<u>In Vitro</u> STUDIES OF THE PERMEABILITY OF TOOTH ROOTS USING RADIOLABELLED MOLECULES AND ELECTRON MICROANALYSIS.

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ABSTRACT

The presence of endodontic-periodontic lesion is well established, but recent studies have suggested that this may be underestimated as a cause of deep dentoalveolar inflammatory lesions. The ability of the dental pulp and periodontal tissue to influence each other is dependent upon communication through dentine and cementum, and accessory canals. The present study was undertaken to determine the ability of molecules to permeate human tooth roots *in vitro*.

A method was devised using freshly-extracted human teeth in which diffusion of three radiolabelled molecules was measured by liquid scintillation spectrometry. The rate of diffusion decreased as the molecular weight increased. The results indicated that the furcation region of tooth roots had the greatest permeability per unit surface area. The tooth roots were permeable to all molecules of physiologic and pathologic autocoids size.

No significant differences between root planed and non-root planed tooth roots were seen, suggesting that cementum is not a major permeability barrier. Coronal dentine was more permeable than root dentine.

Electron microanalysis was used to identify the movement of cobaltous ion through tooth roots from the pulp to the root surface. The electron microanalysis was performed by use of X-ray spectra and X-ray image analysis. Statistical analysis demonstrated a trend for the cervical third of the root to be the most permeable.

The study suggests communication could occur between the dental pulp and the periodontal ligament via the dentine and cementum of the tooth root. The clinical importance of the permeability of tooth roots warrants further investigation.

DECLARATION

This research report is submitted in partial fulfilment for the Degree of master of Dental Surgery in Periodontics at the University of Adelaide.

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

I consent to this research report being made available for photocopying and loan if it is accepted for the award of the degree.

Paul L. McHUGH

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CHAPTER 1. INTRODUCTION.

Dental Pathology is widespread throughout the world's population. Political and Public Health authorities are dedicated to the task of providing dental health for all by the year 2000. Much of the clinical time of dentists is carried out in surgical and preventative philosophies in order to maintain the teeth in good function. This concerns not only the relatively inert materials of enamel, dentine and cementum but also the surrounding structures, the dental pulp, the periodontal ligament, alveolar bone, oral mucosal surfaces. It is apparent that a thorough understanding of the biology of the body is required to complete our knowledge of the functioning human organism.

Current concepts regarding the pathogenesis of periodontal disease, an inflammatory process which slowly destroys the supporting structures of teeth, implicate dental plaque as the primary etiological factor. Plaque accumulation to intact gingivae initiates gingivitis within a few days. This condition is characterized by red swollen gingivae, which bleed on probing. If the plaque is not removed, it is generally assumed that gingivitis slowly progresses to periodontitis, which involves destruction of alveolar bone and periodontal ligament fibres and leads to the formation of periodontal pockets between the tooth and remaining tissues.

Plaque then grows on the tooth surfaces within the pockets from where its removal by brushing or flossing is impossible. Loss of alveolar bone is thought to be a result of the chronic inflammation induced by plaque. The results of recent epidemiological research (Goodson et al 1982, Socransky et al 1984) have led to the questioning of traditional concepts of the nature and pathogenesis of periodontal disease, which has viewed tissue destruction as being a continual slow progressive disease throughout life. It is now clear that periodontal disease progresses through short bursts of acute inflammation with considerable tissue destruction occurring in a short time around isolated teeth.

While systemic factors which compromise the host's defence, polymorphonuclear leukocyte dysfunction, diabetes, stress, etc. may explain why an individual is particularly

susceptible to periodontitis, the enigma that not all teeth in the same mouth are affected by extensive destruction of bone, even though gingivitis may be present throughout the dentition, remains.

The current treatment regime for cases of advanced periodontal disease includes instruction of the patient on plaque control and antibiotic therapy to control pathogenic plaque bacteria, scaling to remove calculus and root planing to remove periodontally affected cementum from the tooth surface, which is contaminated by bacterial products and pathologically altered by exposure to oral fluids.

However, periodontal therapy is not successful in treating all cases of advanced disease (Hirschfeld and Wasserman 1978, Lindhe 1984), nor is current periodontal theory able to explain this phenomenon. Cases described as extreme downhill which fail to respond to every method of periodontal therapy tried are common in dental and periodontal practices.

As a result of recent experiences it is believed that another important factor contributes to the development of deep periodontal defects. This factor involve the recognition of previously undiagnosed pulp pathology. Inflammatory changes within the dental pulp may, via accessory canals and permeation of inflamogens through dentine and cementum, lead to changes within the periodontal structures. The endodontic-periodontal lesion has been described previously by several authors, but the possibility exists that it has been underestimated as a cause of periodontal inflammation.

Inflammation is a complex dynamic vascular, lymphatic and local tissue reaction, consisting of many interdependent cellular and humoral events all related to the elimination of the injurious agent and repair of tissue injury. The messengers of inflammation are the biochemical mediators released which choreograph the inflammation.

A large number of mediators and mediator properties have been identified in the literature over the last 20 years. The continued identification of mediators and their properties will enable us to break the code of inflammation, and understand the messages and signals that are transmitted between the cells. This will increase the

understanding of chronic inflammatory diseases, and provide better determination of the course that they will take.

Central to this theory of pulpal inflammation affecting the periodontal ligament is the transmission of mediators from the pulp tissue to the external surface of the tooth. This study was conducted to determine the possibility of such an interaction between the two compartments, and to assess the size of molecules able to penetrate the dentine and cementum, and to localize the sites of transfer of molecules.

CHAPTER 2. REVIEW OF THE LITERATURE.

2.1. Introduction.

Essential to the hypothesis proposed is communication between the internal and external environments, which in this biological system is through dentine and cementum. The purpose of this detailed review is to allow better understanding of the possible interactions of these structures, and the complex physiology and pathology of tooth structure.

Initially the structure of the root, dentine and cementum, is reviewed, their basic components including chemical and structural analysis. Then the biology of the pulp is reviewed and age changes associated with these structures discussed. Communication between the pulp and the periodontal ligament requires permeation of the hard tissues, or the presence of accessory canals, both of which are discussed including the effects of molecular size, diameter of molecules, the effect of the smear layer, and the role of the dental pulp. Periodontal Disease is associated with profound changes in the structure of cementum, and these are described from a historical perspective up to our present day knowledge.

A renewed area of interest is the effect of microleakage on the dental pulp. It has now become well accepted that all restorations leak to some degree. Leakage of macromolecules and bacteria around restorations can have a profound effect on the health of the dental pulp, by communication through dentinal tubules. Bacteria in caries and under restorations have been an area of concern for restorative dentists for many years. Bacteria have shown varying ability to penetrate dentine, and the host response to their presence, sclerosis and secondary dentine minimize their penetration. Generally bacteria do not appear to penetrate dentine deeply in vital teeth, but their products would appear to be able to diffuse quite readily through dentine, and induce inflammatory changes, which are included in this review.

Endo-Perio interactions have been described in the literature for many years. The ability of the pulpal inflammation induced by caries or necrosis induced in the pulp

chamber to produce changes in the periodontal structures is reviewed, as well as possible mechanisms. In addition the ability of inflammatory change in the periodontium and exposure of tooth roots to the oral environment to cause pulpal inflammation is included in this review.

2.2. Tooth Structure.

2.2.1. Dentine.

Dentine forms the major component of the hard tooth structure, it has similar physical and chemical properties to bone. Dentine in the erupted tooth encloses the dental pulp and provides attachment and support for enamel and cementum. The specialized cells of dentine, odontoblasts, and their cell bodies are arranged in a layer on the pulpal surface of the dentine, and only their cytoplasmic processes are embedded in the mineralised matrix, with each cell giving rise to one process. Osteoblasts are enclosed in their intercellular substance but odontoblasts are located at the periphery of the calcified structure. Unlike enamel, which is very hard and brittle, dentine is subject to slight deformation, is highly elastic, and softer than enamel but harder than bone.

The chemical composition of dentine comprises 30% organic matter and water, and 70% inorganic material (Sicher and Bhaskar 1972). Even after drying at $100^{O}C$, dentine still contains between 5 and 8% water chemically bound to other constituents, and therefore the organic component is approximately 20% (Jenkins 1978). The inorganic components have been shown by X-ray diffraction to be hydroxyapatite, as in other calcified tissues. The organic constituents of dentine consists mainly of collagen, 18% of dry weight and 1.6% collagenous matrix of which the major component is glycoaminoglycan (mostly chrondroitin-4-sulphate) and glycoproteins (Levine 1971).

Dentinal tubules course through dentine in an S shaped path extending peripherally from the odontoblast-predentine junction to the DEJ. These tubules start at right angles to the pulp surface with the first curve directed towards the crown of the teeth. In some areas of the tooth, incisal edges, cusps and in the root the tubules travel in a relatively straight line. The tubules do exhibit minute relatively regular secondary

curvatures that are sinusoidal in shape (Sicher and Bhaskar 1972). Dentine permeability can be attributed to the presence of these tubules, that are formed by deposition and mineralisation of the predentine around the odontoblastic process. Each odontoblast process gives rise to one major tubule, with the tubule branching throughout dentine, the extent of which depends on mineralisation around lateral branches of the odontoblast process (Thomas 1983). Branching of tubules have been described throughout the dentine thickness, but Weber (1983) demonstrated branching was extensive at the DEJ, less in the middle third of dentine and not seen in the pulpal third. These tubular branches could considerably affect the permeability of the tissue.

The external surface area of dentine is about five times that of the pulpal side, and accordingly the tubules are further apart at the peripheral layers and more closely packed near the pulp. The tubules are also wider near the pulp cavity, 2-3*u*m, and become narrower at the DEJ, 0.5-0.9*u*m (Garberoglio and Brannstrom 1976). The number of dentinal tubules ranges from 45-65,000/mm^2 at the pulpal side of human coronal dentine, from 29.5 - 35,000/mm^2 in mid dentine, and 15 - 20,000/mm^2 at the DEJ (Garberoglio and Brannstrom 1976). The distance between the center of adjacent tubules is 15um at the DEJ and 6um at the pulp (Bradford 1958). The average volume of dentine occupied by tubules is calculated to be ten percent, but varies throughout dentine with values of 4% at the DEJ and 28% at the pulp (Garberoglio and Brannstrom 1976).

2.2.1.a. Odontoblastic Processes.

Odontoblastic processes are cytoplasmic extensions of odontoblasts. The contents of these processes are sparse, with prominent structures being microtubules 20-30 nm diameter and filaments 5 to 7.5 nm diameter. Other contents include occasional mitochondrion, some lysosomes and microvesicles, no ribosomes or endoplasmic reticulum are seen (Sicher and Bhaskar 1972).

There has been a debate over whether the odontoblastic process extends throughout the full thickness of dentine for many years, and this debate still exists in the current literature (Thomas 1985). Investigators using scanning and transmission

electron microscopy of mineralised and demineralised tissues in a variety of animal species and ages found the process to be limited to the pulpal third of the tissue (Brannstrom and Garberoglio 1972, Thomas 1979, 1983). Szabo et al (1984), and Fox et al (1984) have claimed to have shown the process in peripheral dentine using SEM, reasoning that improper fixation, and demineralisation for the TEM resulted in failure of other investigators to do so. Thomas (1983) while admitting difficulties with preparing dentine for electron microscopic examination, demonstrated the penetration of the fixative throughout the crown of human teeth, but the odontoblastic process was still confined to inner dentine.

A recent study of 45 premolar teeth from children using TEM and SEM found the odontoblastic process to be identifiable in the inner, middle and peripheral thirds of dentine in crown and root dentine (Frank and Steuer 1988). They suggested the odontoblastic process is difficult to fix in proper conditions and retracts easily if mishandled. The process was seen adjacent to the cemento-dentinal junction (CDJ) in the cervical region. They also described unmyelinated nerve fibres which extend to the peripheral third of the dentine tubule.

