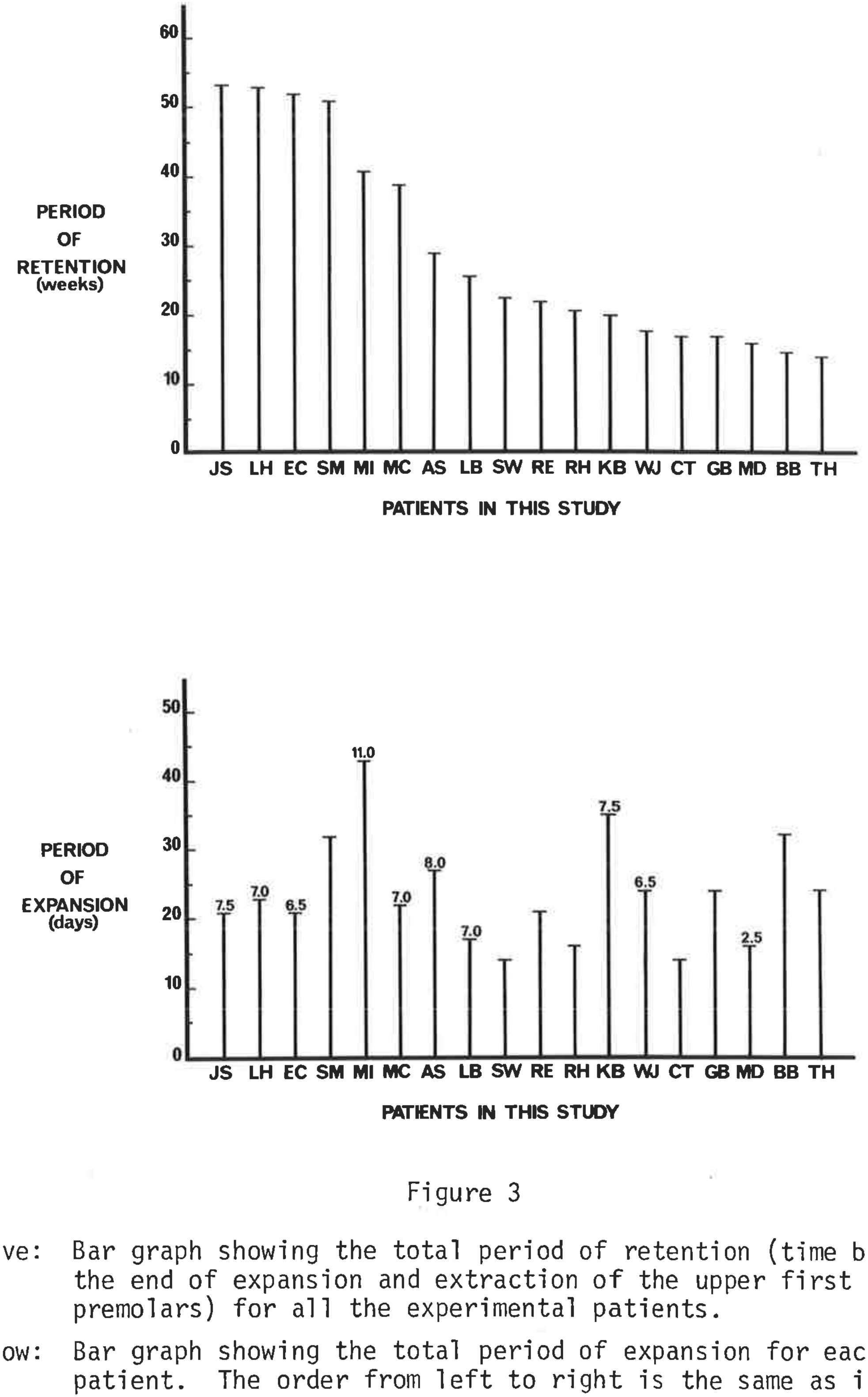


Figure 3

Above: Bar graph showing the total period of retention (time between the end of expansion and extraction of the upper first premolars) for all the experimental patients.

Below: Bar graph showing the total period of expansion for each patient. The order from left to right is the same as in the top graph, illustrating the lack of correlation between the expansion and retention times. The amount of expansion in millimetres for each patient treated by the author is shown at the top of the corresponding bar.

The roots of the upper first premolar teeth from the group of patients who had undergone rapid maxillary expansion were sectioned mesiodistally and the lingual half so produced was sectioned from the crown portion occlusal to the cemento-enamel junction. For most specimens, the buccal half of the root was sectioned buccolingually in the midline prior to cutting off the crown. This division was made to enable histologic examination of one half of the buccal surface and topographical examination of the other half (Figure 4). Direct comparison of features found using each of the two techniques was facilitated in this way. To simplify presentation of the findings and discussion, the buccal segments produced by sectioning have been designated as either mesiobuccal or distobuccal to indicate whether they came from the mesial or distal side of the root.

The selection of buccal segments for histologic or topographic examination was random. All sectioning was done with a Horico 'Thinflex' disc (catalogue number 549/220) in a dental handpiece rotating at approximately 6000 r.p.m. During this procedure an assistant maintained a stream of physiologic saline over the specimen and the operator held the tooth by the crown in a specially modified pair of pliers. Every effort was made to avoid touching or damaging the root surfaces and all specimens were stored resting on gauze, in 70% ethanol, until further preparation could be carried out.

Specimens for examination in the SEM were rendered anorganic to facilitate viewing of the mineral front of the root surface. Previous work reported by Jones and Boyde (1972), Kvam (1972a, 1972b, 1973b) and Harry (1977) indicated that little useful information would be obtained by examining topographically the adhering organic coat on the root surface.

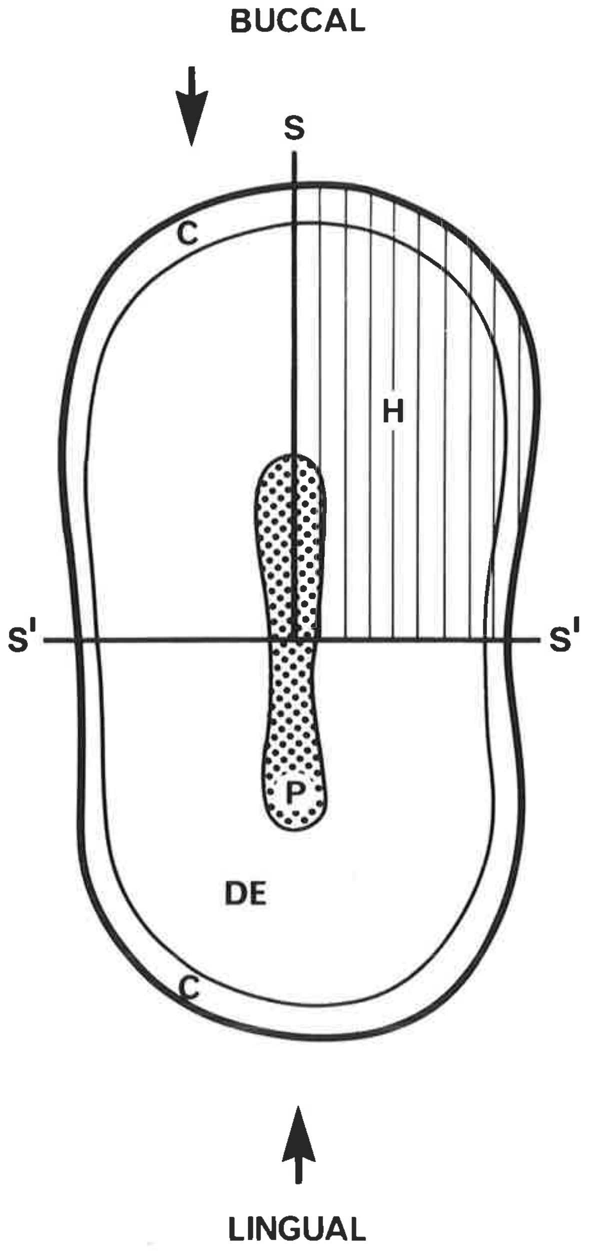


Figure 4

Diagram of a transverse section through the root of an upper first premolar. Experimental teeth were sectioned longitudinally in the planes indicated by S and S'. The resulting segments for topographic examination were mounted with the section edge indicated by S'S' attached to the aluminium stub. In the SEM, the root surfaces of the segments were viewed in the direction indicated by the arrows. The lines in the segment marked H illustrate the plane of sectioning for light microscopy. Pulp (P); Dentine (DE); Cementum (C).

Examination of the histologic sections of corresponding buccal segments enabled an appraisal of the adhering organic layer and its interface with the cementum surface.

To render the specimens anorganic they were placed into 500 ml of 10-13% sodium hypochlorite (Ajax Chemicals, Sydney, Australia) at room temperature. A wire basket was used to suspend the specimens in the sodium hypochlorite while a magnetic stirrer was used to gently swirl the solution. Some specimens were treated in this way for 15 or 20 minutes and a number for 7½ or 10 minutes. All the specimen surfaces appeared to the unaided eye to be similarly free of adhering organic material at the end of these time intervals. Following this treatment in sodium hypochlorite the specimens were rinsed in distilled water. They were then transferred to another wire basket and suspended in three litres of distilled, deionized water in a covered beaker. A magnetic stirrer was used to vigorously swirl the water for 24 hours to wash the specimens. To reduce specimen contamination, Boyde (1979) suggested the use of double distilled water, boiled and cooled to drive off dissolved carbonates just prior to use for specimen washing. In this project, no significant specimen contamination attributable to the water used for washing was observed in the scanning electron microscope when either boiled, double distilled, or unboiled distilled, deionized water was used. Drying of the specimens was carried out after washing, by transfer to 70% alcohol for half an hour then 100% alcohol for one hour, followed by air drying in a covered container.

When dry, specimens were mounted onto aluminium SEM stubs using fast set 'Araldite' epoxy resin (CIBA-Geigy Ltd., Australia). The specimens were then coated with either gold, or carbon followed by gold-palladium in the majority, using respectively a Mini coater (model MCE 200, Commonwealth Scientific Company, Alexandria, Virginia) or a model DV-502 coating unit (Denton Vacuum Inc., U.S.A.). Coating thickness was 20-30 nanometres.

To determine whether any useful information could be obtained by examining organic, decalcified specimens, the buccal segments of two non-experimental first premolar roots from orthodontic patients were placed in formic formate solution (Appendix I) for 24 hours. In addition the lingual and mesiobuccal segments of the left first premolar from patient K.B. were similarly treated for 48 hours and the distobuccal segment of the right premolar from patient S.M. completely decalcified in this way, taking 3 weeks. These specimens were washed, dehydrated, mounted and coated for viewing in the SEM as described previously. It was found that little useful information was obtained by examining these specimens in the SEM even when viewed from the cut edge as the dentine, cementum and periodontal tissue appeared relatively amorphous, lacking in distinctive and recognizable features.

Several of the specimens placed in sodium hypochlorite for 7½ or 10 minutes were examined and recorded in the SEM and then placed in 150 ml of 5% sodium hypochlorite solution in a sealed glass container for 48 hours. During this time most of the metal surface coating became detached from the specimen surface in flakes and sheets. At the end of the 48 hour period, washing, dehydrating, mounting and recoating for examination in the SEM was effected as described previously. This repreparation procedure was carried out to determine if differences in surface details could be found with a short and long deorganifying period and the results will be included in Section 5. The cementum surface was rendered more friable by this second preparation, resulting in increased cracking and areas of cementum flaking off, problems similar to those encountered by Harry (1977). Artefactual damage to the root surface thus occurred but was kept to a minimum by careful handling of the specimens.

After drying and prior to coating one specimen was left overnight in a closed glass dish together with 4 ml of 4% osmium tetroxide in an open receptacle. This was done to assess the effect of this procedure on specimen charging in the SEM. Dr K. Bartusek, Officer-in-charge of the Electron Optical Centre, suggested the use of osmium tetroxide in this way, as he had found that it sometimes reduced the charging of biological specimens in the SEM. The specimen surface was rendered black but coating was not interfered with. There was no noticeable reduction in charging of this specimen. In general, although charging did occur, particularly at sharp points and ridges on the specimens, this was not a problem. The charging did not obscure topographical detail and interpretation was not interfered with.

Examination and recording in the SEM

Examination and recording of prepared and coated specimens was carried out in a scanning electron microscope (Siemens Autoscan, ETEC Inc., U.S.A.) operated at five, ten or twenty kilovolts (Figure 2). An accelerating voltage of 5kV was often used for examination and recording of specimens at low magnifications, to reduce charging. At higher magnifications the definition possible with low accelerating voltages was poor. Another way to reduce the effects of charging is to use backscatter electron imaging. To achieve this the specimen had to be horizontal and raised considerably in the specimen chamber to approximate the detector. The quality of the detector in the chamber was poor and consequently this imaging mode was not used extensively.

To facilitate viewing, an additional specimen stage attachment was sometimes utilized (Figure 5). This was particularly useful for specimens, usually whole roots, mounted vertically with the coronal end attached to the stub. However, the configuration of the stage attachment restricted the degree of freedom within the specimen chamber. This restriction occasionally compromised the optimum working distance and limited the lateral travel and rotation of the specimen possible within the instrument. For this reason, experimental segments were mounted with the proximal cut face (Figure 4) attached to the stub to allow maximum freedom and image definition in the SEM. Mounting in this way restricted the observation of proximal surfaces. Demarcation between buccal or lingual and proximal surfaces was difficult because of the curved nature of the roots. In this study, the buccal and lingual surfaces have been defined as the portions of the root surface seen clearly when segments were examined directly in the SEM.

The inter-radicular surfaces of bifid rooted premolars could not be observed in the SEM except for those from patient T.H. The buccal and lingual roots of the right premolar of patient T.H. were mounted vertically permitting visualization of the inter-radicular root surfaces. The entire left premolar of patient T.H. was examined histologically. The histological sections of buccal segments allowed observation of the lingual facing surface of buccal roots where roots were bifid. In fact, the only completely bifid roots were those of patient T.H. The axial extent of bifurcation of other experimental roots was about ½ mm for the premolars of patient R.H., 1 to 2 mm for E.C., 2 to 3 mm for B.B. and G.B., 3 to 4 mm for L.H. (right premolar only), 4 to 5 mm for L.B. and M.C., and 5 to 6 mm

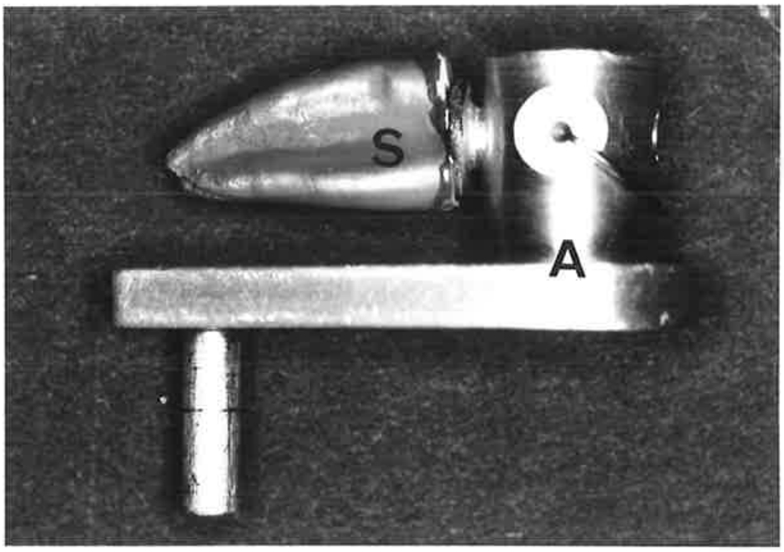


Figure 5

Specimen stage attachment (A) with whole root specimen (S) in position.

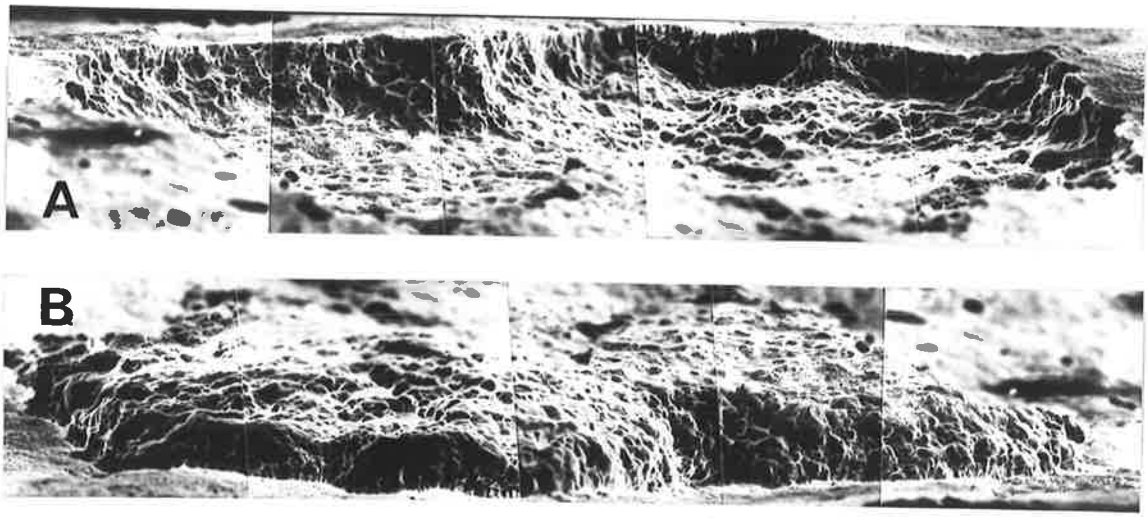


Figure 6

A. Composite of a resorptive excavation from the buccal surface of the right premolar of patient C.T. The approximate width of this defect is 1.8 mm.

B. Same composite as A but inverted to produce the illusion that the defect area is raised.

for J.S. All other premolars were completely single rooted, although generally having the normal cross-sectional configuration of upper first premolars, with long buccolingual dimension and a central mesiodistal constriction (see Figure 4).

Visualization of detail within depressions on a surface was improved by using the derivative amplification facility of the SEM controls. This method enhances the contrast due to fine detail and suppresses gross contrast by electronically amplifying a band of frequencies preferentially. The resulting images lack the three dimensional effect seen in normal scanning modes, but fine surface features are more easily discernible. Utilizing derivative amplification for recording necessitated manual setting of the dark level control to achieve correct exposure of the film.

Ilford FP4:ASA 125 (120 size) film was used for recording (processing details given in Appendix V) with a Singer Graflex camera mounted over the recording screen of the instrument. All photographic parameters were kept constant by the instrument. Data automatically recorded below each negative frame were from left to right, the specimen to negative linear magnification, accelerating voltage, working distance (distance from the final lens aperture to the specimen surface in millimetres), date and frame number. A magnification scale bar was recorded above these. The operator also recorded the tilt angle of the specimen and the condenser lens current. An error of up to 10% in the magnification factor could be expected due to the calibration of the instrument. All magnification figures for illustrations herein presented are the instrument magnification used. Some variation from this stated magnification has occurred in a number of illustrations due to slightly different photographic enlargement. However, the scale bar recorded automatically in the SEM has been printed with the illustrations where possible, or reproduced on the photograph. Composite pictures were made by photographing printed montages with Ilford Pan F film (see Appendix V for processing details) which was then used for printing the final illustration.

Three dimensional imaging

Stereopair photographs were taken with the SEM by photographing an area of the specimen and then tilting it through five to ten degrees about the Y axis of the specimen stage in the evacuated chamber of the instrument. Maintenance of the same field during tilting was achieved by using the X axis stage drive while visualizing in the television monitor to provide completely real-time imaging not possible on the long decay screen monitor. Refocusing was achieved with the Z axis screw micrometer to retain the same magnification. Prints of stereopair photographs were examined in a Geoscope (Stereo Aids, Western Australia) which afforded a 60% field overlap. Height and depth perception was facilitated in this way, making interpretation of topographical features easier. However, it was found by the author that there was not a problem of optically confusing depressions and mounds on the specimen with the two-dimensional image presented by a single photograph (Figure 6). This was because the specimens were always oriented in the SEM chamber in the same way so that with experience features of negative or positive contour were not misinterpreted or confused. Stereopair photographs were used to establish this. In addition, the true contour of some features, whether negative or positive with respect to the surrounding surface, could be checked by viewing them in profile after rotating the specimen in the chamber. Focusing on the extremes of surface features and noting the working distance for these limits, or the Z micrometer setting if this was used to focus at a constant working distance, allowed distinction of raised and depressed areas. Larger features could also be checked by viewing the specimen with a binocular dissecting microscope.

Contour plots

It was felt that hard copy contour plots and profile plots of selected areas could be useful aids in this investigation. Instruments for making contour plots of surface topographical features from stereopair photographs are used extensively in map making. Thus, an attempt was made to produce contour plots from stereopair photographs taken with the SEM. This was done in conjunction with workers at the South Australian Department of Lands Mapping Branch at Netley. Aerial photography for map making generally uses stereopairs taken by a lateral camera shift. The main differences apart from this are the lack of an absolute plane of reference, or reference features of precisely known dimensions, and unknown parallax factors with photographs taken in the microscope. Also, scaling errors introduced by tilting the specimen may produce inaccuracies in plot measurements. As a test specimen, the head of a drawing pin with a raised three-pointed star was mounted on an SEM stub and stereopair photographs of this recorded in the SEM (Figure 7). Stereopair negatives were used to model the specimens in an analytical plotter (Zeiss Planicomp C100 interphased with a Hewlett Packard H.P. 1000 computer). From this model, a hard copy plot (Figure 8) was made of the surface of the drawing pin specimen, using the base of the three-pointed star as an arbitrary absolute plane of reference and the corners of the negatives as fiducial points. The system was scaled using the scale bar on the negatives. The plot gave the highest point in the centre of the star as 0.6 mm. Direct measurement with a vernier caliper indicated that it was in fact approximately 0.6 mm. However, a foreshortening distortion is clearly evident in the plot which could conceivably lead to dimensional

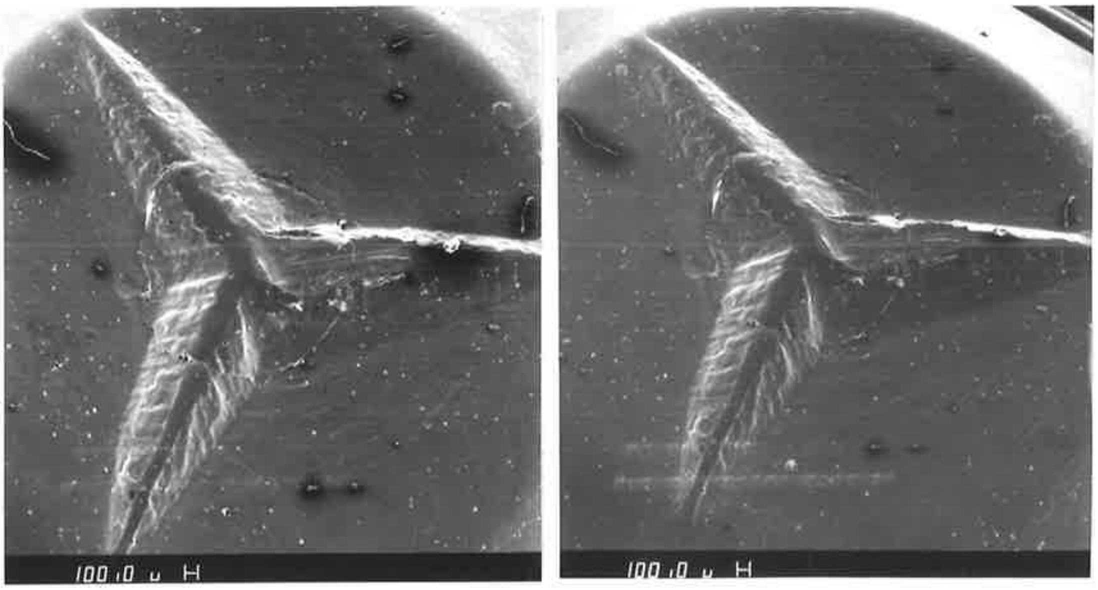


Figure 7

SEM stereopair of drawing pin head. X10

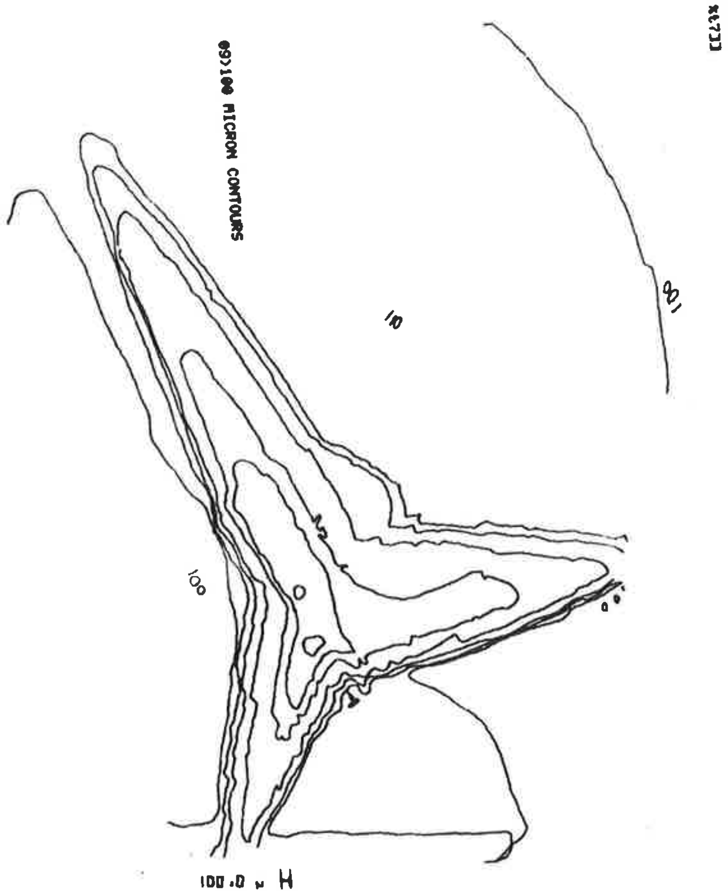


Figure 8

Contour plot of drawing pin head.

errors. It would seem that the tilt of the specimen in the SEM was responsible for this. Within the SEM specimen chamber, tilt of the specimen relative to the electron beam and collector is necessary to achieve a clear picture, particularly in the secondary electron imaging mode. A number of attempts were made to obtain a similar plot from stereopair negatives of an area of root surface topography, using the analytical plotter. These were unsuccessful because of the difficulty of establishing proper reference parameters, particularly an absolute plane of reference. It was felt that such problems could eventually be overcome but that plotting inaccuracy and distortion would still be present, limiting the usefulness of any results. It was possible to model a root surface stereopair in a manually set-up plotter commonly used for mapping. However, despite the fact that a plot could be made, the viewing model for plotting was far from perfect and the result rather crude. It is not suggested that this technique should be dismissed. With further developments and refinement which were unable to be pursued in this project, useful results could emerge.

As a further means of examining specimens coated for the SEM, Nomarski epi-illumination was tried. This technique was found to be unsuitable, however, as the specimen surfaces were curved and too irregular. These specimen characteristics precluded the production of the colour fields associated with small changes in levels on relatively flat surfaces, for which the Nomarski system is generally used.

Estimation of areas of resorption

The total area of resorption observed on the buccal surface of experimental root segments in the SEM was estimated in the following way. Low power composite photographs of the buccal surfaces, which included the proximal surface as seen from the buccal aspect, were recorded and printed.

From these prints, the specimen was cut out with scissors leaving no border and the resulting composite photograph weighed. The areas of resorption (including those in various stages of repair) were then cut out from the composite picture using scissors and the portions of the photograph corresponding to resorption were weighed. The ratio of the 'total weight' to the 'resorbed weight' gave an estimate of the relative amount of surface area involved by resorption for each specimen. The results of these calculations are presented in Section 5.

Preparation of specimens for histologic examination

To evaluate the histologic preparation technique and various staining methods, non-experimental teeth were first prepared for examination. Specimens used were one intact upper premolar root and two buccal half root specimens from upper premolars. The specimens were washed for one hour, after fixation in 10% neutral buffered formosaline They were then transferred to 50 ml of decalcifying medium (either 'Decal', EDTA or formic formate) which was changed every two days. The containers were agitated gently by hand four times daily. Decalcification at room temperature was complete at one week for the intact root in 'Decal' (Mega Chemical Corporation, U.S.A.). The half root specimens took three weeks to decalcify, one in EDTA and one in formic formate (formulae given in Appendix I). The end point of decalcification was determined by radiographing the specimens using a Phillips Oralix X-ray machine (64kV; exposure time 0.4 seconds).

At the completion of decalcification, the specimens were washed in running tap water for one hour and placed in 5% sodium sulphate neutralizing solution overnight. They were then washed in running tap water for one hour, passed through a series of graded alcohols and double embedded in celloidin and paraffin (Appendix II). Following this the specimens were blocked in paraffin in a "Tissue Tek II Tissue Embedding Centre" (Lab-Tek Products, Division Miles Laboratories Inc., Napoville, Illinois).

The blocked specimens were sectioned at 8 micrometres on a Leitz rotary microtome, and the sections flattened by floating on a warm water bath before being transferred to gelatinized slides (Appendix III). The intact root specimen was sectioned longitudinally in a buccolingual direction and the half root specimens were sectioned transversely. The specimen decalcified in formic formate was easier to section without folding or curling, compared with the other two decalcification methods which were tried. The tissue integrity was well preserved by formic formate decalcification.

Stains used were Pollack's trichrome; Weigert's haematoxylin and van Gieson; a modification of Lillie's stain for collagen (Saville Bradbury); Ehrlich's haematoxylin and eosin; and Mayer's haemalum, celestin blue and van Gieson (see Appendix IV for details). Of these only the Saville Bradbury stain was not found to be consistently useful because the depth of colour was difficult to control and often tended to obscure detail. This stain was therefore not used extensively. Pollack's trichrome was found to demonstrate collagen fibres well and was thus very useful for investigating attachment of periodontal soft tissue to the root surfaces. However, this stain did not show cell nuclei clearly and imparted a slightly blurred appearance to sections even when properly focused. This characteristic may have been associated with the section thickness, but was not noticed with the other stains used. The mounting medium used was Pix (dibutyl phthalate in xylene).

Specimens for histological preparation from the experimental teeth represented longitudinal quarter sections of the entire root except for the left premolar of patient T.H. which was prepared *in toto*. These specimens were decalcified in the formic formate solution and sectioned longitudinally in a buccolingual direction.

Ground sections were prepared from some experimental specimens as follows. Specimens were dehydrated by passing through 70%, 90% and 100% alcohol spending two days in each, then drained on tissue paper to absorb surface alcohol and immersed in uncatalysed Biopot resin (Science Aids, Australia) in a small uncovered plastic box. This was subjected to increasing vacuum over half an hour and then left overnight in vacuum. Seven drops of catalyst were added to the resin the next morning and the specimen was removed while the resin and catalyst were stirred carefully, to minimize air incorporation. Full polymerization in air was complete in several hours. At this point the resin block could be removed from the plastic box and reduced to a suitable size around the specimen with a hacksaw. The specimens were then sectioned longitudinally in a buccolingual direction on an Isomet low speed saw (Buehler, U.S.A.). The resulting sections were of the order of 200 micrometres thick.

The cut sections were mounted onto glass slides with biopot resin and reduction of the section thickness was done by hand on increasingly fine grades of wet and dry emery paper (grades 180, 220, 320, 400, 600) with continuous water flow. The resulting section thickness was estimated at 20 micrometres. They were then passed through 95% and two changes of 100% alcohol, brought to xylene and finally coverslips were affixed with Pix.

The number of ground sections obtainable from each specimen was limited to 3 or 4 because of the unavoidable losses associated with the technique. The information derived from the ground sections was similar to that which was obtainable from paraffin embedded sections. Thus only four buccal segments were used for ground section preparation, the remainder of the histological specimens being paraffin embedded and sectioned using the microtome. Ground sections were prepared from the following segments: distobuccal of the right premolar from patients C.T., S.W., B.B. and mesiobuccal of the left premolar from patient R.E.

Recording of histologic sections

Black and white photographic recording of paraffin embedded and ground tissue sections was conducted on a Zeiss Axiomat microscope (West Germany) using a ZG (green) filter. Ilford FP4 plate film (4" x 5" ASA 125) was used and processed in the same way as the FP4 film used for recording with the SEM (Appendix V). For colour recording a number of films were used (listed in Appendix V) with a blue and dydmium correction filter. It was necessary to vary the ASA setting on the Axiomat to correct the automatic exposure for the colour films. In these cases setting the ASA at 50-75% of the stated film rating achieved the necessary increase in exposure time. Kodak photomicrography film (No.2483, ASA 16) gave marginally the best result of the three colour films used (Appendix V). Colour prints were produced using the Cibachrome A (Ilford) print system.

In addition to ordinary transmitted light, phase contrast, darkfield illumination and polarized light were used for examining the paraffin embedded and ground sections. Various effects were obtainable with each of these but for consistency of interpretation ordinary transmitted light proved superior for the assessment and photographic recording of the sections.

Preparation of paraffin sections for viewing in the SEM

A number of selected paraffin sections were reprepared for examination in the SEM after recording in the light microscope. The coverslips and embedding medium were removed by dissolving in xylene. The sections were then passed through three changes of 100% alcohol spending one hour in each and air dried in a covered container. The glass slide was cut back around the section as much as possible and the section was mounted onto an aluminium SEM stub with 'Araldite' epoxy resin (CIBA-Geigy Ltd., Australia). Coating for and recording in the SEM were as described previously.

SECTION 5

FINDINGS

Scanning electron microscopic (SEM) appearance of human bone

The bone specimens from the lingual cortical plate presented a surface characterized by large round vascular openings. The diameter of vascular openings ranged between fifty and two hundred micrometres. Calcified intrinsic matrix bundles were arranged in an orderly fashion with some interlacing evident due to fibrils passing from one bundle to another. The mineralization of the intrinsic matrix bundles generally appeared to be smooth and uniform (Figure 9). The matrix fibre pattern was retained in the anorganic state by the mineral crystals laid within it.

Some areas of the adult cortical bone section, particularly close to the vascular openings, had numerous Sharpey fibre holes (Figure 10) most of which were between four and seven micrometres in diameter. A number had central projections. Forming osteoblast cell lacunae were characterized by round or oval outlines, canalicular openings in their bases and a diameter of ten to fifteen micrometres. Close inspection of the floors of these lacunae often showed a fine fibrillar pattern.

Several areas of resorption were observed (Figure 9). Some of the resorptive Howship's lacunae were considered to be repairing as the lacunar rims were slightly rounded and the floors had a granular appearance at high magnification indicating remineralizing activity.

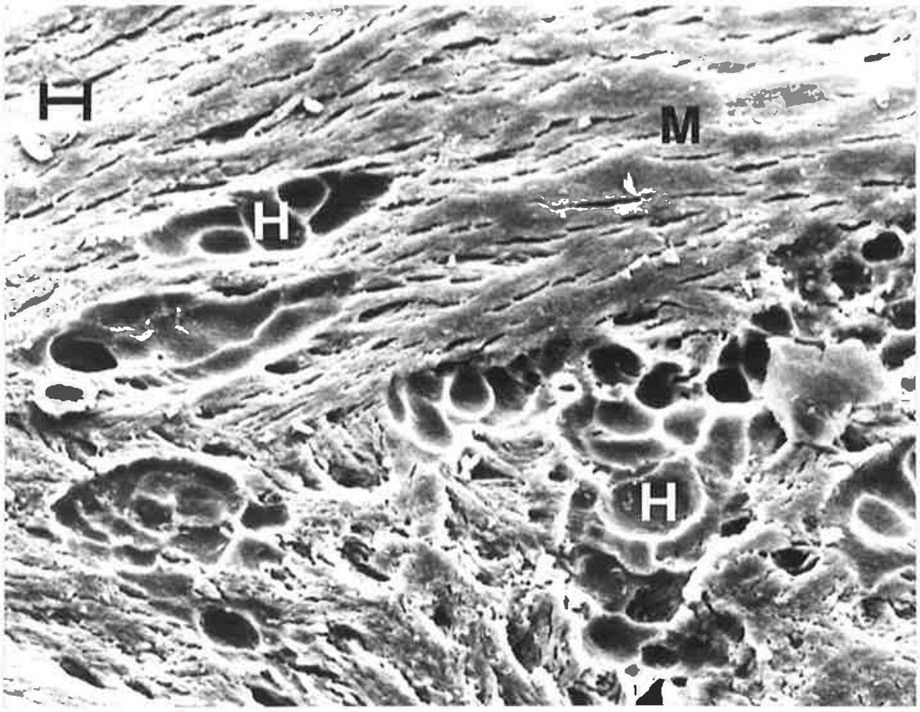


Figure 9

Adult cortical bone showing mineralized intrinsic matrix (M) and resorptive Howship's lacunae (H).
Scale bar : 10 micrometres. X200

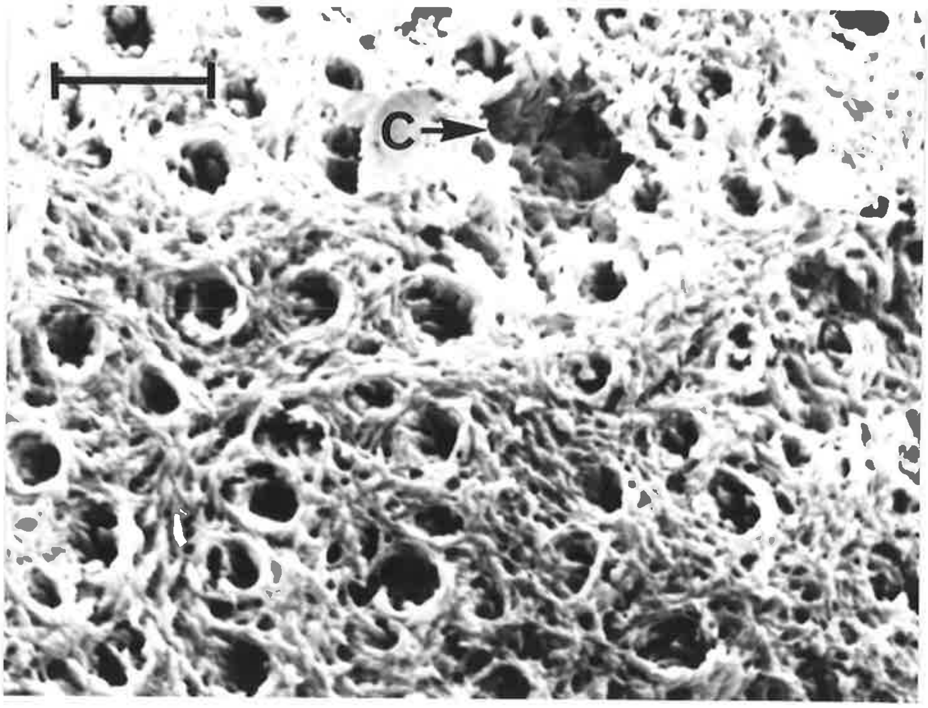


Figure 10

Adult cortical bone with numerous circular Sharpey fibre depressions and an osteoblast lacuna (C).
Scale bar : 10 micrometres. X700

The fetal or primary bone specimens were long bones taken from the lower limb of a twenty week fetus. The surface was seen to be dominated by numerous openings which had a diameter of about 100 micrometres. These were vascular openings and were generally associated with short longitudinal furrows.