An alternative explanation for this discrepancy is that the electron dense structures seen in SEM may not be the odontoblast process. Transmission electron microscopy can distinguish between two different structures when they are seen together. Thomas and Carella (1983) identified a sheet like membraneous structure lining dentinal tubules, which is somewhat similar to a structure seen in bone canaliculi and has been called lamina lamitans. Under SEM examination when the specimen has been air dried this structure becomes shredded and appears to have a fibrillar component; tests of its structure indicate it to have a high content of glycoaminolglycan.

The odontoblast process can fill the dentinal tubule in cross section completely at the dentine-predentine junction (Thomas 1979). As the process passes further up the tubule away from the pulp a distinct separation appears between the process and the

tubule wall. This separation was termed the periodontoblastic space (Frank 1966), and contains granular material and collagen fibrils (Thomas 1979).

2.2.1.b. Peritubular Dentine.

Cross sectional non demineralised specimens show a ring shaped transparent zone around the odontoblastic process, which is easily differentiated from the remaining dentine matrix, and has been termed peritubular dentine. The walls of the tubules and their lateral branches are lined by peritubular dentine, a more mineralised tissue than intertubular dentine. It is thickest in the mid and outer dentine (Atkinson and Harcourt 1961). The narrowing of dentinal tubules occurring with age was interpreted by Bradford (1958) to be due to growth of peritubular dentine, a process accerlerated by attrition or dentinal caries.

2.2.1.c. Intertubular Dentine.

Intertubular dentine comprises the main body of dentine and although highly mineralised, more than 50% of its volume is taken up by the organic matrix, consisting of large numbers of fine collagen fibrils enveloped in a amorphous ground substance (Sicher and Bhaskar 1972).

Throughout life dentinogenesis occurs in two stages. Initially an unmineralised collagenous matrix (predentine) is formed which in the second stage becomes mineralised at a sharp boundary, dentine-predentine junction. The external, first formed part of the dentine called mantle dentine contains coarse fibril bundles which run parallel to the tubules and are formed by pulp cells. The main portion, circumpulpal dentine, the fibres are placed at right angles or obliquely to the tubules and are formed and mineralised by odontoblasts.

Collagen is found within the lumen of dentinal tubules both in the periodontoblastic space and peripheral tubules (Tronstad 1973, Thomas 1979). Intratubular collagen fibres are often quite thick and can be present over considerable distances which may reduce dentine permeability (Tronstad 1973).

Dentinal fluid within tubules has been recognized for many years. Fish (1927) proposed a circulation of dentinal lymph. This fluid has since been found to contain

proteins similar to those of plasma, it has been suggested to be a derivative of capillary transudate or filtrate, i.e. extracellular fluid (Coffey et al 1970) with low K^+, high Na^+ and saturation of Ca^{2+} and $PO4^{2-}$. It contains a host of plasma proteins (Haldi and Wynn 1963, Pashley et al 1981c). In the normal situation the peripheral ends of these tubules remain closed, and little fluid movement will occur across enamel and cementum even though pulpal pressure exceeds atomospheric pressure by 25-30 mm. Hg., tending to filter fluid slowly from pulp to the surface (Berggren and Brannstrom 1965, Brannstrom 1981). During trauma, cavity preparation, or root planing the tubules are opened acutely, fluid flow will occur through the tubules. Under such circumstances with patent tubules the entire contents could be replaced ten times a day (Pashley 1985). Tanaka (1980) using lanthanum tracer studies in developing teeth has confirmed that dentinal fluid originates, at least in part, from terminal capillaries in the pulp and diffuses through periodontoblastic space to the DEJ. The presence of fluid in dentinal tubules is the basis on which the hydrodynamic theory of dentine sensitivity is built (Brannstrom 1966, Brannstrom and Astrom 1972a).

2.2.1.d. Age and Functional Changes in Dentine.

Being a vital tissue the tissues have an ability to react to physiologic and pathologic stimuli. The effects of aging or pathologic influences are expressed by the deposition of new layers of dentine, secondary and tertiary dentine and through alteration of the original dentine, transparent or sclerotic dentine. By comparison of pulp cavities of teeth from young and old subjects it is evident that an inward growth of dentine continues throughout life at the expense of the pulp. Age changes in primary dentine include a gradual obturation of the tubules caused by growth of the peritubular dentine (Stanley et al 1983). The observation of progressive narrowing of dentinal tubules by growth of the peritubular dentine was noted by Bradford (1958). This narrowing was accelerated by attrition or dentinal caries. Mendis and Darling (1979a) indicated that age has little effect on the closure of tubules, showing a closer correlation between attrition and tubule closure than between aging and closure. A more significant factor in tubule closure was the presence of intratubular

mineralisation distinct from the gradual thickening of peritubular dentine with increasing age, seen under dentine covered with corticosteriod and antibiotics with the end result being similar to obturation associated with age (Mjor and Furseth 1968).

Precipitation of minerals within dentinal tubules is fundamentally different from obturation that is produced by growth of peritubular dentine. This type of obturation has been found in the translucent zone of carious dentine (Frank et al 1964), and in attrition (Mendis and Darling 1979b). This appears to be due to intratubular crystallization by precipitation of mineral salts within the tubules, and is a physico-chemical process which may occur *in vivo* and *in vitro*, and are collectively called dentine sclerosis (Stanley et al 1983).

A prominent feature of many older tooth roots is the presence of translucent areas of sclerotic dentine. It is well accepted that the changes in dentine to produce the translucent zones result in the decreased permeability of dentinal tubules. The size of these sclerotic zones is said to increase with age (Johanson 1971, Azaz et al 1977, Vasiliadis et al 1983a, Nitzan et al 1986). Miles (1972) proposed that toxins from diseased periodontal tissues were responsible for sclerotic dentine. Mendis and Darling (1979a) found sclerotic dentine in crowns to be unrelated to age, and showed two types occurred with different tubular contents. Vasiliadis et al (1983a), in canine teeth, found sclerosis of root dentine to increase linearly with age.

Vasiliadas et al (1983b) looked at longitudinal sections of canine teeth from 15 to 70 years of age. In young subjects the dentine was sclerotic near the apex of the tooth, and started at the CDJ. Older subjects had larger areas of sclerosis, and the apex was always sclerotic, and the sclerotic zone was seen to be extending coronally. The sclerotic dentine always appeared in the dentine adjacent cementum first, and extended towards the canal. Transverse sections of these teeth revealed the sclerotic zones appeared first mesially and distally in a butterfly shape (Vasiliadis et al 1983a, Grajower et al 1977). The pattern of distribution of the sclerosis suggests that the process is not simply a result of increasing age, as although the sclerosis started in the periphery of the

tubules, which is the oldest part, the apical dentine is the last dentine to be formed, but the first to become sclerotic.

The nature of the sclerosis has been investigated by LM, SEM, and microradiography. Spherical bodies have been seen within the tubules, which may be nuclei of mineralisation, which eventually occlude the tubule (Brannstrom and Garberoglio 1972c, Vasiliadas et al 1983b). In fracture studies the peritubular dentine, and the occluding material in tubules were almost indistinguishable (Boyde and Lester 1967, Spector and Taylor 1976, Vasiliadis et al 1983b). Vasiliadis et al (1983b) felt their observations indicated the occluding material was not due to centripetal growth of peritubular dentine, but due to growth of calcifying spheres within the tubules.

Vasiliadis et al (1983a,b) believed the sequence of changes in the formation of sclerotic dentine to be as follows. Initially the tubules are narrowed by the formation of peritubular dentine by normal odontoblasts. At a later stage the predentine layer is removed, and the tubules become rapidly occluded. At the end of this final stage there is almost complete mineralisation of the pulpal surface. Two parts are distinguishable in the sclerotic dentine, a central material surrounded by peritubular dentine, both of which are highly mineralised with numerous small packed crystals. This appearance is similar, but finer than the crystals seen in coronal dentine by Mendis and Darling (1979b).

Dead tracts are seen in dried ground sections of normal dentine where the odontoblastic process disintergrates and the empty tubules are filled with air (Sicher and Bhaskar 1972). Following insult, via caries, attrition, abrasion, cavity preparation, or erosion a displacement of odontoblast nuclei may occur, and a marked disarrangement of intratubular structures occurs in conjunction with disintergration of the odontoblast cell (Langeland 1957). This is then followed by the differentiation of new odontoblasts and the production of irregular dentine on the pulpal side (Stanley 1962, Fitzgerald 1979, Brannstrom 1981).

Secondary dentine formation occurs generally throughout life which is separated from the primary dentine by a dark staining line. There does not appear to be

continuity between the tubules and the new tubules are often wavy and less numerous per unit of dentine. It is deposited on all pulpal surfaces however, the rate of individual areas varies (Sicher and Bhaskar 1972). Localized masses of irregular reparative dentine may form beneath dentine affected by various pathological processes, caries, attrition, and also restoration. The tubules of this reparative dentine vary, some regular and others with a tortuous arrangement.

Sometimes the tissue may in parts not have dentinal tubules, cellular inclusion may also be found, especially at the interface between the reparative dentine and adjacent dentine (Mjor 1985). The degree of mineralisation of this reparative dentine varies more than primary dentine, and often contains more organic material. The mineralisation of predentine, without the formation of new matrix is always accompanied by a destruction, or degeneration of the odontoblasts (Mjor 1985).

The odontoblasts which form primary dentine and reparative dentine are different in contrast to the regular (physiological) secondary dentine (Mjor 1985). Two generations of odontoblasts being involved in this phenomenon is important, as there is a barrier formed by a lack of tubular continuity between the two dentines. This would either reduce the permeability of the dentine or make it impermeable, which can be seen under dead tracts reducing their permeability and protecting the pulp from insult and was first described by Fish (1932). Experimental studies on the healing of induced pulpitis (Mjor and Tronstad 1974), have demonstrated the dental pulp has extensive reparative capabilities with the formation of irregular reparative dentine a striking feature of this healing pattern.

Hals (1983) has studied human and red deer coronal dentine using light microscopy and microradiography. Primary and permanent teeth were examined, some displaying heavy attrition. He described the presence of giant tubules in the region of incisal edges and cusp tips in these teeth, with lumina of the tubules was 5-40μm in width. The continuity of these channels throughout the dentine has been demonstrated although they appeared to be blocked by secondary dentine formation, soft tissue was

still evident in some of these tubules. These giant tubules could be important to dentine permeability if they remain patent after tooth eruption.

2.2.2. Cementum.

Cementum is the hard dental tissue covering the anatomic roots of human teeth and furnishes the medium for the attachment of the fibres that bind the tooth to the surrounding structures. The hardness of fully formed cementum is less than that of dentine. Cementum consists of approximately 45-50% inorganic substances in the form of hydroxyapatite, and 50-55% organic material and water, mainly collagen and mucopolysaccharides.

Cells of loose connective tissue come into contact with the dentine of the root surface following the degeneration of epithelial root sheath and differentiate into cuboidal cells, cementoblasts (Sicher and Bhaskar 1972). These cells produce cementum in two consecutive phases. Firstly, uncalcified cementoid is laid down. The second phase transforms cementoid into calcified cementum. Cementum is continually formed on healthy root surfaces, the growth takes place in a rhythmic process with a new layer of cementoid allowing the old one to calcify and therefore a thin layer of cementoid is always seen on the surface which is lined by cementoblasts (Sicher and Bhaskar 1972). Connective tissue fibres of the periodontal ligament pass between the cementoblasts into cementum. These embedded portions are known as Sharpey's fibres.