Fetal osteoblast cell lacunae were similar to those seen in the adult bone specimens, having a circular or ovoid outline, with canalicular openings internally and a fine fibrillar or nodular network on their walls and floors.

There was generally no evidence of an ordered intrinsic matrix. The mineral clusters and particles had a random orientation as seen in Figure 11 except around a few of the vascular openings where the pattern was more ordered and similar to adult bone.

SEM appearance of normal premolar roots

The normal premolar roots examined were obtained from adolescent patients prior to orthodontic treatment. The arrangement of topographical features was found to be similar in all specimens. In the cervical one half to two thirds of the root the surface was composed of close-packed, low Sharpey fibre mounds (Figure 12). These structures were generally round, oblong or hexagonal in outline and were three to eight micrometres in diameter. In a limited area at about the mid-root region of one specimen, less densely packed dish-topped Sharpey fibre mounds were found (Figure 13). The surface in this area had a rough granular texture and a few cell lacunae were observed in the region.

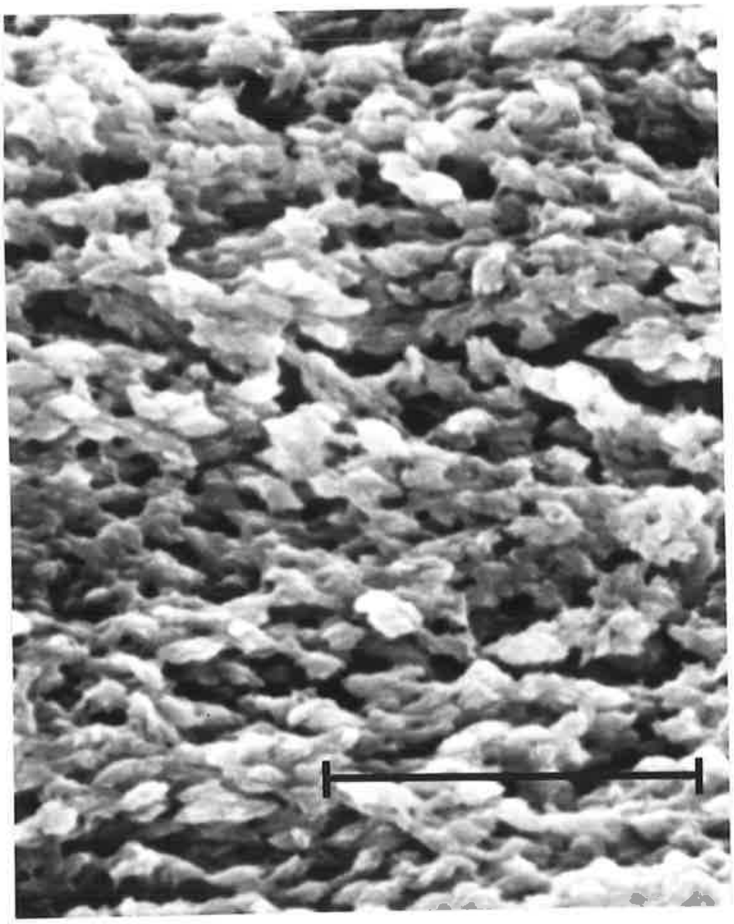


Figure 11

Mineralizing front of fetal bone showing random arrangement of mineral nodules some of which are beginning to coalesce. Scale bar : 10 micrometres. X2000

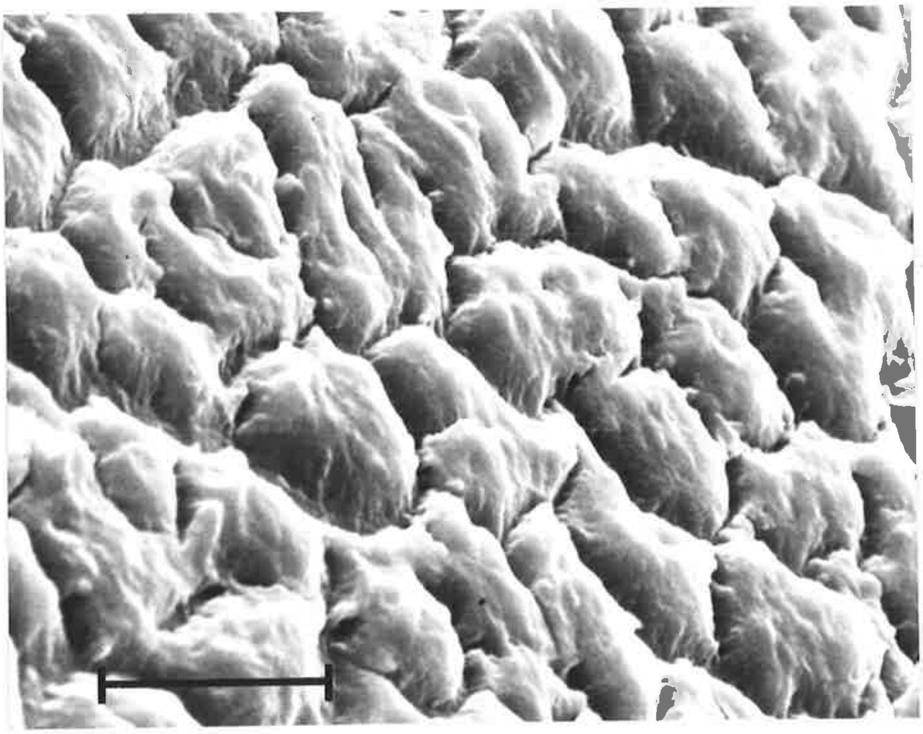


Figure 12

Typical close-packed Sharpey fibre mounds
from cervical acellular cementum.
Scale bar : 10 micrometres. X900

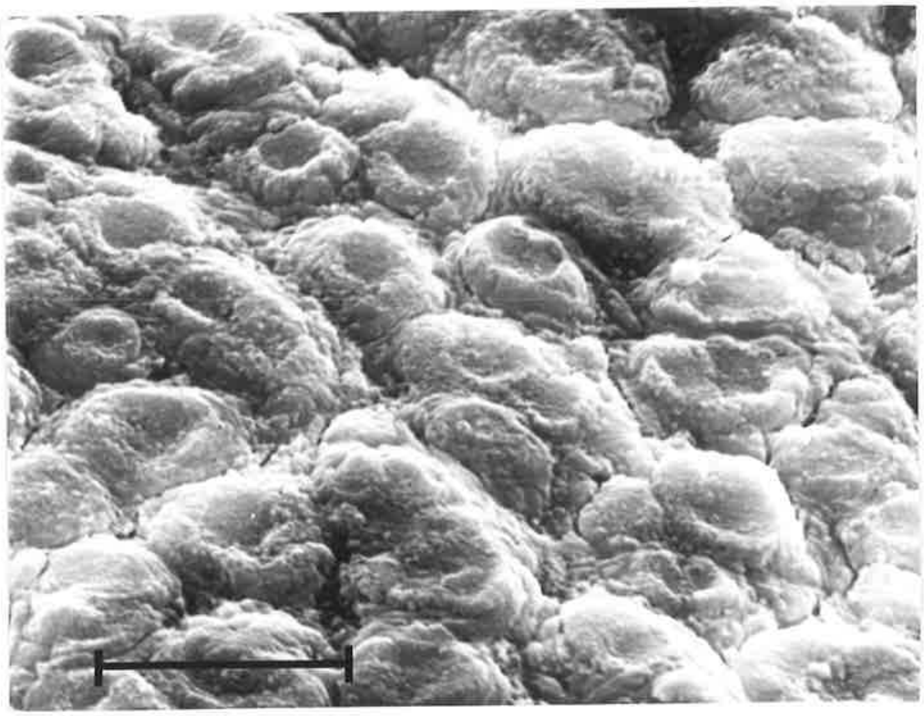


Figure 13

Dish-topped Sharpey fibre mounds from the
mid-root area of one specimen. Granular
surface suggests slow mineralizing activity.
Scale bar : 10 micrometres. X1000

A number of small areas of resorption were found, particularly in the cervical one third and close to the apex of the roots examined. These lesions were usually discrete but sometimes occurred in groups. Various stages of active resorption and repair were found in these defects. The repair tissue had the appearance of mineralizing cellular cementum (Figure 14).

The apical regions of the teeth were covered by cellular cementum having a topography which at low magnifications was undulating and irregular when compared with the cervical areas. The arrangement of the surface features in these apical regions was variable within each specimen. Limited areas of Sharpey fibre mounds similar to those seen in more cervical locations could be found but with a number of cell lacunae evident. The mineral front of the cellular cementum varied from discrete mineral nodules with no apparent orientation (Figure 15) to a more continuous surface exhibiting matrix orientation (Figure 16). Occasionally, relatively flat, featureless areas occurred.

Sharpey fibre depressions or holes with a diameter of four to seven micrometres were noted in cellular cementum, generally close to the apices. The density of these depressions in areas of the surface where they were found varied considerably. In some regions they were close-packed and in other areas more widely dispersed with cell lacunae also evident. Figure 16 shows an area of such close-packed principal periodontal fibre insertion sites with an intervening continuous nodular surface exhibiting orientation around the holes. Cell lacunae could be distinguished by their larger size of 8 to 15 micrometres diameter, generally ovoid shape and saucer-like bases with canalicular openings

Figure 14

Resorptive defect in an area of Sharpey fibre mound acellular cementum. Repair tissue within the defect is mineralizing cellular cementum. X160

Figure 15

Surface of actively mineralizing cellular cementum. Cementoblast lacuna (C). X2000

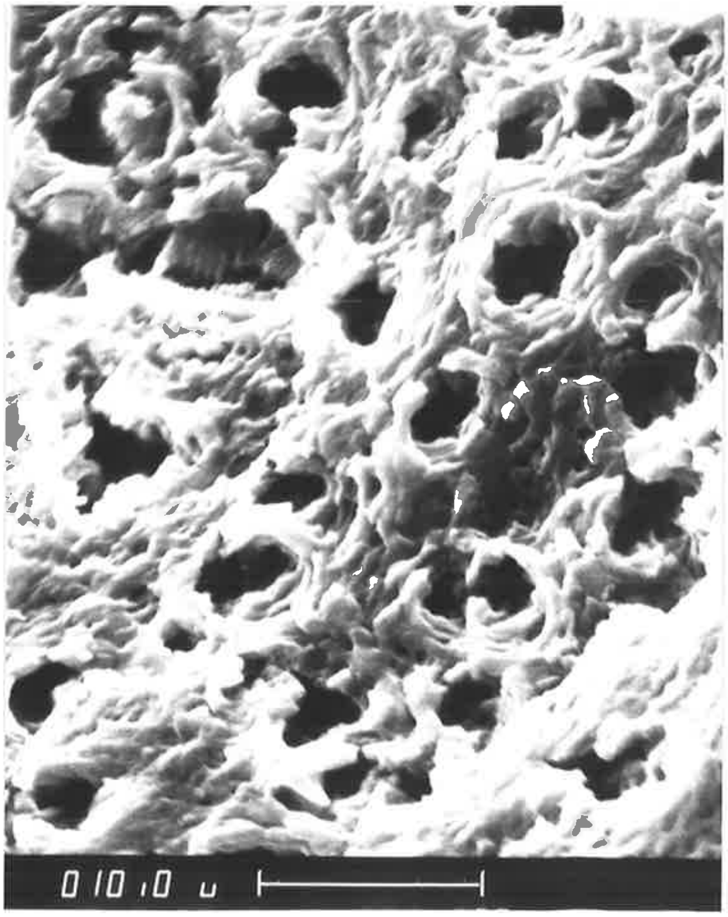


Figure 16

Sharpey fibre holes in a surface showing orientation of the mineralized intrinsic matrix. X1600 present. The floors of cell lacunae exhibited either a homogeneous, nodular, or fibrillar pattern.

The lower first premolars from experimental patients did not show any changes attributable to the orthodontic manipulations in the upper arch. Their overall topography was similar to that described above for normal tooth roots and conformed to general descriptions by Barber (1977), Boyde and Jones (1968) and Jones and Boyde (1972).

Root resorption patterns not associated with RME

The two upper first molars examined were from a female patient. She was ten years of age at the start of headgear treatment, which was aimed at moving the upper first molars distally five to six millimetres to correct their relationship to the lower molars. This treatment was continued for twenty weeks. Information concerning the force used was not available. These first molar teeth were removed for orthodontic reasons twenty months after the cessation of headgear therapy. Macroscopic inspection of the extracted teeth confirmed the radiographic diagnosis that the distobuccal root of the left first molar was almost completely resorbed and that of the right first molar was partially resorbed, probably due to impingement on the unerupted second molar crowns.

The remaining buccal root portion of the left first molar and distobuccal root of the right first molar were prepared for examination in the SEM. The surfaces of the specimens appeared rough and uneven particularly on the buccal and distal aspects. Extensive resorptive excavations on the surface were evident under low magnification. Much of the resorption was repairing with cellular cementum (Figure 17).

At higher magnification, much of the surface, particularly within the resorption areas, was dominated by a profusion of randomly oriented particles. These were either needle-like or cigar-shaped, a few micrometres long and sometimes formed stellate or brush-like patterns (Figure 18). Originally it was thought that these particles represented mineralization crystals. However, examination of later specimens led to the conclusion that they were an artefact. The particles were possibly crystals deposited by the formalin fixative in which the teeth had been kept for some time, or more likely sodium hypochlorite crystals left on the surface by inadequate washing of the specimens after treatment to render the tooth anorganic. Repreparation in sodium hypochlorite, washing for 24 hours and recoating largely eliminated these particles.

An upper lateral incisor which had been undergoing root resorption was examined in the SEM. The resorption was detected radiographically subsequent to trauma and the tooth was removed to allow orthodontic closure of the space. The patient was a female aged eighteen and three quarter years. Examination of the root of the tooth in the SEM revealed extensive resorption in the apical region, a large area of resorption in the cervical one third of the root and several smaller excavations. None of these areas as seen in the SEM was considered to be actively resorbing. Repair appeared to have supervened with tissue resembling mineralizing cellular cementum (Figure 19).

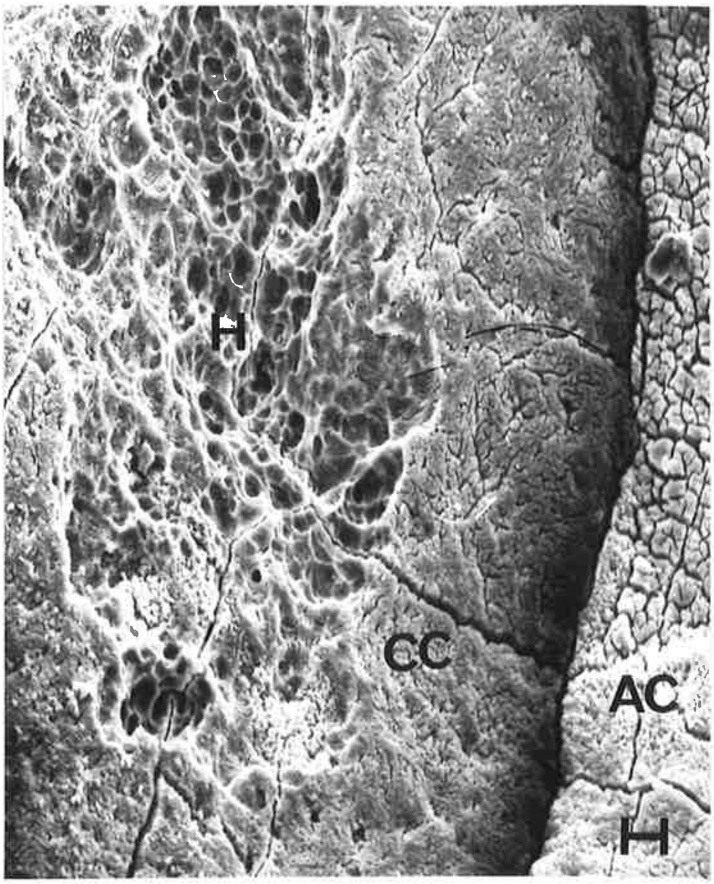


Figure 17

Slowly mineralizing repair cellular cementum (CC) bounded on one side by normal acellular cementum (AC). Resorptive Howship's lacunae (H) show early repair. Area taken from the distobuccal root of an upper right first molar subjected to headgear treatment.
Scale bar : 10 micrometres. X100

Figure 18

Artefact crystalline structures on the surface and within a depression, as seen on an inadequately washed specimen. X1000

An unerupted upper second premolar removed for orthodontic reasons from a twenty-three year old female patient was examined in the SEM. Coating with gold-palladium created a matt, poorly light-reflecting surface making gross surface topography easier to assess with the unaided eye. Following coating an extensive shallow excavation involving one side of the root was evident. The root itself was short and blunt apically.

In the SEM, the extensive excavation on the root presented a surface suggesting a state of early repair. Some parts had mineral clusters on the surface. Howship's lacunae were not numerous and had rounded edges and granular surfaces indicating mineralizing activity. Some areas of the excavation appeared to have completed repair but were at a lower level than the surrounding unaffected cementum. These areas had a relatively flat, featureless topography. Few cell lacunae were evident anywhere. A number of other small resorptive defects were also found on the root surface (Figure 20).

The cementum surrounding areas of resorption had a relatively featureless topography. Generally, a surface of low close-packed mounds without cell lacunae predominated. However, towards the apex a flatter topography was more common (Figure 20).

An upper deciduous canine, removed for orthodontic reasons from the same patient as the unerupted second premolar, was also examined. Radiographically, the root of this tooth appeared to be intact except for some resorption on its distal aspect. In the SEM an extensive resorptive excavation was found on the distal surface of the root of

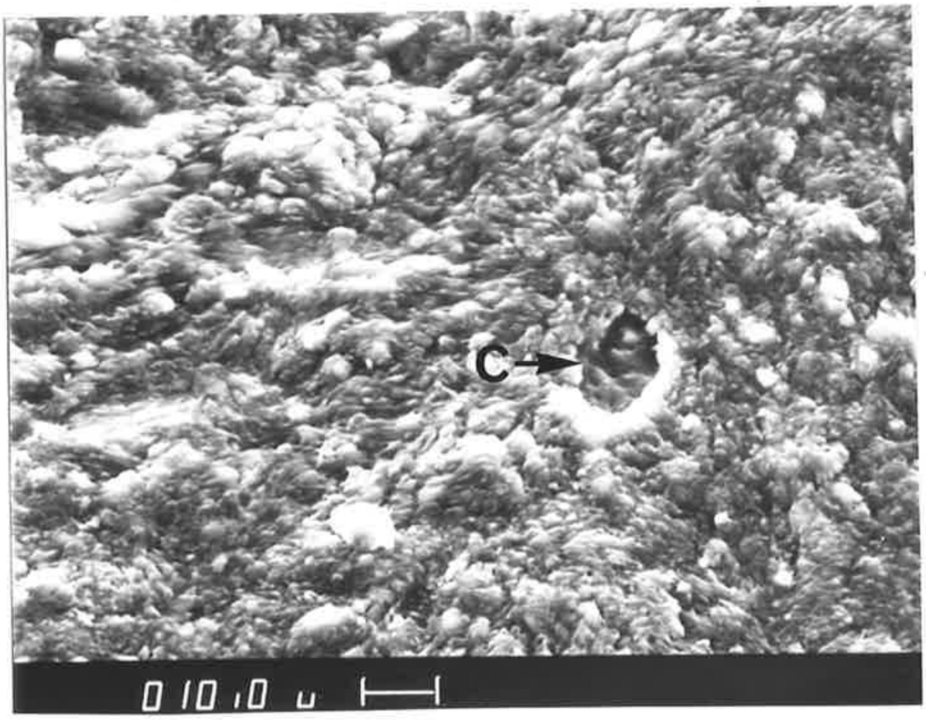


Figure 19

Mineralizing repair cellular cementum in a resorptive defect on an upper lateral incisor. Cementoblast lacuna (C). X400

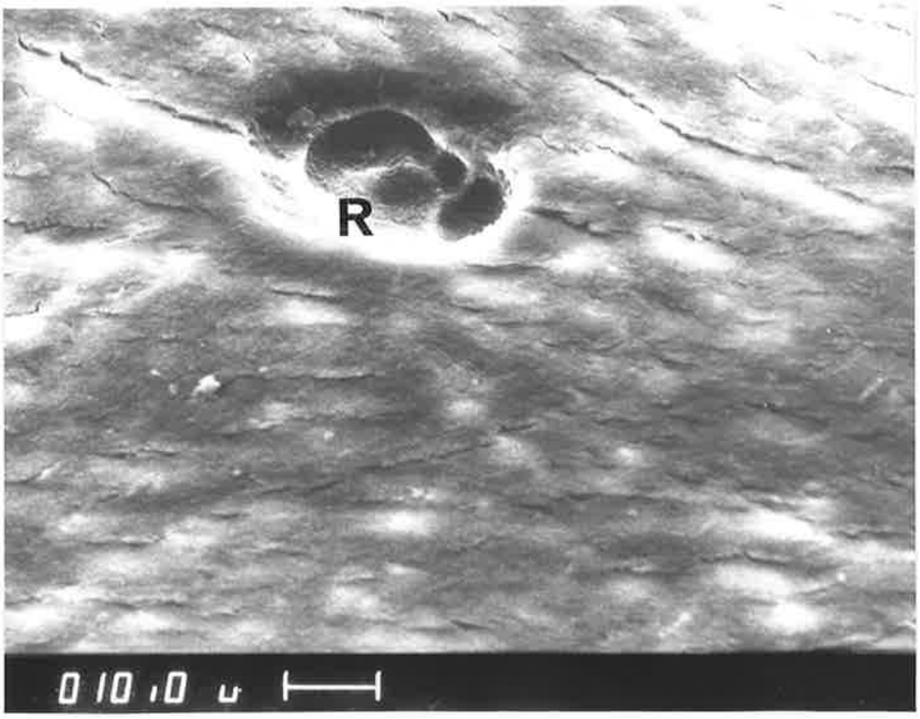


Figure 20

Flat surface topography of apical cellular cementum from an unerupted second premolar. A small area of resorption (R) can be seen. X500 this tooth. Within the excavation no evidence of active resorption was observed and Howship's lacunae appeared only around the periphery. The edges of these lacunae were rounded and their surface texture suggested remineralizing activity. The greater part of the surface within the excavation featured closely packed irregular projections (Figure 21). In some areas a mineralized fibre pattern in the plane of the surface was seen and in these areas cell lacunae were more common than elsewhere, although still not numerous. The fibres formed groups arranged at varying angles in relation to each other (Figure 21).

In the apical region on unresorbed surfaces a limited number of raised annular projections with central depressions varying in diameter from a few micrometres to seven or eight micrometres occurred, adjacent to areas of relatively flat surface topography. These projections were thought to represent principal periodontal fibre insertions in which peripheral mineralization had proceeded beyond the general level of the surrounding mineral front.

The observations made on these non-experimental tooth roots suggested that the topographical presentation of resorption and repair on root surfaces is essentially similar in varying situations. Repair tissue was consistently cellular cementum in these specimens. In addition, some evidence for periodontal fibre insertion in repair cementum was found on the deciduous tooth. Findings from the experimental premolars corroborated these early observations.

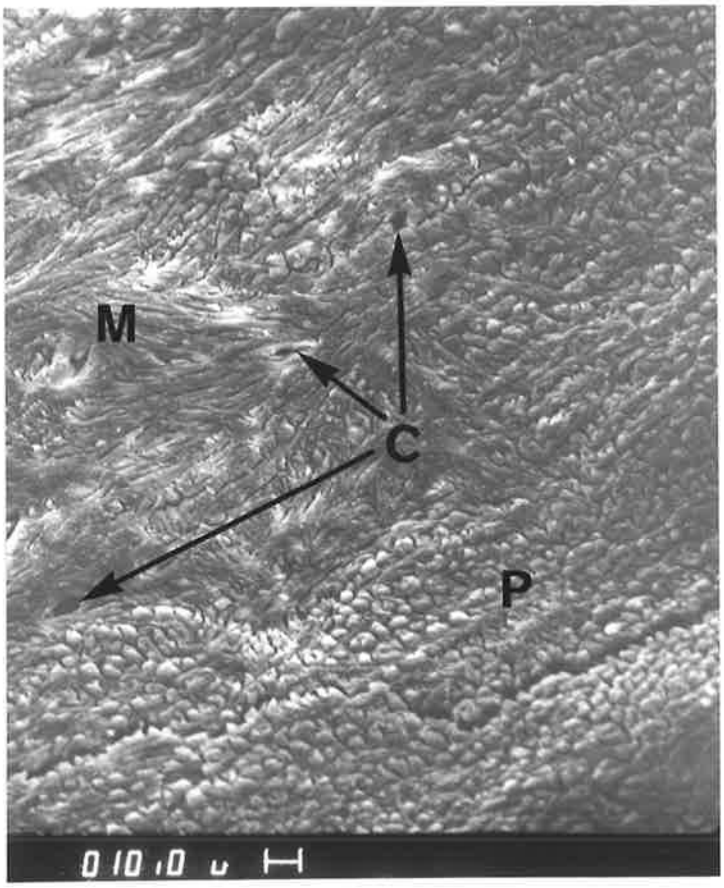


Figure 21

Surface within a large area of previous resorption on a retained upper deciduous canine. Cell lacunae (C) which are beginning to cover over can be seen in an area showing mineralized intrinsic matrix bundles (M). Close-packed, irregular projections (P) which dominate much of the surface are probably associated with periodontal fibre insertions. X200

EXPERIMENTAL ANCHOR PREMOLAR ROOTS

The total patient group of 18 from which material for this study was derived has been divided into three subgroups (Table 3). The division into these subgroups has been made on the basis of the total time span of the retention period following rapid maxillary expansion in each case. The aim of this arbitrary division was to simplify the presentation of the findings and discussion to follow. In addition to a description of observations made in relation to root resorption and its repair on each of the specimens, salient and unusual features seen in apparently unaffected areas, designated as 'normal' in this report, of the tooth roots examined will be included in this section. The results of the scanning electron microscopic examination will be presented first, followed by those of the light microscope histological study. A number of pertinent and supportive observations from the light microscope examination have been included with the findings from the SEM.

Group 1

Patient T.H.

Both upper first premolars from patient T.H. had a bifid root pattern. The roots of the left premolar were decalcified intact and examined histologically. The buccal and lingual roots of the right premolar were examined individually in the SEM.

Well defined areas of resorption were not extensive and

TABLE 3

This table shows the buccal segments which were examined in the SEM

Patient	Total Retention Period (weeks)	Buccal Segment Examined in the SEM	
		Left Premolar	Right Premolar
Group 1.	Patients retained for less than 20 weeks.		
TH*	14	**	Buc
BB*	$14\frac{3}{7}$	DB	MB
MD	16	DB	MB
GB*	17	MB	MB
CT*	17	Buc	MB
WJ	$17\frac{5}{7}$	DB	Buc
Group 2.	Patients retained for a total period between 20 and 30 weeks.		
KB	20	MB	MB
RH*	21	DB	MB
RE*	$21\frac{4}{7}$	DB	MB
SW*	$22\frac{3}{7}$	DB	MB
LB	$25\frac{4}{7}$	MB	MB
AS	29	DB	MB
Group 3.	Patients retained for more than 30 weeks.		
MC	$38\frac{6}{7}$	DB	-
MI	$40\frac{6}{7}$	DB	MB
SM*	51	DB	MB; DB. ***
EC	52	MB	DB
LH	53	MB	DB
JS	$53\frac{2}{7}$	MB	DB

MB: Mesiobuccal; DB: Distobuccal; Buc: Entire buccal surface.

* Patients not treated by the author.

** Total specimen examined histologically.

*** The distobuccal section was fully decalcified and left organic for examination in the SEM.

The buccal segments not examined in the SEM were examined histologically.

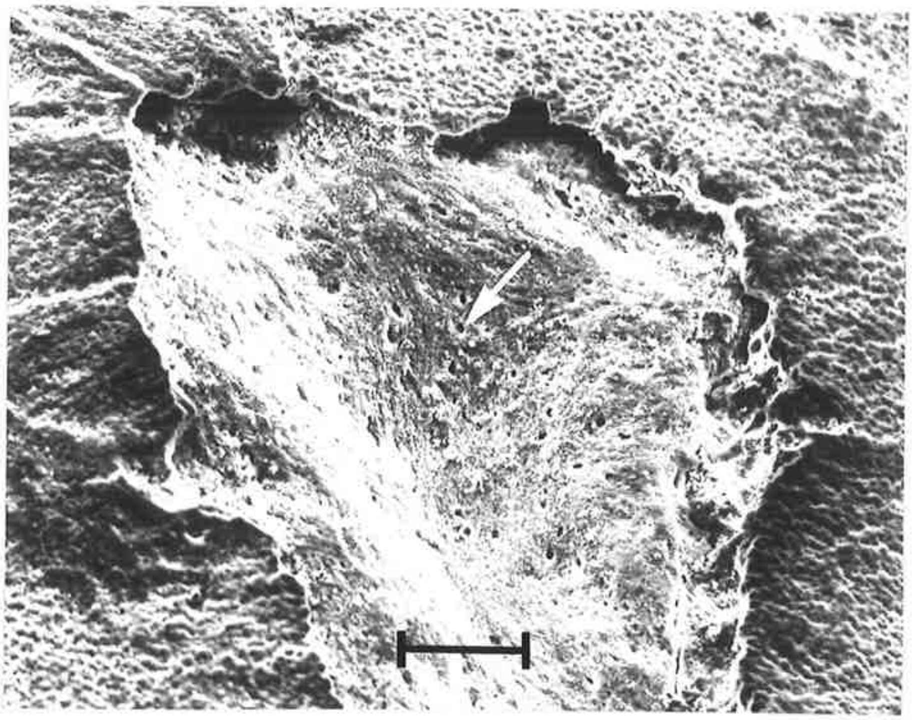
All lingual surfaces except ** were examined in the SEM.

occurred mainly on the buccal aspects of both roots although some small, distinct areas of resorption were seen on every surface of each root. Resorptive activity seemed to be mainly in the apical half to two thirds of each root but some was noted cervically. The areas of resorption all appeared to be repairing. Numerous cell lacunae occurred in the repair mineralizing front of densely packed mineral clusters (Figure 22), presenting a topography similar to that seen on some normal cellular cementum surfaces considered to be actively forming. However, in the repair cellular cementum no principal periodontal fibre insertion sites could be identified. Howship's lacunae were found only at the edges of the resorptive defects, which were often undermined and generally well rounded.

Patient B.B.

The buccal segments of the first premolars taken from the patient B.B. were extensively affected by resorption (Figure 23). In both segments resorption had extended well onto the proximal surfaces. The lesions were generally large, particularly on the left premolar. On this specimen the area affected by resorption appeared to be continuous from the cervical one third to the apical extremity and this was confirmed by the histological examination of the mesiobuccal specimen from the same root. The mesiobuccal segment of the right premolar had numbers of small areas of resorption with diameters of the order of several hundred micrometres in addition to larger but separate defects.

Howship's lacunae, with sharp outlines and smooth floors, were found at the periphery of the larger lesions and dominated some of the



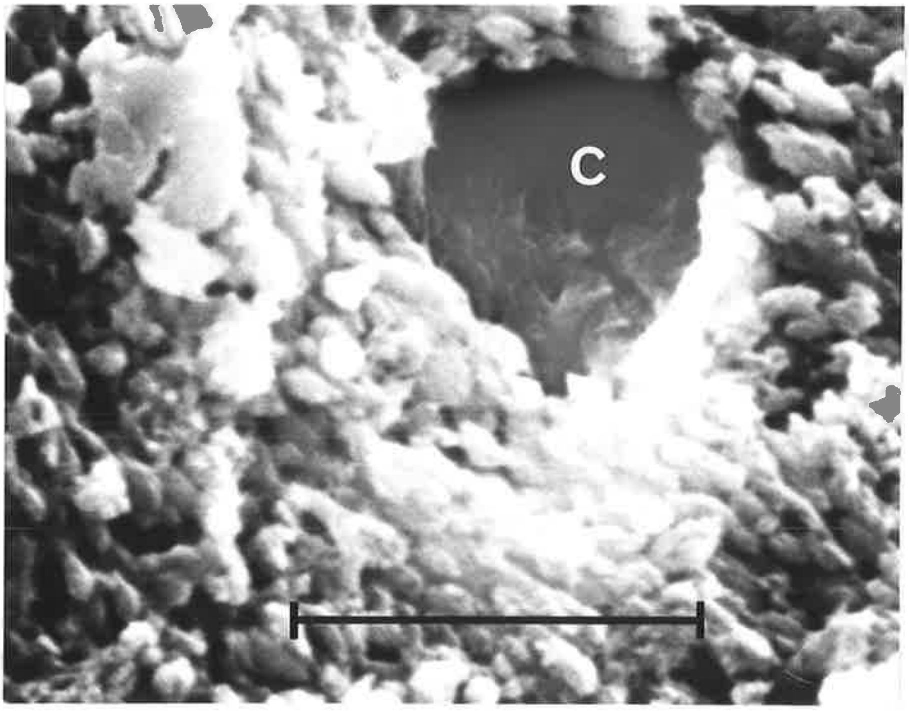


Figure 22

Patient T.H., retention period 14 weeks.

Above: An area of previous resorption repairing with cellular cementum. The arrow indicates a cell lacuna enlarged below. Buccal surface, buccal root.
Scale bar: 100 micrometres. X80

Below: Cell lacuna (C) from the above illustration. The surrounding surface of discrete mineral particles suggests rapid mineralization.
Scale bar: 10 micrometres. X2700

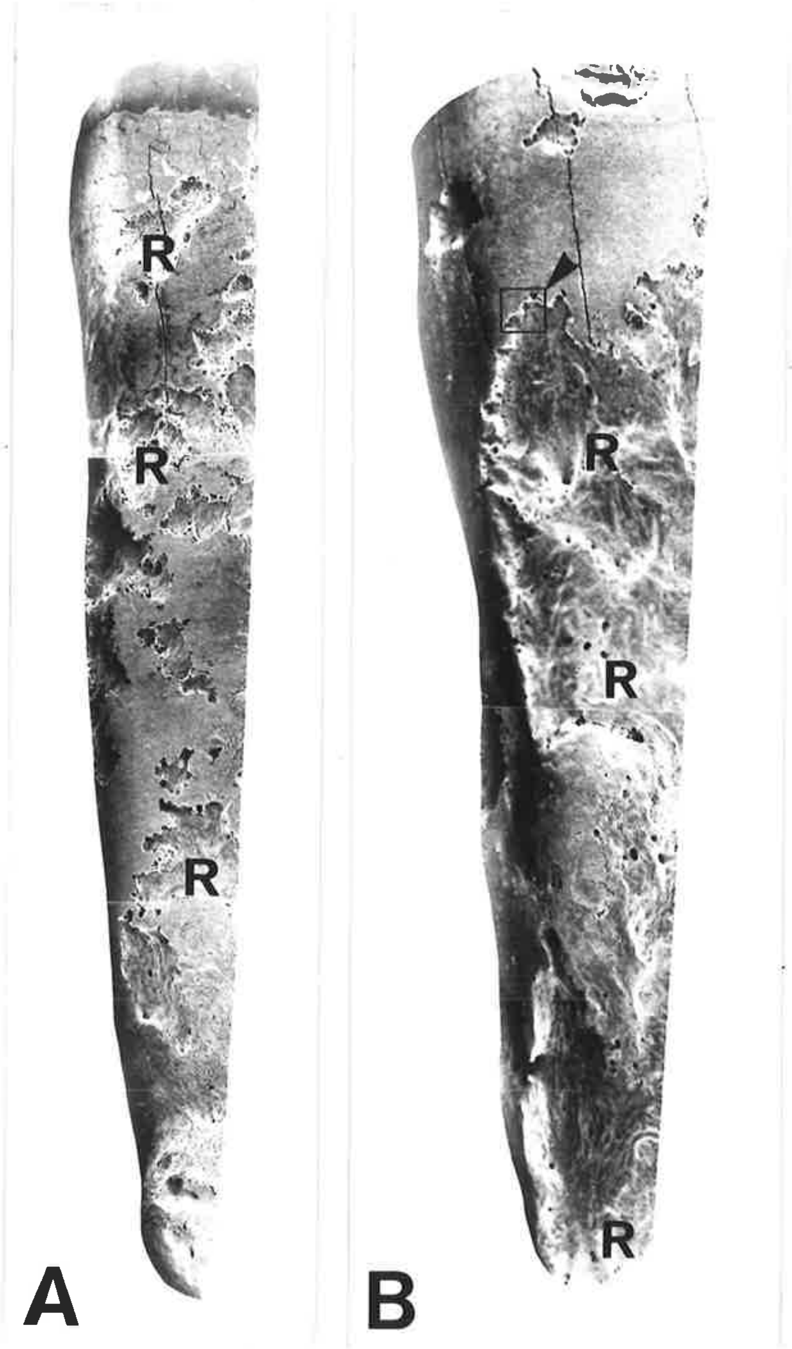


Figure 23

Patient B.B. Retention period 14$\frac{3}{7}$ weeks.

A: Right premolar, mesiobuccal segment showing numerous areas of resorption (R).

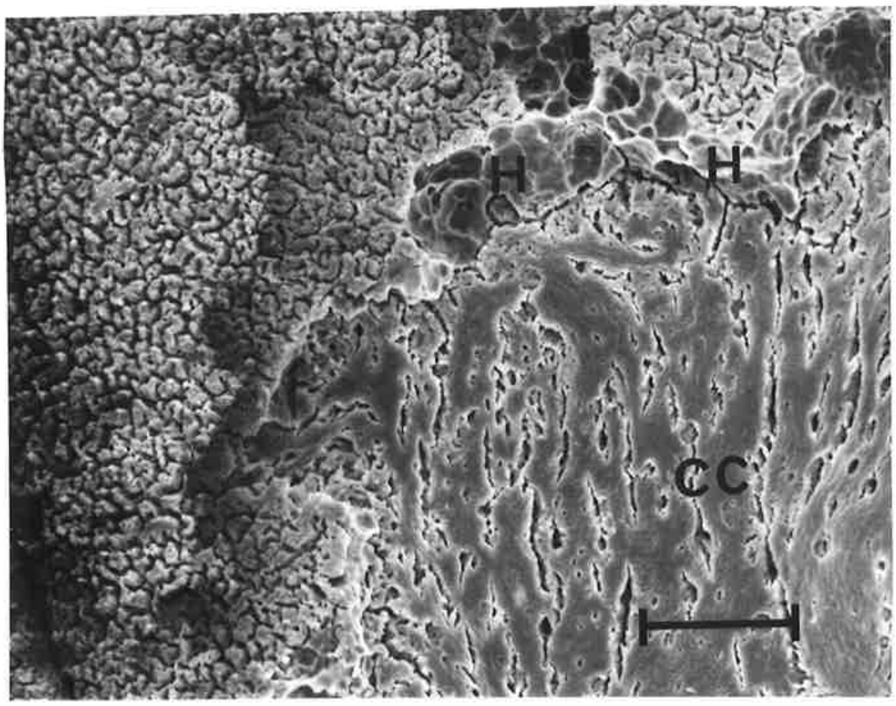
B: Left premolar, distobuccal segment with a large, continuous area of resorption (R). The outlined area is shown in Figure 24. X10 smaller lesions. In general repair was the major feature of the resorptive defects and in places had attained the level of the surrounding surface. On both specimens repair tissue presented a similar topography, having a flat surface interrupted by cell lacunae and roughly parallel cracks, these probably being drying artefacts (Figure 24).

In an area close to the apical extent of the left premolar specimen a field of close-packed circular depressions was observed (Figure 25). It seemed likely from the adjacent histological sections that this was an area of repaired resorption. The depressions in this area were interpreted as Sharpey fibre holes on the basis of their diameter being four to seven micrometres and their close packing. In addition, a lack of canalicular openings internally distinguished them from cementoblast cell lacunae.

The lingual root surfaces of both premolars demonstrated resorptive defects in the apical one third and to a lesser degree the middle one third (Figure 26). The extent of these lesions was minor compared with that seen on the buccal aspects but repair activity was similar.

Patient M.D.

The mesiobuccal segment of the right premolar showed extensive surface resorption along the entire length of the root. The areas of resorption were large and virtually continuous in the apical half. In the cervical half a large area of resorption and several smaller areas were noted. Repair was not a prominent feature anywhere, Howship's



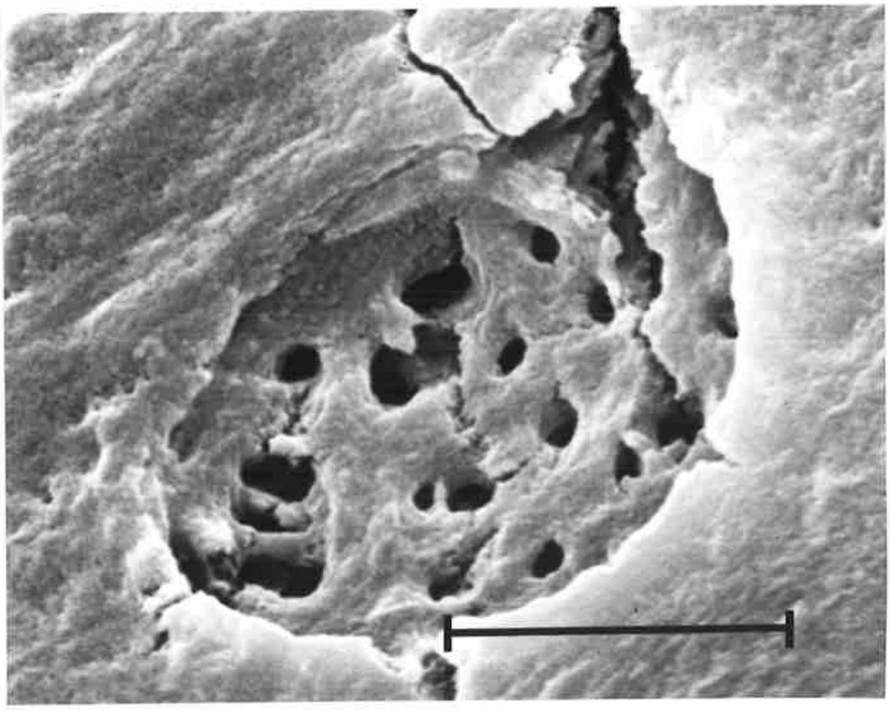


Figure 24

Above: Repair cellular cementum (CC) with a flat topography in an area from Figure 23B. Some Howship's lacunae (H) remain at the periphery of the resorptive defect which is bordered by acellular cementum.
Scale bar: 100 micrometres. X90

Below: A cell lacuna typical of the repair cementum shown above. The surface suggests lack of mineralizing activity.
Scale bar: 10 micrometres. X2300

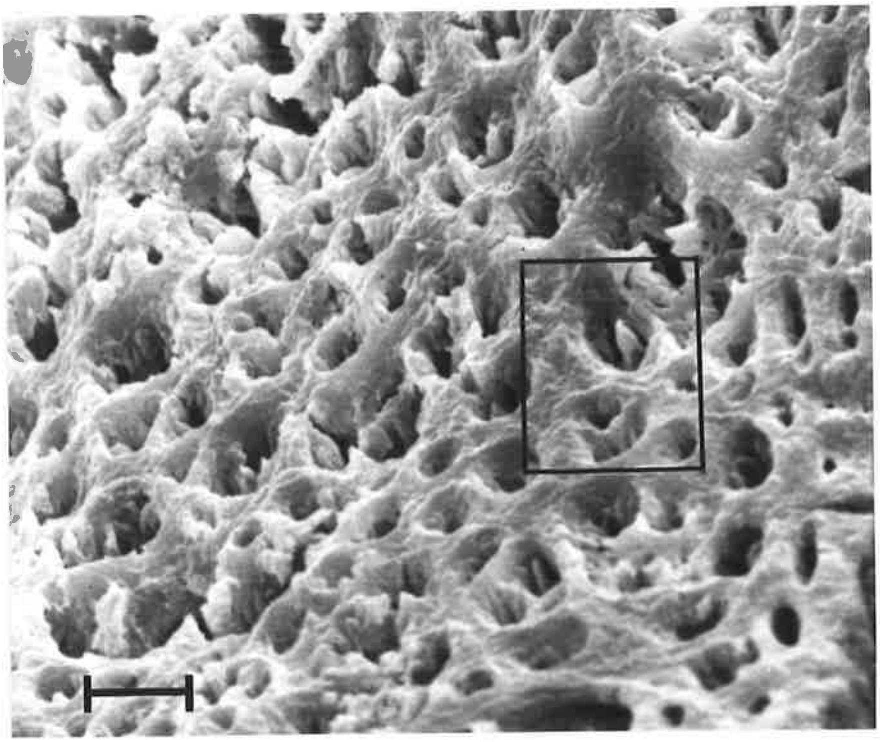


Figure 25

Patient B.B., left premolar, buccal surface.

Above: A surface dominated by Sharpey fibre depressions in an area, possibly previously resorbed, near the apex.
Scale bar: 10 micrometres. X400

Below: Stereopair of the area outlined above. Tilt difference 8^0. X2800

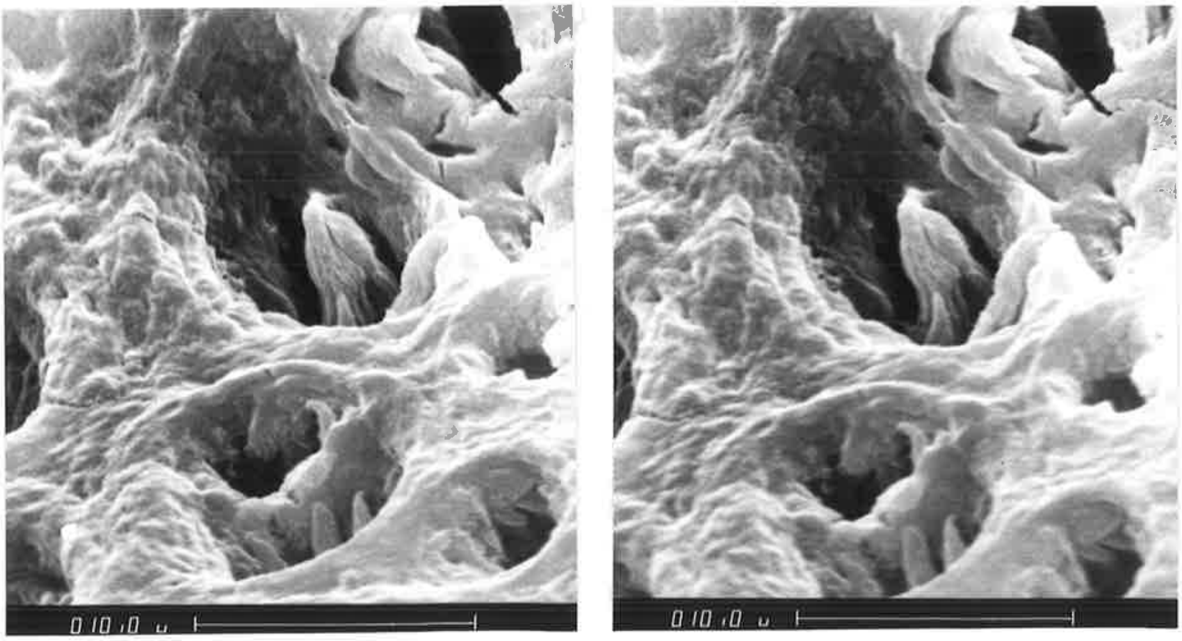


Figure 26

Minimal areas of resorption (R) on the lingual segment of the right premolar from patient B.B. typify the amount of damage generally encountered on lingual surfaces in this study. X10 lacunae dominating the defects. These findings were confirmed by the histological sections. The Howship's lacunae generally had smooth floors and slightly rounded edges (Figure 27). Resorption was relatively shallow although penetrating into dentine, but isolated 'punched-out' pockets of deeper resorption were found within the larger areas. It was difficult to definitely judge the status of Howship's lacunae as resting or resorbing but resorptive activity seemed to be occurring at the periphery of the lesions, evidenced by sharper margins of the Howship's lacunae.

Repair tissue was not advanced with respect to restoring the defects and appeared to be slowly mineralizing cellular cementum, having a relatively homogeneous surface due to confluence within the mineral front.

The lingual surface exhibited only minor resorptive activity near the apex and one area cervically. The pattern of resorption and repair was similar to that noted on the buccal aspect. The apex did not appear to have been blunted or shortened by resorption.

The distobuccal segment of the left premolar was extensively resorbed only in the apical half. The cervical half of this specimen had a number of small, discrete lesions. The pattern of resorption and repair was similar to that described for the right premolar, except that large areas of Howship's lacunae on the left premolar had rounded edges suggestive of early repair.

The lingual surface of the left premolar was affected by resorption in a relatively minor way. The left and right lingual

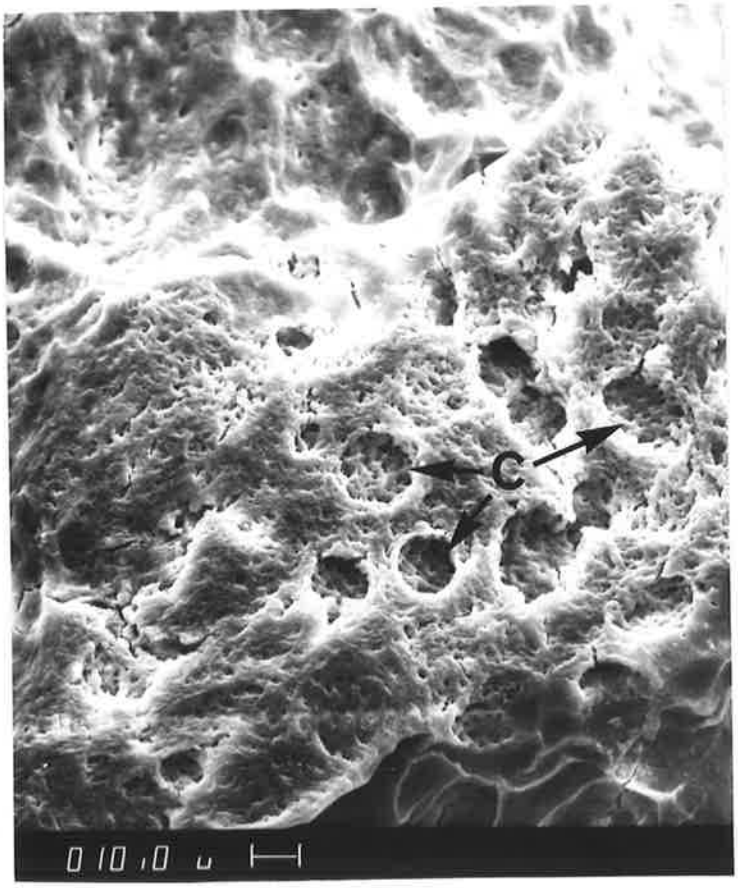


Figure 27

Patient M.D., retention period 16 weeks.

Howship's lacunae bordering cellular cementum in the apical half of the right premolar. The Howship's lacunae in the lower area of the illustration have sharp margins and smooth floors indicating active resorption. Cell lacunae (C). X300 surfaces were similar in the gross distribution and extent of resorption. However, the apex of the left tooth on the lingual surface appeared to have been blunted slightly by resorption as Howship's lacunae dominated the apical extremity.

Patient G.B.

The mesiobuccal segments of both upper premolars from patient G.B. were examined in the SEM. Large areas of resorption were seen along the entire length of both specimens extending onto the mesial aspect in each. Repair was the dominant feature of most defects but open Howship's lacunae at the periphery were also frequently observed.

The pattern of the repair tissue was more variable than that described for patient B.B. and generally did not present such a flat topography. The mineral front of the repair tissue was composed of circular or fusiform particles having an apparently random distribution around surface depressions (Figure 28). The mineral particles were usually discrete but in some areas demonstrated varying degrees of coalescence to produce a more continuous although nodular surface topography.

The lingual surfaces were much less affected by resorption, but demonstrated similar features of repair as did the buccal surfaces of these teeth. In an area of shallow resorption on the lingual surface close to the apex of the right premolar, a number of holes were observed in resorbing Howship's lacunae (Figure 29). These holes were 2 to 3 micrometres in diameter with rolled peripheries, circular on the outside and crenated or folded on the inner margins. It was felt

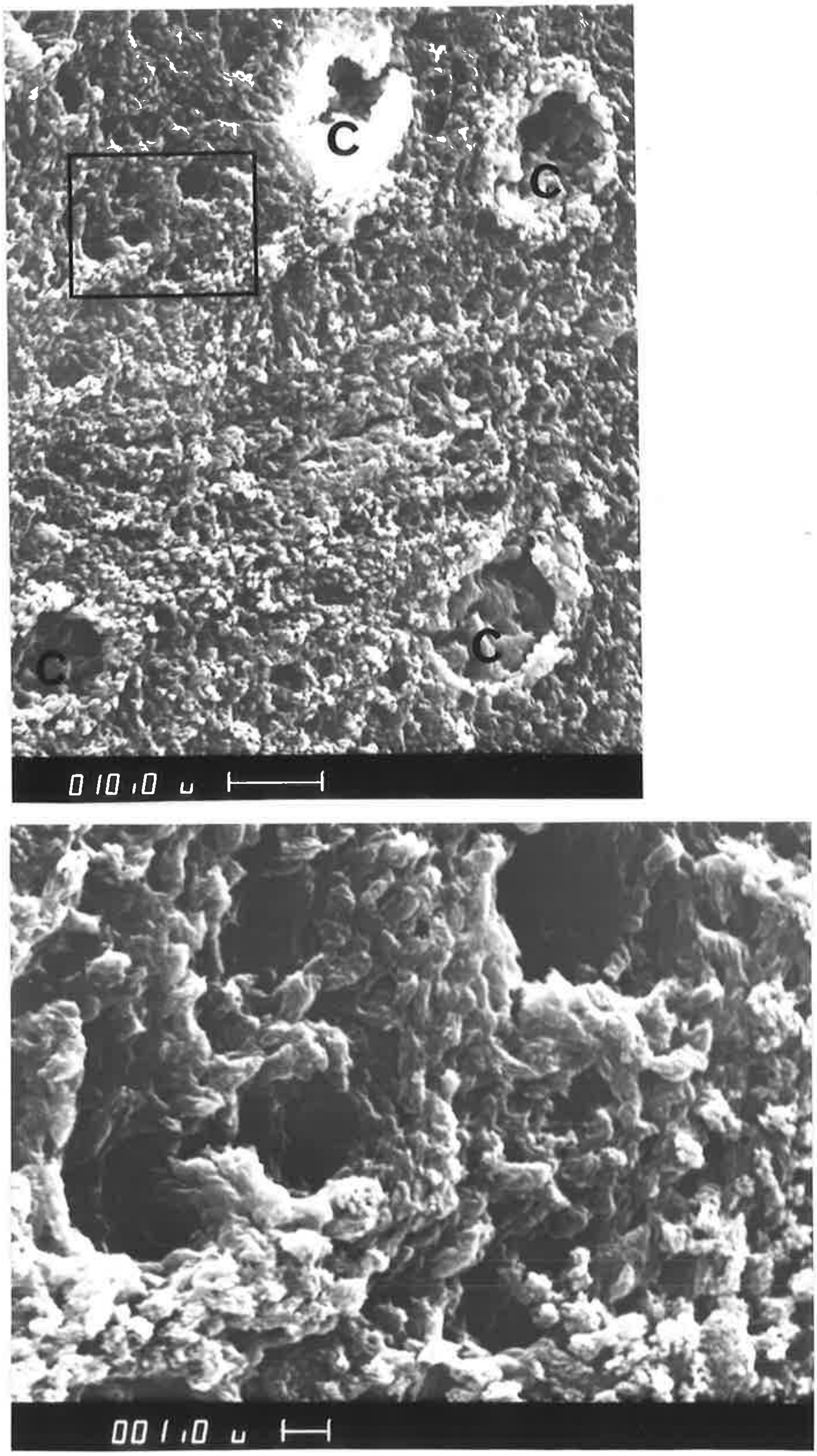


Figure 28

Patient G.B., retention period 17 weeks. Left Premolar.

Above: Typical surface of mineralizing repair cellular cementum from the buccal surface. Cell lacunae (C). X700

Below: Enlargement of the outlined area, with holes which are possibly Sharpey fibre holes. X3000

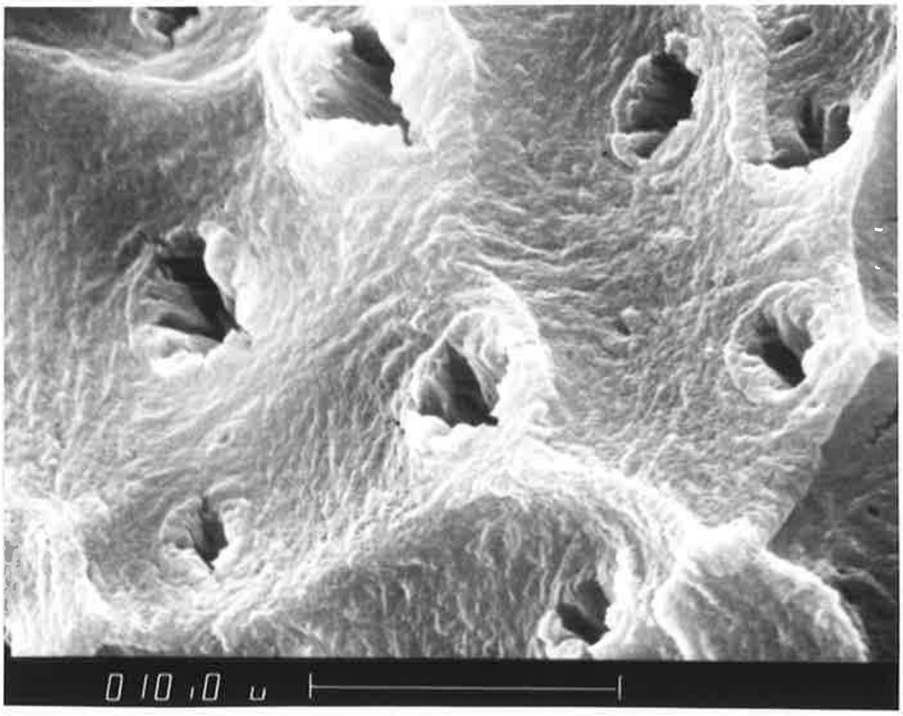


Figure 29

Patient G.B., right premolar, lingual surface.

Sharpey fibre holes exposed by resorption confined within cellular cementum near the apex, an unusual observation in the SEM. X2000

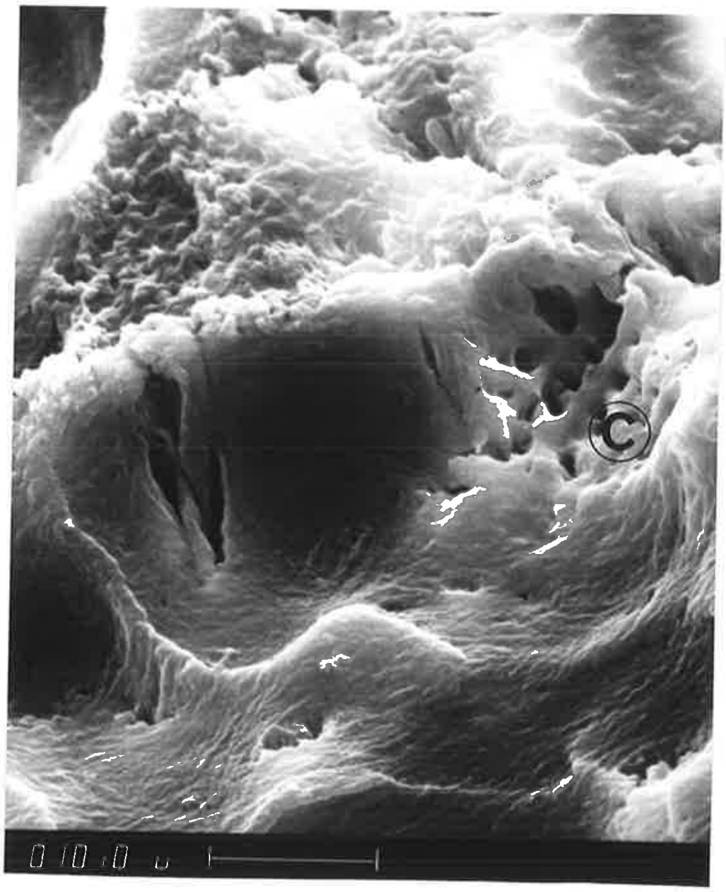


Figure 30

A cementocyte lacuna (C) exposed by resorption presents a smooth surface between canalicular openings. From the same area as Figure 29. X1200 that these holes represented principal periodontal fibre tracts rather than dentinal tubule openings as the resorption appeared to be confined to cementum at this level. In addition these openings were wider than dentinal tubules and were similar to some Sharpey fibre holes seen on intact cementum surfaces elsewhere (Figure 56). Cementocyte cell lacunae exposed by resorption were occasionally seen (Figure 30) and these had a smooth surface between their canalicular openings.

Cementoblast and cementocyte cell lacunae were identified topographically by their size which varied from 10 to 15 micrometres in diameter, circular to ovoid shape and the presence of canalicular openings. Smaller circular depressions were also found in the mineral front of the repair tissue on these specimens (Figure 28) but their distribution and occurrence did not follow any definable pattern. It was not possible to view the walls and floors of all of these depressions or holes but it was assumed that some were cell lacunae being covered over. Many, however, because of the well rounded shape, close proximity to each other, size and lack of observable canalicular openings were thought to represent principal periodontal fibre insertions sites.

Patient C.T.

Only the distobuccal segment of the right first premolar from patient C.T. was prepared for histologic examination. The remaining segments were examined in the SEM. The buccal surface of the left premolar exhibited extensive resorption along its entire length except for the cervical one quarter (Figure 31). The mesiobuccal segment of the right premolar, however, was affected only in its apical half to one third. The pattern of resorption and repair was similar to that

Figure 31

Patient C.T., retention period 17 weeks.
Left premolar.

Extensive resorption evident on this full buccal segment exemplifies the area of root surface involved by resorption on specimens in this study. X10 described for other specimens in this group. The mineral front of repair cellular cementum observed, showed a range from discrete mineral clusters without orientation to a more homogeneous surface.

The lingual surfaces of both teeth exhibited only minor resorptive damage both cervically and apically. In the apical region of the left premolar lingual surface, circular depressions of 4 to 7 micrometres diameter were observed (Figure 32) which were clearly distinct from cell lacunae, having a central opening from which radiated spoke-like extensions connected to the outer margin. These features were considered to be Sharpey fibre depressions presenting an unusual type of topography.

Patient W.J.

The entire buccal half of the right premolar from W.J. was examined in the SEM. The apex appeared to have been blunted slightly by resorption. Two large resorption areas and a number of smaller areas were seen on the buccal surface (Figure 33). Repair was a feature of the larger defects with resorption appearing to continue around their peripheries and also apically on the root. The smaller defects appeared to be continuing to resorb. The repair tissue was cellular cementum having a coarse fibrous surface texture arranged around circular depressions most of which were identified as cell lacunae (Figure 34). A number of the holes observed may have represented Sharpey fibre depressions but could not be definitely identified as such because their internal topography could not be seen.

The right buccal section was originally prepared with 10 minutes

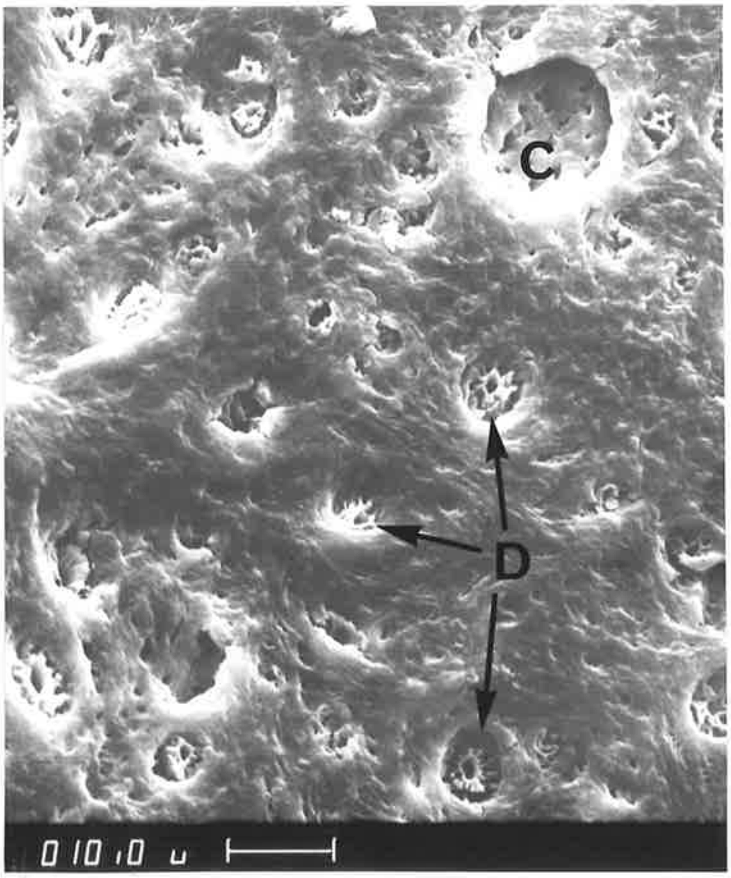


Figure 32

Patient C.T., left premolar, lingual surface.

Sharpey fibre depressions (D) of an unusual configuration within normal cellular cementum. Cell lacuna (C). X700

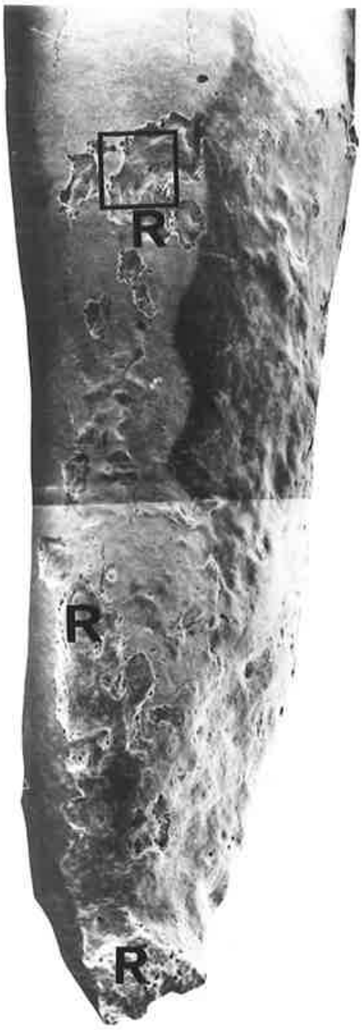


Figure 33

Patient W.J., retention period $17\frac{5}{7}$ weeks, right premolar.

Resorption (R) is evident over the length of the buccal surface and appears to have blunted the apex. Area outlined is shown in Figure 34. X10

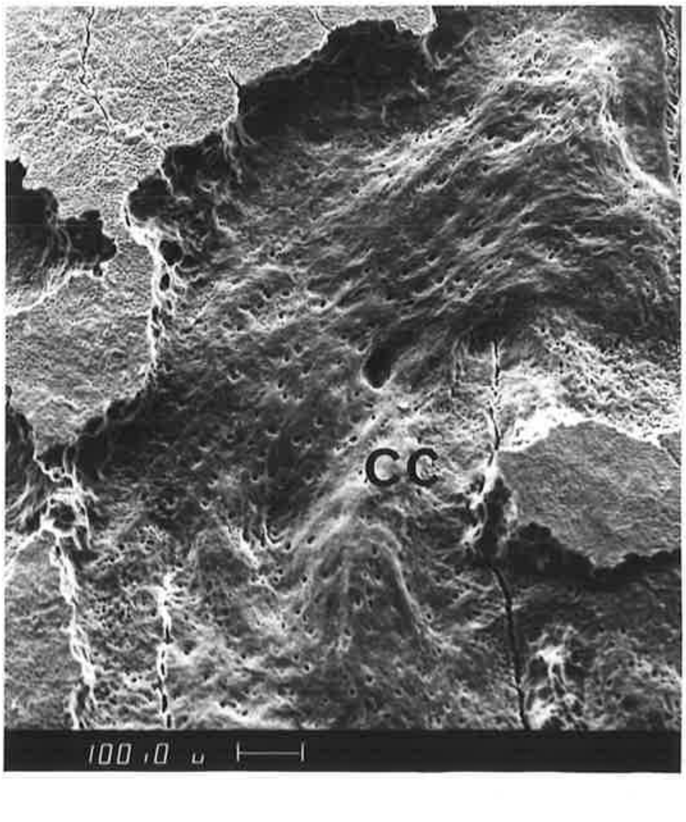


Figure 34

The extent of repair with cellular cementum (CC) in the area outlined in Figure 33 can be seen. X50 exposure to sodium hypochlorite (10-13%). After examination in the SEM, repreparation by exposure to 5% sodium hypochlorite for 48 hours followed by drying and recoating was undertaken. Most of the original conductive coating was removed by re-exposure to sodium hypochlorite. The repreparation increased the surface cracking due to drying and rendered the specimen more fragile and prone to damage and flaking of the surface with light knocks.

The surface topography was changed little by repreparation in some places, possibly where the first coating was not removed. Figure 35 shows two different cell lacunae from the same area of repair cementum in the large cervical resorption area. The fine fibrous texture seen prior to repreparation was lost after this procedure, due to increased deorganification. Similarly the normal acellular mound cementum became smoother in appearance after repreparation. Whether the organic matrix removed by repreparation was mineralized to any degree is uncertain.

The lingual surface exhibited relatively minor resorption, this being most extensive in the apical region. The pattern of resorption and repair was similar to that on the buccal surface.

The distobuccal segment of the left premolar had an uneven, undulating surface over most of its length. Resorption areas bounded by Howship's lacunae were observed (Figure 36), but these were smaller and fewer in number than on most of the other specimens in this group. The reason for the uneven surface was the presence of areas of cellular cementum some of which were found in the cervical half of the specimen. As this portion of the root is usually covered by acellular Sharpey

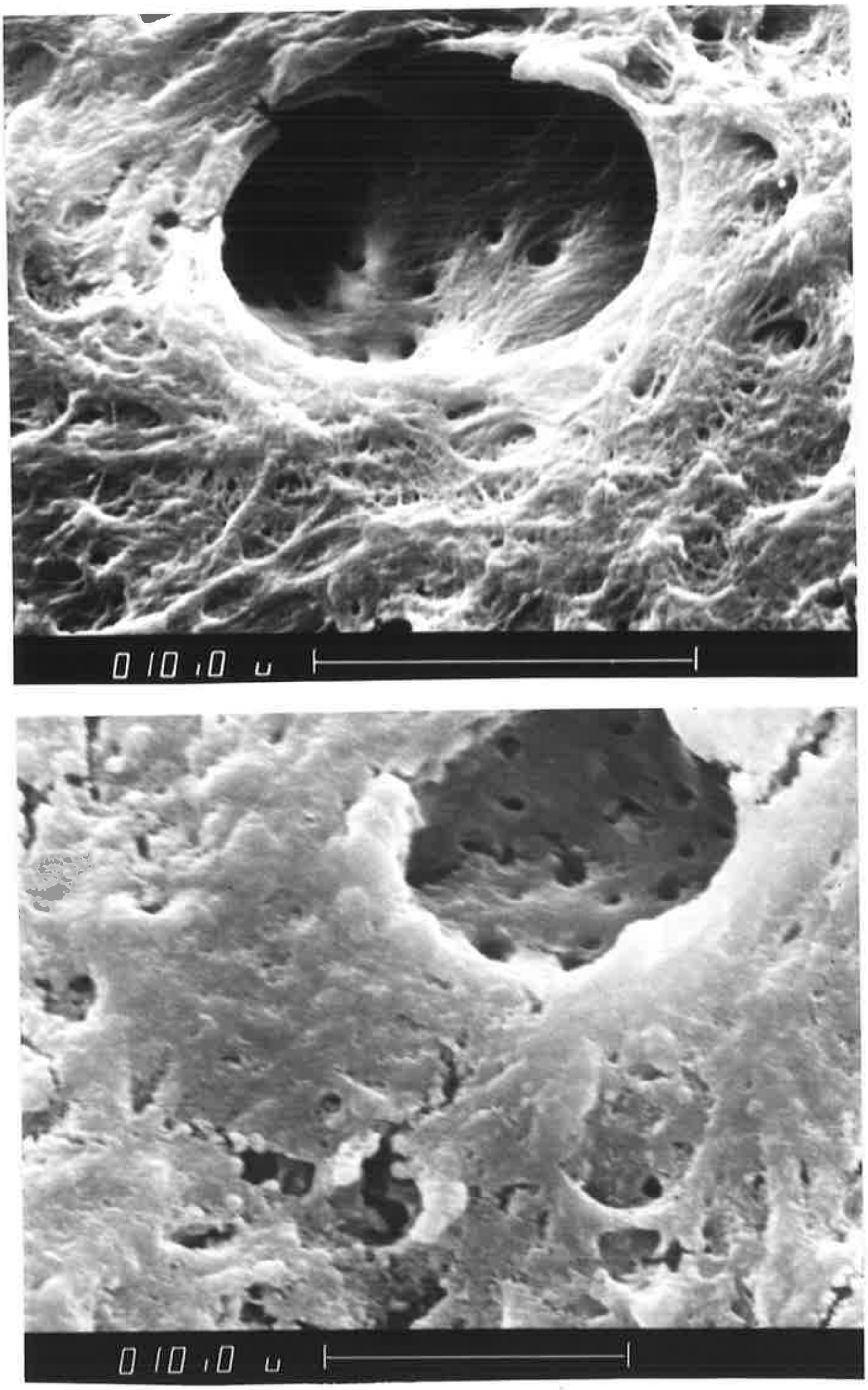


Figure 35

Two different cell lacunae from the same area of repair cementum are illustrated before (above, X2500) and after (below, X2000) specimen repreparation. The fine fibrous surface texture has been removed by repreparation.

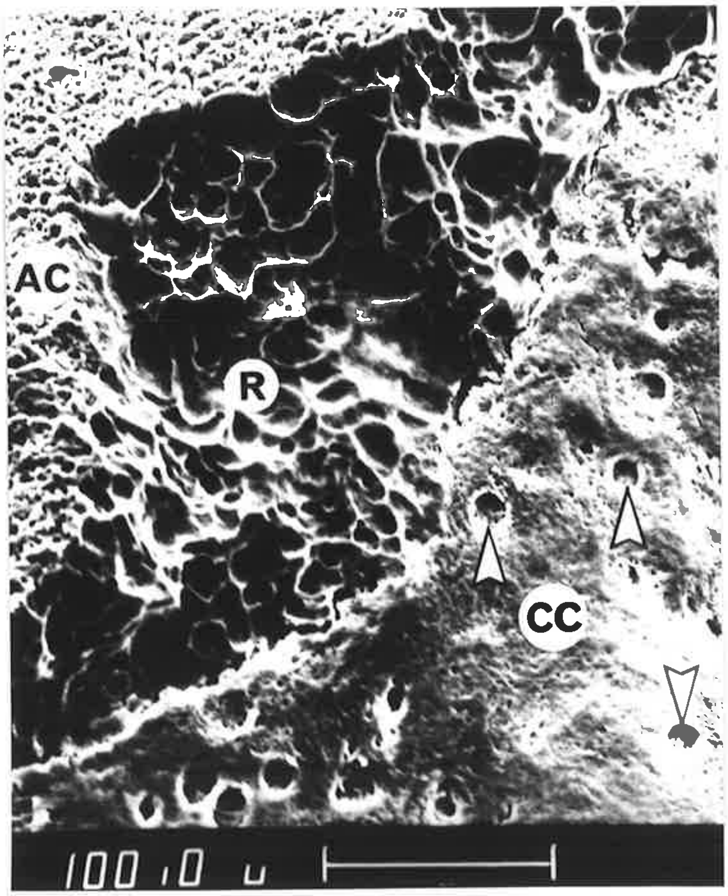


Figure 36

Patient W.J., retention period $17\frac{5}{7}$ weeks.