Cementum is differentiated into two kinds morphologically, acellular and cellular cementum. Acellular dentine covers the root dentine from the CEJ to the apex. The cementum is thinnest at the CEJ, 20-50um, and thickest toward the apex, 150-200um. (Sicher and Bhaskar 1972). It consists of only calcified intercellular substance and embedded Sharpey fibres with cementoblasts on its surface. The intercellular substance is comprised of ground substance and collagenous fibrils, which are perpendicular to the Sharpey fibres and parallel to the cementum surface (Sicher and Bhaskar 1972).

Cementocytes, the cells included in the cellular cementum are similar to osteocytes lying in lacunae in cementum. The cell body has numerous long processes radiating from it, which may branch and anastomose with those of neighboring cells. Most of these processes are directed towards the periodontal surface. At a depth of 60 microns or more, cementocytes appear to be degenerating (Sicher and Bhaskar 1972). Both acellular and cellular cementum are separated by incremental lines into layers, indicating periodic formation. Where cementum is relatively thin, Sharpey fibres can be observed crossing the entire thickness of cementum, as more cementum is layed down the deeper layers of the Sharpey fibres become more obscure, and the attachment proper seems confined to the most superficial cementum.

Ketterl (1983) distinguishes four kinds of cementum: a) acellular afibrillar cementum, which can be detected only under the EM and formed exclusively prior to and during tooth eruption. b) acellular fibrillar cementum, also known as fibrous or primary cementum, is found predominately in the coronal part but also occurs in several separate layers in the apical area. c) cellular fibrillar cementum, d) intermediate cementum which was considered to be the product of a disturbance of development. Cellular fibrillar cementum is permeable in both directions in young people, but its permeability declines in old age, and acellular fibrillar cementum was reported by Stones (1934) to be permeable only in young teeth. The intermediate cementum is a homogenous zone of approximately 10 um wide (Blackwood 1957) at the junction of dentine and cementum. Protoplasmic communications at this junction were visualized by Blackwood (1957), where the orientation of these protoplasmic bodies are predominantly at right angles to the dentine tubules. The mineralisation of the intermediate cementum and the innermost layer of aprismatic enamel in the crowns has been shown to be very similar (Lindskog 1982b). In an SEM study the advancing mineralization front of the intermediate cementum was covered by the root sheath, which appears to take an active part in the formation of intermediate cementum (Lindskog 1982a).

Of great interest is the relationship between cementum and enamel at the cervical region of the teeth which is variable (Sicher and Bhaskar 1972, Muller and Van Wyk 1984). In 30% of teeth cementum meets the cervical end of enamel in a sharp line where both substances of these taper to a knife edge, while 60% of teeth cementum overlaps the cervical and of enamel for a short distance. This leaves 10% of teeth where various other aberration maybe seen. The enamel epithelium may during tooth formation cover the cervical part of the root and does not separate at the proper time and prevents the formation of cementum, leaving a zone of root devoid of cementum (Sicher and Bhaskar 1972).

Recent SEM studies have shown the hard tissue relationship at the CEJ to be unpredictably irregular and the previous ground sections through the CEJ do not necessarily reflect the true structural features (Schroeder and Scherle 1988). They found in a 150μm distance, and certainly over larger distances the CEJ relationship may vary unpredictably and indicated three relationship types. There was a variation in CEJ relationship seen between incisor, premolar, and molar tooth groups. Their data indicated premolars have 68% edge to edge, 30% cementum overlap, and 1% dentine exposure at the CEJ, while molars had 43%, 52%, and 5% respectively in young healthy teeth. The study also indicated dentine exposure occurs more frequently on buccal and distal surfaces. In this study the CEJ was covered by healthy gingival tissues, and the cementum-enamel relationship may change with time due to the continual formation of cementum.

Permanent teeth have a smooth dentine surface upon which the cementum is deposited. The attachment to the dentine is firm although the nature of this attachment is not fully understood (Sicher and Bhaskar 1972). The cementodentinal junction is not distinguishable under the electron microscope as collagen fibrils of the mature structures intertwine and there is no structural change demarcating the two tissues (Selvig 1965).

2.2.3. The Dental Pulp.

The pulp is a specialized loose connective tissue, consisting of specialized cells, fibroblasts, vascular and neural elements, and intercellular substance. The mature dental pulp has the appearance of embryonic connective tissue, but with a very specialized cells at its periphery. Trowbridge (1984) mentions the special environment of the pulp enclosed within dentine, means that the tissues ability to respond to vasodilation by increasing volume is restricted, because all of the tissue constituents are relatively incompressible. The dental pulp tissue is responsible for the formation of dentine, and retains the ability to form dentine throughout life.

The dental pulp has been shown in embryonic studies to be derived from neural crestal cells, and the dental papilla, which forms the mature pulp, forms as the neural crestal cells proliferate and condense adjacent to the dental lamina. This occurs in the eighth week of development in the incisors (Sicher and Bhaskar 1972). The rapid proliferation of the epithelial elements gives the tooth germ a bell shaped structure, and the future pulp is well defined in its outline. The mature pulp can be divided into several morphologic layers, odontoblast layer, cell-poor zone, cell-rich zone and the pulp proper.

Odontoblasts make up the outermost stratum of cells in healthy pulp, located immediately below the predentine with odontoblastic processes extending into the dentinal tubules. Odontoblasts are highly differentiated connective tissue cells, and are columnar in shape, with an oval nucleus. Capillaries and nerve endings may also be seen in this layer (Trowbridge 1984). The height of the cells varies dependent on their location within the pulp, with the coronal cells being tall columnar cells. In light microscope sections they appear to be several layers of cells thick as the nuclei are staggered due to the varied height of these cells. The cells in the radicular pulp are more cuboidal, and the cells of the root may be quite flattened, especially near the apex (Trowbridge 1984, Sicher and Bhaskar 1972, Ogilvie and Schaeffer 1976).

Immediately subjacent to the odontoblast layer a cell free layer can be found particularly in the coronal pulp, this is also known as the zone of Weil, or

subodontoblastic plexus. It is narrow, approximately 40 microns in width, and has a low cell density of cells (Trowbridge 1984). This layer contains a network of nerve fibres, mostly unmyelinated originating from deeper layers and passing into the odontoblast layer, and has a number of capillaries traversing its width. The appearance of this layer is dependent on the functional status of the pulp, and is not clearly seen in inflammation or when the pulp is laying down reparative dentine (Ingle 1976).

Below the cell free layer is a layer containing a high proportion of fibroblasts compared to other areas of the pulp. Also found frequently in this area are defense orientated cells, macrophages, lymphocytes, or plasma cells. Gotjamanos (1969) suggested the peripheral migration of cells from the central regions were responsible for the cell-rich zone. The importance of these cells becomes evident when there has been damage to the odontoblastic layer causing cell death, as they are replaced by cells migrating from the cell rich zone, which is probably the first step in the regeneration of the odontoblasts (Stanley 1962, Stanley et al 1975, Fitzgerald 1979, Brannstrom 1981).

The pulp proper is the central mass of the pulp which has the appearance of embryonic tissue. It contains the larger blood vessels and nerve fibres which are abundant, and the connective tissue consists of fibroblasts, collagen, and ground substance. Also present are cells known as fibrocytes particularly as the pulp ages, and are thought to help maintain collagen fibres (Trowbridge 1984). Other cells present are a number of pluripotential cells, lymphocytes and polymorphonuclear leukocytes in small numbers, which probably performing a surveillance function rather than responding to inflammatory stimuli.

The relatively small cellular composition of the dental pulp has been shown in *in vitro* studies to have a relatively low oxygen consumption rate compared to other tissues (Fisher 1967, Sasaki 1959, Hamersky et al 1980), with the greatest metabolism being in the odontoblast layer. Fisher and Walters (1968) have shown the pulp has the ability to operate anerobically via a phosphogluconate shunt type of carbohydrate metabolism. This would be beneficial to the pulp being a rather long end arterial system. The pulp is also subjected to periods of decreased perfusion from vasoconstrictor in local anesthetic

solutions (Kim et al 1984), and also during parafunctional activity (Clarke and Carey 1985). At these times the pulp would benefit if able to function under ischemic conditions.

In studying the microcirculatory contribution to the physiology of the pulp it is important to remember the dental pulp is encased in a rigid dentine structure and is therefore in a unique environment. Various methods have been used to study the pulpal blood vessels in humans and experimental animals since the turn of the century. The pulp appears to consist of small vessels with arteriolar diameters below 100 microns, and venules below 200 microns in diameter (Kim 1985a,b). The flow rate of these vessels concurs with arteriole and venules of other tissues (Kim 1985a,b). The main feeding arterioles enter the root canal through the apical foramina and appear to be slightly off center and run longitudinally to the coronal pulp (Takahashi 1985). Capillaries branch off from the arterioles at right angles, which then ramify below the odontoblast layer to form a dense subodontoblastic capillary plexus. In the root canal region these small vessels form fine cross-fenced capillary network, while in the pulp horn region a more loop network is seen (Takahashi et al 1982). A continuous progression was visualized from arterioles to capillaries to venules. Venules occupy the greater area in the central portion of the pulp, where they appear as thin walled vessels which are larger than the arterioles.

The pulp features numerous arteriovenous anastomoses, venous-venous anastomoses and U-turn loop arterioles. Using microspheres Fan et al (1979) demonstrated the shunts were more prevalent in the apical half of the tooth. Takahashi (1985) believed these probably participate in the regulation of blood flow in the pulp. There is a regional difference in the vascular network arrangement in the pulp, with the coronal area having a denser capillary network than the root canal, and the peripheral area is occupied by dense capillaries while the central area is occupied by mainly large vessels (Takahashi et al 1982). Kim (1985b) found the capillary blood flow in the coronal portion is twice that in the root of the tooth.

An adequate microcirculation is required by the pulp to transport nutrients to, and remove wastes from the tissues, to ensure its well being. This is dependent upon the functional parameters, blood flow, blood volume, and capillary permeability (Kim 1985a). He found the pulpal blood flow in young dogs to be 40-50 ml per min. per 100 grams, meaning the pulp has the highest blood flow value per unit weight of tissue among the oral tissues, but the flow is still less than most visceral organs. Chien (1985) although admitting that the rate of oxygen consumption of the pulp is unknown, believed this relatively high rate of blood flow was probably a luxury perfusion for the pulp at rest.

During the preparation of the rat incisor the drilling of the tooth opens the apical arteriovenous anastomoses, possibly due to pulpal pressure increase, and the opening of shunts maintains the blood flow under certain conditions (Kim 1985b). Noradrenalin administered by arterial route close to the pulp resulted in a decrease in pulpal blood flow, which was reversed by the injection of an alpha-blocker phenoxybenamine, as it did to the effect of cervical sympathetic stimulation (Kim et al 1980). Isoproterenol a beta-agonist when administered intra-arterially caused a reduction in pulpal blood flow, which was abolished by the use of the beta-blocker propanolol (Kim et al 1980). The authors found in the use of isoproterenol there was arterial dilation with a transient increase in arterial volume flow followed by a decrease, with a decrease in venular flow due to venule constriction. This was explained by the pulp being a low compliance system, and a transient dilation of the feeding arterioles lead to increased tissue pressure which produced a venule constriction. This was supported by the simultaneous injection of noradrenalin and isoproterenol causing no significant change in pulpal blood flow due to the action of one cancelling the other. Electrical stimulation of cervical sympathetic nerves has been studied by non invasive means, microsphere injection and radioisotope washout, and was seen to cause a reduction in pulpal blood flow (Edwall and Kindlova 1971, Tonder and Naess 1978). Heyeraas (1985) explained the drop in pulpal blood flow was due to a drop in extrapulpal perfusion pressure. This

was due to the surrounding tissues response to the vasodilators and therefore "stealing" the blood flow to the pulp.