Normal acellular cementum (AC) can be seen falling away at the periphery of a resorptive defect (R). Repair with slowly mineralizing cellular cementum (CC) is occurring centrally in the defect, a common finding in this study. A number of cell lacunae are indicated by arrows. Left premolar, buccal surface. X110 fibre mound cementum it was considered likely that exuberant cellular cementum here represented a repair response. Sharpey fibre depressions were found in some areas of the exuberant cellular cementum on the root surface.

Resorption was not prominent on the lingual surface of the left premolar. One area in the cervical one third demonstrated two parallel ridges of cellular cementum approximately 500 micrometres long and 50 micrometres wide in a circumjacent surface of cellular cementum with Sharpey fibre holes. This total area was surrounded by normal acellular Sharpey fibre mound cementum and could thus have been a repair response, which had completely restored a resorptive defect,or a proliferative response to tension on the lingual side. The cellular cementum in the apical region of the lingual surface (Figure 37) exemplified the similarity of this tissue with human lamellar bone.

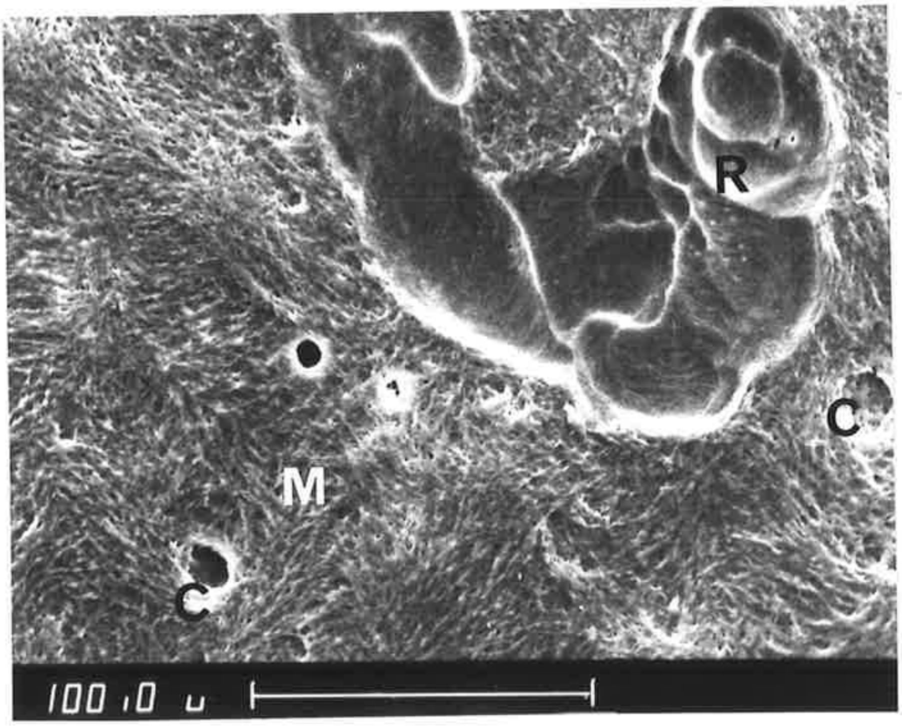


Figure 37

A surface of normal cellular cementum demonstrating the similarity of this tissue to adult lamellar bone. The intrinsic matrix (M) forms an interweaving pattern. Cell lacunae (C), Area of resorption (R). X200

Group 2

Patients in the second group were retained for periods varying between 20 and 30 weeks.

Patient K.B.

The left first premolar of patient K.B. was partially decalcified in formic formate for 48 hours prior to sectioning. This was done to assess whether additional information about reattachment of the soft tissue periodontal fibres could be obtained in this way without deorganification. It was found that this method afforded no useful information, even when the segment was examined from the flat sectioned face.

The right first premolar exhibited areas of resorption along its entire mesiobuccal segment. The surface of the Howship's lacunae and unaffected cementum was dominated by spherical structures as illustrated in Figure 38. The ubiquitous distribution of these nodules suggested that they were an artefact, but no reasonable explanation for their deposition could be proposed. Indeed, they closely resembled structures seen in a small number of other repairing resorption areas and on some areas of mineralizing acellular cementum found by Barber (1977) and also found occasionally in this study. Barber (1977) designated these structures as mineral calcospherites "building up" the surface, but apparently did not encounter them commonly. The size of the nodules was about 1 micrometre in diameter and their shape was spherical. They were not found on any other specimen in such profusion and the lingual surface of this tooth which was virtually unaffected by

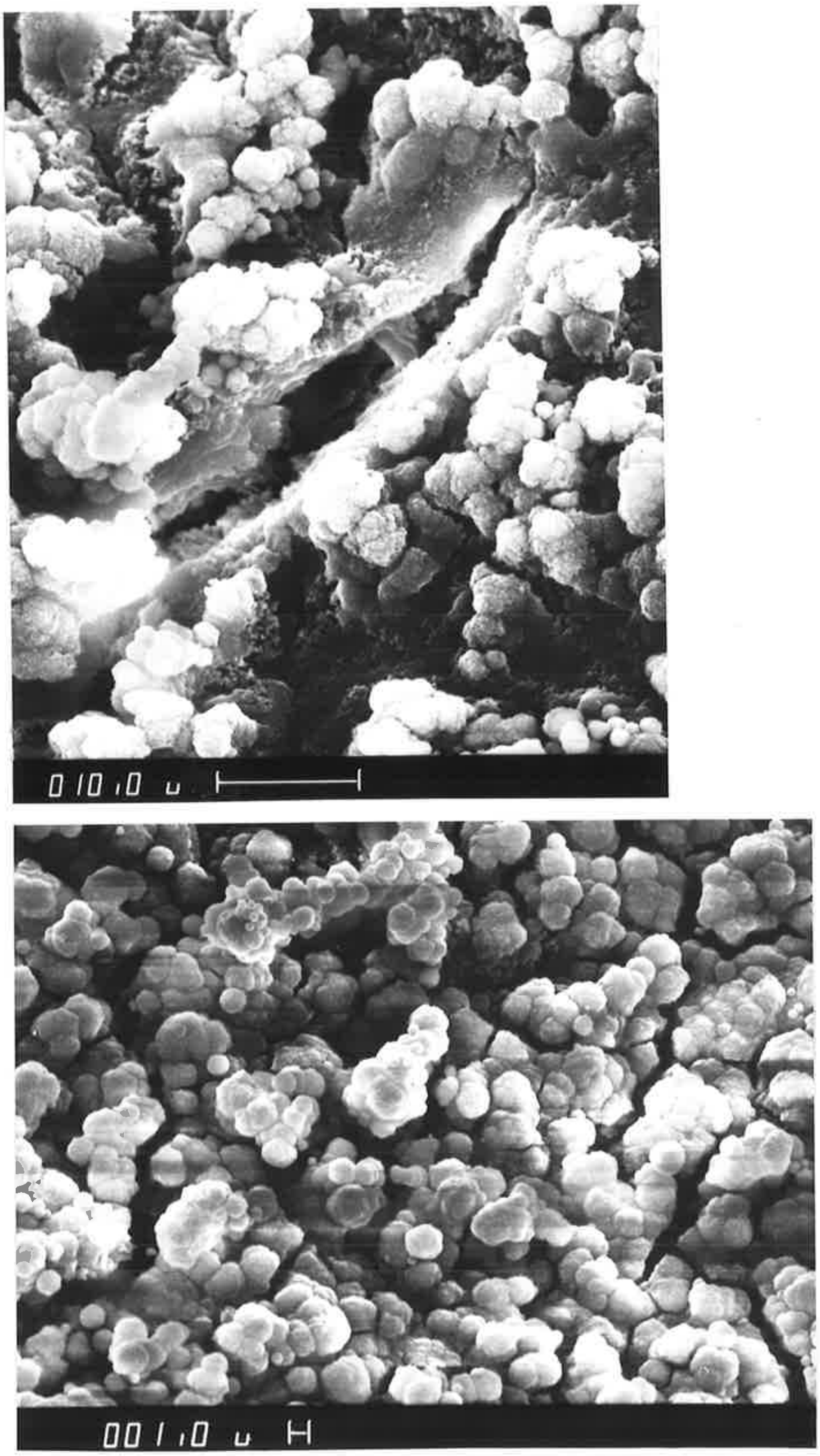


Figure 38

Patient K.B., retention period 20 weeks.

This nodular mineralizing topography, seen on the buccal surface of the right premolar, was not commonly encountered in the present study.

Above: In an area of resorption. X1000
Below: On normal acellular cementum. X1100

resorption did not exhibit them at all. The nodules did not correspond precisely to the description by Boyde and Hobdell (1969b) of the mineral particles at periosteal surfaces of primary bone or to the microcalcospherites in cementum illustrated by Boyde and Jones (1968). The former particles were irregular and about 1/3 micrometre in diameter and the latter more fusiform in shape.

The right first premolar mesiobuccal segment was reprepared in 5% sodium hypochlorite, recoated and examined again. Repreparation eliminated the spherical surface nodules found previously. Repair tissue observed in the resorptive defects was found to be cellular cementum having a flat surface topography between cell lacunae, indicating a slowly mineralizing surface.

Patient R.H.

The composite view of both the distobuccal and lingual segments of the left premolar shown in Figure 39 illustrate the extensive resorption found on the buccal as compared with the lingual surface. The right premolar was similarly affected by resorption except in the middle one third of the mesiobuccal segment where only two small defects were observed.

The topography of repair cellular cementum was variable and indicated differing rates of mineralization both within different resorptive defects (Figure 40) and occasionally within the same defect. In large areas of repair the more central surfaces were suggestive of slower mineralization, having a more confluent mineral front.

On all specimens in this investigation, at low magnification,

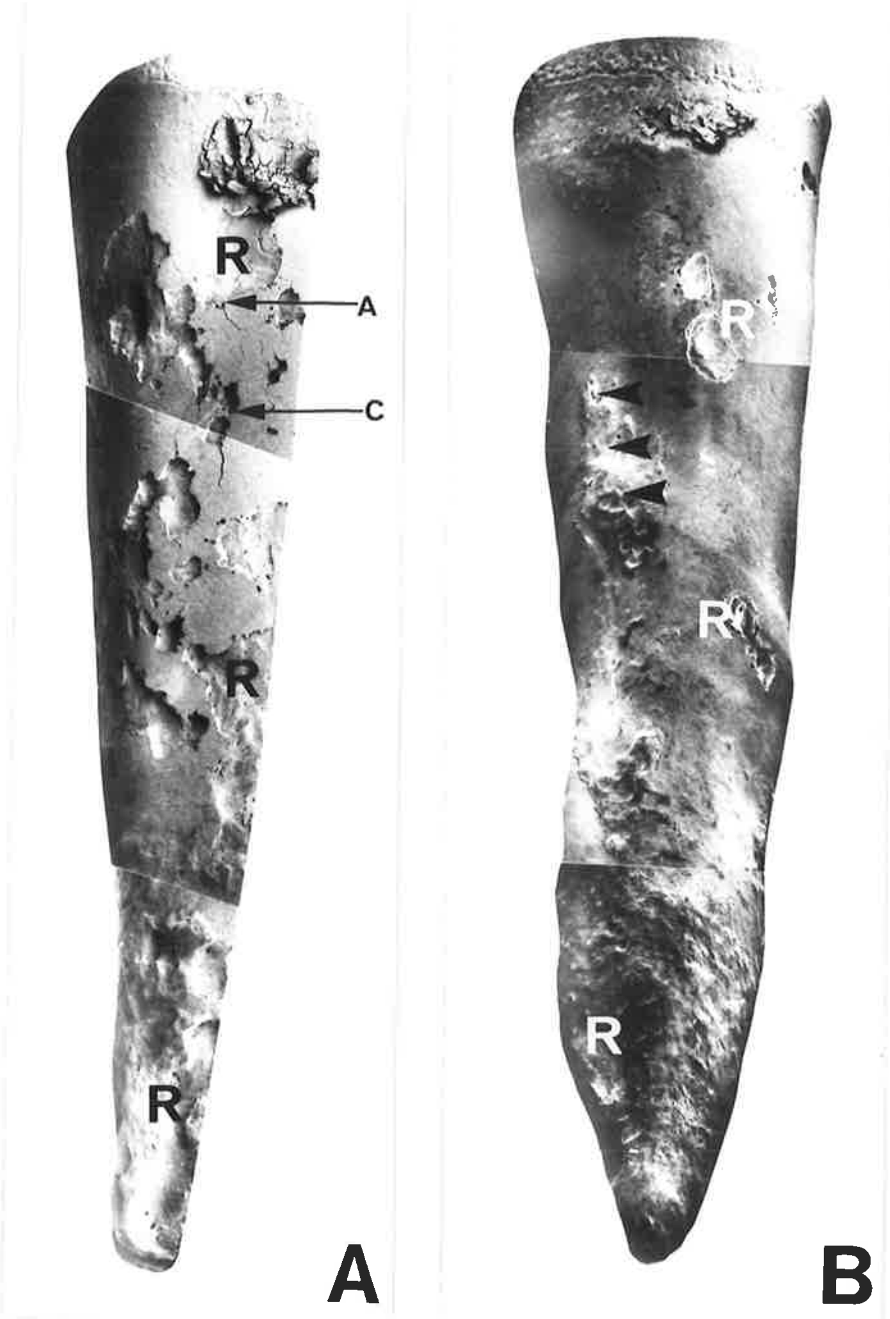


Figure 39

Patient R.H., retention period 21 weeks. Left premolar.

A: Distobuccal segment showing extensive resorption (R). A and C refer to the areas shown in Figure 40. X10

B: Lingual surface showing small areas of resorption (R). Arrows indicate an area of exuberant cellular cementum in a surface of normal acellular cementum. X10

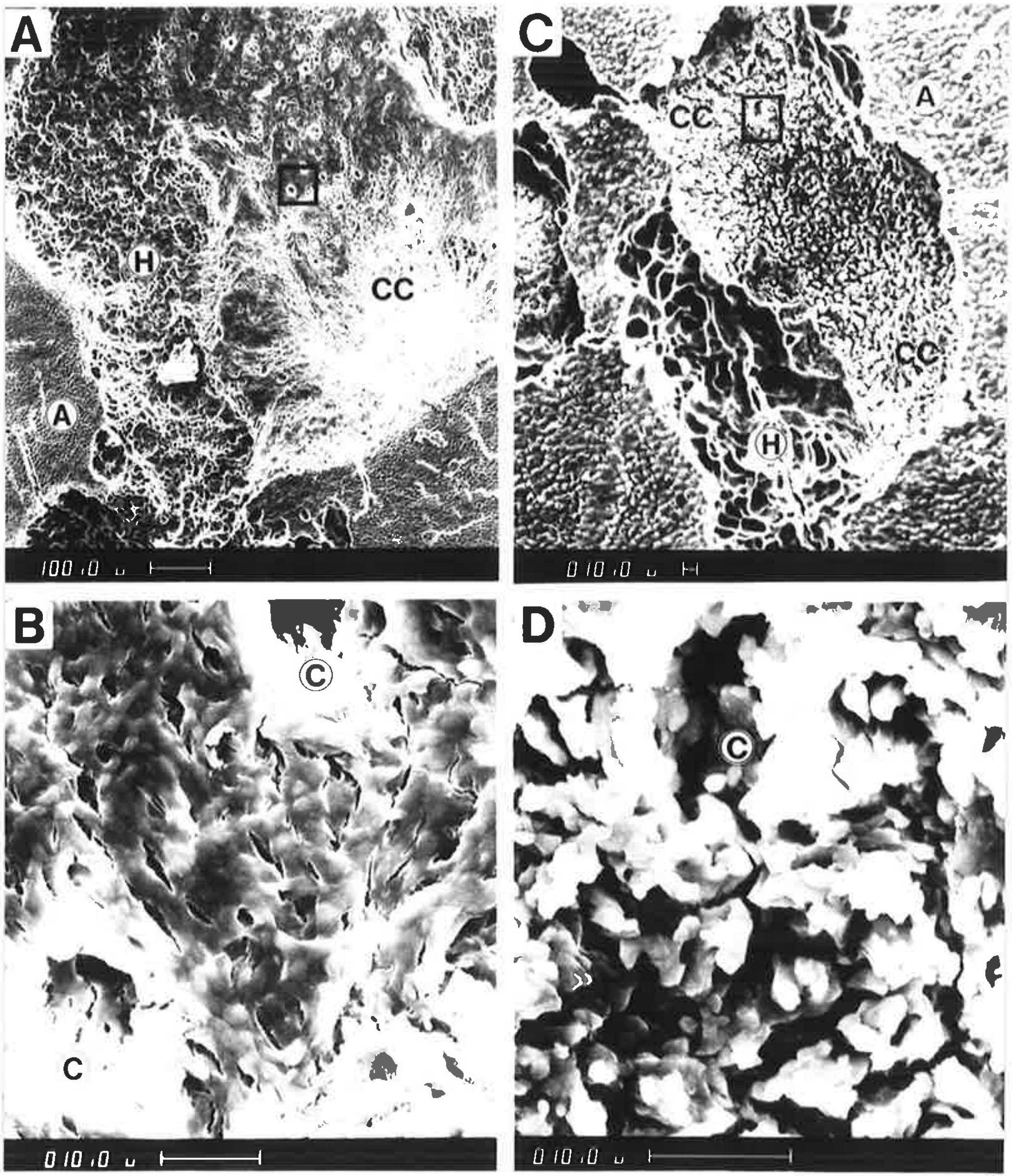


Figure 40

Comparison of repair cementum topography in different areas of resorption from the same specimen. A and C show areas of resorption from the locations indicated in Figure 39. Detail of the repair cellular cementum (CC) topography is shown in B and D respectively. In B, a flat topography suggests no mineralizing activity and in D a nodular surface indicates active mineralization. Cell lacunae (C), Multiple Howship's lacunae (H), Normal acellular cementum (A).

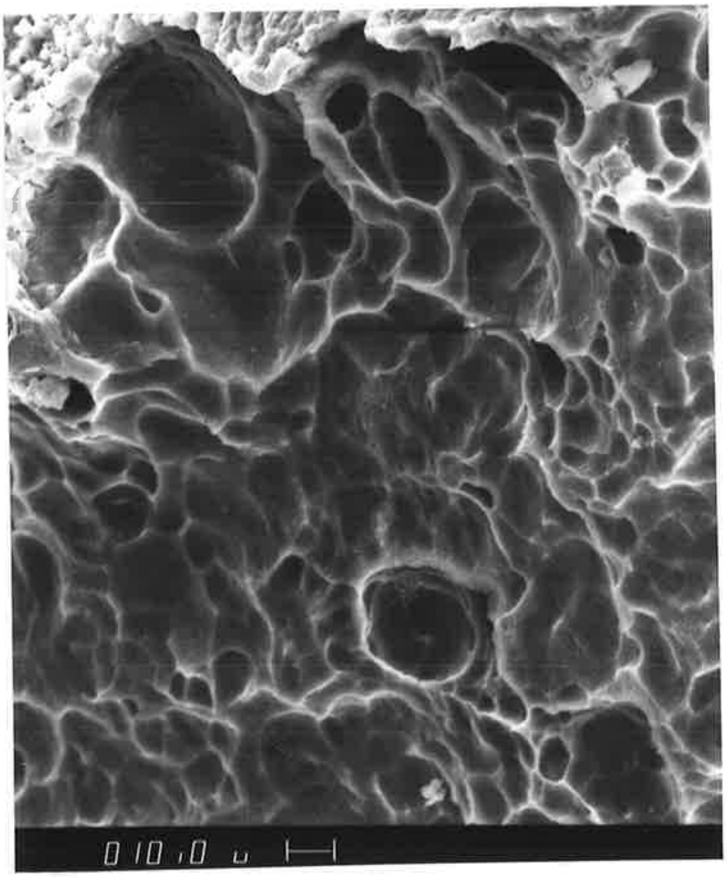
Howship's lacunae often appeared smooth but at higher power were seen to have a granular textured surface (Figure 41). The small holes within the Howship's lacunae were probably dentinal tubule openings although they did not demonstrate the resistance of peritubular dentine to resorption as reported by Barber (1978), Boyde and Lester (1967b), and Boyde and Jones (1972).

The extent of repair within resorptive areas on the specimens from patient R.H. was variable. It is possible that some defects may have completely repaired making their identification in the SEM as areas of previous resorption difficult. Sharp demarcation of different surface topographies such as exuberant cellular cementum surrounded by normal Sharpey fibre mound acellular cementum in the cervical half of a specimen may be indicative of such repaired areas or of a proliferative response to tension when found on the lingual side (Figure 39).

Sharpey fibre depressions providing evidence of periodontal fibre insertion into repair cementum were found in a number of locations. The position of the previous resorption on the root did not influence the occurrence of these depressions. Sharpey fibre depressions were generally found in repair cellular cementum which appeared to be rapidly mineralizing. An example of such depressions is shown in Figure 42, taken from repair tissue at the edge of a large cervical resorption area on the buccal aspect of the right premolar. Cell lacunae were not numerous in areas of Sharpey fibre depressions.

Patient R.E.

The pattern of resorption was similar on both first premolars from this patient. The buccal segment specimens exhibited large areas



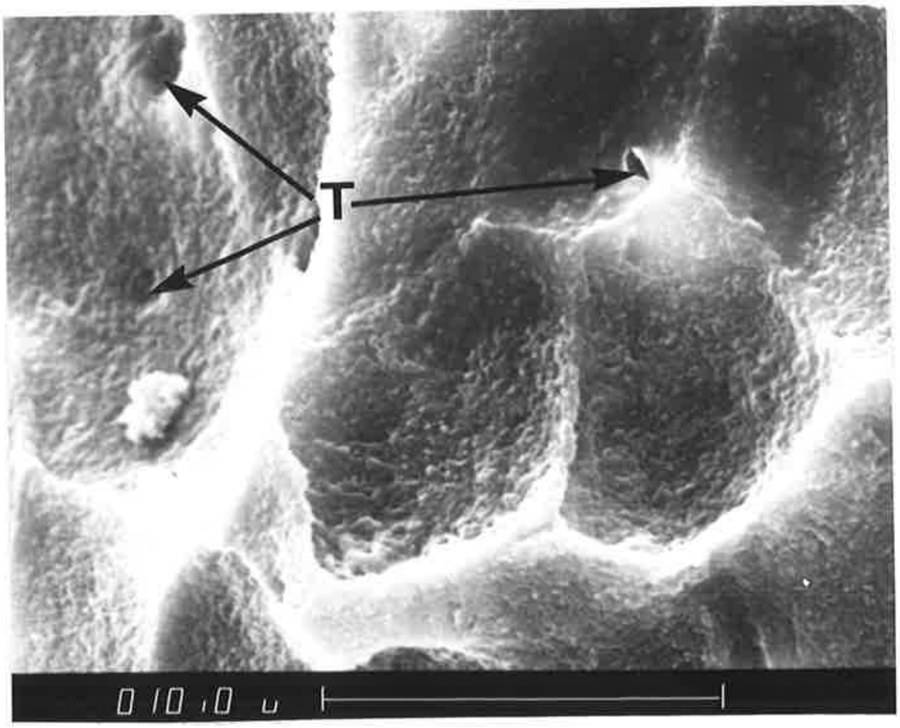


Figure 41

Patient R.H., right premolar, lingual surface.

Above: Howship's lacunae seen at low magnification in the SEM often showed a smooth surface similar to this. X300

Below: Higher magnification revealed a granular textured surface in Howship's lacunae. Small holes (T) are probably dentinal tubule openings as the resorption illustrated had penetrated cementum. X2600

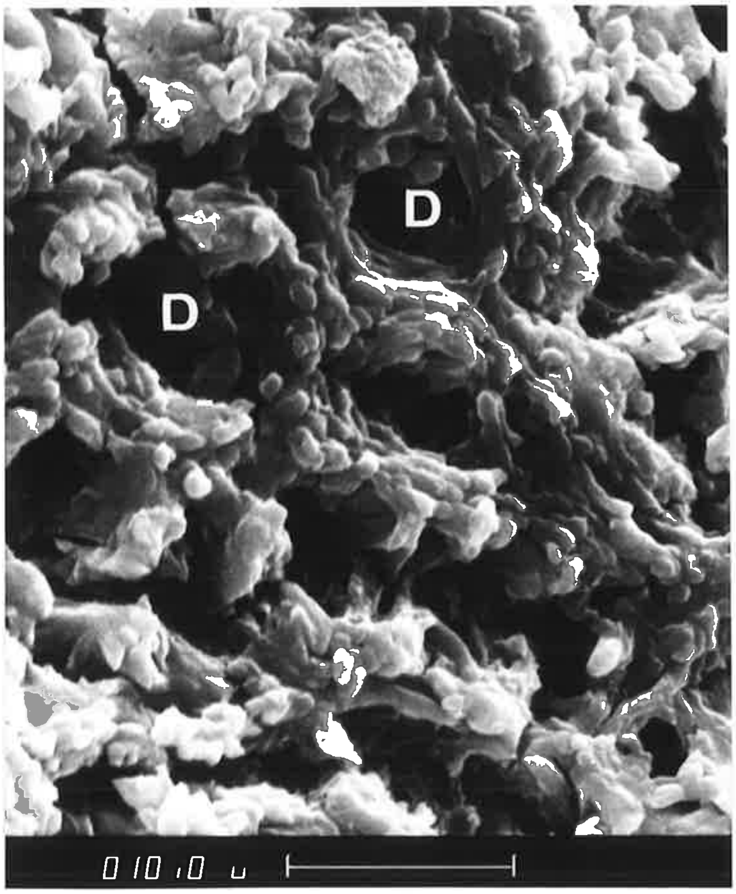


Figure 42

Depressions (D) in the mineral front of repair cellular cementum from a large cervical resorption area on the buccal surface of the right premolar. Patient R.H. These depressions resemble principal fibre bundle insertion sites. X1500 of resorption in the cervical and apical one third but little apparent damage in the middle one third. The lingual surfaces were almost unaffected by resorption, minor defects being observed only in the cervical and apical areas.

Repair with cellular cementum had advanced to various levels in most of the resorption areas observed. Howship's lacunae dominating the perimeter of large resorptive defects had smooth or finely granular floors and sharp to slightly rounded outlines. The topography of the repair tissue was generally characteristic of slowly mineralizing cellular cementum (Figure 43). The cell lacunae had smooth surfaces between canalicular openings and the surrounding mineral front was confluent. A small number of repairing areas appeared to be more rapidly mineralizing as shown by discrete mineral nodules which were arranged in an apparently random fashion between cell lacunae (Figure 43). The internal surfaces of cell lacunae reflected the activity of the mineral front also (Figure 43).

The unaffected cementum surface, both lingual and buccal, on the teeth from patient R.E. were dominated by acellular cementum except in limited areas of the apical one quarter where cellular cementum prevailed. The type of acellular surface demonstrated by Figure 44, with mounds of irregular outline separated relatively evenly by a fibrous textured surface, was dominant in the cervical two thirds of these teeth. This type of surface was not uncommon on other teeth in this investigation, and as such has been considered to be a normal manifestation of acellular Sharpey fibre mound cementum.

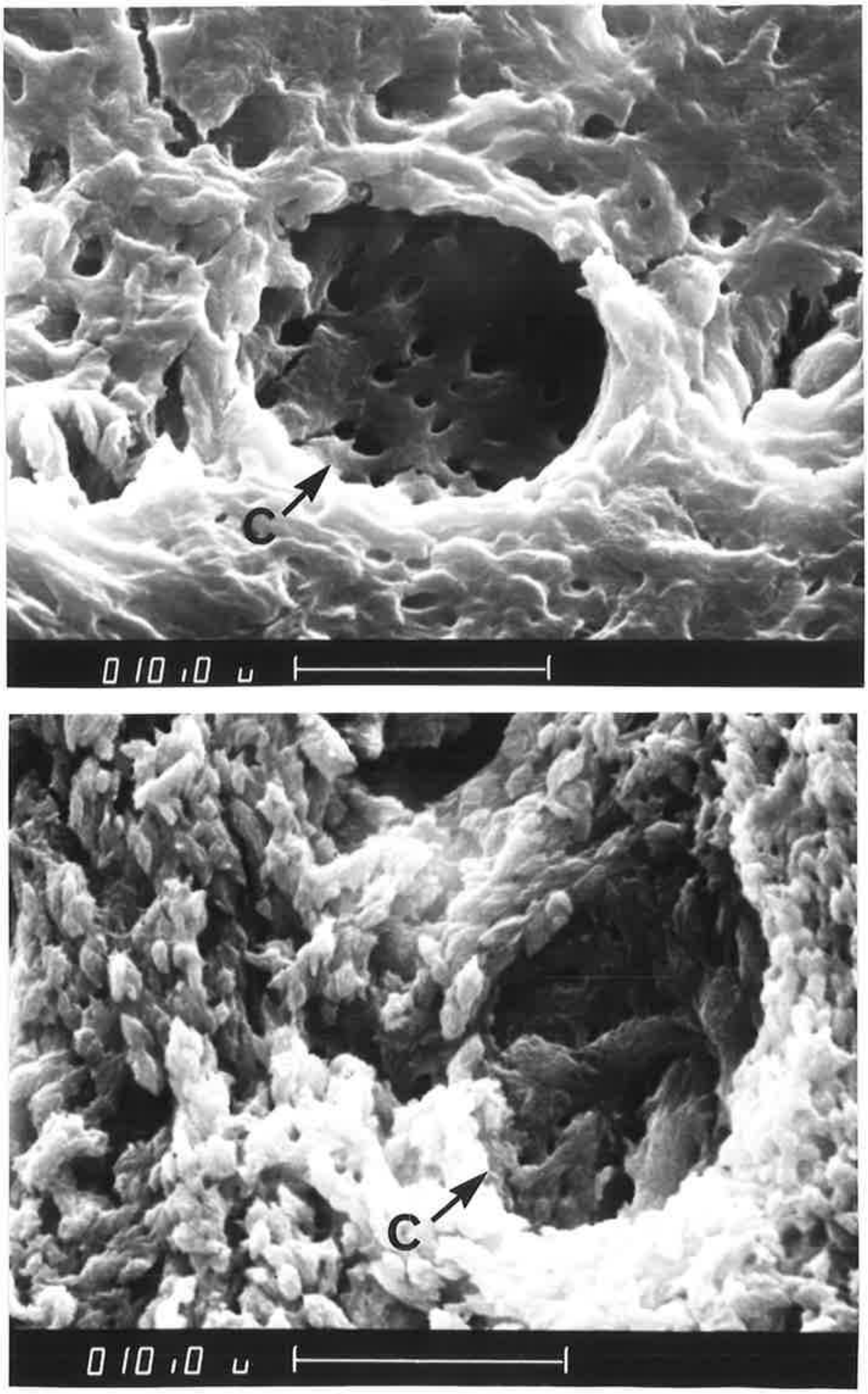


Figure 43

Patient R.E., retention period $21\frac{4}{7}$ weeks. Right premolar.

Cell lacunae (C) from repair tissue illustrating different surface topographies suggestive of different rates of mineralization.

Above: Slow or resting mineralizing activity is indicated by a confluent surface. X1700

Below: Discrete mineral particles indicate active mineralization. X1700

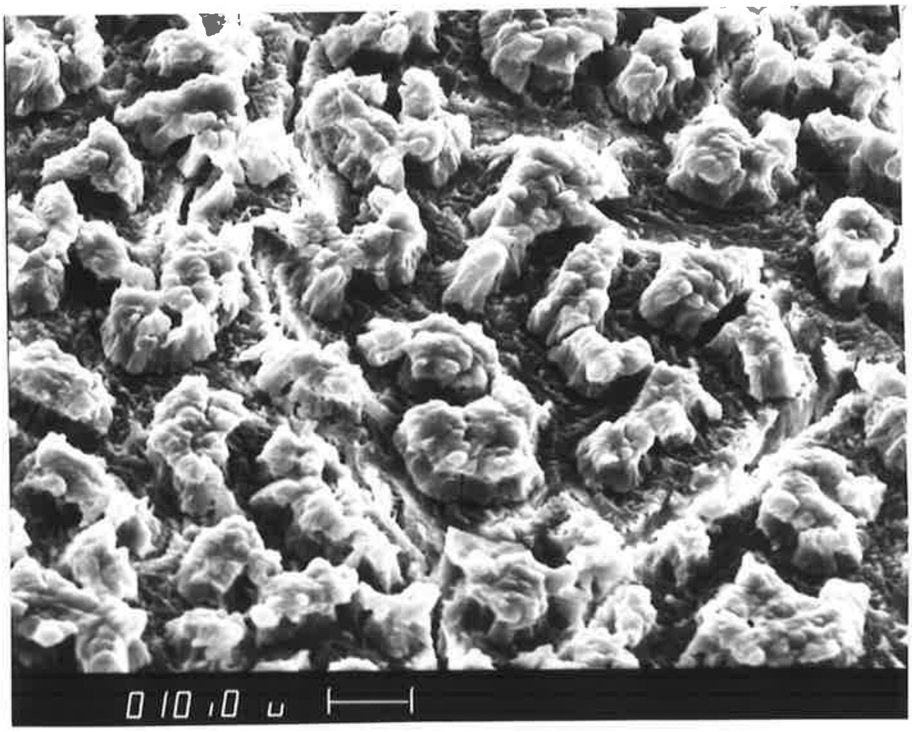


Figure 44

Acellular cementum topography typical of the cervical two thirds of the premolars from patient R.E. Raised areas are separated by a surface with a fibrous texture which is probably cementum matrix. X500

Patient S.W.

The buccal surface of first premolars from patient S.W. showed the effects of resorption most extensively in the apical half. The lingual surfaces were largely unaffected except for the apical region where minor resorptive activity was apparent.

On the buccal specimens, repair by cellular cementum was relatively more extensive in area than on specimens retained for shorter time periods, but exhibited a similar range of topography. Extensive areas of open Howship's lacunae occurred only in the apical region of these first premolars. Repair in many areas appeared to be close to re-establishing root contour and in a number of areas had slightly exceeded the surrounding surface level (Figure 45). Where no Howship's lacunae remained to define an area as previously resorbed, a number of features could be used to detect repaired defects. Firstly, discrete, well defined areas of cellular cementum surrounded by normal acellular cementum were likely to be repaired resorption areas, particularly if the levels of the cellular cementum and surrounding cementum differed slightly. In addition, examination of the sectioned edge of the root segment in the SEM revealed a characteristic reversal line at the limit of resorption, where the plane of section passed through the lesions. The area of cellular cementum in Figure 45 was designated as repair by both the preceding criteria.

Figure 46 shows Howship's lacunae near the apex of the left premolar on the buccal surface. The edges of the resorptive lacunae seen in this figure are rounded over by a nodular deposit thought to be due to mineralizing activity. Such a deposit was not commonly observed

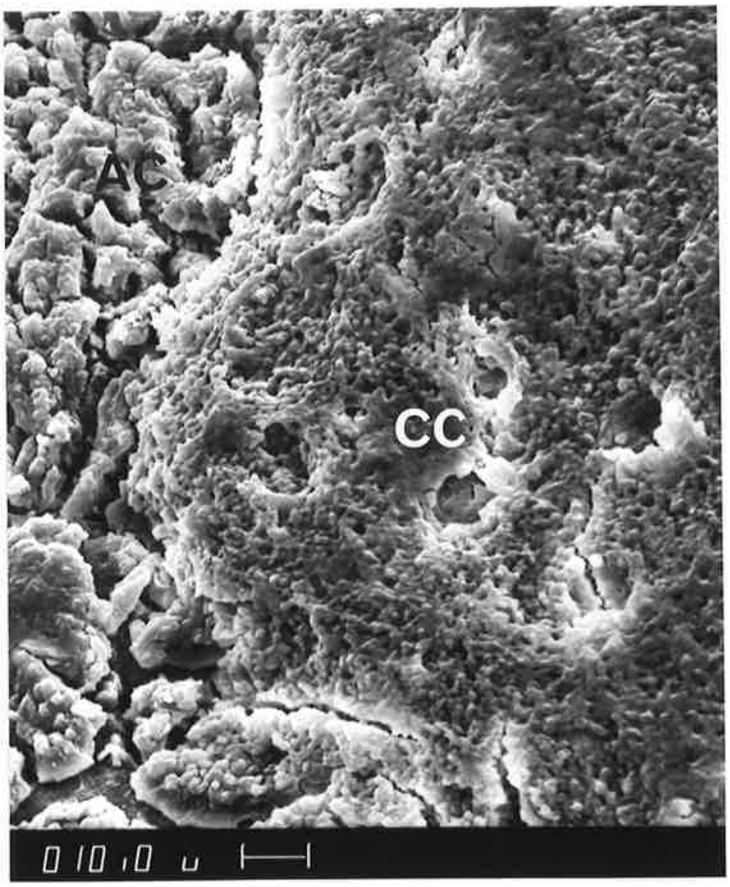


Figure 45

Patient S.W., retention period $22\frac{3}{7}$ weeks.

Repair cellular cementum (CC) has slightly exceeded the level of adjacent acellular cementum (AC). Buccal surface of the left premolar. X400

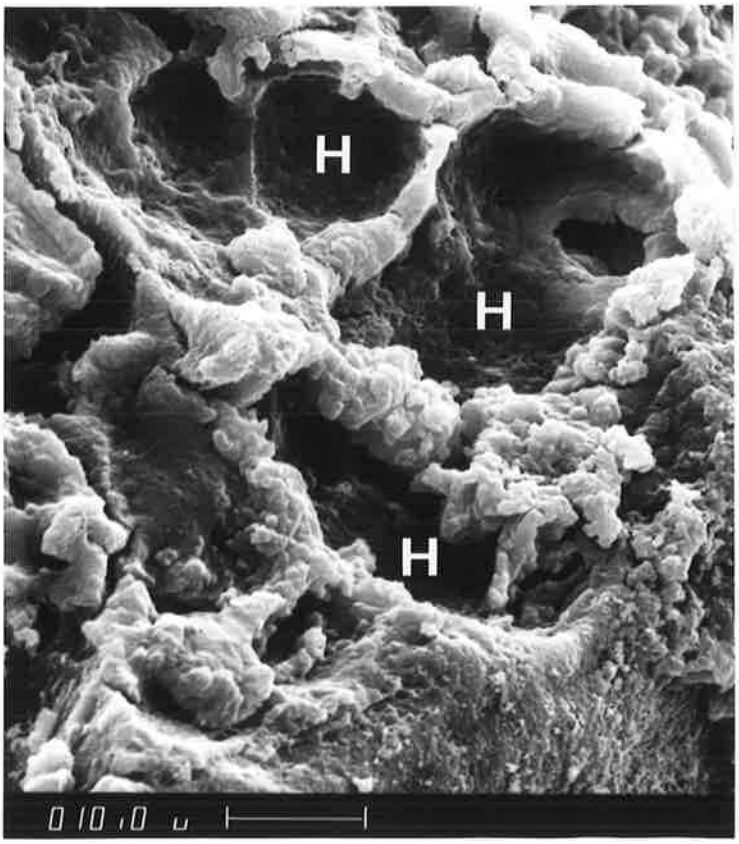


Figure 46

The margins of these Howship's lacunae (H) are rounded by a nodular deposit in a manner not frequently encountered. This deposit probably represents mineralizing activity. X1000 in this study. Limited surfaces within resorptive defects elsewhere on this specimen also showed a nodular deposit similar to that observed on the right premolar of patient K.B.

Patient L.B.

The mesiobuccal segment of the left first premolar was extensively resorbed in the apical one third and two large areas of resorption were found in the cervical half. Repair was occurring by apparently slowly mineralizing cellular cementum which was not advanced within any of the large resorptive areas. The Howship's lacunae generally had slightly rounded edges with smooth floors making a definitive interpretation of their state of activity difficult. Along the sectioned edge, exuberant cellular cementum was found (Figure 47) adjoining acellular Sharpey fibre mound cementum in the cervical half of the root. Within this cellular cementum Sharpey fibre holes were found (Figure 48). It is possible that this tissue was repair cementum. Although examination of the sectioned edge did not show a resorptive reversal line at the level of this feature, histological examination of the distobuccal segment revealed extensive resorptive lesions corresponding to this cellular cementum observed topographically. Histologically, repair cellular cementum in these resorptive defects appeared to have nearly re-established root contour and thus the cellular cementum observed adjacent to the section edge at this level in the SEM probably represents exuberant repair tissue at the margin of areas of repaired resorption.

After initial recording in the SEM this specimen was treated with 5% sodium hypochlorite for 48 hours, dehydrated and recoated with

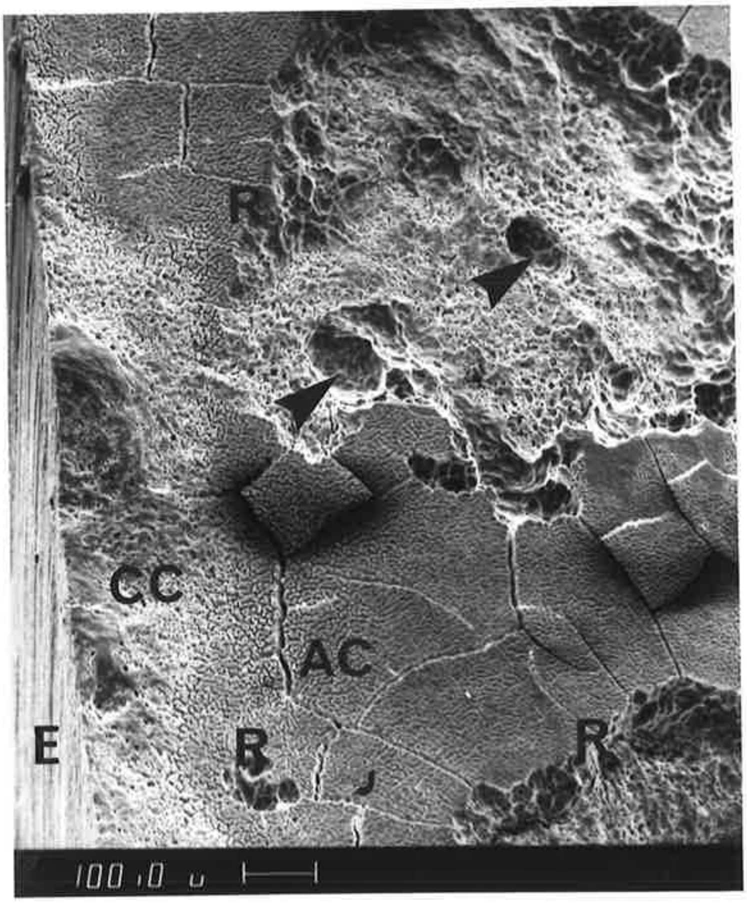


Figure 47

Patient L.B., retention period $25\frac{4}{7}$ weeks. Left premolar.

Three areas of resorption (R) and a region of exuberant cellular cementum (CC) can be seen adjacent to the section edge (E). The arrows in the resorption area at the top of the illustration indicate small areas of resorption in the slowly mineralizing repair cementum in this lesion. The normal acellular cementum (AC) exhibits surface cracks due to drying. X50

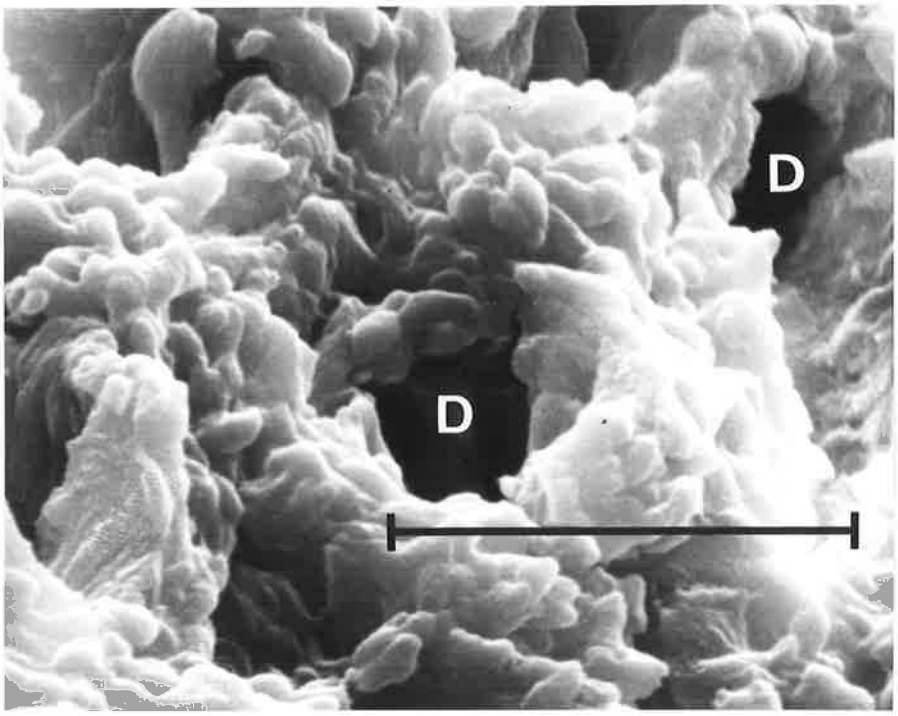


Figure 48

Sharpey fibre depressions (D) from the exuberant cellular cementum along the section edge seen in Figure 47.
Scale bar: 10 micrometres. X3100

carbon and gold-palladium. Subsequent examination in the SEM revealed that large areas of acellular cementum in the cervical region had flaked off leaving a featureless surface exposed. Some of the remaining acellular cementum exhibited cracking while other areas appeared similar to those seen prior to repreparation. Needle shaped, crystal-like artefacts identical to those seen on an early test specimen (Figure 18) were found dispersed over the entire surface, including the exposed section edge of the specimen. This reinforced the interpretation of these as artefacts, probably sodium hypochlorite crystals. The main topographical features were otherwise unaltered by repreparation.

The lingual surfaces of both premolars were largely unaffected by resorption. Both apices appeared blunted but only the right tooth exhibited resorptive Howship's lacunae at the apex.

The mesiobuccal segment of the right premolar showed more extensive resorption than seen on the left but only in the apical region were large areas with open Howship's lacunae found. Elsewhere repair was well advanced, generally occurring with rapidly mineralizing cellular cementum especially at the advancing periphery of repair tissue. Root contour was nearly re-established in many areas and in some the repair was beginning to over-contour the surface (Figure 49). Recognition of such areas as repair was afforded by following the outlines of resorptive areas, indicated by Howship's lacunae persisting at the periphery of the lesions in some locations. Figure 50 shows Sharpey fibre depressions found in the repair cementum seen in Figure 49.

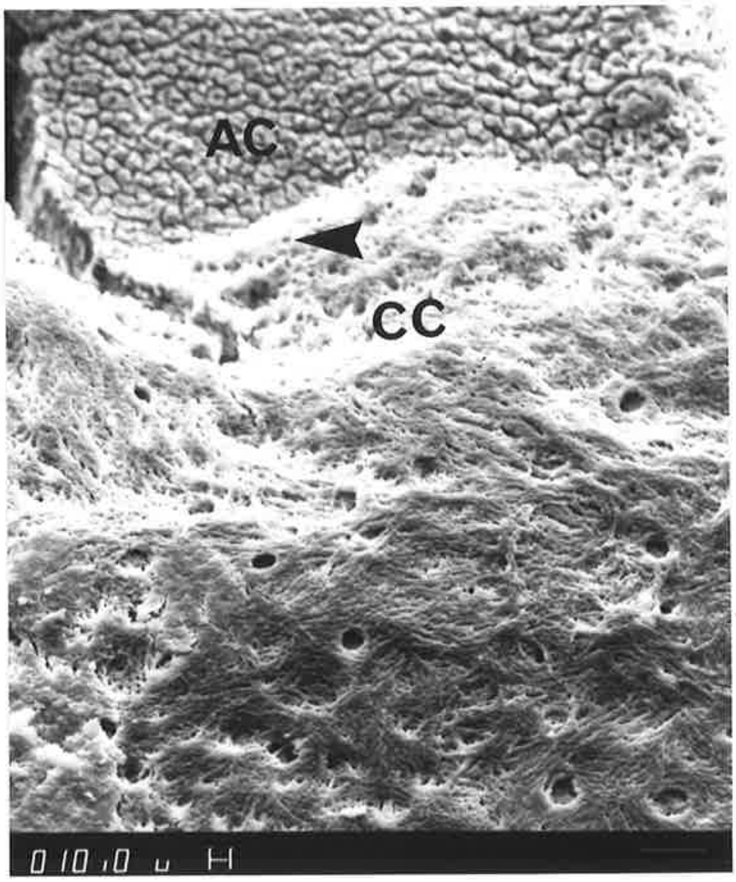


Figure 49

Repair cellular cementum (CC) beginning to cover normal acellular cementum (AC) in the middle third of the buccal surface of the right premolar, patient L.B. Arrow shows the area from which Figure 50 is taken. X100

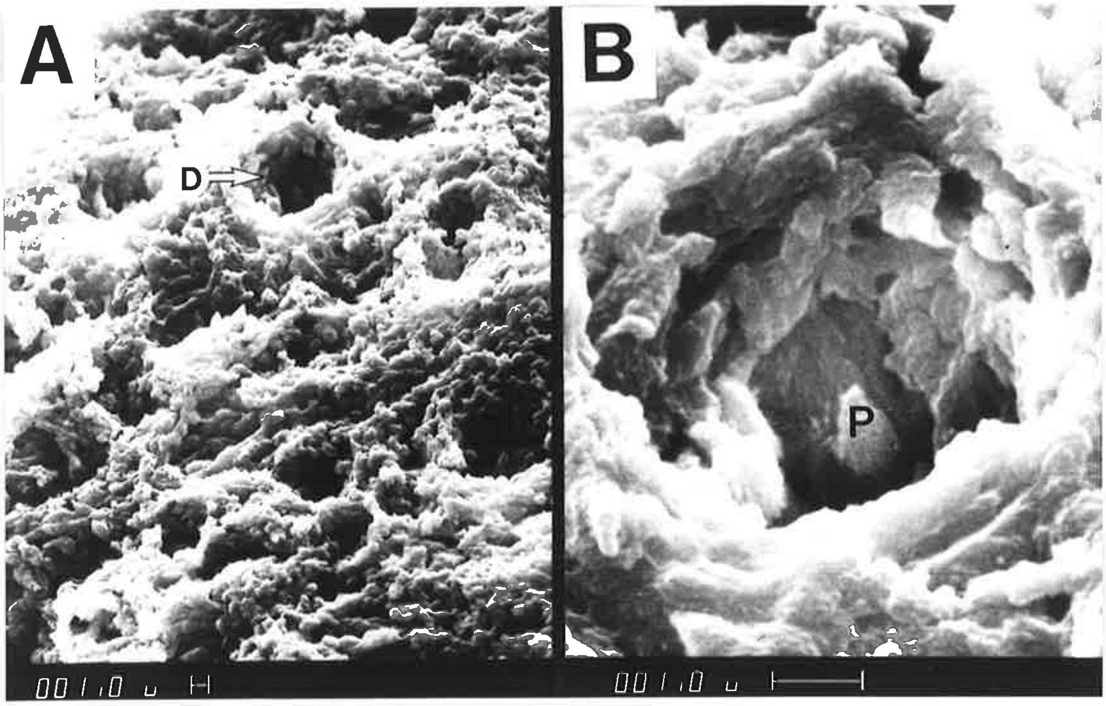


Figure 50

Patient L.B., right premolar, mesiobuccal segment.

A: Sharpey fibre depressions at the periphery of the repair cellular cementum shown in Figure 49. Such depressions were not a common finding in repair tissue and most often occurred at the rapidly mineralizing periphery as illustrated here. The depression marked D is shown in B at higher magnification. X2000

B: This Sharpey fibre depression shows distinct mineral particles in its wall and a small projection (P) from the floor. X7000

Patient A.S.

Both premolars from this patient were dominated by cellular cementum in their apical halves. This was sharply demarcated from the cervical acellular cementum and had the characteristic rough surfaced appearance at low magnification. In some locations, particularly towards the mid-root area, the cellular cementum appeared to be mineralizing over acellular cementum. This was demonstrated by islands of acellular cementum surrounded by apparently exuberant cellular cementum.

Histological examination of the mesiobuccal segment of the left premolar showed an area of resorption in the mid-root region repairing with cellular cementum. The repair tissue was exuberant, overcontouring the root surface and covering adjacent acellular cementum (Figure 51). Such repair activity may explain in part the topographic observation of cellular cementum covering acellular cementum. In addition, normal cellular cementum could be observed covering acellular cementum at the coronal extremity of the cellular cementum, in histological sections. The light microscope examination confirmed that repair was well advanced on the specimens from patient A.S.

In the SEM, the lingual surfaces showed little sign of resorptive damage. The distobuccal segment of the left premolar was not as heavily affected by resorption as the mesiobuccal segment of the right tooth. Active resorption was found at the apex in both teeth and at the periphery of resorptive defects. Repair was generally well advanced with cellular cementum and in some areas had overcontoured the root surface (Figure 52). The topography of repair cellular cementum was

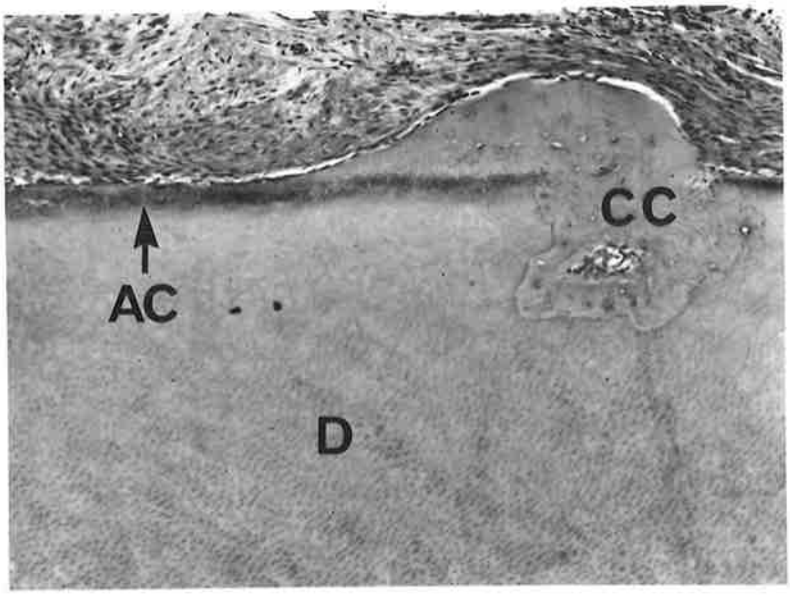


Figure 51

Patient A.S., retention period 29 weeks.

Histologic appearance of exuberant repair cellular cementum (CC) which has overcontoured the surface and covered adjacent acellular cementum (AC). Dentine (D). Left premolar, mesiobuccal segment.
Stain: Haematoxylin and eosin. X100

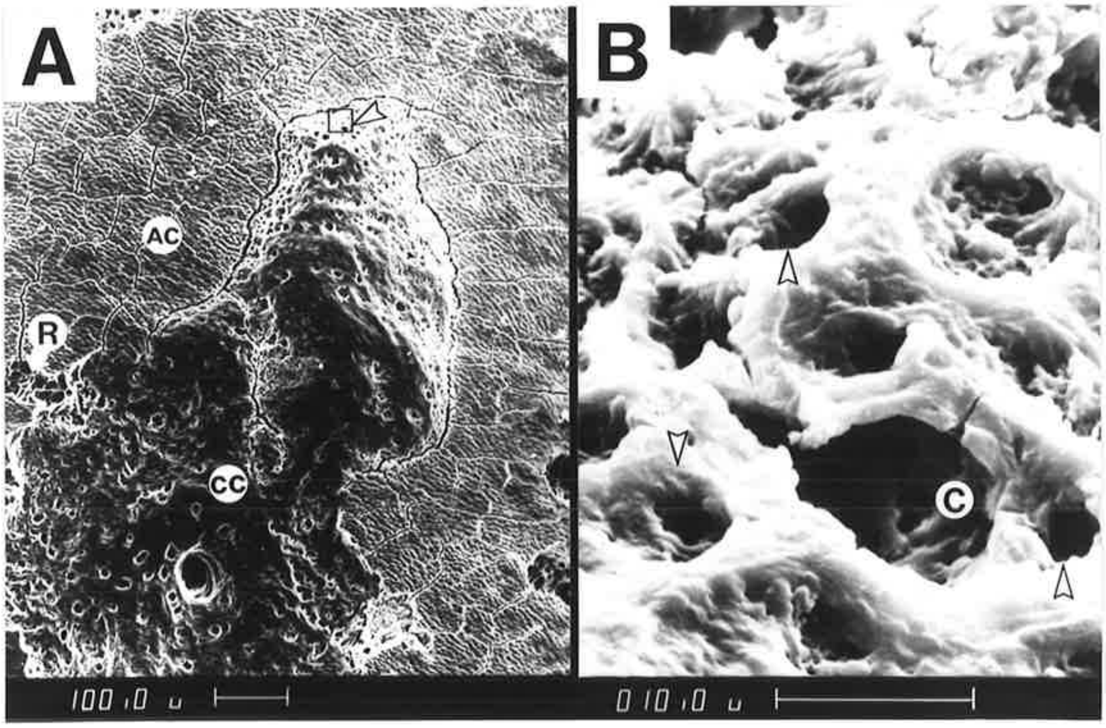


Figure 52

Topographic appearance of an area of exuberant repair cellular cementum (CC) from the distobuccal segment, left premolar, Patient A.S.

A: Resorptive Howship's lacunae can be seen at R. Normal acellular cementum (AC). X60

B: Area of repair cementum outlined and arrowed in A with a cell lacuna (C) and depressions (arrowed) which may be Sharpey fibre depressions. Surface suggests lack of mineralizing activity. X300

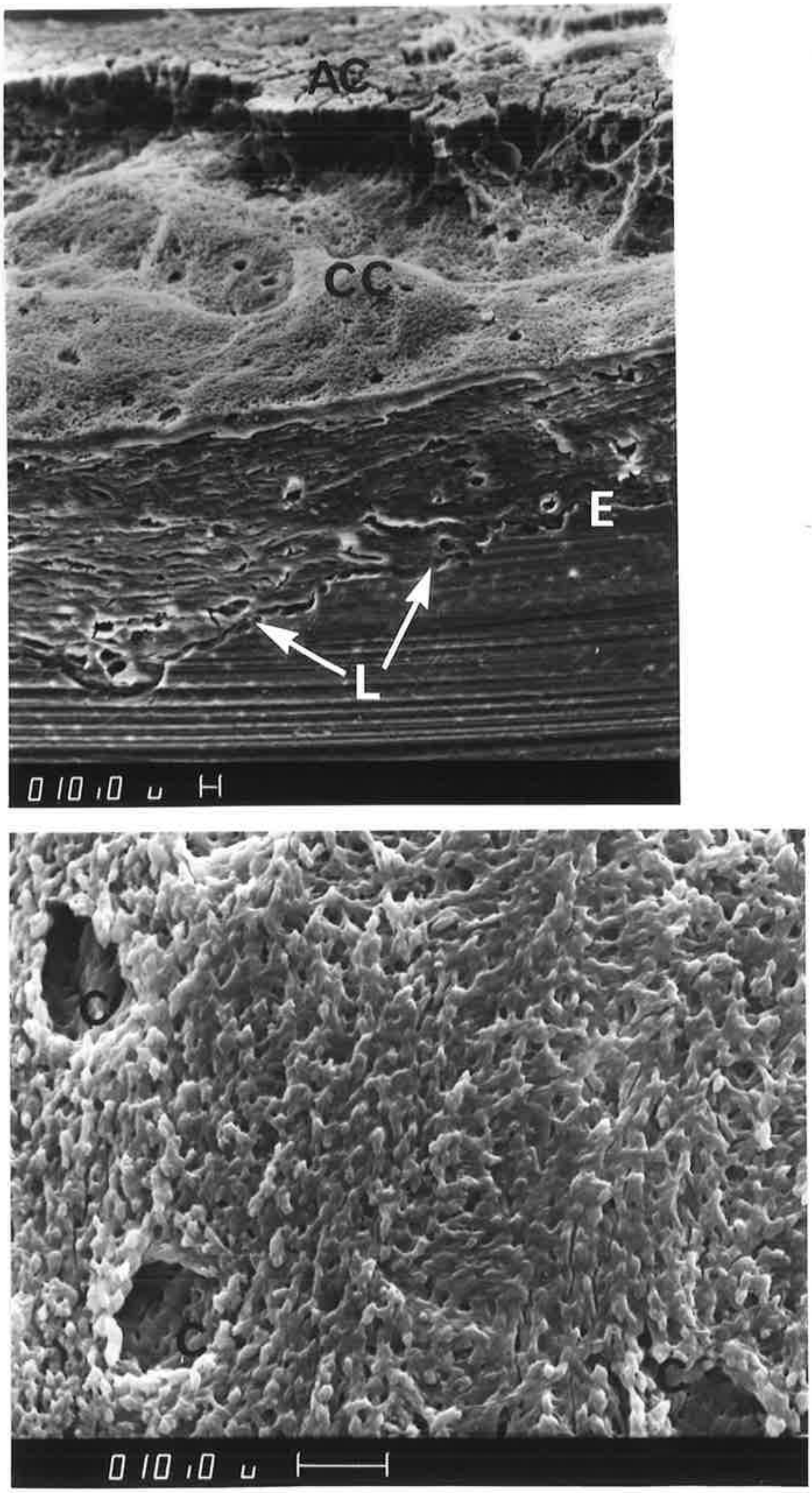


Figure 53

Patient A.S., left premolar, buccal surface.

Above: The level of repair with cellular cementum (CC) is apparent in this view from the section edge (E). The scalloped reversal line (L) can be seen. Normal acellular cementum (AC). X100

Below: The surface of the repair tissue shown above is illustrated. The mineral particles have coalesced but no matrix pattern is evident. A slow or resting state of tissue apposition is indicated. Cell lacunae (C). X600 variable, exhibiting a range from that suggesting rapid mineralization (Figure 53) to surfaces apparently more slowly mineralizing (Figure 52). Features possibly representing principal periodontal fibre bundle insertion sites such as those seen in Figure 52 were not common. Viewing the specimens from the sectioned edge demonstrated the extent of resorption and repair in defects transected by sectioning (Figure 53).

Group 3

Patient M.C.

For orthodontic reasons only the left first premolar of patient M.C. was removed. The lingual section had a wide, open apical foramen, approximately 500 micrometres in diameter, indicating continuing root development.

The distobuccal segment showed several large areas of resorption in the cervical and middle thirds and some smaller areas apically. Repair with cellular cementum had extended to the margin of most defects although few areas had re-established root contour. Rapid mineralization signified by random, discrete mineral nodules was found at the edge of repair tissue in some lesions. In some locations Howship's lacunae were seen at the periphery of resorptive lesions. An interesting repair tissue topography was found in a resorptive defect in the apical region (Figure 54). The topography of this repair tissue was similar to the unaffected cementum surface immediately coronal to the defect. In addition, this repair tissue resembled the appearance of some areas found within normal cellular cementum with periodontal fibre bundle insertion sites on the lingual surface (Figure 54). Interestingly, comparison of the repair tissue topography with normal acellular cementum revealed a reversed type of similarity between the two surfaces (Figure 54) the mounds of the acellular cementum corresponding to the depressions in the repair tissue. It is possible, that some of the depressions seen in the repair tissue were periodontal fibre bundle insertion sites.

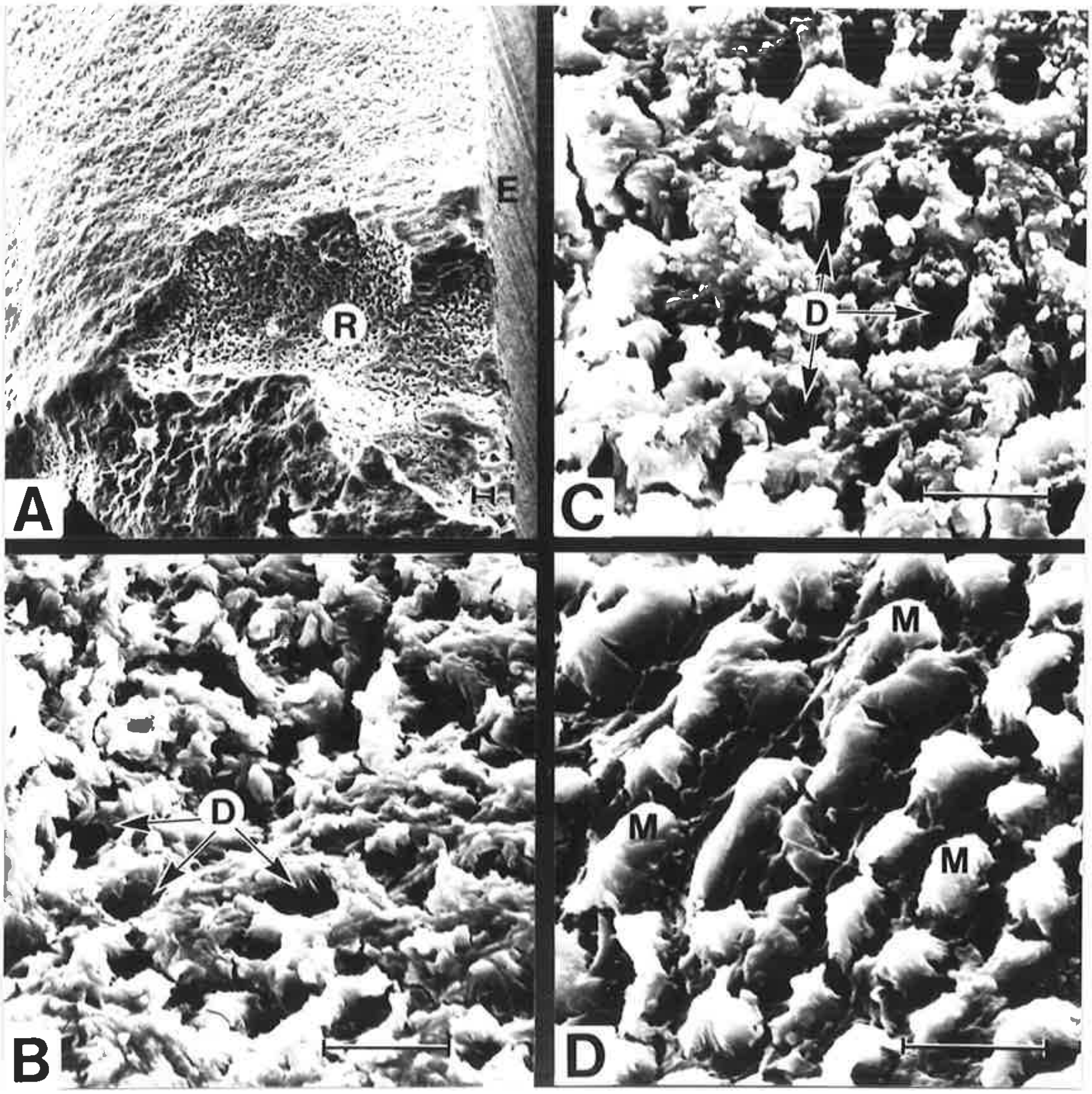


Figure 54

A comparison of repair tissue topography with normal tissue from the same tooth root. Patient M.C., retention period 38$\frac{6}{7}$ weeks.

A: An area of resorption (R) on the buccal surface in the apical $\frac{1}{3}$. Section edge, E. X100

B: Mineral front of the repair tissue from the lesion shown in A. Depressions (D) resemble Sharpey fibre holes. X1300

C: Sharpey fibre depressions (D) in normal apical cementum on the lingual surface. The surface nodules indicate mineralizing activity. X1400

D: Normal cervical acellular cementum from the buccal surface showing Sharpey fibre mounds (M) separated by a fibrous textured surface. X1500

Scale bars: 10 micrometres.

Patient M.I.

The lingual surfaces of both first premolars were apparently unaffected by resorption. In contrast, the buccal segments of both teeth showed many resorptive defects along their entire lengths. The distobuccal segment of the left premolar exhibited smaller areas of root surface resorption than the mesiobuccal segment of the right tooth. The level of repair within the defects was generally more advanced on the left tooth. Repair was by cellular cementum and followed the pattern described for other specimens. Features interpreted as Sharpey fibre holes were observed in some locations in repair tissue, but as found on other specimens these topographical indications of fibre bundle insertions were not a numerous or consistently distributed feature.

Patient S.M.

This patient has been included in Group 3 because the time from the end of expansion to the extraction of the first premolars was 51 weeks. However, fixed retention was in place for only the first 23 weeks of this period after which no appliances were used until removal of the first premolars 28 weeks later.

Both the lingual surfaces had a resorptive defect of several hundred micrometres in diameter in the cervical region but only that on the left tooth was repairing.

The mesiobuccal segment of the right premolar showed some active resorption areas on the mesial proximal surface at about mid-root level. Resorption on the buccal surface was found apically and in the middle area adjacent to the section of the specimen. By viewing the mid-buccal section edge, the extent of repair in these areas of resorption could be seen. Repair was well advanced in general, having re-established root contour in some places and overcontoured in others. Again, cellular cementum appeared to be the exclusive repair tissue, only varying in its rate of mineralization. Holes possibly representing fibre bundle insertions in the mineral front were found in repair tissue which had matched the level of the surrounding root surface.

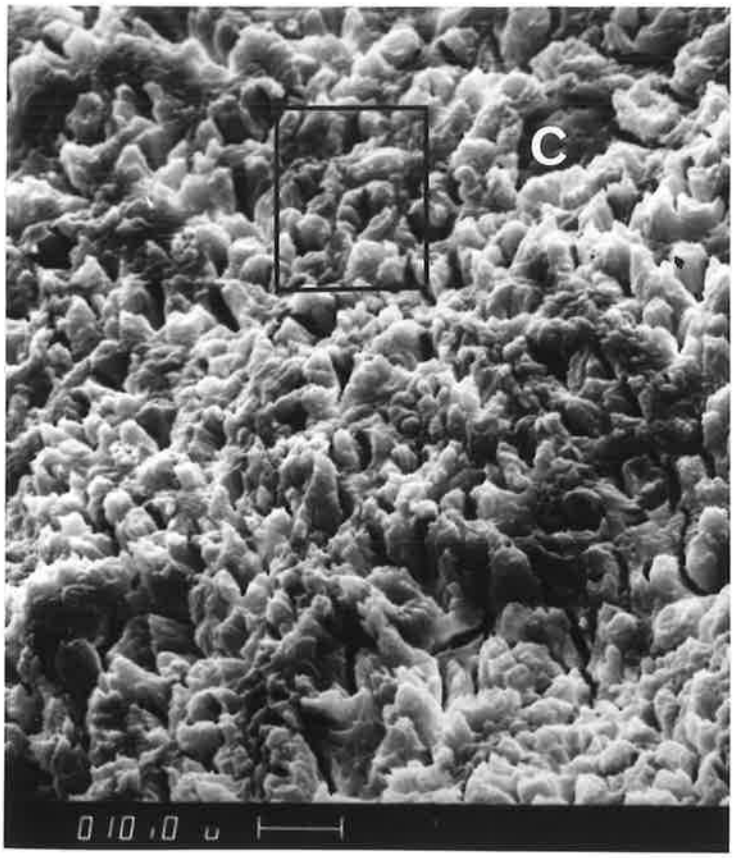
The distobuccal segment of the left premolar had a large area of resorption cervically with smaller areas close by. The apical two thirds was dominated by an area of well demarcated uneven cellular cementum adjacent the section edge (Figure 55). By viewing the section edge it could be seen that this cellular cementum was actually repair tissue in an extensive resorptive defect. This finding was confirmed by the histological examination of the distobuccal segment. No Howship's lacunae remained at the periphery of the repair tissue which had apparently completely filled the lesion. Few Sharpey fibre holes were evident, but one area of normal cementum close to the apex had principal fibre insertion sites of an interesting configuration (Figure 56). These sites were slit-like openings with crenated inner margins and rolled peripheries and resembled Sharpey fibre tracts exposed by resorption which were observed elsewhere (Figure 29). Repair in the obvious cervical resorption areas was not as advanced as on the right tooth but exhibited a range of topographies indicating slowly to rapidly mineralizing cellular cementum. In some areas, apparent fibre insertion sites were found (Figure 57). Figure 58 illustrates the early rapidly mineralizing repair of a small cervical resorption area on this specimen.

Figure 55

Patient S.M., retention period 51 weeks.

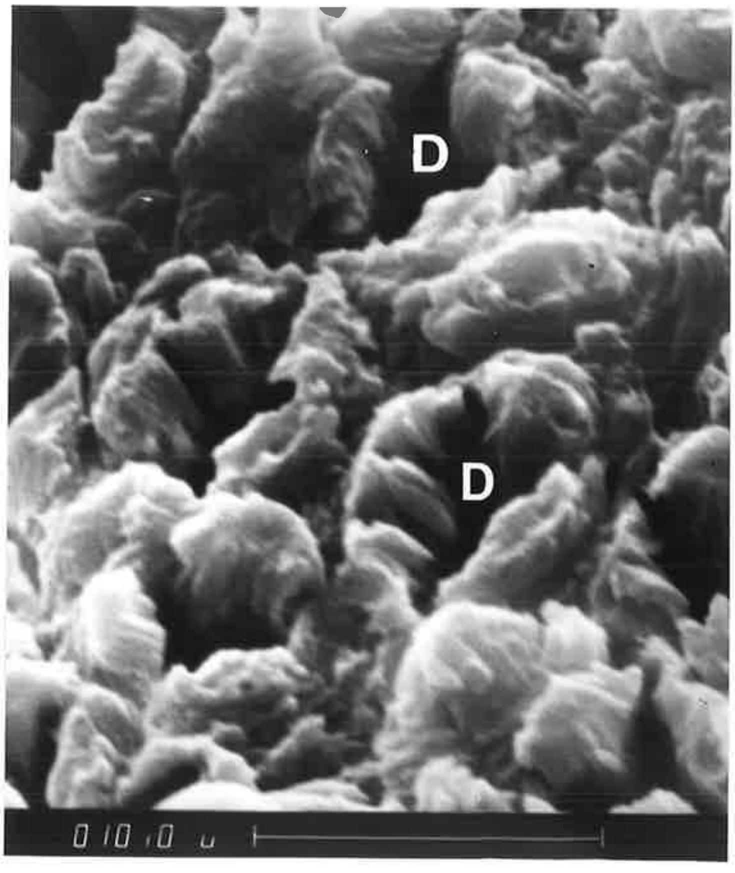
Distobuccal segment of the left premolar with obvious resorption cervically. The area (R) delineated by arrows was determined to be repair tissue by examining the section edge, although no evidence of the previous resorption was found on the root surface. X10

Figure 56



Patient S.M., left premolar.

Unusual Sharpey fibre holes or depressions in normal apical cellular cementum on the buccal surface. The outlined area is enlarged below. Cell lacuna (C). X600



The configuration of the Sharpey fibre depressions (D) from the area outlined above resemble those in Figure 29 which were exposed by resorption. X2600

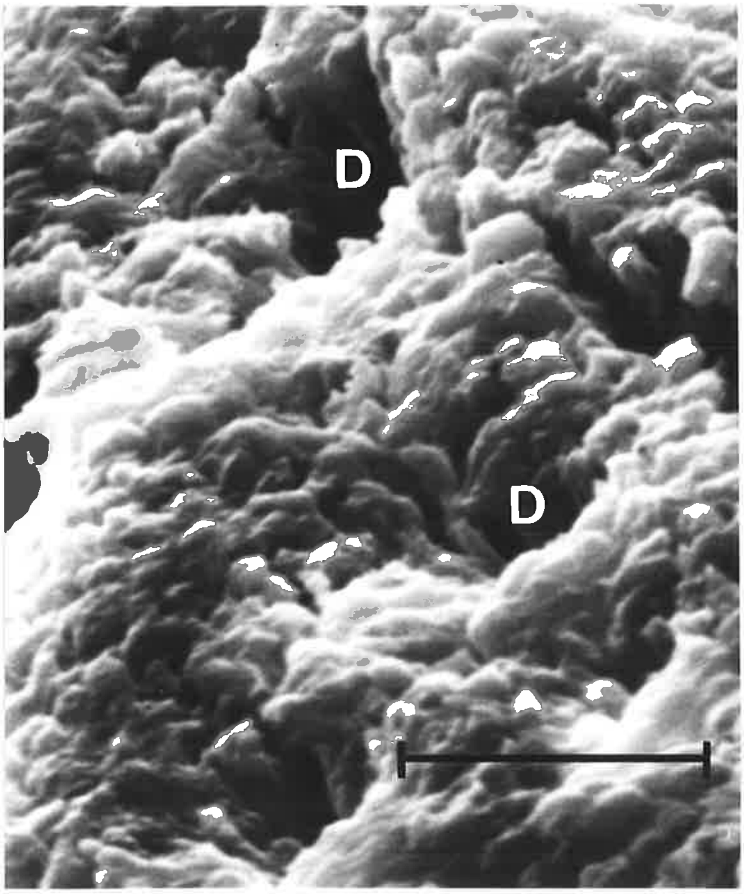


Figure 57

Sharpey fibre depressions (D) found in an area of repair tissue in a cervical resorption area.