The chemical mediators of inflammation, histamine, bradykinin, prostaglandins, 5-hydroxytryptamine, and other vasoactive agents are released during cell injury. Changes seen in inflammation are vasodilatation, vascular stasis and increased permeability. Pulpal blood flow changes in dogs corresponds to the degree of inflammation. Experimental pulp inflammation in dogs after three days increased the blood flow by 35%, but after seven days the blood flow had decreased by 28% compared to the control (Kim 1985a). Histological studies demonstrated the teeth after three days to have classical chronic inflammation, and the seven day experimental teeth to have an area of necrosis. Interestingly no changes in the blood flow were seen in the apical region of the inflamed pulp. Heyeraas (1985) using micropipette technique reported no change in apical areas in response to coronal inflammation. These observations do not support the strangulation of the pulp concept, and the inflammation is appears to be a local phenomenon. Heyeraas (1985) reported an increase in pulp pressure of 8-10 mm.Hg. in the inflamed area compared to the adjacent area so the increase in pulp pressure may be localized, and not necessarily spread to the rest of the pulp. A circumferential spread of inflammation was the finding of Van Hassel's experiments (1971) on pulp tissue.

Kim (1985b) presents a hypothetical model for pulpal death. He believes small insults such as incipient caries and slow progressing caries allow the pulp time to cope with the insult by the deposition of dentine. The sudden drilling of the tooth such as full crown preparation causes more sudden and widespread changes, often leading to necrosis. Kim (1985b) and Heyeraas (1985) find that local insult causes local inflammation, with the surrounding regions showing no signs of inflammatory change. The associated oedema in the localized area causes an increase in local pulp pressure, which in a low compliance environment would lead quickly to the pressure exceeding that of the venule, causing passive compression of the draining venule (Kim 1985b). The resultant stagnation would lead to in a build-up of metabolic products which

contribute further to vasodilation and gradual spreading of the inflammation. The work of Pashley (1979) on pulpal blood flow supports the concept of the reduction of blood flow leading to the accumulation of potentially toxic or injurious agents locally leading to further pulpal damage. The concept then is one of total pulp necrosis being the result of accumulating local micro necroses, while Kim (1985b) and Chien (1985) concede much more information is required about the pulpal blood flow.

The vascular network of vessels has the function of providing nutrients to the dentine as well as the pulp tissue itself. This small thin structure has multiple arterial and venous vessels passing through the apical foramen (Kramer 1960, Takahashi et al 1982). Under normal circumstances much of this network lies dormant and collapsed (Langeland 1959, Scheinin 1963). In multi-rooted teeth there are anastomoses between the vessels of each root (Ogilvie and Schaeffer 1976). Kramer (1960) demonstrated venous drainage in many multi-rooted teeth through the furcation or a lateral canal high on the root surface.

2.2.3.a. Age and Functional Changes in Dental Pulp.

There are structural features in older pulps not seen in many younger specimens, and whether these changes are purely physiological response to aging or pathologic responses to continuing pulpal insult is still not totally clear. For example the subtitle age changes is found in both the physiology and pathology chapters of endodontic textbooks (Ogilvie and Schaeffer 1976, Simon 1984, Trowbridge 1984). One endodontic textbook says that although aging is not strictly a pathological process it is considered in a chapter on pathology because of its effect on the ability of the pulp to react to stimuli, and therefore the patient's age should be taken into account when making a diagnosis (Simon 1984).

The size of the pulp chamber gradually reduces throughout life due to the laying down of secondary dentine. Fibrosis of the pulp is seen due to decreased cellularity and increased collagen within the pulp (Sicher and Bhaskar 1972). Odontoblasts are seen to decrease in size and number, and may disappear altogether in some areas (Bernick and Nedelman 1975). They also noticed the fibrosis appeared to be related to the pathways

of degenerated vessels or nerves, and felt the thick collagen may act as a foci for calcification. The vascular and nerve supply are seen to decrease both in number and size (Sicher and Bhaskar 1972, Takahashi 1985). The reduced blood supply to the pulp will affect the ability of the tissue to respond to injury. There is an increase in the incidence of pulp stones and dystrophic calcifications with age. The diffuse calcifications are found mainly in the apical areas of the pulp, and have been described as following nerve fibres and blood vessels (Sicher and Bhaskar 1972).

Calcifications in the pulp are classified as true denticles, false denticles, and diffuse calcifications (Sicher and Bhaskar 1972). True denticles consist of dentine showing dentinal tubules and odontoblasts, and are comparatively rare, compared to the false denticles. False denticles have no dentine structure, and consist of concentric layers of calcified tissue, the center of which is associated with the remnants of necrotic and calcified cells, or thrombus in blood vessels (Sicher and Bhaskar 1972, Simon 1984). These calcifications are seen in both erupted and embedded teeth, subjected to environmental effects and aging. Tooth morphology may be changed by alterations in response to the environment in the oral cavity, including caries, periodontal disease, restorative treatments, attrition, abrasion, and erosion.

Pulp calcifications have been found in embryonic, deciduous, permanent teeth, erupted and unerupted teeth (Sicher and Bhaskar 1972, Thoma 1940). The incidence and influence of age on pulp calcifications appears unclear. Some authors consider it a natural event (Sicher and Bhaskar 1972, James et al 1959, Seltzer and Bender 1984), while others classify it as a pathological process related to local environmental influences (Sayegh and Reed 1968, Bernick 1967, Patterson and Mitchell 1965). Nitzan et al (1986) looked at 52 impacted canines between the ages of 11 and 76, and assessed them for histologic changes due to age. They separated the changes into concentric denticles, including false and true denticles, diffuse calcifications, and measured the width of dentine, predentine, and cementum. All the concentric denticles were of the false variety, and were found in 56% of the pulps. There was a constant incidence and size indicating no relationship with age. In contrast, diffuse calcifications had a low

incidence, 10%, in the young age group and increased sharply after the age of 25 years, and were seen with a frequency of 50% in the older age groups. Nitzan et al (1986) had no explanation for this sudden steep increase in diffuse calcifications. No attempt was made to correlate any increase in size with age. They believed that the calcifications should be looked at separately to correlate calcification changes to age, and environmental factors.

2.3. Permeability.

The major channels for solute diffusion across dentine are the dentinal tubules, which may be considered as cylindrical water filled tubules, each surrounded by a relatively impermeable mineralised matrix (Pashley et al 1978c). This structure acts as a diffusion barrier, where movement across it may occur by either diffusion, or filtration.

Diffusion is the movement of molecules from one location to another by random molecular motion (Vander et al 1980). By diffusion a gas or a substance in solution expands to fill all of the available volume. All ions or molecules are in continuous random movement, and in regions where they are abundant they frequently collide and therefore tend to spread from areas of high concentration to areas of lower concentration, until spread uniformly. The magnitude of the deficiency tendency from one area to another is proportionate to the difference in concentration of the substance in the two areas, that is the concentration or chemical gradient. The speed at which these molecules move, and therefore collide and diffuse is dependent upon the molecular size, with the smaller molecules moving at a greater speed than their larger counter parts. Diffusion of ions is also affected by their electrical charge, as whenever a difference in electrical potential exists between two areas, the ions will move towards the area of opposite charge (Ganong 1975). Diffusion occurs not only within the fluid compartment, but also between compartments provided the barrier between the compartments is permeable to the solute. The permeability of the barrier will affect the diffusion rate.

Filtration is the process by which fluid is forced through a membrane or other barrier due to a difference in hydrostatic pressure on the two sides (Ganong 1975). The amount of fluid filtered in a given interval is proportionate to the differences in pressure between the two compartments and the surface area of the membrane. Molecules which are smaller in diameter than the pore size or sieve size of the barrier pass through with the fluid, and larger molecules are retained.

2.3.1. Dentine Permeability.

Since the beginning of the century diffusion, osmosis, or electrochemical gradients have been mentioned in the literature in relation to the dispersion of particles through dentine. The term "dental lymph" was first coined by Fish (1927a,b), suggesting the fluid could flow in either direction according to the surrounding pressures. In an attempt to explain the sensory mechanism of dentine the hydrodynamics of the dentinal fluid has been examined by Brannstrom (1966), Brannstrom et al (1967, 1968), Polhagen and Brannstrom (1971), Johnson et al (1973), and Linden and Brannstrom (1976). By the loss of fluid from the external surface, due to capillary forces, the rate of flow from the dentinal tubules has been calculated *in vitro* (Berggren and Brannstrom 1965, Linden and Brannstrom 1976). The hydrodynamic theory of dentine sensitivity states that the fluid movement across dentine in either direction induces pain (Brannstrom and Astrom 1972).

Early experiments on the permeability of tooth crowns to various dyes demonstrated dentine was readily permeable from both pulp and dentinoenamel junction, with enamel being less permeable than dentine, and that this permeability decreased with age (Fish 1927a, Bodecker and Lefkowitz 1937, Ross 1941). The early experiments were done by viewing ground specimens (Fish 1927a,b, Bodecker and Lefkowitz 1937). A cavity was cut in the dentine, *in vivo*, and a dye was placed and remained *in situ* for periods of 5 to 28 days before the specimens were examined. The rate of diffusion of dentinal fluid was believed to be slow, hence the long staining periods. The stain was found to be confined to the cut tubules in the area of the cavity

and a few adjacent tubules. Bodecker and Lefkowitz (1937) noted the vital staining had permeated into uncut tubules adjacent to the pulp chamber.

Lefkowitz (1943) was the first to estimate the rate movement of a silver stain, argyrol, into the pulps of dogs through small holes in the cervical regions of the teeth which were extracted at various times. After thirteen minutes, half the distance between pulp and enamel had been stained. The stain reached the enamel in 17 minutes, and after 28 minutes half the dentine within the tooth had been stained. A cross section of one of these teeth demonstrated the dye to be in the dentinal tubules. The dye was also observed in the organic matrix of enamel in both the area of the cut tubules and the area of secondary staining.

Experiments using solutions of radioactive urea, thiourea, and acetamide confirmed dentine to be highly permeable (Wainwright and Lemonine 1950, Wainwright 1954, Barber and Massler 1964). Wainwright (1954) placed radioactive substances inside extracted teeth kept at body temperature, and in a humid chamber in an attempt to reproduce *in vivo* conditions. Following sectioning and autoradiogrgaphy the whole thickness of dentine, 3 to 4 mm, was shown to be permeated within 20-30 minutes. Wainwright and Belgorod (1955) demonstrated C^{14}-acetamide penetrated through dentine, average thicknes 4.1 mm., and faintly through enamel in twenty two minutes.

2.3.1.a. The Dentinal Fluid.

The dentinal fluid has been investigated by several authors. Haldi and Wynn (1963) concluded the pulp fluid could be regarded as a capillary transudate or filtrate. Coffey et al (1970) after centrifuging teeth to collect dentinal fluid from tooth crowns analyzed the fluid by electron microprobe which indicated the fluid was not cytoplasm but extracellular fluid with a protein concentration of 20% that of blood plasma. Fluid collected from dentine tubules at the external surface must have permeated dentine before it can be collected, and dentine could restrict the free diffusion of protein molecules. The results of Pashley et al (1981a) indicate there is little or no molecular sieving, as the pulp fluid collected from direct pulp exposure was essentially the same as

fluid collected on the dentinal surface. Using intra-arterial infusion of histamine they found the major barrier for plasma protein permeation to be the capillary endothelium rather than the dentine per se. Histamine resulted in a change in capillary permeability, which was reflected in the fluid collected on the dentinal surface. The barrier appears to be the capillary rather than the odontoblasts.

In vitro studies such as Stevenson (1965) which demonstrated the presence of fluid on exposed dentinal surface may have limited relevance to the *in vivo* situation. These experiments do not reveal any outward movement in the dentine due to physiologic pressure gradient. The work of Beveridge and Brown (1965), Haldi and Wynn (1963) on teeth in dogs *in vivo* demonstrated a colorless fluid rising in a hole drilled near the pulp indicating an outflow of fluid from the pulpal tissue. Odontoblast aspiration has been explained by the pressure gradient between the pulp and the surface of the dentine (Brannstrom 1962). From their experiments in young patients the mean intrapulpal pressure of 28 mm Hg was estimated by Beveridge and Brown (1965).