Left premolar, patient S.M.

Scale bar: 10 micrometres. X1500

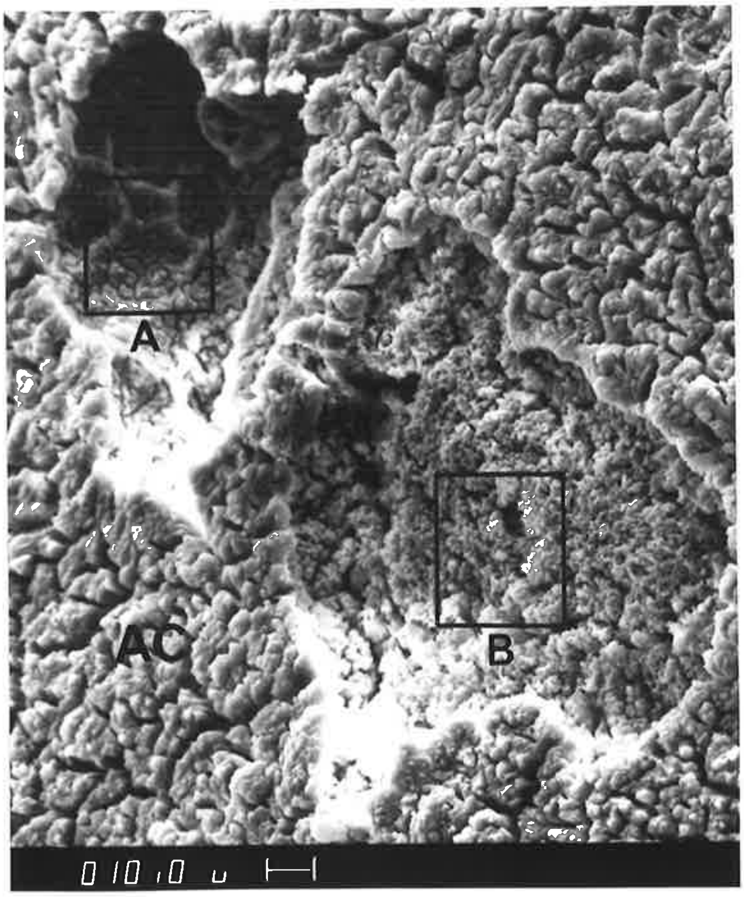
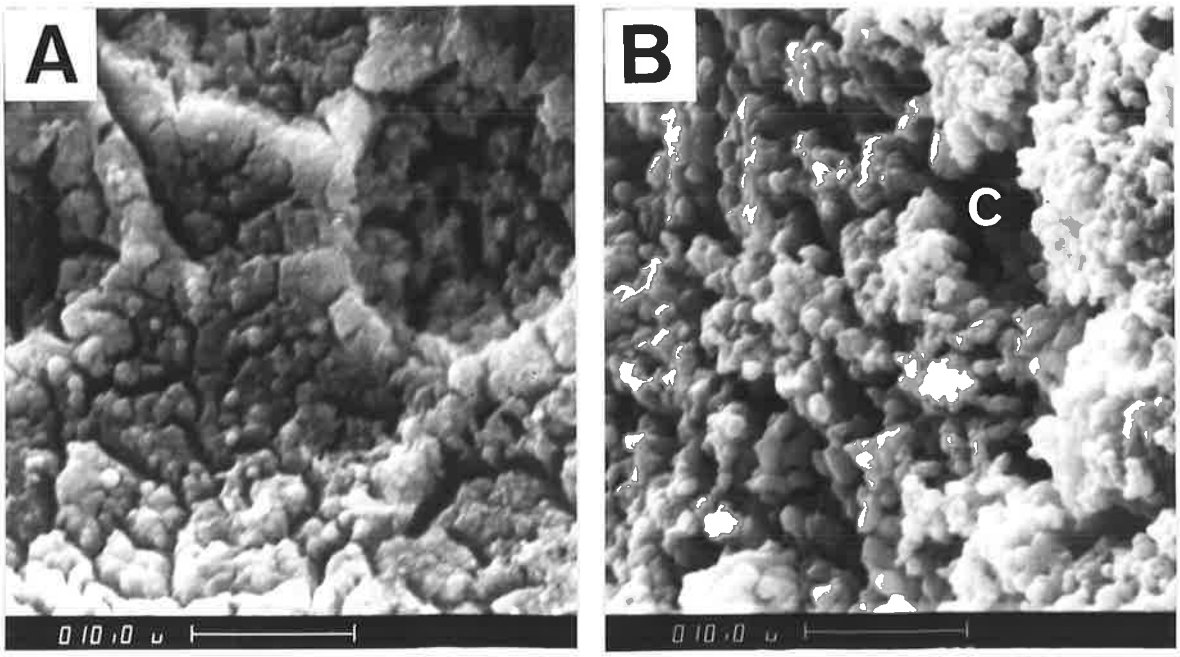


Figure 58

Patient S.M., left premolar, buccal surface.

Above: Two small areas of resorption in the cervical region. Acellular cementum surrounds the defects. X300

Below: The areas outlined above are shown at higher magnification to illustrate the mineralizing front of this early repair. In B a cell lacuna is marked C. X1600



Patient E.C.

As with other experimental teeth in this study, the lingual surfaces of the first premolars from patient E.C. were little affected by resorption (Figure 59). The lingual surface of the right premolar exhibited a number of unusual features in the cervical half. By viewing these features in profile it could be seen that they were elongated protuberances angled slightly away from the root surface (Figure 60). Their topography suggested rapid mineralization and the surrounding surface resembled areas of cellular cementum with few distinguishable cell lacunae but many depressions possibly representing periodontal fibre bundle insertion sites (Figure 60). These unusual topographical features in an area of normal acellular cementum may have been produced as a response to tension on the lingual side. Interpretation of these features as a reparative response cannot be unequivocally substantiated. However, this possibility should not be dismissed entirely. Much of the acellular cementum surface on both lingual specimens did not display typical mounds and appeared to be mineralizing, indicated by a nodular surface.

The buccal segments of both teeth were heavily affected by resorption (Figure 59). Repair in most of the defects had extended to the margins, with only a few Howship's lacunae evident at random locations peripherally (Figure 61). In limited areas of some repair surfaces, holes attributed to inserting periodontal fibre bundles occurred (Figure 61). As in other specimens, these depressions were not predominant and did not exhibit a predictable pattern of occurrence except that they tended to be found towards the lateral extent of repair tissue and in more rapidly mineralizing surfaces.

Figure 59

Patient E.C., retention period 52 weeks. Left premolar.

A: Numerous areas of resorption (R) along the length of the mesiobuccal segment, still evident 1 year after expansion although repair was generally extensive. X10

B: One small resorptive defect (R) on the lingual surface. The apical foramen (F) can be seen in this segment. X10

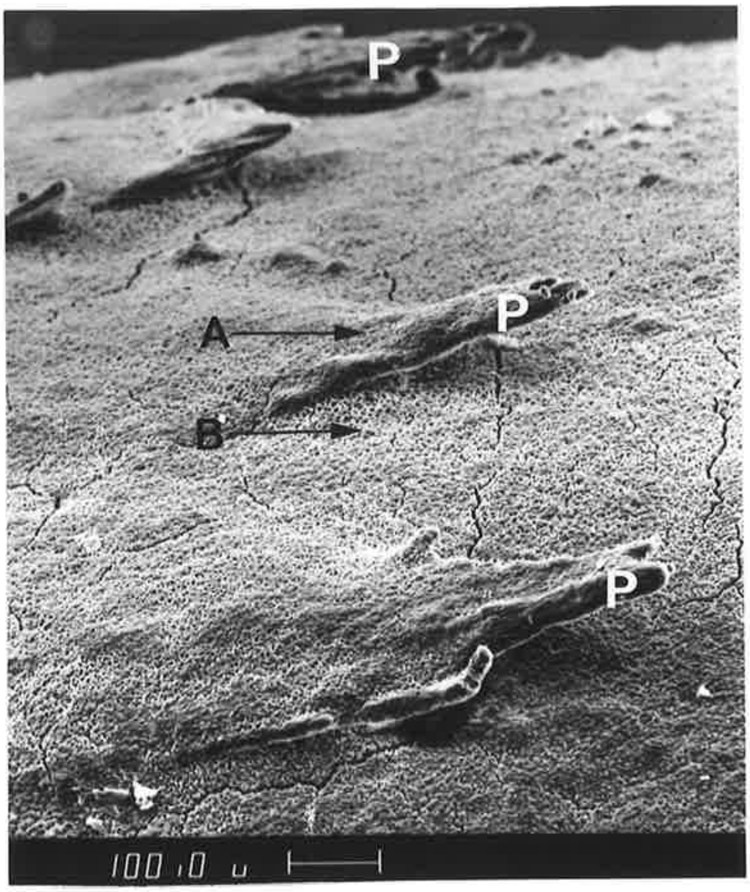
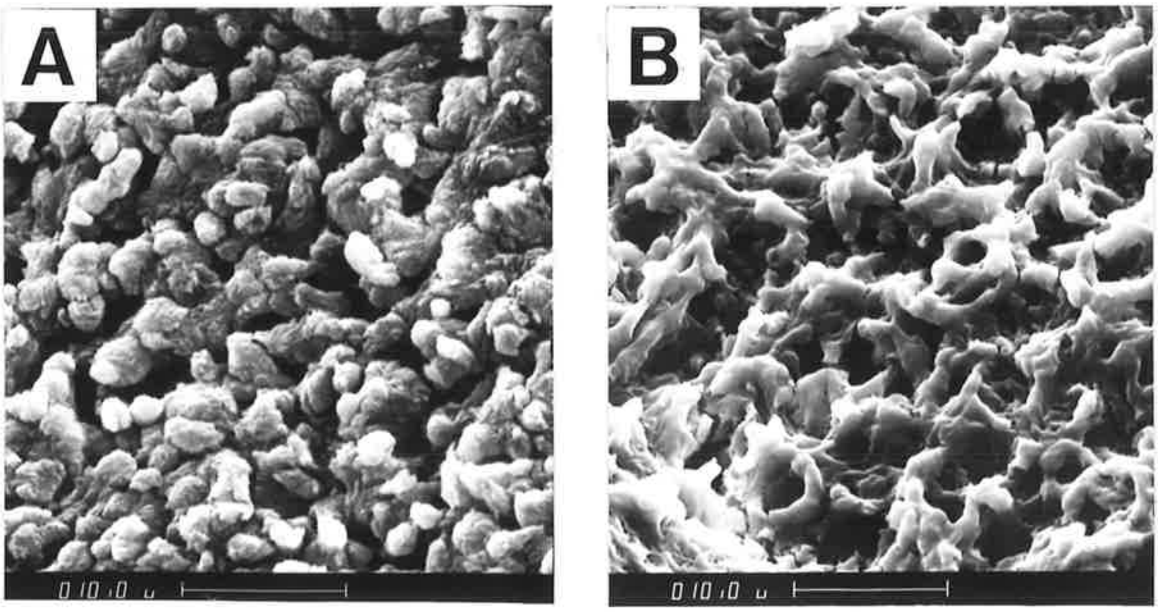


Figure 60

Patient E.C., right premolar, lingual surface.

Above: Projections (P), seen in the cervical third, which are possibly an unusual response to tension. Surfaces from regions A and B are shown below. X60

Below: The surface in A was typical of the body of the projections and indicated mineralizing activity. X1600
The surface immediately surrounding projections, seen in B, resembles some non-mineralizing cellular cementum surfaces seen in this study. X1600



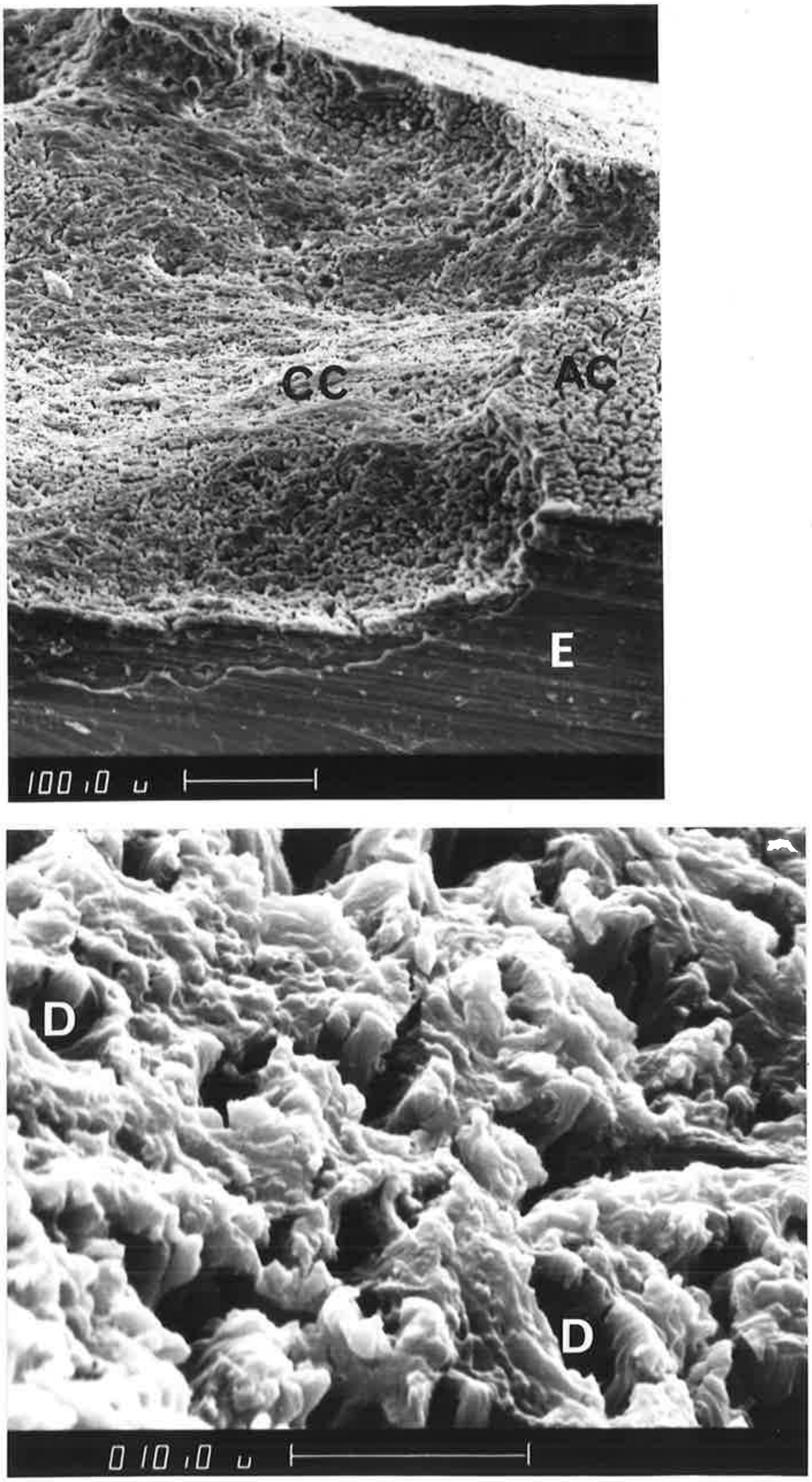


Figure 61

Patient E.C., left premolar, buccal surface.

Above: Repair cellular cementum (CC) viewed from the section edge (E). Although root outline is not restored, repair has extended well on to the margin of the lesion. Normal acellular cementum (AC). X100

Below: The surface of the repair cementum illustrated above, with possibly Sharpey fibre depressions (D). X1700

Patient L.H.

The pattern of resorption and repair described for other specimens in this third group was repeated for Patient L.H. The lingual surfaces were virtually unaffected while the buccal segments showed extensive resorption, with repair extending to the periphery of most defects and re-establishing root contour in some areas (Figure 62).

The root surface specimens for examination in the SEM from this patient were prepared by exposure to 10% sodium hypochlorite for only 7½ minutes. This left a surface topography which was essentially similar to other specimens exposed to sodium hypochlorite for longer periods, but with a fine fibrous texture (Figure 63). The buccal and lingual segments from the left premolar were examined, recorded and then reprepared in 5% sodium hypochlorite for 48 hours. During this period the conductive coating flaked off the surface and after drying and recoating it was found that the fibrous texture had been removed from the surface without any gross topographical changes having occurred (Figure 63).

Not all areas exhibited a fine textural coating initially as can be seen from Figure 64. This shows the central reparative tissue in a cervical resorptive defect on the buccal surface of the left tooth. The repair tissue surface is homogeneous except for circular openings about 5 micrometres in diameter some of which may represent fibre bundle insertion sites. Towards the edge of this repair tissue discrete mineral particles were observed, suggesting more rapid mineralization. In another resorptive defect on the buccal surface a small area of resorption of cellular cementum repair tissue was noted, an uncommon finding in this study.

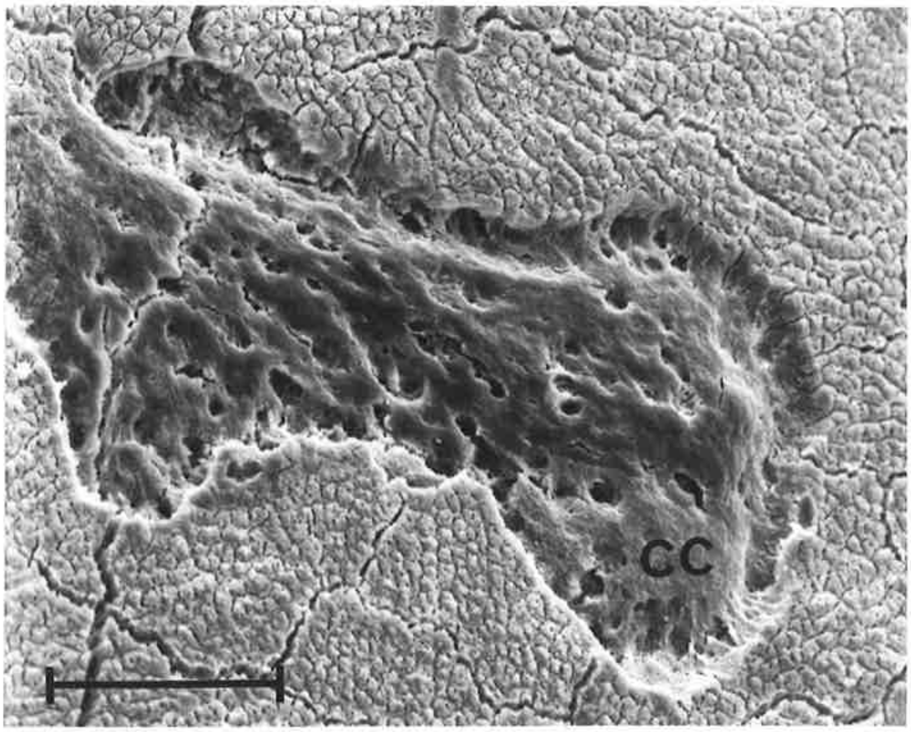


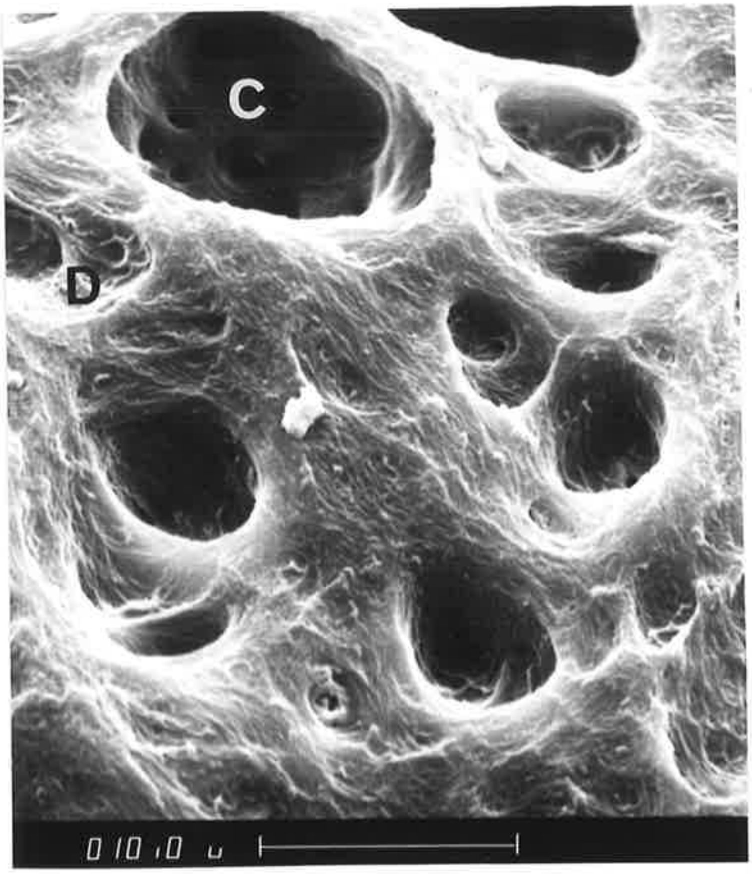
Figure 62

Patient L.H., retention period 53 weeks. Left premolar, buccal surface.

Resorption area repaired with cellular cementum (CC). Root contour is close to being re-established except at the periphery. The surface of the repair tissue is confluent around cell lacunae suggesting a lack of mineralizing activity. The surface surrounding surface is acellular, Sharpey fibre mound cementum.

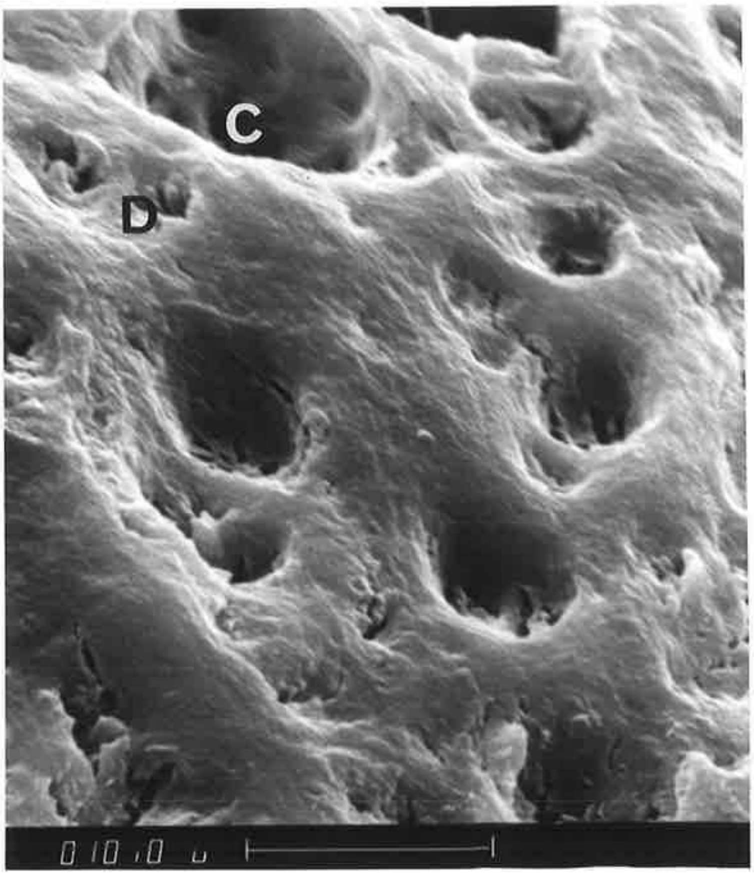
Scale bar: 100 micrometres. X150

Figure 63



Patient L.H., left premolar, buccal surface.

Repair cementum, which does not appear to be actively mineralizing, from an area of resorption, prior to specimen repreparation. A fine fibrous texture is evident on the surface. Cell lacuna (C). Other depressions are most likely Sharpey fibre depressions. Compare D with similar features in Figure 32. X1800



Same area as that illustrated above, after specimen re-preparation. Gross features remain the same but the fibrous texture has been lost. Compare D with Sharpey fibre depressions in Figures 29 and 56 and with D above. This comparison suggests that organic tissue imparted a radiating spoke-like appearance to these depressions. X1800

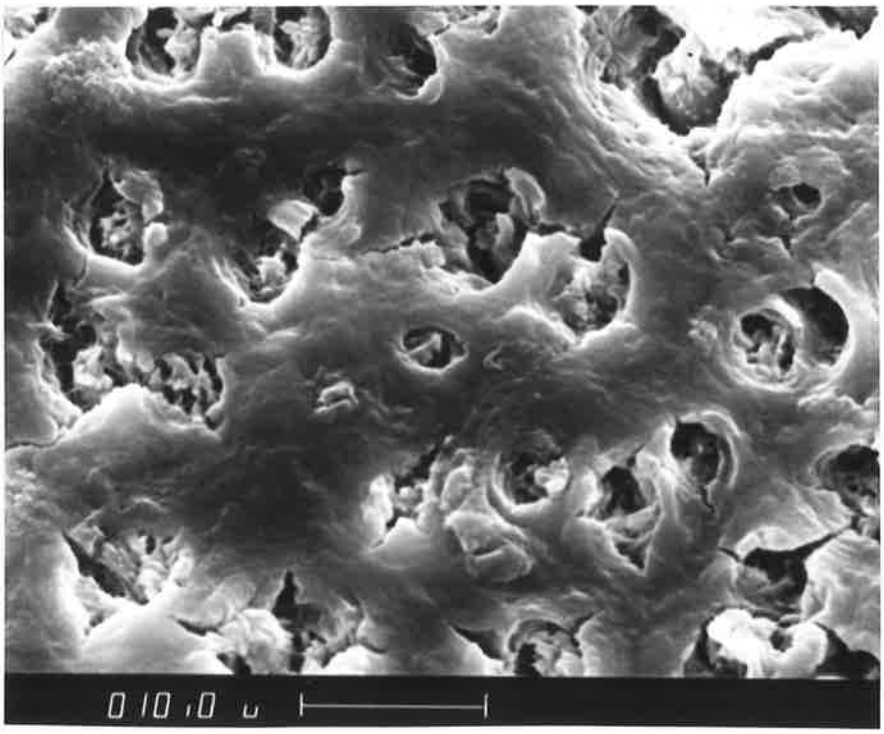


Figure 64

A resting surface in the central area of repair cellular cementum from a cervical resorptive defect on the buccal surface of the left premolar, patient L.H. Repair in the defect was well advanced and this central area demonstrates a homogeneous surface between depressions which resemble Sharpey fibre holes. X1200

The lingual surface of the right premolar displayed Sharpey fibre depressions similar to a number described and illustrated (Figure 32) on the lingual surface of the left premolar of patient C.T.

Patient J.S.

Acellular cementum, generally with typical Sharpey fibre mounds, predominated over the cervical three quarters of the maxillary first premolars from patient J.S. The apices of both teeth were square in outline but did not appear to have been resorbed as Howship's lacunae were not in evidence at the apex. The lingual surfaces of both teeth showed a number of small resorptive areas along their length and the left tooth had a large resorptive area in its apical third. Repair was occurring except in some of the smaller areas.

The buccal segments both had a similar distribution of resorption, with a large cervical area and smaller areas in the middle and apical regions. Repair was generally well advanced (Figure 65) with cellular cementum, demonstrating a range of topographies paralleling that described for repair tissue on other specimens. Features possibly representing fibre bundle insertion sites were occasionally found in repair cementum, particularly at its advancing periphery. The size, relative proximity and lack of canalicular openings in these holes indicated that they were not cell lacunae.

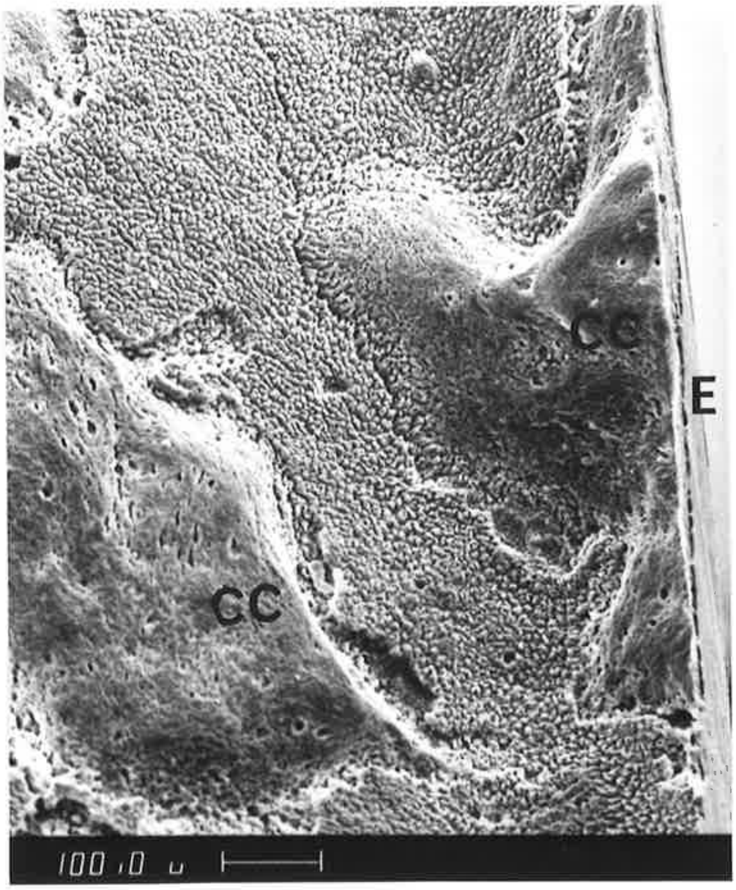


Figure 65

Patient J.S., retention period 53$\frac{2}{7}$ weeks.

Repair cellular cementum (CC) has over-contoured the surface adjacent the section edge (E) and almost completely filled a neighbouring resorptive defect. Left premolar, mid-root region of the buccal surface. X70

Areas of resorption

Table 4 sets out the estimated area of resorption on the buccal root segments observed in the SEM. Generally, segments from both the left and right premolars of patients were examined in the SEM. In these cases, the area of resorption on both premolars has been averaged and this figure used as the estimate for that patient. Figure 66 illustrates these results graphically, showing a lack of association between the period of retention and the area of resorption. The group averages presented in Table 4 point to the fact that significant variation in the area of resorption did not occur with the retention periods used in this study. In addition, no definite age or sex relationship with the area of resorption could be established.

TABLE 4

Estimate of the area of resorption on the buccal surface of experimental premolar root segments observed in the SEM, to the nearest round figure.

Patient		% area of resorption
Group 1		
T.H.		12
B.B.		37
M.D.		45
G.B.		16
C.T.		22
W.J.		17
	Average*	25
Group 2		
K.B.		31
R.H.		26
R.E.		17
S.W.		28
L.B.		24
A.S.		19
	Average	24
Group 3		
M.C.		24
M.I.		22
S.M.		25
E.C.		30
L.H.		23
J.S.		16
	Average	23

* Average for Group 1 excluding patient M.D. is 21%.

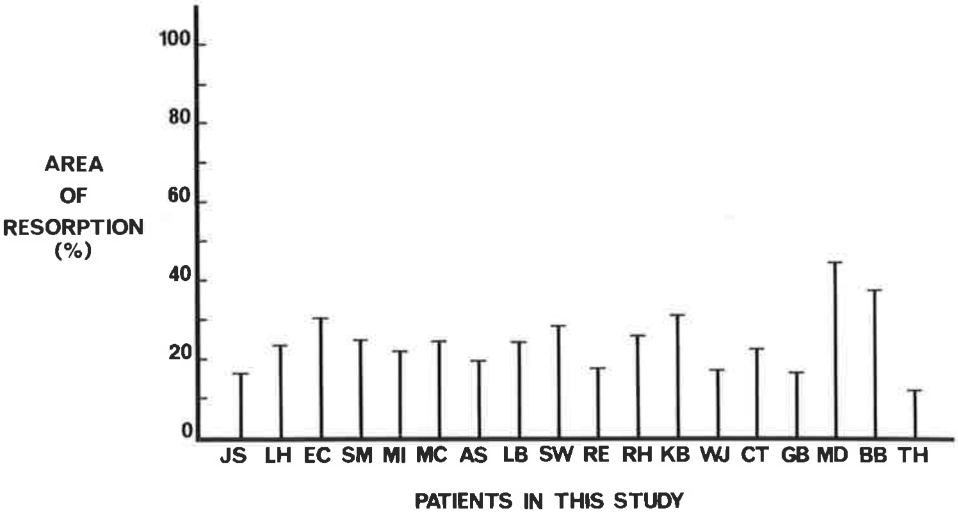


Figure 66

Bar graph depicting the area of resorption, observed on the buccal segments of specimens viewed in the SEM, as a percentage of the total segment area seen. Retention periods are in order from left to right : longest to shortest.

HISTOLOGICAL EXAMINATION

The experimental tooth segments examined histologically may be ascertained from Table 3, as all buccal segments not examined in the SEM were sectioned for study in the light microscope. One experimental tooth, namely the left premolar from patient T.H., was left intact for histological preparation. The lingual surfaces of all other anchor premolars were assessed in the SEM.

The ground sections clearly demonstrated the Tomes' granular layer in the dentine adjacent to the cemento-dentinal junction (Figure 67). As the ground sections were relatively thick and unstained, soft tissue elements could not be resolved well and it was found that phase contrast and dark field illumination did not improve the clarity of these features. Resorption was readily apparent and was seen to vary considerably in depth of penetration although the plane of section in relation to a resorbed area must be considered as influencing this observation. As a means of studying repair in root surface defects, ground sections were not found to be useful and the technique was abandoned for the majority of this study. However, methods of mechanical reduction to produce thinner ground sections and staining of the resulting sections could prove more valuable but were not undertaken in this study.

The majority of histological specimens were paraffin embedded, sectioned and stained as described in Section 4. The observations made in the light microscope were found to directly compliment the findings from specimens examined in the SEM.

Acellular cementum covered the cervical half to two-thirds of all sections. The remaining portion of the root surface in each case was

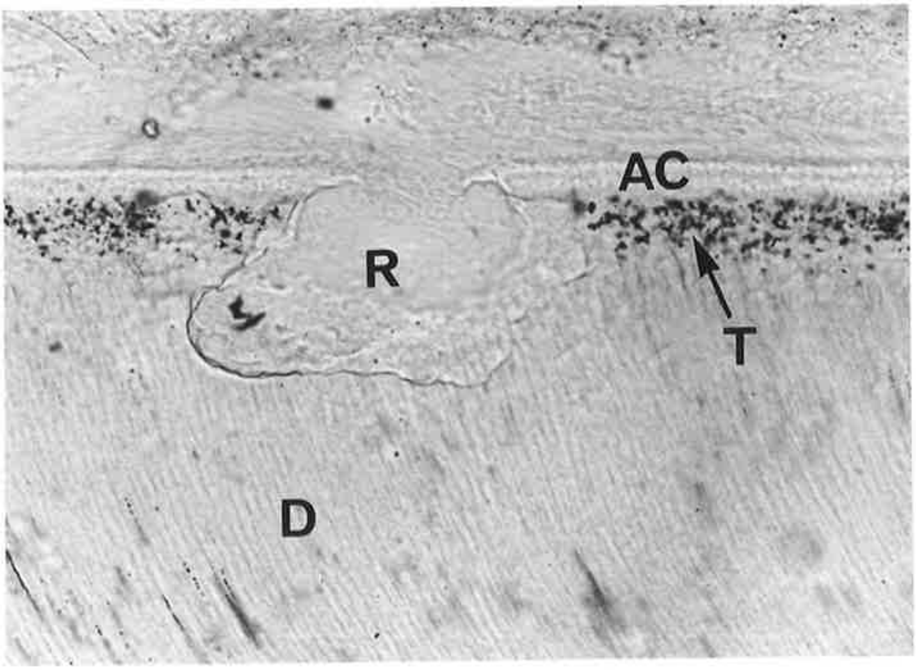


Figure 67

An area of resorption (R) penetrating dentine (D). Tomes' granular layer (T), Acellular cementum (AC).

Patient C.T., right premolar. Retention period 17 weeks.

Ground section. X100

covered by cellular cementum, having an irregular surface in comparison to the straight periodontal surface of the acellular cementum. The thickness of cellular cementum was quite variable unlike the acellular cementum which formed a uniform covering on the cervical end of the roots. The plane of section affected the thickness of cementum seen, particularly the apical cellular cementum, as the sections approached the proximal side of the root. In some cases sections eventually were taken through cementum only, because of the curvature onto the proximal surface. Thus, observations on cementum thickness and depth of penetration of resorption must be rationalized with respect to the plane of section variable. This variable affected the apical structures to a greater extent so that a relatively uniform thickness of cervical acellular cementum was maintained in sections for examination.

The histological picture of acellular and cellular cementum was characteristic. In addition to uniform thickness and a straight smooth periodontal surface, acellular cementum displayed closely packed horizontal striations these being the Sharpey fibres of inserting principal periodontal fibre bundles. The Sharpey fibres appeared to branch, forming the matrix of the acellular cementum (Figure 68). Vertically arranged and parallel incremental lines could sometimes be discerned in acellular cementum at low magnification, unlike those in cellular cementum which were not evenly spaced or straight. Cellular cementum was also defined by the presence of cementocyte lacunae, the number of which was variable. Sharpey fibre tracts could be seen in some locations in cellular cementum but were never a dominating feature (see Figure 72).

Extensive resorption was a feature of all buccal segments investigated histologically, corresponding directly with observations

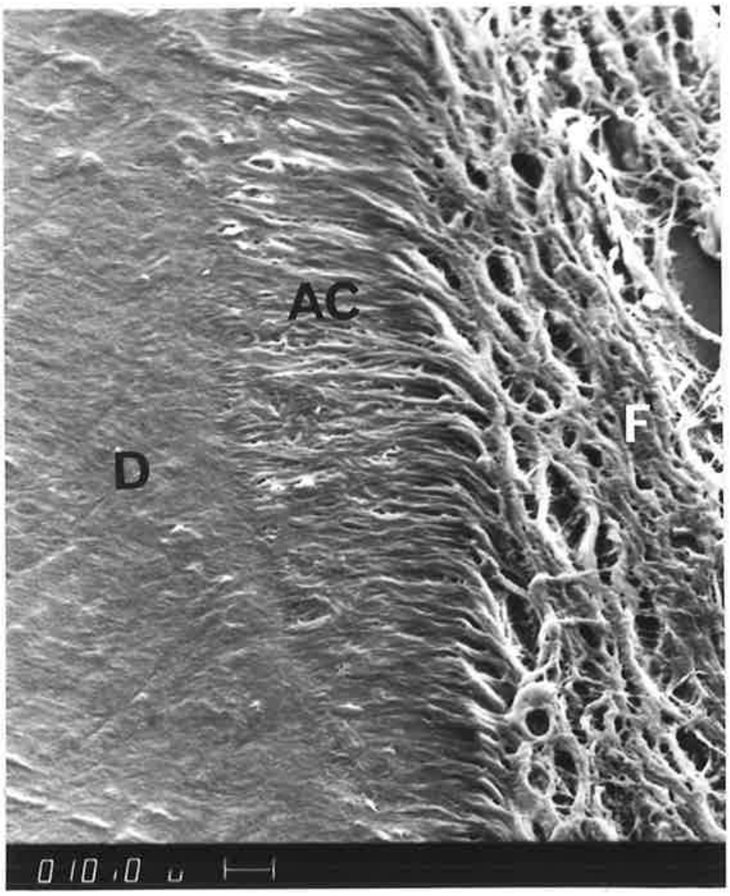


Figure 68

Patient T.H., retention period 14 weeks.

Retained periodontal fibres (F) can be seen inserting into normal, unaffected acellular cementum (AC) to form its matrix.

Histological section from the left premolar examined in the SEM. X300 in the SEM. The axial extent of resorption varied considerably but generally involved dentine. The overall impression was that the resorption was laterally rather than axially expansive and in no specimen was it considered that pulp exposure had occurred or would occur.

Marked undermining was evident in many areas of resorption (Figure 69). Although accurate measurement of the extent of resorption observed histologically was not possible, there did not seem to be greater resorption either in surface area or depth of penetration in those specimens retained for longer periods.

Repair activity was a feature of the majority of resorptive areas. Where repair was observed it was always with cellular cementum, although the number of cementocyte cell lacunae was variable. Indeed, in some sections it appeared that the tissue between incremental lines in repair tissue was acellular, but examination of serial sections revealed cementocytes within the tissue layer. The incremental lines observed in repair cellular cementum (Figure 70) indicated that its deposition was phasic in nature.

The extent of repair within resorption areas varied both on the same tooth and between different teeth. However, a general trend towards proportionately more repair in longer retained specimens was noted (Figure 71). Thus overall, repair was closer to completely filling in resorptive lesions in the longer retained specimens, although isolated areas in specimens retained for the shorter periods in this study were completely repaired. A tendency towards exuberance of repair tissue was apparent and a number of repair areas were overcontoured and occasionally repair cellular cementum covered the adjacent previously unaffected root surface.

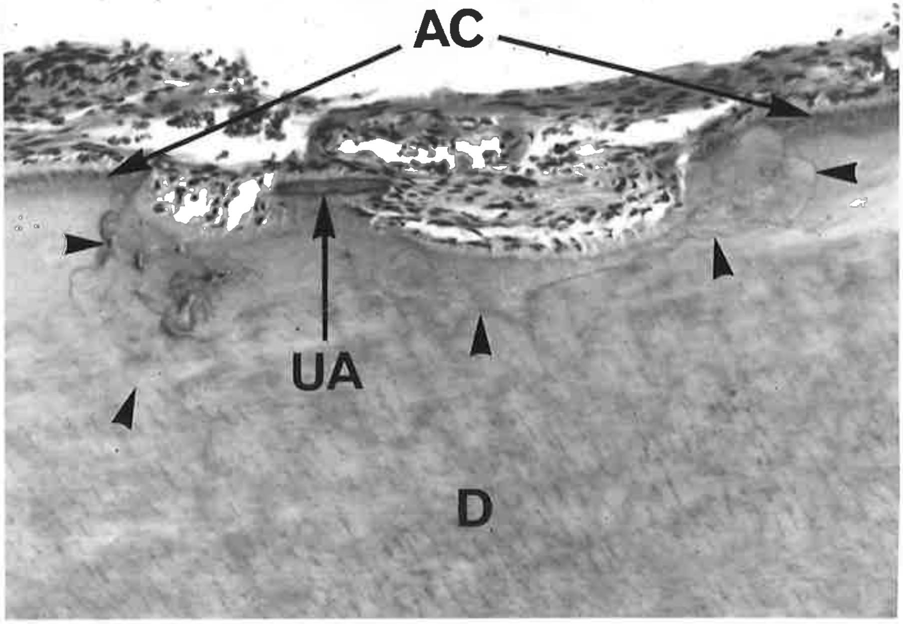


Figure 69

An area of resorption partially repaired with cellular cementum. The undermining character of the resorption is demonstrated by the island of unresorbed acellular cementum (UA). The outline of the resorptive excavation is indicated by arrows. Dentine (D), Acellular cementum (AC).

Left premolar, patient R.H., retention period 21 weeks.
Haematoxylin and eosin. X170

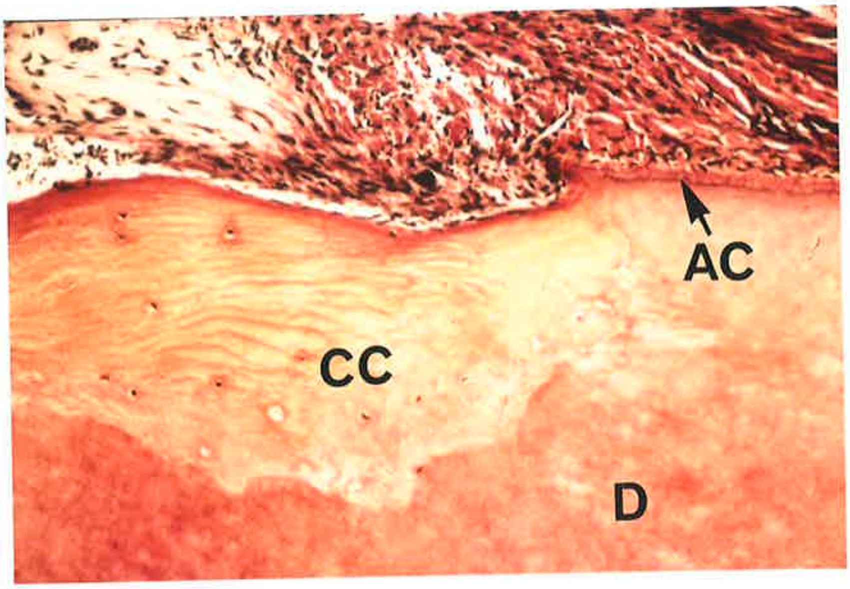


Figure 70

Patient J.S., retention period $53\frac{2}{7}$ weeks.

Repair cellular cementum (CC) with incremental lines clearly visible indicating the periodic nature of its apposition. Repair has nearly re-established root contour.
Dentine (D), Acellular cementum (AC).
Weigert's haematoxylin and eosin. X160

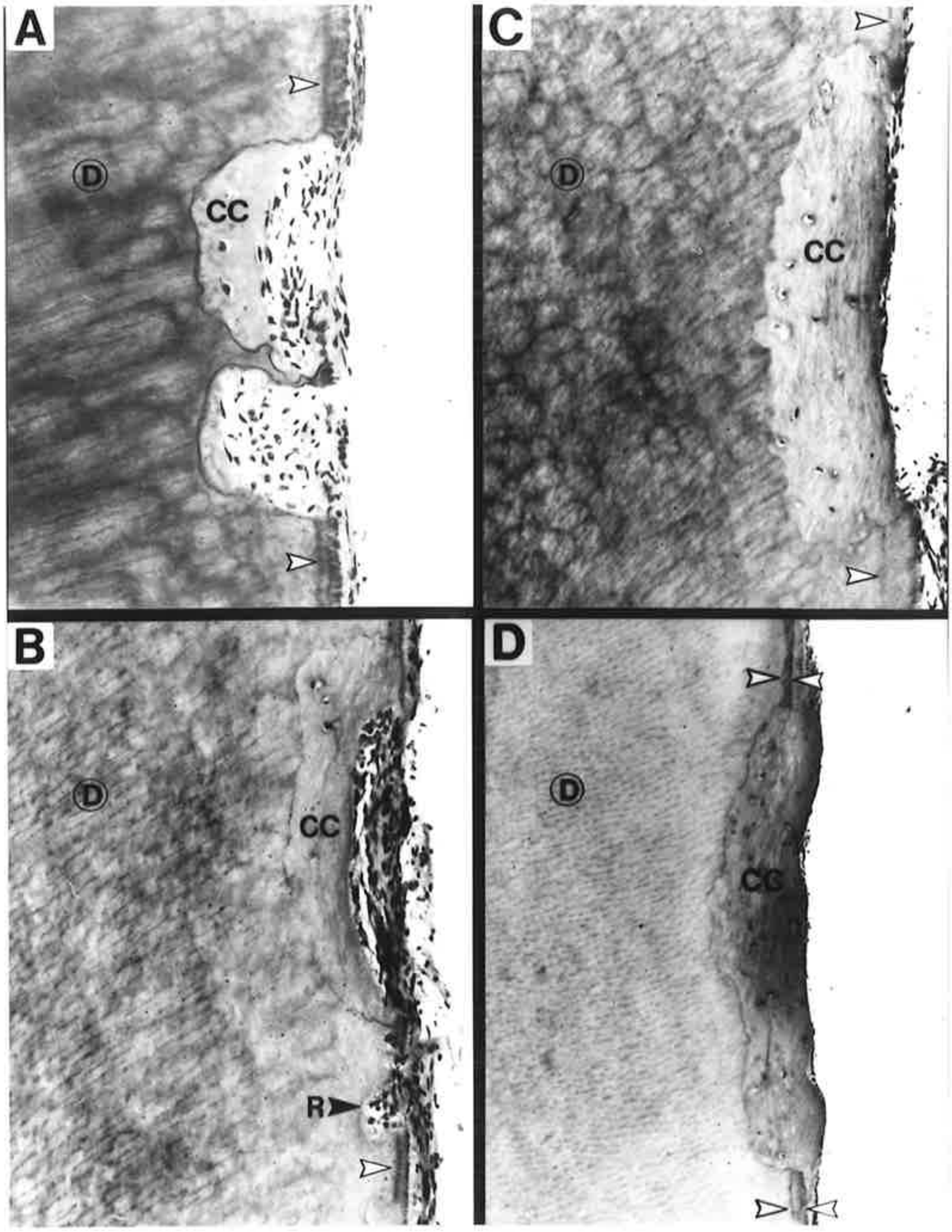


Figure 71

Four levels of resorption and repair are illustrated.

A: Resorption and early repair. Patient B.B., left premolar. Retention period 14$\frac{3}{7}$ weeks. Haematoxylin and eosin. X250

B: Resorption (R) and further repair. Patient R.H., left premolar. Retention period 21 weeks. Haematoxylin and eosin. X190

C: Repair re-establishing root outline. Patient J.S., left premolar. Retention period 53$\frac{2}{7}$ weeks. Wiegert's haematoxylin and van Gieson. X160

D: Exuberant repair beginning to cover adjacent cementum. Patient S.M., left premolar. Retention period 51 weeks. Haematoxylin and eosin. X100

Repair cellular cementum (CC), Dentine (D). Normal acellular cementum is indicated by white-on-black arrows.

In the apical areas of some specimens where the cellular cementum was thick, characteristic scalloped reversal lines within cementum indicated that resorption had occurred within cementum only. Often these areas of resorption were repaired restoring root outline (Figure 72) and in some instances overcontouring the surface.

Periodontal soft tissue was best retained in more deeply penetrating incompletely repaired lesions (Figure 71). Shallow expansive areas of resorption and completely repaired areas did not retain soft tissue to a marked extent. It appeared from the tissue retained on the surface of repair cementum that there was continuity of the fibrous matrix of the cementum and periodontal tissue. However, the pattern of orientation of the fibres was often parallel to the surface and distinct principal fibre bundles directed horizontally or obliquely away from the surface were not in evidence generally. Some repair cementum did display fibres and fibre bundles at right angles to the surface, particularly where repair was less advanced (Figures 73 and 74). An assessment of the entire course of such fibres was not possible due to the disruption caused by tooth extraction. Fibres attached to repair cementum did not produce close-packed fibre tracts as seen in acellular cementum even where root outline was re-established.

Importantly, the type of repair tissue did not differ over the length of the root. Thus large areas of resorbed cervical acellular cementum were replaced with cellular cementum. The repair cellular cementum was entirely similar to the normal cellular cementum seen on the tooth roots examined.

The pre-cementum on the surface of repair cementum showed flattened cementoblast cell nuclei. Very few multinucleate cells were

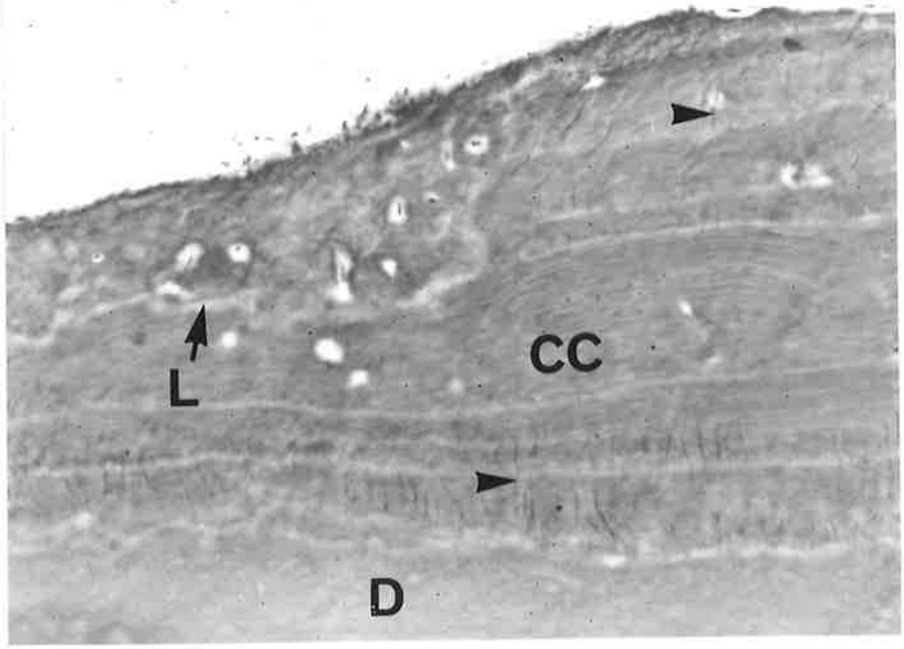


Figure 72

An area of repaired resorption within cellular cementum (CC) which displays indistinct Sharpey fibre tracts (arrowed) in some locations. Resorptive reversal line (L), Dentine (D).

Left premolar, patient L.B. Retention period 25$\frac{4}{7}$ weeks.

Pollack's trichrome. X180

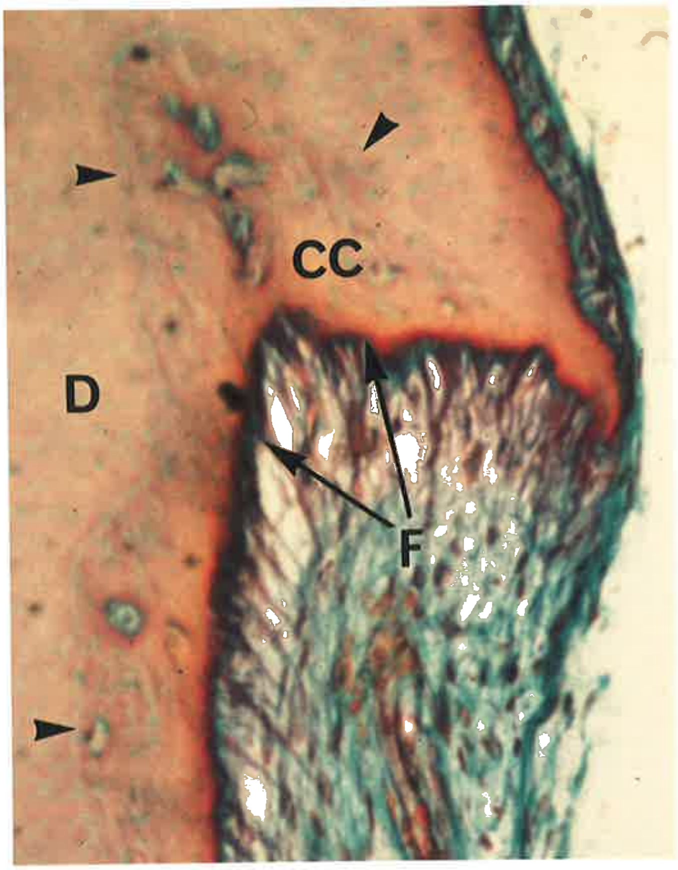


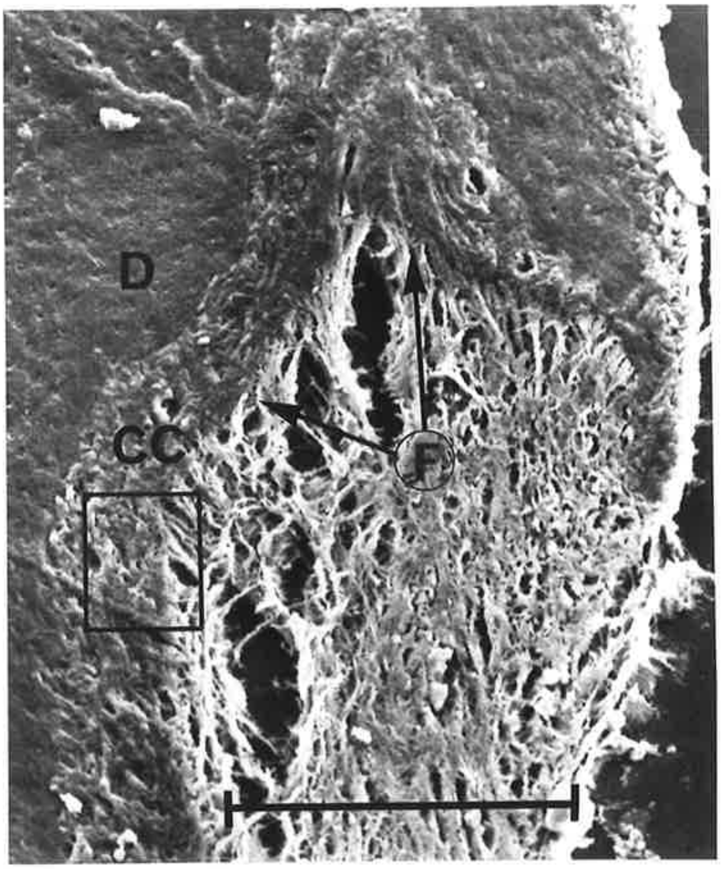
Figure 73

An area of resorption, repairing with cellular cementum (CC). Fibres (F) appear to attach to the surface of the repair tissue. The resorptive reversal line is indicated by arrows. Dentine (D).

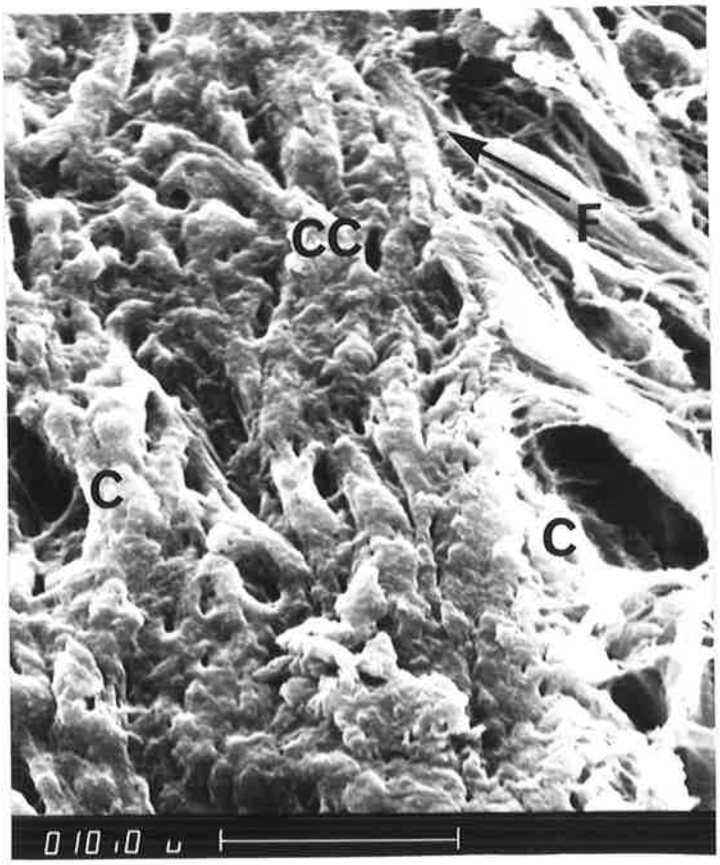
Patient A.S., right premolar, retention period 29 weeks.

Pollack's trichrome. X200

Figure 74



Topographical view of the repairing resorption area seen in Figure 73 from an adjacent section. Repair cellular cementum (CC), Dentine (D), Periodontal fibres (F).
Scale bar: 100 micrometres.
X140



Area outlined above showing fibres (F) inserting into repair cellular cementum (CC). Two cell lacunae, C, are evident, one on the surface of the mineralizing repair tissue. X1700 observed in or adjacent to resorbed areas. Next to the surface of unrepaired resorbed areas, round to oval cell nuclei were often prominent and sometimes close together but not discernibly in multinucleate cells although cell outlines were not distinct. It was possible that these cell nuclei belonged to active odontoclastic cells. An interesting observation on many of the "raw" resorbed dentine surfaces was the presence of fibre bundles extending at right angles from the surface (Figure 75 and 76).

The lingual facing surface of the buccal section of bifid rooted specimens, seen in histological sections, revealed that resorption occurred on these surfaces. This resorption was never extensive and was usually limited to the apical one third area which in most cases was the limit of the furcation. The pattern of repair in these areas was the same as that on the buccal surfaces.

The entire left premolar of patient T.H. was sectioned and examined histologically. The premolars of this patient were the only completely two-rooted experimental teeth in this study. Extensive resorption of the buccal facing aspect of the lingual root had been repaired with cellular cementum. Resorption on the buccal surface of the buccal root had occurred at the mid-root and apical areas but repair was well advanced.

In a number of specimens, fragments of alveolar bone were found attached to the root surface by retained periodontal soft tissues. These adherant bone fragments were found in the crestal region (Figure 76) and in two cases were associated with resorptive defects in the middle to apical third of the roots.

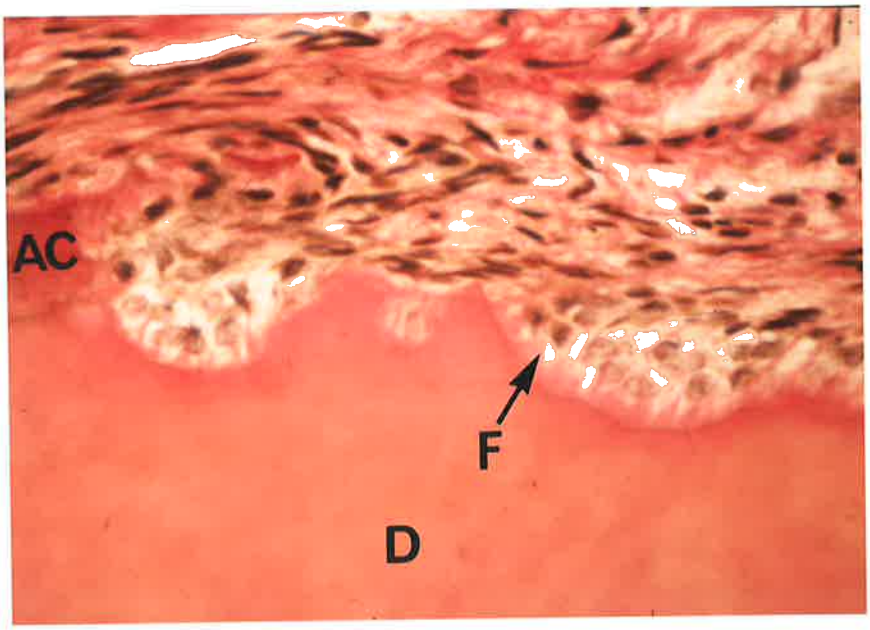


Figure 75

An area of resorption showing round to oval nuclei adjacent to the resorbed surface. Fibre bundles (F) extend from the surface. Dentine (D), Acellular cementum (AC).

Patient M.D., retention period 16 weeks. Right premolar.

Wiegert's haematoxylin and van Gieson. X250

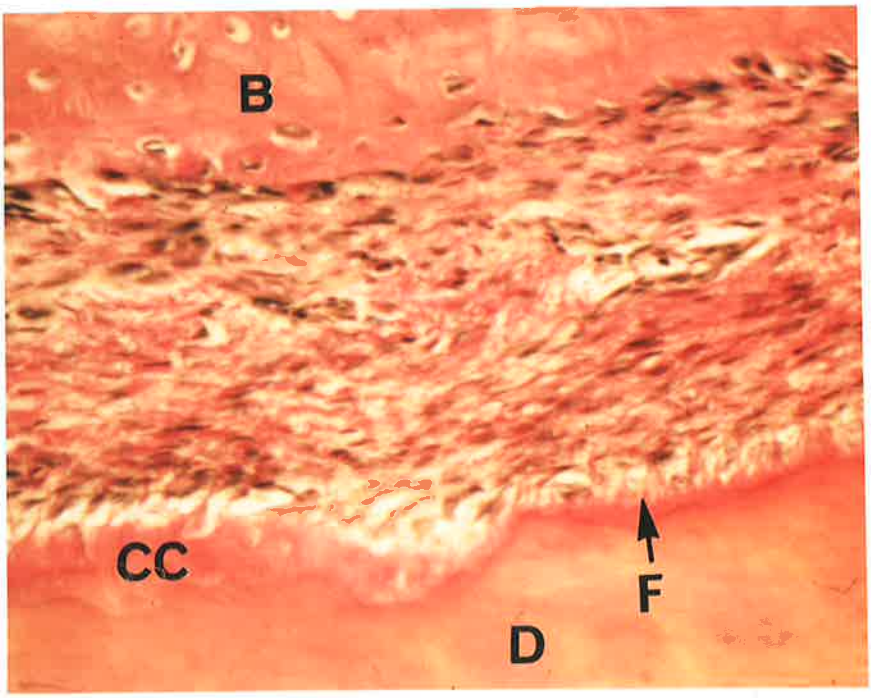


Figure 76

Patient M.D., retention period 16 weeks. Left premolar.

A sliver of crestal alveolar bone (B) retained adjacent to an extensively resorbed surface. Fibres (F) extend from the resorbed dentine (D). Repair with cellular cementum (CC) has commenced at one location.

Weigert's haematoxylin and van Gieson. X250

RADIOGRAPHIC ASSESSMENT

Standard long cone periapical radiographs of the upper first premolars were obtained prior to expansion and again just before extraction for all patients treated by the author. None of the later radiographs demonstrated the root surface resorption which was found topographically on these teeth. A comparison of the two radiographs in each case revealed no discernible changes for patients J.S., E.C., M.D., A.S. and K.B. Of the remainder, patient L.B. exhibited minor apical root loss of the order of 2mm or less for both roots of the right premolar and the palatal root of the left tooth (Figure 77). These teeth also exhibited a slightly widened periodontal ligament space, as did the right premolar of patient M.I., and indistinct lamina dura in the radiographs taken just prior to extraction. Minor apical resorption was also apparent on the right premolar of W.J. and on both roots of the left premolar of M.C. Some surface irregularity and widening of the periodontal ligament space was detected on both roots of the right premolar of L.H.

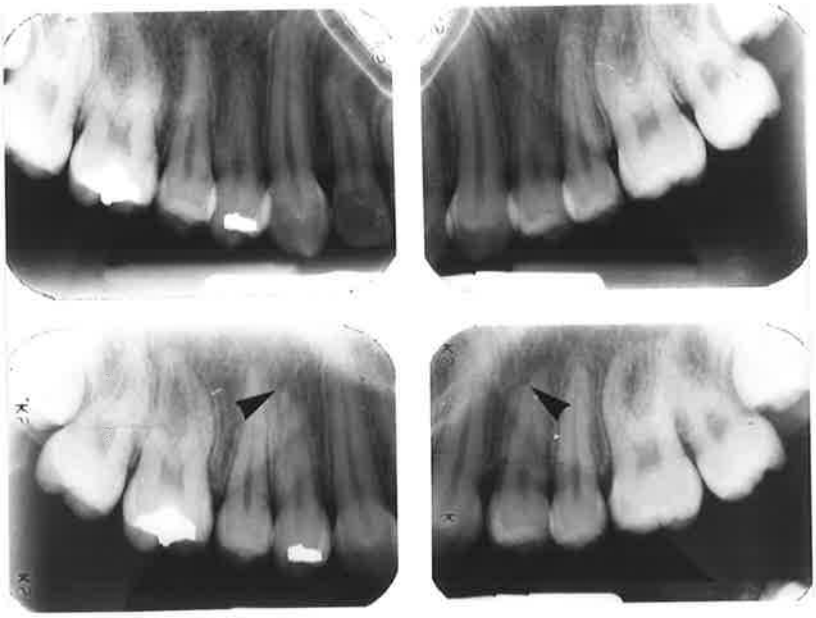


Figure 77

Patient L.B., retention period $25\frac{4}{7}$ weeks.

Long cone periapical radiographs showing upper first premolars prior to expansion above, and at the end of retention below. Only minor apical root loss, indicated by the arrows, is evident on the radiographs.

SECTION 6

DISCUSSION

The human fetal and adult bone specimens examined presented a range of topographical features similar to that seen in areas of normal cellular cementum on the tooth roots examined. These similarities point to cellular cementum being a tissue like bone in many respects and, importantly, displaying topographical differences in its mineral front which relate to its rate of formation. The reasons for the difference between the pattern of mineralization in fetal and adult bone are thought to include the speed of formation of matrix, the organization of the collagen, the proportion of mucopolysaccharide and the rapidity of calcification and its relationship to the matrix (Boyde, 1972; Boyde and Jones, 1972). Boyde (1972) pointed out that there appeared to be a spectrum of bone types from frankly fetal bone to adult lamellar bone. He stated that resting surfaces of fetal bone could be difficult to distinguish from resting adult bone surfaces and this was borne out by the bone specimens examined in this study. Normal and repair cellular cementum surfaces observed in this study exhibited a wide variety of topographical patterns which could be described as part of a spectrum virtually identical to that proposed by Boyde (1972) for bone.

Acellular cementum would appear to be unique in that it is formed almost entirely by mineralized Sharpey fibres with very little intrinsic matrix (Boyde and Jones, 1972; Jones and Boyde, 1972; Jones and Boyde, 1974; Kvam, 1973a). Furseth (1974) proposed the presence of an intrinsic matrix component in acellular cementum, based on the finding of fibres arranged at different angles in human acellular cementum seen in the transmission electron microscope, a finding supported by Selvig (1965). In the present study, observations of histologic sections prepared for and examined in the SEM showed that branching of inserting principal fibre bundles formed the matrix of acellular cementum. Although Sharpey fibre mounds have been demonstrated on bony surfaces (Boyde, 1972; Russell and Kapur, 1977), bone tissue having a purely extrinsic matrix has not been described and therefore acellular cementum, unlike cellular cementum, has no counterpart in bone.

Other surface features displaying distinct similarity in bone and cementum were the cell lacunae, Sharpey fibre depressions or holes and resorption lacunae. Both osteoblast and cementoblast cell lacunae, designated blast cells in this study because of their position in the surface and not beneath it, displayed similarities in size, shape and presence of canalicular openings. The lacunar surfaces showed a uniform texture particularly in areas of slow or resting mineralization (designated as Type III lacunae by Toda et al, 1974) and a fibrillar (Type I, Toda et al, 1974) or nodular (Type II, Toda et al, 1974) pattern in actively mineralizing surfaces.

The Sharpey fibre depressions seen in the adult bone examined were similar to many observed in cementum. In the bone specimens in this study these depressions probably represent tendon insertion sites from the medial pterygoid muscle. The Sharpey fibre depressions in cementum were variable in their internal patterns, depending on the rate and state of formation of the surrounding mineralization front, and were found only in cellular cementum. These principal periodontal fibre bundle insertion sites were not numerous in cellular cementum overall, but did tend to occur together in limited areas often with few cell lacunae. The range of appearance of Sharpey fibre depressions in cementum found in this study is supported by the collected illustrations of these features presented by other workers (Boyde and Jones, 1968; Jones and Boyde, 1972; Harry, 1977; Barber, 1978). The unusual principal fibre insertion sites with crenated or spoke-like inner configuration (Figures 29 and 32), encountered on a few specimens, closely resemble some Sharpey fibre depressions seen topographically by Boyde and Jones (1968) in rat cementum, Boyde and Jones (1972) in human Sharpey fibre bone, Harry (1977) in human cementum and histologically by Selvig (1965) in human cementum.

Occasionally within Sharpey fibre holes central projections were observed similar to those shown by Russell and Kapur (1977) in Sharpey fibre holes of bone adjacent to a subperiosteal implant. These projections may represent calcification within individual fibres or fibrils as described by Selvig (1964) or may simply be detached mineral structures which had fallen into the holes during specimen preparation. The latter explanation seems more likely as the overall impression was that Sharpey fibres in cellular cementum tended to mineralize from the periphery inwards as proposed by Boyde and Jones (1972).

The relative paucity of Sharpey fibres in cellular cementum contrasts with the cervical acellular cementum which is dominated by Sharpey fibre mounds and suggests that the role of cellular cementum in the mechanical support of the tooth is limited. The main function of cellular cementum is likely to be in adaptation to changing physiological demands, tooth drift and eruption. Support for this contention is provided by the process of continual apposition of cementum described by numerous writers including Gottlieb (1942 and 1943), Kerr (1961) and

Armitage (1976). This phenomenon appears to occur most rapidly on the cellular cementum (Furseth and Mjör, 1973) as evidenced by its greater thickness compared with acellular cementum, a feature which was observed histologically in this study. Stallard (1963) found that stimulating the eruption rate of molars in experimental mice resulted in an increased rate of cellular cementum formation and in some areas addition of cellular cementum over previously uniform acellular cementum. This finding further supports the role of cellular cementum proposed here.

The relatively homogeneous surface of acellular cementum generally seen in the SEM suggests a surface either resting or mineralizing only very slowly. In addition, cellular cementum was sometimes found to be covering over acellular cementum at the latter's apical extremity. A further indication of the function of cellular cementum is the temporal sequence of its formation in relation to acellular cementum which forms initially prior to the tooth erupting into functional occlusion. Idiopathic resorption areas described by Henry and Weinmann (1951) and Jones and Boyde (1972) were cited as occurring most frequently in the apical areas of the teeth, a fact which may be interpreted as being consistent with the proposed function of cellular cementum in this region.

A few areas of normal acellular cementum observed on some experimental specimens were characterized by the presence of spherical nodules, ranging in diameter between ¼ and 1 micrometre. An extreme example was found on a specimen from patient K.B. (Figure 38). Such nodules were also found occasionally in relation to normal cellular and acellular cementum and resorptive lacunae in early repair. Barber (1978) described and illustrated similar features on acellular cementum and in resorptive excavations. He concluded that the nodules were mineralizing calcospherites but did not often encounter such evenly proportioned spherical particles in any location. Generally in this study, the mineral front of depository cellular cementum displayed cigar-shaped or fusiform particles with an uneven surface and occasionally structures which appeared to be formed of distinct clusters of smaller mineral particles similar to microcalcospherites described by Jones and Boyde (1972). Various degrees of confluence between particles were found to occur in different surfaces, possibly relating to the rate of mineralization at the time of extraction. It is apparent that the presentation and configuration of mineral nodules on surfaces in this study varied considerably but conformed overall to descriptions and illustrations by Boyde and Jones (1968), Jones and Boyde (1972) and Barber (1978).

It was interesting to note that repreparation of the right premolar mesiobuccal specimen from patient K.B. removed the densely packed spherical structures leaving a relatively homogeneous surface texture overall. That these particles were an artefact was suggested by their profuse nature on this specimen and no other, including the lingual segment of the same root. However, the particles' similarity to mineralizing nodules elsewhere indicated that they were calcospherites. Some reservation must therefore be held about conclusions regarding surface activity based on the presence and appearance of mineralizing nodules and particles seen topographically. It would appear that small variations in the technique of specimen preparation for examination in the SEM have different effects on the specimens, particularly where discrete particles may be on the surface and easily removed by preparation. This variation is probably most critical for rapidly forming surfaces where separate mineral particles occur largely unrelated to the collagen matrix (Jones and Boyde, 1972). In fact, Boyde and Sela (1978) have described mineral particle clusters and detached calcospherites recovered from sodium hypochlorite solutions used to deorganify various animal dental and osseous tissues.

Evidence that surfaces rendered anorganic and examined in the SEM do not necessarily represent the outermost layer of the mineral front is provided by comparison with transmission electron microscope studies. Selvig (1965) demonstrated hydroxyapatite crystals with dimensions not greater than 400 x 200 x 20 Angstrom units at the surface of cellular and acellular cementum on human teeth, a finding which he equated with continuous cementum apposition. It is apparent that such small, discrete, initial mineralization crystals would probably be lost during specimen preparation and even if they were not, their resolution in the SEM would be difficult. Thus specimens prepared for topographical examination must be considered as demonstrating relatively gross features which remained a part of the surface after specimen preparation.