The outward displacement of tubule contents produced by a pressure gradient has been used to explain histologic observations of Johnson et al (1973). Johnson et al (1973) constructed an *in vitro* experiment to test the effect of a physiologic pressure gradient, 30 mm Hg., on fluid flow from dentinal tubules, fractured and cut, and to see if such a flow might produce aspiration of nuclei into the tubules. All teeth with fractured surface had an outflow of liquid, in contrast to the cut dentinal surface and the intact enamel, which recorded no fluid flow. All teeth subjected to pressure, had under histologic examination, nuclei or their remnants in the tubules.

The effect of bacterial products and inflammatory messengers in dentine on the pulp depend on the concentration of substances in the interstitial fluid, which is determined by a balance of two factors in the dental pulp. The first is the rate of permeation of the solute across the dentine, and the second is the rate at which the substance is removed by the pulpal blood flow (Pashley 1979). The dentinal fluid can be regarded as a reservoir or compartment coupled in series to the pulp.

2.3.1.b. Movement of Molecules.

Movement of solutes across the dentine surface may occur by either diffusion or filtration under pressure. The movement under filtration pressure, depends on bulk fluid movement of solute and solvent across dentine. The term hydraulic conductance has been used by Reeder et al (1978) as a measure of the ease with which fluid permeates dentine, during experiments. Previous studies had attempted to quantitate fluid movement through dentine, without clear definition of surface area and thickness of dentine (Brannstrom et al 1968, Johnson et al 1973, Anderson et al 1967). Reeder et al (1978) said the reason for not measuring fluid flow through cut dentine surfaces was due to a lack of sensitivity in the system used by other investigators (Johnson et al 1973, Stevenson 1965). Changes in dentine thickness demonstrated an exponential change in hydraulic conductance (Reeder et al 1978). They concluded sanded dentine surfaces have a low but measurable hydraulic conductance. Filtration of fluid across dentine obeys entirely different laws from those of diffusion and can be expressed by Poiseuille-Hagen equations (Pashley 1985). Importantly the filtration was shown to vary with the fourth power of the radius rather than the square of the radius as for diffusion, and the driving force is the pressure gradient rather than a concentration gradient.

The *in vivo* fluid movement across dog dentine was calculated by Pashley et al (1981a). Exposed dentine had conical chambers cemented on to its surface and was connected via micropipette to a pressure bottle on an attempt to quantitate bulk fluid movement through dentine *in vivo*. Hydraulic conductance indicates the ease with which a fluid moves across a filtration barrier, and is dependent upon the dentine surface area and the pressure gradient. This pressure has been estimated using techniques of direct penetration to the pulp (Brown and Beveridge 1966), while the above technique of Pashley kept the dentine intact. The pulp pressure ranged from 11 to 35 mm Hg with a mean of 24 mm Hg which are similar to those reported by Brown and Beveridge (1966). A question that seems to have been unanswered is the effect of the preparation technique, removal of buccal enamel and dentine (Pashley et al 1981a,c) or boring a hole into the pulp tissue (Brown and Beveridge 1966), on the status

of the pulpal tissue, with respect to increasing pulp pressure as a result of inflammation induced by tissue damage.

A knowledge of intra pulpal pressure enabled Pashley et al (1981b,c) to calculate the hydraulic conductance *in vivo* of dog dentine. The dog molar hydraulic conductance was found to be 1.35 x 10^{-2}ul $cm^{-2}min^{-1}cmH_2O^{-1}$ which was higher than human dentine *in vitro* (Reeder et al 1978), but closer to experiments of Pashley et al (1978a) 8.1 x 10^{-3}, in crown segments which, like the latter study, had coronal pulps and odontoblasts present. The lack of agreement with Johnson et al (1973) was put down to the lack of sensitivity of the Johnson et al measurement techniques. The density and diameter of tubules in dogs and humans is similar (Forssell-Ahlberg et al 1975) and one could therefore expect agreement.

The use of dentine disc in split chambers was designed as a versatile method for studying dentine permeability (Outhwaite et al 1974). Using this technique the effect of various parameters were studied on dentine permeability to radioactive iodide (Outhwaite et al 1976). Doubling the surface area doubled the permeability, and a temperature increase of 10 degrees C doubled the rate of iodide permeation. The effect of thickness was most important as reductions in dentine thickness increased permeability 6 to 8 times. Reduction from the occlusal side resulted in a much greater increase in permeability than reduction from the pulpal side, also when the disc were spilt in half to two 0.5mm. thick discs the bottom disc was more than twice as permeable as the top half. This is in agreement with the decrease in diameter of the dentinal tubules from the pulpal surface to the enamel surface (Garberoglio and Brannstrom 1976). Post extraction time seemed to have only a minor effect on permeability (Outhwaite et al 1976). The dentinal tubules used are only from the pulpal third of dentine (Brannstrom and Gararoglio 1972) and in these discs the authors felt most of the odontoblastic processes would have been removed and the effect of odontoblastic processes is unknown. This means thin dentine is more permeable than thick dentine, full crown preparations are more permeable than buccal pits and deeply excavated dentine is more permeable than dentine at the DEJ (Pashley 1985). Large regional

differences in permeability of dentine discs was seen, with the highest values at the periphery, and lower at the centre of the disc (Pashley et al 1987). The highest permeability was over the pulp horns, and even though the dentine discs were of uniform thickness they may not all have the same tubule lengths.

Merchant et al (1977) compared the rate of iodide permeation of dentine by diffusion and filtration. Dentine discs were prepared as described by Outhwaite et al (1974). The application of filtration pressure 240 cm of H_2O (176 mm Hg) doubled the rate of iodide permeation, after acid etching the surfaces the same pressure caused a 32 fold increase in permeation. Also a diffusional equilibrium required 20 minutes in contrast to the filtration pressure group, which reached a steady state after two minutes.

The effect of molecular size on the permeability in human dentine *in vitro* was studied (Pashley et al 1977, Pashley and Livingston 1978). The results indicated a decrease in permeability with increasing size, but all substances tested were able to penetrate dentine. The substances tested in order of size were water, urea, fluoride, lidocaine, glucose, sucrose, inulin, dextran, polyvinylpyrrolindone and albumin. A 100-fold decrease in permeability coefficients resulted from a 19-fold increase in molecular radius from 1.9Å (H_2O) to 37Å (^{131}I-albumin) (Pashley and Livingston 1978). The effect of odontoblasts on permeability can only be speculated on from these *in vitro* results. Pashley et al (1977) tested the permeability of dentine *in vitro* to several isotopes by preparing an occlusal cavity in freshly extracted teeth. The relative rate of permeation were ^{3}H-water > ^{131}I > ^{99m}Tc > ^{14}C-urea. The iodide and pertechnate were both negative ions with radii between water (1.97Å) and urea (2.70Å). In contrast the molecular weights for these compounds water 22, urea 62, iodide 131, and pertechnate 163 indicating that this alone is insufficient to predict dentine permeability. Pashley et al (1977) also measured the effect of the presence of pulpal tissue on permeability of iodide in freshly extracted teeth and found more rapid permeation of iodide once the pulpal tissue had been removed. The tissue vitality must be questioned in such an experiment, and therefore its application to the *in vivo* situation.

Pashley and Livingstone (1978) also found the permeability of fluoride and chlorhexidine to be much lower than expected for their molecular weight or size, suggesting that they are both bound to dentine, therefore preventing their permeation. Pashley et al (1977) found the molecular radius $^{3}H_2O$ 1.97Å; ^{131}I 2.58Å; $^{99m}TcO_4^-$ 2.55Å; and ^{14}C-Urea 2.70Å (Renkin 1954, Wolff 1964) matches the dentine permeability. The two negatively charged tracers had intermediate permeability between water and urea. The authors felt this may reflect size is more important than charge as the permeability varies inversely with molecular radius, but is dependent on interactions between the permeating molecules and dentine.

Hume (1984) placed tritiated ZNOE restoration in occlusal cavities of third molars and measured the permeation and release of ^{3}H-Eugenol. He detected no ^{3}H-Eugenol in the first hour, but pulpal ^{3}H-Eugenol outflow increased over the next few hours. It reached a peak during the first day, and this level was maintained for about a week. The time required and size of the peak had an inverse relationship to the dentine thickness. Compared to ^{3}H-Eugenol release from the free occlusal surface bathed in phosphate buffered saline the dentine provided a major diffusion barrier. He was able to quantify the Eugenol concentration within the different levels of the dentine, demonstrating a concentration gradient occurred between the zinc oxide-eugenol material and the pulp.

On freshly extracted human molar teeth the release of tritiated-triamcinalone from Ledermix cement was quantified by Hume and Kenney (1981). Triamcinalone placed on class 1 cavity will diffuse through dentine to the pulp. The release of triamcinalone from small pellets of Ledermix cement placed into aqueous medium was compared to the release and diffusion through dentine. The results allowed the conclusion that dentine provided only a minimal barrier to the diffusion of triamcinalone.

Human tooth roots have been shown to release corticosteriod and tetracycline in an *in vitro* model (Abbott et al 1988). They found dentine to be of greater importance for molecule release than the apical foramen. This was supported by the suppression of

inflammatory root resorption *in vivo* (Pierce et al 1987). They used a similar paste in the pulp chamber to reduce inflammation on the external root surface.

Using freshly extracted human teeth Wach et al (1955) placed S^{35} labelled penicillin G in the pulp chambers and found all teeth demonstrated some permeation from the pulp outwards. They noted all specimens had a decreased or absent uptake of labelled penicillin in the apical region. The authors also reported a lack of penetration of intact enamel and cementum. The apical dentine was reported to be transparent suggesting dentinal sclerosis, and hence a reason for the lack of penetration in that area.

Dentine permeability in root canals, after debridement and use of medicaments was demonstrated using isotopes of iodine, sodium, sulphur, and phosphorus (Marshall et al 1960). Among their findings were that the cervical and mid root dentine areas were permeable to all isotopes and isotopes failed to pass beyond the DEJ from either inside the canal outwards or from the external surface inwards. Debridement, reaming of the canal, had a slight decrease in the permeability of the dentine and the use of sodium hypochlorite and hydrogen peroxide both increased the permeability of the dentine.

Hampson and Atkinson (1964) looked at the relationship between various drugs used in endodontic treatment and the permeability of the dentine using human incisors and canines removed for extensive caries. The root canals were treated and enlarged, the apex sealed with wax, and after treating the canal with one of a wide range of drugs, the permeability was tested by placing radioactive iodine or sulphur into the canal for 24 hours before sectioning the teeth for autoradiography. Their results confirm the observations of Wach et al (1955) and Marshall et al (1960) that apical dentine is impermeable. The reasons submitted for this were the apical region being smaller in size therefore less material in this region, or more probably the isotope solution never reached this part of the canal. They also reported the presence of transparent dentine in this region, which may by its reduced tubule diameter prevent permeation of dyes.

Avny et al (1973) demonstrated *in vitro* diffusion of parachlorophenol by autoradiographic technique in extracted human incisors. After standard endodontic

preparation, and removal of soft tissue debris with sodium hypochlorite and hydrogen peroxide irrigation, aqueous parachlorophenol or camphorated parachlorophenol was sealed in the root canal for 48 hours. There was generalized permeation of dentine by aqueous parachlorophenol while camphorated parachlorophenol had limited permeability of 0.2mm. from pulpal wall in crown and 0.25 mm. in middle third and only 0.05mm. in the apical third.

Electrical current has been used in dentistry to facilitate the movement of ionized substances through dentine, particularly in the field of desensitization of hypersensitive dentine. Using the dentine disc model, Pashley et al (1978b) applied an electric current to the disc to calculate the effect on permeation of iodide ions and ^{14}C-lidocaine. The application of a 0.1 mA current with the negative electrode in the iodide solution produced a decrease in time to reach the steady state permeation by 4 times, with a 0.5 mA current steady state diffusion was reached five times as quickly, and at 1.5 mA a ten times reduction in time was observed to reach steady state diffusion. Reversal of the applicated current produced a reduction of permeation.

Pashley et al (1984c) studied the effect of air drying *in vitro* for 0, 0.5, 2, 5 minutes in human dentine permeability using dentine discs by changes in hydraulic conductance. If the tubules were filled with water, there was no effect of evaporation in dentine permeability. Using phosphate buffered saline, a time–dependent decrease in permeability was seen, with a 30% reduction over 5 minutes. When 1.5% albumin solution was used a large reduction of permeability was seen, about 50% reduction over 5 minutes. The authors noted the effect was more marked in unetched dentine. The results implicate the presence of salts and proteins as responsible for up to 50% reduction in dentine hydraulic conductance. If the surface was acid etched after 5 minutes of air drying the permeability returned to previous levels suggesting the material occluding dentine is acid labile and present in or near the tooth surface. These findings support Brannstrom's (1981) observations of prolonged air blasts decreasing sensitivity clinically.

Pashley et al (1985) studied the effect of cavity varnishes and bases on dentine permeability using isotope permeation and hydraulic conductance. They concluded bases were more effective than varnishes in reducing permeability. Two separate applications of varnish were superior to one application while there was little correlation between thickness of bases and reduction of permeability. The effect of calcium hydroxide on dentine permeability in human dentine discs *in vitro* was studied by Pashley et al (1986). The calcium hydroxide paste produced a 50% reduction in dentine permeability in dentine discs with smear layer and a 75% reduction if smear layer was previously removed, but this was removed by acid etching for two minutes with 6% citric acid indicating calcium hydroxide offers little protection to acid challenge. Their scanning electron microscopy results demonstrated fine amorphous deposit on the intertubular matrix as well as within the tubule orifices. Pashley et al (1984b) studied the effect of desensitizing dentrifices *in vitro*, finding all dentrifices caused a decrease in hydraulic conductance of dentine, even if the active ingredient was omitted suggesting the abrasive action of the dentrifice may be more important in decreasing sensitivity, by producing a smear layer on the root surface.

Pashley et al (1983c) studied the effect of temperature on hydraulic conductance of dentine. Varying the temperature from 10-50 degrees Celsius in acid etched and unetched dentine resulted in an 1.8 fold increase in fluid flow in unetched dentine, and four times in acid etched dentine. This magnitude, 1.8, was similar to the decrease in viscosity over the same temperature range. The additional increment in hydraulic conductance in acid etched dentine was due to the thermal expansion of tubule diameter. These *in vitro* experiments do not have any direct clinical application as temperature changes *in vivo* are transient, and often only to a small area of the tooth. This thermal gradient may diffuse across the tooth. This would produce changes in tubule and pulp chamber volume, producing pressure which would result in fluid movement in dentinal tubules (Beveridge and Brown 1965, Van Hassel and Brown 1969), and *in vivo* studies of hydraulic pressure in the dental pulp of humans provides a powerful tool in the study of normal and physiologic tooth function.

Little experimental work has been conducted in hydraulic conductance of root dentine outside of effects of various periodontal therapies (see 2.3.5.). SEM and hydraulic conductance experiments were conducted on root dentine slices (Fogel et al 1988). Their results indicated the permeability of the root dentine decreased with the distance from the pulp and dentine slice thickness. The SEM study found good correlation between tubule density and diameter, and root dentine permeability. The relatively low hydraulic conductance indicated it was a significant barrier to fluid movement across the root structure, and demonstrated root dentine to be less permeable than crown dentine. An interesting finding was during thickness reductions of the root slices, cementum was removed with little effect on hydraulic conductance, and peripheral dentine appeared as a more significant barrier.

If fluid movement is the stimulus underlying dentinal sensitivity as indicated by the hydrodynamic theory, then certain physical factors will regulate the fluid flow through the tubules. Poiseiulle's Law allows an equation to determine the rate of flow through long narrow tubes, dentinal tubules (Ganong 1975):

$$Jv = \frac{Pr^4}{8nl}$$

Jv= Volume flow rate in $cm^3.sec^{-1}$

P = hydrostatic pressure dyne cm^{-2}

n = viscosity in dyne $sec.cm^{-2}$

l = length of tube in cm.

r = radius of tube in cm.

Therefore small changes in the tubule radius may produce large changes in the rate of fluid movement. Michelich et al (1978) used filtration and surface tension techniques to measure the functional tubule radii of dentine discs and compare these with anatomic radii determined with scanning electron microscopy.

Garberoglio and Brannstrom (1976) have measured the anatomic radii using scanning electron microscopy micrographs. These micrographs have shown that dentine tubules can contain fibrillar structures, odontoblastic processes, irregular radii and occluding debris. Scanning electron microscopy of fractured dentine at 1-1.5*u*m from the pulp have been shown to have anatomic radii of 0.45-0.75*u*m. (Garberoglio and

Brannstrom 1976). Michelich et al (1978) concluded the functional radii of dentine discs ranged from 5 to 40 % of anatomic radii, calculated by filtration techniques, giving a more accurate reflection of the true functional tubular radius which corresponds with the narrowest radius within the tubule length. Acid etched dentine discs have a mean anatomic radius of 1.58*u*m., a value much greater than the functionally determined radius. A view of the scanning electron microscopy of the fractured surface shows the acid etched surface, produced a funnelling effect which extended below the surface for only a short distance, as well as removing surface debris (Johnson and Brannstrom 1974). Bur cut dentine results in closure of tubules, and is associated with a less dentine sensitivity, while acid etching ground dentine removed occluding debris from the tubules and made them more sensitive, similar to fractured dentine (Johnson and Brannstrom 1974).

The resistance to fluid flow in human dentine could be viewed as resistances coupled in series (Pashley et al 1978 a). The surface of the dentine can be open or partially occluded by surface debris, surface resistance. There may be mineralised fibrils and nodules within the tubules (Brannstrom and Garberoglio 1972, Garberoglio and Brannstrom 1976) which could cause intratubular resistance. The odontoblast process or cell body could modify the space for fluid to move on the pulpal surface, pulpal resistance. Each of these was percieved to have an effect on the filtration through dentine (Pashley et al 1978a), but the most important was the surface debris which accounted for 86% of the total resistance, 6% in tubule, 7% in the odontoblastic end.

It is the smallest diameter that determines the rate of fluid movement through the tubules, the functional radius. Dentine is not a uniform structure with smooth bore tubules, but its width varies during its length (Thomas 1983). It is lined by sheath like structure, crossed by collagenous fibres, with occasional mineralised deposits jutting from the walls narrowing the tubule and odontoblastic process. There was a variation in fluid movement seen dependent on positive or negative pressure applied, with negative pressures having reduced hydraulic conductance, which Pashley et al (1978) explained as movement of odontoblasts either towards or away from the pulpal end of tubules.

2.3.1.c. The Effect of the Smear Layer.

The smear layer is a layer of microcrystalline debris produced when dentine is cut with any instrument due to microscopic particles produced as the dentine matrix shatters which settle on the dentine surface (Eicke et al 1970). Its thickness varies depending on the type of cutting instrument, pressure and amount of coolant. Eicke et al (1970) were one of the first groups to describe the smear layer. Olgart et al (1974) reported a number of orifices of cut dentinal tubules were plugged with debris. Jodaikin and Austin (1981) said the organic component consisted of coagulated dentine proteins, saliva, serum, and microorganisms, and the inorganic component derived from minerals and possibly, inorganic contaminants. The size of the microcrystalline debris is extremely small, below the resolving power of the scanning electron microscopy (Pashley et al 1981b). Hence the appearance of a homogenous smooth amorphous surface which obscures the underlying dentine tubule orifices. This description was supported by Dippel et al (1985), where they mention to avoid cracks in the smear layer under scanning electron microscopy the specimens should be dehydrated with the critical point drying method. The smear layer has been shown to be comprised of aggregates of globular subunits approximately 0.05 - 0.1 *u*m in diameter, and compacted on the smear surface so that individual particles cannot be detected (Pashley et al 1988). Several *in vitro* studies have indicated that dentine occluded by grinding debris does not permit fluid to move as readily as it does across fractured or acid etched dentine (Stevenson 1965, Johnson et al 1973, Pashley et al 1978a, Reeder et al 1978).

The nature of the dentine surface and it's effects on the permeability coefficients of $^{3}H_2O$, ^{131}I-Albumin were established *in vitro* by Pashley et al (1978c), using the dentine disc model of Outhwaite et al (1974). The four surfaces investigated were; the glossy surface of the diamond saw, the rough finish of a tungsten fissure bur, the bur surface treated with citric acid for 2 minute on both pulpal and enamel sides, and the acid treated surface treated with 3% oxalate solution on enamel side for two minutes. The burnishing action of the diamond saw produces a highly polished surface and under scanning electron microscopy it appears uniform with no apertures. The tungsten

carbide bur removed the highly uniform layer, but the tubules remained covered by debris. There was no significant difference between the two surfaces with regard to permeability coefficients.

Citric acid treatment removes the crystalline surface debris and peritubular dentine to a maximum depth of 30 *u*m from the surface (Lee et al 1973, Johnson and Brannstrom 1974). After etching of the surface, the dentine viewed under the scanning electron microscopy has a funnel appearance going into the tubules, due to the removal of peritubular dentine on the surface of the dentine. This occurs only for 20-30 microns from the surface (Michelich et al 1978, Pashley et al 1978, 1981, 1983, Dippel et al 1985). Below this the tubule diameter remains in its normal range, and it is this diameter that effects the filtration and permeability of the dentine.

The removal of the smear layer produced a significant increase in permeability and the ratio between $^{3}H_2O$ and ^{131}I-Albumin decreased indicating the unblocking of the tubules had a greater effect on albumin (Pashley and Livingstone 1978). Treatment of the enamel surface with 3% oxalate produced a significant reduction of diffusional surface area similar to the cut surface, but the albumin:water permeability ratio was similar to acid etched surface, and under scanning electron microscopy these show crystalline debris in the surface, but tubule apertures were still evident.

Pashley et al (1981b) looked at the effect of removal of the smear layer on dentine permeability. They etched the dentine discs on both sides using a solution of 6% citric acid for 5-60 seconds to remove the surface debris, with most of the smear layer removed after 30 seconds. At 60 seconds practically all the smear layer was removed as well as peritubular dentine on the surface. Pashley et al (1981b) calculated filtration rates for these discs with significant increases in filtration after 5, and 15 seconds compared to intact smear layer. Fifteen seconds of 6% citric acid treatment increased the filtration rate 20 times. Pashley et al (1978a) had previously reported that 86% of the total resistance to fluid flow across dentine was due to smear layer. Acid etching for more than 15 seconds produced no significant increase in filtration. This can be explained that after 5 seconds of acid treatment the superficial smear layer was

removed, and the opening of the tubule orifices was relatively small, 0.7-1.0um. This was similar to the diameter found throughout the length of the tubules (Pashley et al 1981b), and it is the smallest diameter that determines the rate of fluid movement through the tubules (Michelich et al 1978), which explained the lack of significant increase in filtration with etching periods longer than 15 seconds (Pashley et al 1981b).

Dippel et al (1984) compared the method of cavity preparation with diamond and tungsten carbide burs over a range of speeds, finding that all produced considerable smear layer. Diamond burs produced a continuous smear layer over the 25,000-80,000 rpm range, with steel burs the smear layer was most continuous at 25,000 rpm. Dippel et al (1984) used 50% citric acid solution for up to ten minutes to etch the surface, and calculated the effect on permeability as a percentage of ^{3}H-sorbitol in a steady state diffusion system. They found a significant increase in permeability after etching for all of the cutting instruments used. They calculated the 40% reduction in permeability due to the smear layer was comparable to that of Pashley et al (1978a), because in their experiments they had etched both sides of the dentine discs.