The assessment of the state of resorptive lacunae as actively resorbing, resting or beginning to remineralize is all the more tenuous in the light of this discussion. Jones and Boyde (1972) indicated that early repair occurs with clusters of mineral particles on the surface. However, it is apparent that these may be lost during specimen preparation. In addition, repreparation of some specimens in this study indicated that a short deorganification period may leave a fine reticular fibrous coating on the surface in places, which can be removed by further treatment in sodium hypochlorite. This finding is further evidence of the different results obtainable with slightly different times of preparation. Jones and Boyde (1972) stated that active resorption on tooth roots is characterized by smooth Howship's lacunae. Contrastingly, Boyde (1972) stated that initial remineralization occurring in ground substance of resorbed bone surfaces would produce a smooth surface. Boyde and Lester (1967b) proposed that demineralization was the first stage in resorption. Presumably then, resorption might be characterized topographically by a slightly rough or textured surface as illustrated by Kvam (1972b, 1972c). Based on the results of slightly varying preparation times found in this study therefore, the commonly observed appearance of Howship's lacunae with a fine, uneven texture at high magnifications cannot be related unequivocally to active resorption or repair.

Boyde and Jones (1968) alluded further to the difficulty of interpreting surface activity topographically. They drew attention to the similarity of surfaces on which matrix production was proceeding and those on which it had stopped but mineralization had not been completed. The static 'frozen' image, seen in the SEM, of the dynamic surface of cementum must obviously be interpreted with caution. The resolution afforded by the light microscope is insufficient to clarify these uncertain interpretations made topographically. Ultrastructural investigation must be used on undecalcified tissue to define more accurately the precise activity at the cementum surface. However, it seems that the overall pattern of resorption and repair on specimens in this investigation can be faithfully interpreted using the topographical and histological techniques employed.

A few areas of resorption were found on the adult bone specimens examined. These areas were characterized by aggregations of Howship's lacunae which were essentially similar to those in resorption areas on the tooth roots examined. The main difference was that the resorption observed on bone was much shallower and was not associated with reparative tissue. This difference may be related to the physiological homeostatic function and remodelling characteristic of bone.

The upper lateral incisor examined in the SEM had a number of areas of previous resorption. In some locations repair activity within these areas resembled mineralizing primary bone. Within the resorption areas repair had produced mounds in some places and saucer-like depressions with raised rims in others. It is possible that these features represent principal periodontal fibre insertion sites, particularly the depressions resembling dish-topped Sharpey fibre projections described by Jones and Boyde (1972). This finding indicates that periodontal fibre reattachment can occur in an area of previous resorption on a non-experimental tooth.

Flat, featureless cementum surface topography was seen in a number of places on the root of the unerupted upper second premolar examined. This surface suggested a resting, uniformly mineralized tissue and the paucity of topographical features indicated either absent or reduced principal periodontal fibre insertions. The Sharpey fibre mounds which did occur were generally very low probably due to a lack of functional stimulation, as Stern (1964) proposed that the calcified projections associated with inserting periodontal fibres were formed in response to functional pull.

Within the resorptive excavation on the deciduous canine examined, areas of closely packed irregular projections indicated periodontal fibre attachment. Repair was dominant within the resorptive excavation but generally appeared to be inactive although root outline was not re-established. A topography very similar to adult lamellar bone was found in some places in repair tissue, consisting of a few cell lacunae in a surface dominated by mineralized bundles with preferred orientations.

The bundles interchanged fibres with each other. Furseth (1968) noted areas of cellular cementum repair associated with resorption on human deciduous teeth. A number of photomicrographs published by Furseth (1968) suggest a type of periodontal attachment to resorbed dentine and repair cementum on deciduous teeth, although he did not specifically discuss this.

In the experimental premolar series a striking feature was the extensive surface resorption particularly affecting the buccal surfaces. Such root surface damage had been expected, based on the previous report of Barber (1978) who found similar resorptive lesions on RME anchor premolars. In addition some resorption was also found on the lingual surfaces, generally in the apical region. This latter finding may be explained by a tendency for the anchor teeth to tip during expansion and retention and possibly by tension on the lingual side, as suggested by Barber (1978). Tension generated resorption may occur in the same way that excessive orthodontic extrusive forces may cause apical resorption via stretched fibre bundles (Reitan, 1969). By comparison, features which could possibly have been a proliferative response to tension were also observed on some lingual surfaces. In support of this interpretation, Reitan (1969) stated that cellular cementum formation could be elicited on the tension side of orthodontically rotated teeth. Thus, it is possible that different tissue reactions on the root surface may be generated by essentially the same stimulus, although the evidence for this is purely circumstantial and requires more detailed investigation.

The fact that resorption also continued from the buccal onto the proximal surfaces is hardly surprising since the root surface is curved and pressure buccally will also be directed against the buccal aspect of the proximal surfaces. In addition, a rotated tooth will present a greater proportion of one proximal surface buccally when *in situ*. Of the patients treated by the author, only M.I. and J.S. had slight rotations of their first premolars resulting in more of the anatomical mesial surfaces being directed buccally.

The dominant feature of most areas of resorption was found to be repair. One important observation made in all the experimental material was that repair was always by cellular cementum. This finding was substantiated by the light microscope histological examination. Topographically, designation of the repair tissue as cellular cementum was based on the presence of cementoblast cell lacunae in the mineral front and the similarity of the repair tissue with cellular cementum observed on normal root surfaces. In the light microscope, cementocyte lacunae within repair tissue indicated that it was cellular cementum. In some histological sections, tissue between incremental lines displayed no cell lacunae but observation of serial sections revealed this to be an effect of the plane of the section only. In no instance, either topographically, or in the light microscope, was repair tissue found resembling the acellular cementum which dominated the cervical half to two thirds of each root specimen examined.

The incremental lines in repair cellular cementum indicate the phasic nature of its deposition. These lines are produced when collagen matrix production slows temporarily but mineralization proceeds. This fact points again to the difficulty of assessing the state of activity of cementum surfaces examined in the SEM, as evidence of continuing mineralization may be lost during specimen preparation.

On the buccal surfaces, the resorption was not predominant in any one location overall. Thus, middle, apical and cervical areas of the buccal surfaces were severely affected by resorption. Dentine was generally involved in the resorption but the main direction of advancement of established lesions appeared to be circumferential expansion. This was evidenced by the large areas of root surface destroyed by resorption and the absence of pulpal involvement, which was confirmed in the light microscope, suggesting limited axial penetration. An impression gained from the experimental series was that often, small areas of resorption would coalesce to form larger areas.

Undermining of the surface by resorption was also a feature. This could relate to the resistance to resorption of normal root surface cementoid or pre-cementum, suggested by numbers of writers (Furseth and Mjör, 1973; Reitan, 1969, 1974). Rygh (1977) also pointed out that the periodontal ligament collagen adjacent to cementum is more mature than that adjacent the alveolar bone and as such is more resistant to degradation. However, he stated that this mature collagen adjacent to cementum would disappear in some places after hyalinization thus removing a possible protective barrier against root resorption.

Mineralized dentine may be more susceptible to resorption than cementum due to the lower mineral content and tubular structure of the former tissue. This proposed differential susceptibility to resorption of dentine and cementum may also be greater near the cemento-dentinal junction because of the Tomes' granular layer and interglobular dentine in this area. The Tomes' granular layer has been attributed to interglobular dentine which consists of spaces of deficient mineralization within the dentine (Mjör, 1973). Another suggestion is that Tomes' granular layer represents random looping of the dentinal tubules in the first formed dentine (Osborn and Ten Cate, 1976). In addition, a structureless hyaline layer has been described between cementum and dentine and recent work indicates that this is a product of the root sheath cells which acts as a 'glue' between cementum and dentine (Osborn and Ten Cate, 1976). This layer would also be hypomineralized. The intermediate cementum at the cemento-dentinal junction in premolars and molars is another hypomineralized layer (Osborn and Ten Cate, 1976) which together with those described above could account in part for the undermining character of root surface resorption as this would proceed more readily along these layers.

The very nature of root surface resorption being undermining may partially explain its peripheral rather than axial expansive nature seen on anchor premolars. It seems reasonable to assume that the heavy forces used in expansion create large areas of hyalinization in the periodontal ligament on the buccal pressure side. Reitan (1969) stated that the magnitude of the force essentially determines the duration of hyalinized areas, which are also termed cell-free zones. Therefore the hyalinized areas produced by rapid maxillary expansion would probably be of long duration, as the force levels are continually within the heavy orthopaedic range during expansion and for some weeks afterwards (Isaacson and Ingram, 1964; Zimring and Isaacson, 1965). These forces are much greater than normal orthodontic tooth moving forces (Gianelly and Goldman, 1971) and apparently do not produce significant tooth movement or alveolar bone resorption in the time period during which they are present. It is also apparent, however, that these forces produce extensive resorption of root structure. This root resorption is most likely to be initiated adjacent to the hyalinized zones of the periodontal ligament. Progress of the resorption by undermining would then proceed in a manner similar to that described by Reitan (1969) in relation to alveolar bone during some types of orthodontic tooth movement and Rygh (1977) in relation to tooth roots following experimental orthodontic manipulation in rats. Such root resorption would progressively relieve the compression in the hyalinized areas. However, because of the curved and somewhat irregular nature of the root surface, the pressure would be distributed around the periphery of resorptive excavations which would thus tend to expand at their extremities. The central areas of these lesions in this case would not be under pressure and thus free to repair. Although this sequence of events is theoretical, it fits well with the results observed in this study.

The total area involved in resorption on the buccal aspect of anchor premolars showed some variation between antimeric teeth and also different patients. However, no time-related trend could be seen in the material overall. Indeed, patient J.S. (retained for $53\frac{2}{7}$ weeks) displayed a smaller total area of resorption (16%) than patients with much shorter retention periods (average for Group 1 was 25%). It should be pointed out that the light microscopic examination confirmed this finding which topographically may have been obscured by repair concealing areas of previous resorption.

Barber (1978) in his study of root resorption following RME in man indicated that active resorption was continuing up to 36 weeks after expansion and during fixed retention. This assessment was based on the presence of open, sharply defined Howship's lacunae at the borders of resorption areas, a finding confirmed in the present study. Barber (1978) interpreted this apparent continued resorption to indicate the presence of a significant relapse force. Reitan (1964), based on a study in dogs, concluded that once root resorption was started by strong continuous orthodontic forces, much lighter pressure could maintain or increase the resorption process. If Reitan's (1964) contention is true in humans, the magnitude of the force continuing the resorption process in Barber's (1978) material might have been relatively small and possibly not capable of causing significant relapse of the expansion.

Interestingly, a subjective assessment of Barber's (1978) illustrations leads to the conclusion that the extension of resorption was most significant between the end of expansion and three months into retention. As the shortest period of retention in the present study was 14 weeks, the apparent lack of correlation between the retention period and the amount of resorption is thus supported by Barber's (1978) work.

Although it is likely that some resorption occurs after about 3 months retention, the presence of open Howship's lacunae should not be taken to indicate a definite and significant ongoing resorptive process. Timms and Moss (1971) found areas of resorption and repair in the apical third of teeth extracted after retention periods of 21, 22 and 23 months. However, they did not discuss the amount of either repair or resorption.

Further evidence that active resorption slows significantly after about 3 months retention was provided by the extent of repair. A trend to increased filling of resorptive defects over the span of retention periods studied was evident. Repair was observed in its early stages to commence centrally within the lesions and gradually to expand peripherally with new repair tissue as well as continuing to build outwards on previously deposited repair cementum. This trend of an increasing proportion of repair tissue with longer retention periods resulted in very few open Howship's lacunae on the longest retained specimens. It should be appreciated that this time sequence of events in repair has been assessed from observing the patterns on the different specimens in this study.

The conclusion that the presence of open Howship's lacunae indicate active resorption and thus a relapse tendency (Barber, 1978) is unsatisfactory on two grounds. Firstly, resorption shows only relatively minor advances after 3 months retention and secondly, repair progressively restores the resorptive defects during a period when active peripheral extension of the resorption has been claimed to be present (Barber, 1978). Inherent in this line of reasoning also is a questioning of the topographical indications of active resorption and early repair as discussed previously. The differentiation of active and resting Howship's lacunae does not seem possible on a topographic basis because of their similar surface features. The possibility that a surface may be resorbed but not repairing (i.e. resting) should be considered. Such a situation must prevail at some stage to form a reversal line between a resorbed surface and repair tissue and is analogous to the formation of incremental lines in cementum due to the periodic nature of its formation.

From the information gained in this investigation it appears that significant relapse forces may operate for up to 3 months after RME. Beyond this time in retention, strong relapse forces directed against the buccal root surfaces of anchor teeth are probably not present. Zimring and Isaacson (1965) found that residual loads acting on the expansion appliance dissipated within five to seven weeks during initial retention. Any continuing resorption could be maintained by light and diminishing medially directed forces. Occlusal forces may even be enough to perpetuate the small amount of ongoing resorption which probably occurs, as generally the teeth are held in an over-corrected position, often resulting in abnormal occlusal relationships. Haas (1980) pointed out that persistence of forces tending to collapse the expanded maxillae during fixed retention could result in "...the maxillae moving through the stabilized teeth". The fact that this does not appear to occur supports the proposal that residual loads and relapse forces are dissipated well within the first 3 months after expansion. How then does relapse occur? It would seem from studies by Thörne (1960) and Krebs (1964) that apical base and nasal cavity width gains during expansion are in reality not lost. Reports of the relapse which can occur over a long term (Stockfisch, 1969; Timms, 1976; Linder-Aronson and Lindgren, 1979) generally relate to the dental arch alone. Some loss of dento-alveolar width should be expected because of the dental over-correction during expansion and possibly because some buccal tooth tipping may occur during RME. However, the recommendation of a safe minimum period of retention based on the results of this study would be unwise. Previously, recommendations for retention periods have varied up to several years (Timms, 1976; Timms and Moss, 1971) with the least being three months (Thörne, 1956).

The histological examination in the light microscope revealed very few distinct multinucleate odontoclastic cells in relation to resorbed surfaces. A similar lack of these cells was reported by Oppenheim (1942) in relation to root resorption associated with orthodontic treatment in human material and by Furseth (1968) in relation to the resorption of human deciduous teeth. It is possible that the apparent absence of multinucleate odontoclasts reflects a resting state at the termination of resorptive activity. On the other hand it may be that a proportion of the round and oval nuclei often observed adjacent to resorbed surfaces in the histological study may belong to uninucleate odontoclasts or indeed to multinucleate cells with indistinct cell outlines. It seems more likely however that these nuclei belong to cementoblasts. The flattened or fusiform nuclei also observed on resorptive surfaces and repair cementum are probably associated with fibroblast type cells or frank cementoblasts. Fibroblast-like or osteoblast-like cells have been described in close proximity to osteoclasts at resorbing bone surfaces by Heersche (1978) and Kurihara and Enlow (1980a). Heersche (1978) ascribes to these cells a collagen phagocytic role but Kurihara and Enlow (1980a) suggest that they are secretory and involved in the production of an adhesive attachment to resorbed alveolar bone. To help resolve the question of the nature of the cells observed in this investigation ultrastructural and stereologic study is required.

Stern (1964) proposed that cementoblasts may be involved in cementolysis as well as cementogenesis and fibrillogenesis and Gianelly and Goldman (1971) postulated that cementocytes might be converted into resorbing cells in some circumstances. Cementocyte lacunae exposed by resorption were rarely observed in this study probably because resorption had generally penetrated through cementum into dentine. The configuration of exposed cementocyte lacunae suggested that they were not involved in resorption. A similar finding and conclusion was arrived at by Boyde and Jones (1972) in relation to osteocytes. However, this conclusion cannot be considered unequivocal as cells which modulate to undertake resorption would probably create Howship's lacunae, so that topographical identification of the original cell type would not be possible.

In this study, a precise analysis of the area of resorption on each specimen was neither possible nor warranted. The number of patients from whom specimens were obtained would invalidate statistical analysis because not only were retention times different for each patient, a major consideration in the study protocol, but age, sex, skeletal and dental maturation and the amount of expansion varied considerably. Importantly, however, the results showed that large areas of root surface were involved by resorption in every specimen examined. Previous studies of root resorption associated with orthodontic treatment have concentrated on apical root loss because it can be readily detected on radiographs. In this study, almost no apical root loss was detected. Gianelly and Goldman, (1971) stated that moderate root shortening was probably "harmless" in the long term, confirming the impression gained from the earlier work of Phillips (1955). However, Reitan (1969) stated that only apical root loss could "imperil the stability and normal function of the tooth" when compared to surface resorption in the cervical and middle areas of the tooth. Reitan assumed that the repair of resorptive defects which he observed on root surfaces was sufficient indication that regeneration of a normal functioning supportive periodontal attachment would occur. This assumption is invalid, particularly in areas where so-called 100% Sharpey fibre acellular cementum has been lost.

The extent of surface resorption observed on specimens in this study could hardly be termed insignificant. In relative terms the estimated areas involved on individual teeth conservatively equate with a one millimetre loss of crestal alveolar bone support around the entire tooth or a two millimetre apical root loss. In terms of tooth attachment, the apical region of the tooth is probably far less important than the cervical half to two thirds, as discussed previously. Thus, as resorption affected the cervical as much as the apical areas, the comparison with loss of alveolar bone support is more valid. Although the loss of one millimetre of crestal bone may seem small, Jacobson (1952) and Sjølien and Zachrisson (1973) pointed out that the long-term significance of such reduction is not well established but is probably greater than a similar degree of apical root loss. Also, the effect of extensive resorption of the root surface close to the cervical extent of the attachment apparatus has not been investigated at all. Consideration should be given to the fact that an intact, horizontally or obliquely orientated fibre system may play a role in resisting the apical migration of the epithelial attachment in periodontal disease. It is salutory to realise that despite the extensive research into the clinical effects of RME no work has been done to elucidate the long-term functional status and indeed survival of the anchor teeth.

The fact that the surface resorption generated during expansion and retention on anchor premolars may be deleterious to their function and survival is obvious. To assist in determining the significance of this resorption the exact nature of the repair which has been observed to be a significant and progressive feature of the resorptive defects must be studied. This investigation has yielded a number of important findings in relation to repair. The first of these is that the repair tissue observed was always cellular cementum, regardless of the location on the root of the resorption. This repair cellular cementum appeared topographically and histologically identical with normal apical cellular cementum, displaying a similar range of surface features indicative of its rapidity and state of formation. Early repair and tissue at the periphery of areas of advancing repair displayed the characteristics of rapid mineralization, namely randomly orientated, discrete mineral nodules. The surface of later more advanced repair tissue, found initially centrally in the lesions, was more homogeneous and sometimes displayed a fibre pattern indicating a slowly mineralizing or resting surface. On longer retained specimens particularly, repair was often exuberant, over-contouring the surface in relation to the surrounding level and sometimes covering normal cementum at the edge of the defect. A 'functional' type of repair as described by Orban (1928a), Gottlieb (1928) and Armitage (1976) was suggested in a small number of resorption areas by retained fragments of alveolar bone which were observed in the light microscope. Generally, however, repair would appear to progress toward fully restoring root contour.

Importantly, Sharpey fibre depressions in repair cementum were sparse and inconsistent in their presence. Where they did occur they were generally in groups and often towards the advancing edge of repair. In fact their distribution and occurrence was not unlike that in normal apical cellular cementum. No Sharpey fibre mounds were observed at all on repair tissue. The significance of these findings is twofold. Firstly, the proposal that cervical acellular cementum is unique and cannot be reformed (Slavkin, 1976; Stahl, 1977) is supported for the circumstances prevailing in this study. Secondly, if as seems likely it is the cervical 100% Sharpey fibre acellular cementum which is primarily responsible for tooth attachment (Selvig, 1965), the resorption observed in this study could result in the support for the tooth being seriously compromised indefinitely. It is prudent to point out here that contemporary concepts of tooth support propose that the collagen fibres of the periodontal ligament provide attachment for the tooth to alveolar bone and only participate in the cushioning of the functioning tooth (Melcher and Walker, 1976).

The light microscopic examination of the histological sections gave rise to a number of interesting observations. Resorbed and unrepaired dentine often exhibited fibre bundles at right angles to its surface. The fibres from these bundles intermingled with the periodontal fibres not far from the root surface. Disruption of the periodontal ligament due to extraction makes definite interpretation of fibre direction and pattern difficult and so conclusions about the periodontal attachment must be guarded. However, it is possible that as dentine is resorbed, part of its fibre matrix is retained and serves as an ongoing source of direct attachment. This would parallel the situation of continuous attachment to alveolar bone during orthodontic tooth movement (Gianelly and Goldman, 1971) and also Kurihara and Enlow's (1980a) Type I continuous attachment to resorptive and depository alveolar bone surfaces. Kraw and Enlow (1967) pointed out that the continuous attachment seen in areas of resorbing alveolar bone was not identical to that provided by inserting principal periodontal fibres forming Sharpey fibres in bone.

Kurihara and Enlow (1980a) also described adhesion of periodontal fibrils to resorptive alveolar bone surfaces which they designated Type III (discontinuous) attachment. They illustrated, using one low power photomicrograph, a Type III attachment in an area of resorbed dentine from a rat. This dentine attachment was not discussed in the text of their report and no convincing evidence was presented that it involved the initial secretion of a "strandlike material" by ligament cells as they claimed.

Isolated illustrations of a possible fibre attachment to resorbed or resorbing dentine have occurred elsewhere in the literature. (Orban, 1928a; Azuma, 1970; Dragoo and Sullivan, 1973). However, the significance and even the existence of such periodontal attachment to tooth surfaces in areas of active resorption has received negligible attention and comment. It is interesting to note in the context of a continuing periodontal attachment that Rygh (1973) has shown that the continuity between fibrils and cementum is not disrupted by hyalinization of the periodontal ligament due to orthodontic forces except where foci of heavy pressure or prolonged hyalinization occur.

The existence of a direct attachment to resorbing dentine could explain the occurrence of Sharpey fibre depressions observed occasionally in the advancing repair tissue. Mineralization may proceed around the attaching fibre bundles, initially forming surface depressions similar to Sharpey fibre holes. Later, as repair progresses outwards the depressions may be mineralized as the fibres become incorporated as cementum matrix.

Although Sharpey fibre depressions, suggesting principal periodontal fibre insertions, were not a feature of repair surfaces and particularly more advanced repair, reattachment as a possibility is not precluded. Indeed, observation in the light microscope suggested that fibres were attached to repair cementum. It is difficult to establish the precise nature of this fibre attachment at the light microscopic level even using polarized light. The magnification and resolution afforded by the transmission electron microscope is necessary to clarify the relationship of fibres to the repair tissue surface.

The possibility exists of the attachment being an adhesive type similar to that described by Stahl, Slavkin, Yamada and Levine (1972) and Kurihara and Enlow (1980a). Importantly however, whether the attachment observed in the light microscope was direct or adhesive in nature, the orientation of the periodontal fibres adjacent to most repair tissue especially the more advanced areas, appeared to be parallel to the root surface vertically without horizontally or obliquely directed principal fibre bundles. Although this assessment is based on observation of the disrupted periodontal ligament tissues adhering to the root surface, it matches descriptions of periodontal reattachments by Orban (1928b); Linghorne and O'Connell (1950); Morris (1969); Stahl et al (1972); and Stahl (1977). A vertically orientated fibre attachment would be unlikely to provide the same mechanical attachment and support for the tooth as the normal horizontal and obliquely directed fibres (Orban, 1928b). However, in the long term, with normal functional stimulation, the fibres involved in reattachment may be reorientated as suggested by Stahl et al (1972). In this study there was no evidence of any such adaptations of attachment occurring and this could conceivably be related to the altered functional stimulation of the anchor teeth due to the fixed rigid retention of the expansion.

The large areas of root surface involved by resorption suggest that significant amounts of periodontal ligament are hyalinized by the expansion procedure. The effect of this hyalinization on the periodontal ligament may have a bearing on the pattern of repair observed, particularly in the early stages. It is surprising that ankylosis did not seem to occur during repair on any specimen. Ten Cate (1976) suggested that a critical factor for the production of ankylosis was the number of ligament cells, capable of regenerating a new ligament, which remain after periodontal injury. He proposed that if the number of these cells was insufficient, repopulation from the adjacent bone marrow could result in the formation of bone tissue and ankylosis, a sequel also suggested by Melcher (1970) and Line, Polson and Zander (1974). Apparently therefore, despite the large areas of hyalinization and thus loss of cells caused by RME and retention, the periodontal ligament is capable of repairing itself.

Whether regeneration in the strictest sense occurs has not so far been demonstrated.

Certain patients in this study were of particular interest because their expansion and retention regimes were slightly different to the majority (see Table 2). On specimens from these patients the amount of resorption and its pattern of distribution was found to be essentially the same as on other specimens. However, repair tissue seemed to be slightly further advanced towards restoring the defects on the roots from patients B.B., R.H. and S.M. when compared to other roots removed at similar periods after expansion. This subjective appraisal suggests that more normal functional stimulation associated with removable retainers or no appliance retention may hasten the repair process to a small degree. A study involving a larger, matched population is necessary to test this proposal which is, however, supported by Reitan (1969). He found that repair cementum formation occurred more rapidly on teeth provided with physiologic stimulation after orthodontic movement.

Patient M.D. is of interest because of the large area of resorption compared to other specimens in this study. Patient M.D. was a female of advanced skeletal age which mitigated against an orthopaedic expansion. Expansion was assessed clinically as being mainly dento-alveolar rather than skeletal and for this reason was not prolonged. A relatively small interpremolar width increase of 2.5 mm associated with a total appliance activation of 5.0 mm was achieved. The forces attendant to RME were, however, still present and probably higher than normal because of the lack of a skeletal response. It is not surprising therefore that a greater amount of resorption was observed on specimens from this patient. Little repair was found, probably because the high forces persisted for much longer than normal, perpetuating large areas of hyalinization which virtually precluded cementum repair. It seems that skeletal and local tissue responses to RME may be affected by the physiologic maturity of the patient, as patient M.D. was the most skeletally mature of the author's group in this study. Indeed, a clinical impression expressed by Sims (1980) is that RME patients in the age group up to about 14 years require significantly less time in retention to achieve stability than do older patients. A large matched and controlled patient sample is required to test Sims' (1980) proposal which could be related to the amount and duration of relapse forces following RME which in turn would influence the extent and timing of root resorption and repair. A case similar to patient M.D. was cited by Harry (1977) who found resorption involving about one third of the buccal root surface of an anchor premolar from an RME patient who exhibited arch expansion of dental origin only. This degree of resorption was more extensive than that found by Harry (1977) on another anchor premolar root from a patient who demonstrated skeletal expansion with RME.

The estimated area of resorption on the buccal surface of the buccal root of patient T.H. examined in the SEM was markedly lower than other specimens in this study. If the buccal surface of the lingual root was included in the assessment of the area of resorption, the percentage figure for this patient would approach more closely that determined for other specimens. From the histological investigation of the antimeric premolar it was evident that extensive resorption had occurred on the buccal aspect of the lingual root but was virtually completely repaired with cellular cementum.

It is salutory that in neither this study, nor those of Harry

(1977) or Barber (1978) was the extensive root surface resorption which was discovered in the SEM detected on radiographs. Henry and Weinmann (1951), on the other hand, claimed that a resorption area 0.734 mm long and 0.104 mm deep, the average size of lesions found in their investigation, could be readily seen on a radiograph under ideal conditions. Although these workers performed a limited investigation on extracted teeth to arrive at this conclusion, the *in situ* equivalent was not demonstrated. Indeed, the 'ideal situation' would seem to be the critical factor as pointed out by Black (1965). In a radiographic study of root resorption Black (1965) found that the position of small resorption areas were critical to their projection on the image. He also found no correlation between resorption observed histologically and that seen radiographically. These findings support the conclusion that radiographs are of doubtful use in demonstrating the surface resorption found in this study.

SECTION 7

CONCLUSIONS

1. The active and resting mineralizing fronts of human tooth root cellular cementum are similar and in some cases indistinguishable from such fronts which occur in human adult and fetal bone. Descriptions of cellular cementum as being very like bone are thus justified. Indeed, the mechanism of mineralization of these tissues is probably essentially the same, apparent topographical differences being due to rate of formation or functional demand.

2. Forces causing extensive tooth root resorption, associated with rapid maxillary expansion in man, appear to be most significant for up to three months during fixed retention following expansion, in skeletally immature patients.

3. Repair of root surface resorption was always by cellular cementum indicating that typical cervical acellular cementum is not regenerated in the circumstances attendant to RME.

4. Periodontal fibre attachment to resorbed dentine and repair cementum does occur although a description of its exact nature requires further study at the ultrastructural level.

5. Routine periapical radiographs do not show evidence of the extensive root surface resorption found on anchor premolar tooth roots following RME and retention.

SECTION 8

APPENDIX I

FIXATION

NEUTRAL BUFFERED FORMOSALINE : FORMULA

40% Formaldehyde	200ml
Distilled water	1800ml
Sodium dihydrogen phosphate	8.0gm
Disodium hydrogen orthophosphate	13.0gm

DECALCIFYING MEDIA : FORMULAE

EDTA decalcifying medium

500ml water

add 37.5gm PVP (polyvinyl pyrrolidone) and dissolve.

add 50gm EDTA.

Formic/formate decalcifying medium

Water	1650ml
Sodium formate	68gm
Formic acid	340ml

APPENDIX II

TISSUE PROCESSING AND EMBEDDING

After neutralization in 5% sodium thiosulphate the decalcified sections were treated according to the following stages.

1. Wash in running water for 1 hour then in a 37°C oven:-
2. Placed in 70% alcohol overnight.
3. 80% alcohol for 1 hour.
4. 90% alcohol for 1 hour.
5. 95% alcohol for 1 hour.
6. Three changes of absolute alcohol for 1 hour each.
7. One part absolute alcohol, 1 part methyl salicylate for 1 hour.
8. Methyl salicylate with 0.5% celloidin for 2 days.
9. Methyl salicylate with 1% celloidin for 2 days.
10. Methyl salicylate 2/3 with paraffin wax 1/3 for 1 hour.
11. Methyl salicylate 1/2 with paraffin wax 1/2 for 1 hour.
12. Methyl salicylate 1/3 with paraffin wax 2/3 for 1 hour.
13. Two changes of paraffin wax for 2 hours each.
14. Further change of paraffin wax overnight.

Then vacuum in clean paraffin wax at 60°C for 15 minutes and block using Tissue Tek II Embedding Centre.

APPENDIX III

PREPARATION OF GELATINIZED SLIDES

1. Soak clean slides in dichromate cleaning solution, wash in running tap water for at least 6 hours and then rinse thoroughly in distilled water.
2. Allow slides to drain for 2 to three seconds.
3. Dip into a subbing solution prepared as follows:-
   (a) Dissolve completely in 1 litre of warm distilled water, 5gm U.S.P. gelatin or Knox unflavoured gelatin.
   (b) Add 0.5gm chrome alum.
   (c) Cool and filter through Whatman No. 1 filter paper.
   (d) Store at 5°C for 48 hours.
4. After slides have been dipped into subbing solution, set vertically in racks in a dust free atmosphere and allow to dry.

Dichromate Cleaning Solution

Formula.

Potassium dichromate 100gm
Conc. sulphuric acid 100ml
Distilled water to make up to 1 litre.

Made up as follows:-

Dissolve the potassium dichromate in 3/4 total volume of water, add the sulphuric acid slowly while stirring constantly and make up to 1 litre with distilled water.

APPENDIX IV

STAINING TECHNIQUES

POLLACK'S TRICHROME

Formula.

Acid fuchsin	0.5 gm
Ponceau 2R	1.0 gm
Light green, SF yellowish	0.45gm
Orange G	0.75gm
Phosphotungstic acid, C.P.	1.5 gm
Phosphomolybdic acid, C.P.	1.5 gm
Glacial acetic acid	3.0 gm
Alcohol, 50% to make	300.0 ml

Made up as follows:-

First, the glacial acetic acid is added to the alcohol. Of this acidified alcohol, 50ml portions are prepared in four beakers: in the first beaker, acid fuchsin and ponceau 2R are dissolved; in the second, light green; in the third, orange G and phosphotungstic acid; and in the fourth, phosphomolybdic acid. The rest of the acidified alcohol is used to rinse out the beakers and to make up the volume. All the ingredients dissolve easily except for the phosphomolybdic acid, which will dissolve after slight warming of the beaker.

The mixture is then filtered and ready for use.

(After Luna, 1968).

Staining Procedure:-

1. Bring sections to water.
2. Flood with Pollack's trichrome for 30 seconds.
3. Rinse with tap water.
4. Dehydrate through alcohols and mount.

WEIGERT'S HAEMATOXYLIN AND VAN GIESON

Weigert's Haematoxylin Formula.

(a)	Haematoxylin	1gm
	Absolute alcohol	100ml
(b)	30% aqueous solution of ferric chloride	4ml
	Distilled water	100ml
	Hydrochloric acid	1ml

Mix equal parts of (a) and (b) immediately before use (add (b) to (a)).

Solution (a) should be allowed to ripen for one week prior to use.

(After Culling, 1974).

Van Gieson Formula.

Acid fuchsin, 1% aqueous	2.5ml
Picric acid, saturated aqueous	97.5ml

Ready for use. (After Luna, 1968).

Staining Procedure:-

1. Bring sections to water.
2. Weigert's haematoxylin solution for 10 minutes.
3. Wash in distilled water.
4. Van Gieson's solution for 1 to 3 minutes.
5. Dehydrate in alcohols and mount.

(After Luna, 1968).

SAVILLE BRADBURY - a modification of Lillie's stain for collagen

Staining Procedure:-

1. Bring sections to water.
2. Mordant 2 minutes in saturated aqueous picric acid.
3. Wash in water 30 seconds.
4. Stand 2½ minutes in 1% Ponceau 2R (Brilliant Crystal Scarlet) in 1% Acetic acid.
5. Rinse in water.
6. Mordant 4 minutes in 1.5% ferric chloride solution (fresh).
7. Wash in water.
8. Stain in 0.1% methyl blue in 1% acetic acid for 1 minute.
9. Rinse in 1% acetone for 30 seconds.
10. Dehydrate in 100% acetone for 30 seconds.
11. Acetone - xylene 50:50 for 30 seconds.
12. Clear in xylene.
13. Mount sections.

EHRLICH'S HAEMATOXYLIN AND EOSIN

Ehrlich's Haematoxylin Formula:

Haematoxylin crystals	4.0gm
Alcohol, 95%	200.0ml
Ammonium or potassium alum	6.0gm
Distilled water	200.0ml
Glycerine	200.0ml
Glacial acetic acid	20.0ml

Made up as follows:

Dissolve the haematoxylin in the alcohol and the alum in the distilled water and mix. After these are in complete solution add the glycerine and acetic acid.

(After Luna, 1968).

Eosin Stock Solution:

Eosin Y, water soluble	1.0gm
Distilled water	20.0ml

Dissolve and add:

Alcohol, 95%	80.0ml

Eosin Working Solution:

Eosin stock solution	1 part
Alcohol, 80%	3 parts

Make working solution just before use and add 0.5ml of glacial acetic acid to each 100ml of stain.

(After Luna, 1968).

Staining Procedure:-

1. Bring sections to water.
2. Leave in Ehrlich's haematoxylin solution overnight.
3. Place in running water for 5 minutes.
4. Differentiate in 0.2% acid-alcohol (HCl in 70% alcohol)
5. Place in running water for 5 minutes to blue.
6. Flood with eosin for 3 minutes.
7. Wash in water quickly.
8. Dehydrate in alcohols and mount.

MAYER'S HAEMALUM, CELESIN BLUE AND VAN GIESON

Mayer's Haemalum Formula:

Haematoxylin	1.0gm
Distilled Water	1000ml
Ammonium or potassium alum	50.0gm
Sodium iodate	0.2gm
Citric acid	1.0gm
Chloral hydrate	50.0gm

(After Culling, 1974).

Made up as follows:-

Dissolve the haematoxylin in the distilled water. Add the alum and shake to dissolve. Then add the sodium iodate, citric acid and chloral hydrate and shake until dissolved.

Celestin Blue

Made up as follows:-

Dissolve the 2.5gm of iron alum in 50ml of distilled water overnight at room temperature, and to this add 0.25gm of celestin blue. Boil for 3 minutes and filter when cool. Add 7ml of glycerine.

Van Gieson - as for Weigert's haematoxylin and van Gieson.

Staining Procedure:-

1. Bring sections to water.
2. Stain with celestin blue for 3 to five minutes.
3. Rinse in tap water.
4. Flood with Mayer's haemalum for 5 minutes.
5. Wash in tap water for 3 minutes.
6. Flood with van Gieson for 3 minutes.
7. Rinse in tap water.
8. Dehydrate through alcohols and mount.

APPENDIX V

FILM PROCESSING

Ilford FP4 : ASA 125. 120 size roll film and 4" x 5" plate film.

1. Develop for 3½ minutes in Ilford ID2 diluted 1:3 with water.
2. Wash in tap water once.
3. Fix for 5 minutes in Ilford Hypam Rapid Fixer diluted 1:4 with water.
4. Wash in running tap water for 10 minutes.

Ilford PAN F : ASA 50. 35mm roll, black and white film.

1. Develop for 4½ minutes in undiluted Ilford Microphen developer.
2. Wash in tap water once.
3. Fix for 5 minutes in Ilford Hypam Rapid fixer diluted 1:4 with water.
4. Wash in running tap water for 10 minutes.

COLOUR FILMS used for recording histological sections.

1. Kodak Photomicrography (No. 2483). ASA 16.

   Processed by Kodak Australia (Melbourne). Process E4.
2. Kodak Ektachrome 200 Daylight.
3. Kodak Ektachrome 64 Daylight.

   2,3, processed by Group Colour (Gilberton, Adelaide) using E6 process.
4. Fujicolor F-II colour print film, ASA 100.

BLACK AND WHITE PRINTS

Papers used: Ilford, Ilfospeed photographic paper (medium wt)
numbers 1 to 5.

Processing:

1. Develop in Ilfospeed paper developer diluted 1:9 with water.
2. Wash quickly in running tap water.
3. Fix for 2 minutes in Ilfospeed paper fixed diluted 1:3 with water.
4. Wash in swirling tap water for 5 minutes.

SECTION 9

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