The effect of smear layer removal *in vivo* was studied by acid etching cavities cut in the buccal aspect of dog first molars by cementing a conical chamber to the cavity and applying 200cm of water filtration pressure to the chamber prior to and after acid etching (Pashley et al 1983a). The acid etching of dentine resulted in a five fold increase in permeability. There was some variation with the lower the initial hydraulic conductance, the higher the conductance after acid etching, which possibly reflects the degree of smear layer produced during cavity preparation. With the increase in the ability of the fluid to cross dentine after acid etching the effect on intrapulpal pressure was of interest to see if the permeating fluid was removed as rapidly as it penetrates. Pashley et al (1983a) penetrated into the pulps during their experiments and found only a slight increase in hydraulic conductance, which was not significant. The absence of elevations in tissue pressure in the pulp following acid etching and filtration indicates the microvascular system of the pulp is effective at removing filtered fluid, and hence the rate of removal by the pulp circulation is not the rate limiting step.

2.3.1.d. The Role of the Dental Pulp.

The importance of pulpal blood flow was eloquently demonstrated by Pashley (1979), particularly with respect to a substance that has permeated dentine. The concentration of the solute will not increase in the pulp if the rate of removal of the substance from the pulp via it's micro circulation is greater than its permeation. If the rate of permeation is greater than its removal there will be a concentration of the solute in the pulp circulation, and the response that it produces will be greater or a dose-response relationship. If the response produced a further decrease in the circulation then a positive feedback system may be generated (Pashley 1979). Pashley (1979) believed that dentine can be viewed as a compartment coupled in series to the pulp.

A study was designed to test what was the effect of increased dentine permeation due to increased surface area, decreased thickness, acid etching of dentine, or a given constant permeation of dentine, how much reduction in blood flow in the pulp was required before the concentration of toxins in the pulp interstitial fluid rises to deleterious levels (Pashley 1979). The *in vivo* experiment used the permeation of ^{131}I in dog molar teeth. ^{131}I was detected in plasma within fifteen minutes, and continued to rise during the experiment while the activity in the lingual chamber was much lower and increased at a very slow rate suggesting most of ^{131}I was removed from the pulpal circulation. After four hours the animal was sacrificed and an immediate rise in the lingual chamber was seen. In another part of the experiment adrenalin 1:10,000 concentration was added to the buccal chamber with ^{131}I, 3.5 hours after the experiment commenced. This produced a plateau in the systemic circulation and rapid rise in ^{131}I effluent in the lingual chamber, due to an increase in the pulp chamber as a result of decreased blood flow to the pulp.

There is an inter-relationship between substances permeating dentine and the rate of removal by the circulation. While normal healthy pulp can efficiently remove substances as they permeate to the pulp, anything that reduces pulp blood flow substantially, occlusal trauma, orthodontics, restorative work, may allow an accumulation of solute in the pulp interstitial fluid. This also included the effect of

adrenalin in local anesthetic in the area where there has been an accumulation of toxins from carious dentine, or a leaking restoration.

Pashley and Whitford (1980) reported on the reflection coefficients of human dentine *in vitro*, that is the extent that the membranes are permeable to solute, affects the effective osmotic pressure across the membrane. The magnitude of the coefficient varies from 0-1.0 with a score of 0 indicating lack of discrimination between solute and water and 1.0 indicating complete solute impermeability. They calculated the reflection coefficients of urea, NaCl, $CaCl_2$, $AlCl_3$, glucose and sucrose were about 0.0001. This means the effective osmotic pressures of these solutions is 10^{-4} of the theoretical osmotic pressures, indicating that dentine was very permeable to the substances studied.

Using isotope clearance and fluid filtration techniques Pashley et al (1983b) measured longitudinally the dentine permeability in cavities prepared in dogs which were measured weekly. The reaction of the dental pulp to restorative procedures and materials have traditionally been investigated by the presence or absence of histologic changes during experimental periods (Langeland 1957, Swerdlow and Stanley 1958, 1959, Seltzer et al 1961, Vojinovic et al 1973, Heys et al 1977). The term dentine-pulp complex used by some workers implies a single entity because they recognize that dentine changes, probably of pulpal origin occur following exposure of tubules by wear, and by caries (Gwinett and Johnson 1978, Mendis and Darling 1979b, Brannstrom and Garberoglio 1980). Exposed dentine is thought to respond by forming sclerotic zones or irritation dentine. Both sclerotic dentine and irritation dentine are thought to be less permeable than normal dentine, but this was disputed by some workers (Langeland 1963, Tronstad and Langeland 1971). The time for these to form is thought to be a period of weeks. Acute responses reported histologically are displacement of odontoblasts and nuclei into tubules (Brannstrom 1962, Langeland 1957).

Pashley et al (1983b) found a 76% drop in hydraulic conductance in seven days, with a further decrease of 18% over the second week. The permeability of dentine to ^{99m}Tc demonstrated a 60-89% decrease in the first week with further, but smaller decreases during the second week. The importance of a vital pulp was demonstrated in

that teeth with pulps removed had an increase in permeability during experimental period. This was presumably due to degeneration of odontoblasts and their processes. Small constrictions, anywhere throughout the length of the tubules as described by Tronstad (1973), and Mendis and Darling (1979b) reduce permeability of dentine (Pashley et al 1978c, Michelich et al 1980, Greenhill and Pashley 1981). The time period for their development is unknown, but could be due to the precipitation of calcium salts in tubules. In further experiments the change in hydraulic conductance over six hour period measured hourly found the permeability to progressively decrease every hour for the five hours of measurement following cavity preparation (Pashley et al 1984a). At the end of the study, the permeability figures were about 20 percent of zero time values, and again this was not seen in pulpless teeth. They also found the decrease in permeability could be delayed, but not prevented, by applying 200 cm H_2O filtration gradient across dentine.

Plasma, serum and whole mixed saliva are all capable of causing immediate reductions in dentine permeability (Pashley et al 1982). Individual plasma protein fractions and several different types of bacteria were also effective in reducing hydraulic conductance. Fresh plasma produced the greatest reduction, 77%, with the loss of fibrinogen and clotting factors the hydraulic conductance fell to 54%. Solutions with 5% fibrinogen were used in this study but plasma contains 0.1-0.7% fibrinogen. With a very high molecular weight, 340,000 daltons, it does slowly permeate capillary endothelium and has been identified in dentinal fluid (Haldi and Wynn 1963). Another source of macromolecules is saliva, and many salivary proteins are highly charged glycoproteins which exist in extended forms, producing increased viscosity. The authors (Pashley et al 1982) observed a reduction in viscosity of saliva after permeation of dentine, indicating adsorption of glycoproteins. The protein adsorbing properties of hydroxyapatite are well known. Pashley et al (1982) observed individual plasma protein fractions and several different bacteria also reduce hydraulic conductance. These observations may explain the "spontaneous" reduction in dentine sensitivity seen by clinicans following periodontal therapy.

Potts et al (1985) used a technique for measuring movement of circulating molecules across the odontoblast-dentinal complex in dogs into a prepared buccal chamber. Using tritiated water the movement depended on the dentine thickness. No counts were observed in the first 2.1 minutes in the flow cell with dentine thickness of 0.4-1.1 mm., but were detectable by 7.0 minutes. Alpha-aminoisobutyric acid, is reported as a non-metabolisable analogue of analine which is rapidly transported across mammalian cell membranes via the neutral amino acid transport system (Guidotti et al 1978). Tritiated alpha-aminoisobutryic acid crossed the thinner dentine areas (ave 0.35mm thick) in five minutes. At all time intervals, the permeability of the odontoblast-dentine complex was lower than tritiated water and its concentration in plasma more than dentinal fluid. The findings confirm those of Pashley et al (1981c, 1981d), that the dentinal fluid has a protein level about 20% of plasma, in the movement across dentine *in vitro*. Pashley et al (1981c) found intrapulpal pressure to be 32.6 cm of water while Potts et al (1985) found the value to be higher. Potts et al (1985) believed this to be due to the thicker dentinal walls and the area used to calculate the permeability differed as they used the area of the pulp chamber while Pashley et al (1981c) used the area of chamber floor.

2.3.2. Enamel Permeability.

Small quantities of watery fluid was seen to constantly pass through the enamel of extracted teeth by Bergman (1963). He attached a piece of capillary tubing to the teeth and noted a flow of water along the capillary at a rate equivalent to 0.1mm per hour. No difference was noted whether the pulp and dentine were removed or left intact. In an extension of this study, Bergman (1963) covered extracted teeth with immersion oil, and examined these microscopically. Within 2-3 hours droplets of fluid, 2-4 *u*m in diameter collected under the oil over the entire enamel surface. Adjacent to cracks, tufts, and lamellae the largest drops were seen. These droplets have also been detected *in vivo* in teeth covered in oil. Bergman et al (1966) collected fluid from teeth by placing teeth in sealed plastic bag for 48 hours and collected an average of 0.004*u*l/tooth.

Experiments with stains show enamel to be much less permeable than dentine, but is permeable under certain circumstances. Stains placed in the pulp of young dogs were found to pass through to the enamel surface (Fish 1927a). In older dogs the stain did not permeate so freely, with the outer layers and in some areas the whole thickness of enamel was unstained. Fish (1927a) also found if stains were applied to the external surface of the tooth in young dogs it penetrated enamel and dentine, but in older dogs there was no penetration through the outer surface. From his microscopic examinations the main areas of diffusion appeared to be the interprismatic substance, tufts and enamel.

Berggren and Hedstrom (1951) placed methylene blue in holes drilled into dentine in human teeth, finding penetration of pulp occurred rapidly and the rest of the dentine then stained from the pulp. However a limited amount of stain penetrated enamel along tufts and lamellae or in their vicinity. The staining did not reach the surface of enamel and was irregular. Bergren and Hedstrom (1951) reported he often found a sharp line at the junction of stained and unstained enamel clearly showing an impermeable barrier in the outer part of enamel.

Results tend to suggest the enamel is relatively impermeable to fairly large organic dye molecules. The results of experiments with radioactive ions such as ^{45}Ca, $H^{32}PO_4$, ^{131}I and ^{14}C-urea have demonstrated permeability of the enamel. Sogannes and Shaw (1952) using ^{32}P in rhesus monkeys observed two gradients in teeth, one decreasing from the internal surface to the external dentine, the other increasing from the internal to external enamel. They found the elevated surface activity of enamel can be eliminated by isolating the teeth from saliva. In younger monkeys the salivary ^{32}P is the main source of radioactivity in enamel, whereas blood borne ^{32}P is the main source of radioactivity in dentine. In contrast Wassermann et al (1941) found if the enamel was covered by an impermeable cap, the enamel still takes up the same amount of phosphorus suggesting that the ^{32}P presence in enamel is not due to saliva. The teeth were said to be crowned with a metal cap but no mention of cement used, and our current knowledge would suggest that there may have been leakage around the crown.

Freshly extracted human teeth were painted with the radioactive compounds of nicotinamide, urea, thiourea, and acetamide, kept in a humid atmosphere at 37 degrees prior to sectioning and autoradiography (Wainwright 1954). The substances except nicotinamide which was somewhat slower, penetrated the full thickness of the enamel within 30 minutes. Wainwright and Belgorod (1955) used the same compounds and placed them in prepared pulp chambers of teeth. All these substances penetrated dentine rapidly, reaching the dentinoenamel junction in about twenty minutes. ^{14}C-Acetamide penetrated through enamel in 22 minutes. Wainwright and Lemonie (1950) applied ^{14}C-urea to the external surface of extracted teeth and measured its permeability by autoradiography. They found diffuse penetration of enamel without the necessity of cracks or lamellae, taking approximately 10 minutes to occur. Wainwright (1951) using isotopes of Ca, Zn, and Ag demonstrated these elements could penetrate defects in enamel, but not intact enamel. The preparation of sections in this case may have produced smearing of radiolabelled substances.

Enamel is known to be permeable to water (Berggren and Hedstrom 1951), and is thought to be a semipermeable membrane. Fosdick et al (1959) demonstrated the effect of age on enamel permeability. They found that older teeth were much less permeable than freshly erupted teeth. They also reported impacted old teeth, not exposed to the mouth and therefore oral fluids, were as permeable as freshly erupted teeth. This indicated that the fall in permeability with age involves deposition of material from oral fluids, particularly further mineralisation of the surface enamel.

Brannstrom and Lind (1965) studied the response of the pulp to early enamel caries, white spots without cavitation, and indicated bacterial products can diffuse through the inner enamel, unaffected by caries. Histologically the number of odontoblasts in the affected region were reduced and in some nuclei were seen in the dentinal tubules. The border of dentine had a calciotraumatic line present frequently, and lymphocytes accumulated in a small area of the pulp immediately under the affected odontoblasts.

Poole et al (1963) reported anions do not pass through enamel as readily as water or cations, suggesting that enamel behaves as if it possessed a negative charge. Borggreven et al (1977) developed a method to determine quantitatively and simultaneously the transport of different compounds, ^{3}H-sorbitol, ^{14}C- glycerol, $^{36}Cl^{-}$ and $^{86}Rb^{+}$, through enamel. There was great variation in the diffusion coefficients of identical compounds in different slices of enamel. These differences may be caused by differences between the individual animals, or variation in the composition of enamel in various parts of the tooth. They also found dental enamel to behave as an ion-selective membrane with fixed negative charges. The diffusion coefficients for sorbitol and glycerol were significantly higher than water, indicating enamel may have a molecular sieve ranging over nearly two orders of magnitude for different preparations of enamel. They also noted an ion selective behaviour with cations more mobile than anions. Borggreven et al (1981) in further experiments with radiochemical methods found the transport of $^{45}Ca^{2+}$ ions altered the fixed charge of enamel from negative to more positive values with no influence on the permeability of Rb and Cl ions.

Scholberg et al (1984) presented an electrochemical method to measure enamel permeability as the diffusion process is a slow one because of the low permeability of enamel. The electrochemical method however can only measure the permeability of ions, and not other molecules. They concluded enamel had an ionic matrix, with ion selectivity caused by the charge on the interface between the ionic matrix and the solution, and an ion charge transfer system by means of so called hopping (bridgehead) mechanism. Enamel has a double humped distribution of its structure in region of 0.5-10nm. (Morenco and Zahradnik, 1973).

Hoppenbrouwers et al (1986) investigated the permeability of dental enamel of erupted and unerupted human premolars *in vitro* by an electrochemical method to determine the resistivity of successive 100*u*m thick layers. The electrical resistance of halved tooth crowns which from Scholberg et al (1984) is inversely related to the permeability, was measured before and after removal of successive enamel layers. The resistivity of the successive enamel layers increased from the dentinoenamel junction

toward the outer surface in approximately the same way in erupted and unerupted premolars, except the outer most layer of 100 to 200um. thickness. The resistivity of this outer most layer of the dental enamel in the erupted teeth was higher than that of the unerupted teeth. Indicating the resistivity of this layer has been increased after eruption, probably due to post-eruptive mineralisation. They also reported that the teeth or parts of teeth after eruption encounter less favorable conditions in the mouth the resistivity of the outer layer of enamel decreases. A high resistivity corresponds with a low rate of diffusion, and thus with a low permeability of the dental enamel.

2.3.3. Cementum Permeability.

While much attention has been given to investigating the enamel and dentine of teeth, comparatively little experimental work has been done on cementum permeability. There are several problems in studying this particular hard tissue, firstly it is very difficult if not impossible to isolate the material from the other dental hard tissue, secondly it is only present in relatively thin layers over the anatomical root of the tooth. The presence of very fibrous attachment to cementum makes the surface difficult to study. Similar tissue pressures between the pulp and periodontal ligament compartments exist, except when the periodontal ligament is under pressure, or tension, or when the pulpal tissue is inflamed when hydrostatic presure differences between compartments would likely be transient. The vitality and permeability of cementum remains somewhat in question.

The work of Fish (1927a,b) is also pertinent to this field with his demonstration of the penetration by methylene blue from the pulp to dentinoenamel junction and dentinocementum junction in just 24 hours. One of the first attempts to study the permeability of cementum *in vivo* was done by Stones (1934) in three parts. Initially dyes, methyl blue, eosin, acid fuschin, St Louis green, chlorazol sky blue, trypan blue, were applied to the external surfaces of roots in dogs by cotton pellets, and held in position by a cap for up to 48 hours. Acellular cementum was found to be permeable only in young dogs, and in these young animals the permeation was of a diffuse nature with no definite channels seen, that is not along Sharpey fibres. Older adult dogs

acellular cementum was very impervious. Cellular cementum was stained in the cementocytes and their processes. In young animals all layers of cells up to and adjoining dentine were impregnated, while in older animals only certain cells usually the outer layers were stained. The dyes did not seem to penetrate cementum into the dentine tubules. When the stain was placed in the root canals of dogs permeability of acellular cementum was only observed in very young animals. As the animals aged and cementum thickened it became very resistant to the passage of dyes from within, even though the tubules were heavily impregnated with the stain. Stain placed in the pulp chamber and canals of young animals resulted in the dye crossing dentine through the tubules and right through the cellular cementum staining numerous cementum cells and their processes, and into the periodontal membrane. In young animals, cellular cementum is permeable through the intercommunicating processes of the cementum cells, both from the dentinal tubules and the periodontal membrane, but in older animals only the periodontal membrane route is important (Stones 1934). The lacunae of the inner part of the cementum do not become stained. Lindhe (1984) says the presence of cementocytes in cellular cementum allows the transportation of nutrients through the cementum, and contributes to the maintenance of the vitality of this mineralised tissue.

Linden (1968) used an oil immersion technique on tooth roots to study the permeability to physiological saline placed in the pulp chamber by detecting a colour change to alcoholic toluidine blue solution. In young teeth extracted for orthodontic reasons permeaton of the cementum was noticed after 2-4 minutes, starting in the cervical region and spreading to the apex. In contrast old teeth started later usually after 30-60 minutes and was slower to spread and less uniform. Teeth with apical transparency and dentine had a very slow rate of penetration. He found by studying ground sections that the penetration in the acellular cementum was generalised, but did show some prevalence for Sharpey's fibres. In cellular cementum the lacunae and canniliculi were the main pathways for the fluid, although a diffuse penetration as seen in the acellular cementum was also seen. Further experimentation demonstrated the

reduced permeability of older teeth, which was related to the transparent areas, which were related to changes in the dentine not the cementum. The cementum was always permeable to water and physiological saline in any teeth tested (Linden 1968).

Using ^{35}S labelled penicillin in freshly extracted teeth Wach et al (1955) studied the penetration of this antibiotic over a period of five days. They reported the cementum showed no uptake of radiolabelled material. They felt diffusion of penicillin occurred through dentinal tubules and expected no penetration of cementum, enamel, and transparent dentine as they have no tubules. Bennett and Miles (1955) in a simlar study using penicillin felt that a highly mineralized layer in the borderline between dentine and cementum may act as a semi-permeable membrane.

Marshall et al (1960) using freshly extracted single rooted teeth evaluated the permeability of tooth roots to isotopes, ^{22}Na, ^{131}I, ^{35}S as sulphate, ^{32}P as phosphate over a twenty four hour period by autoradiography. They found the dentinocemental junction acted as a barrier to the passage of isotopes from inside the canal outwards and from outside the canal inwards. The apical dentine was less than half as permeable as the mid root or cervical dentine, and often appeared transparent in reflected light. They also found a range of results using Na, I, and PO_4^{2-}, indicating that they may be chemically reactive with dentine. They suggested more consistent results could be obtained by using a non ionizing isotope, such as the rapid penetration of enamel using ^{14}C-urea in approximately ten minutes (Wainwright and Lemonie 1950).

Sognnaes and Shaw (1952) infused ^{32}P into the blood stream and found the radioactivity of whole dentine in pulpless teeth was on an average one ninth that of intact pulp teeth. All dentine contains measurable amounts of ^{32}P even with the absence of pulp tissue. Some of these exceeded those in enamel indicating that it is not due to salivary ^{32}P but more likely this may have originated from the blood supply surrounding the teeth, and possibly through cementum. Wassermann et al (1941) measured the uptake of ^{32}P from intravenous injection over a twenty four hour period. They also demonstrated that pulpless teeth took up phosphorus by the way of the

dentinocemental junction. The authors found about one tenth of the amount of phosphorus taken up by dentine entered the pulpless teeth through cementum.

Avny et al (1973) studied the effect of the medicaments aqueous and camphorated parachlorophenol in endodontics, particular their penetration of tooth roots. They found aqueous parachlorophenol penetrated from the dentine at least to the dentinocemental junction in all areas of the root.

Abbott et al (1988) studied the release and diffusion of triamcinalone and demeclocycline through tooth roots. In one of the experiments he studied the release of these tritiated compounds with the apex open against the apex being sealed. The rate of release was found to be slightly faster when the apices were left open, but the difference between the mean rates of release of demeclocycline were not significant at anytime interval. The triamcinalone had significantly more release in the open apex at the one and three hour sample times but after this the differences were not significant. From these experiments it appeared the major route of supply of these drugs to the periodontal membrane is via diffusion through the dentinal tubules and cementum. Triamcinalone was seen to have a greater release than demeclocycline at all time intervals. This may be due to the binding of demeclocycline to tooth structure as has been widely reported in the literature (Baker et al 1983, Terranova et al 1986, Wikesjo et al 1986).

Studying the effect of cementum on the diffusion of these same compounds, the root surfaces were compared with and without cementum in teeth with apical seals (Abbott et al 1988b). The diffusion through dentine only, where the cementum had been removed, was significantly faster than when the cementum was present. The importance is that cementum is not a barrier to diffusion of these materials, but did delay the appearance of triamcinalone and demeclocycline on the external surface. Abbott et al (1988a) concluded the results indicated the major supply route for components of Ledermix paste to the periodontal tissues is via the dentinal tubules, and made no comment on the cementum. A further study (Abbott et al 1989) was performed on tooth roots where the cementum was removed with a high speed bur, and

produced significant cementum effects. The authors acknowledged that some peripheral dentine was removed during this procedure. This peripheral dentine has been reported to be important in assessing root permeability (Fogel et al 1988). Abbott et al (1989) concluded cementum was not a total barrier.

Andreasen (1985) stated that the cementum acted as an insulating layer against the penetration of bacterial products from the root canal and dentinal tubules into the periodontal ligament. The removal or loss of cementum would also be expected to allow more diffusion of materials from the periodontal tissues into the pulp, suggesting this may lead to inflammation or necrosis of the pulp depending on the area of cementum lost, initial status of the pulp, and materials available for diffusion. This concept of a cementum barrier was not supported by Fogel et al (1988), Abbott et al (1988,1989).

Root canal treatment was performed on the mesial root of the lower left molar in rats to study the effect on the cementum, described as necrosis, over a period of 90 days (Erausquin and Muruzabal 1967). They found in the short term specimens the nuclei of the cementocytes were found to be shunken, and had nuclear changes evident of necrosis. In the long term specimens, the cementum lacunae were empty. The area of cementum with cellular changes in cementocytes was limited to a relatively thin layer adjoining the dentine, or in others the changes were seen in the full thickness of cementum. Where the full thickness of cementum had changes, a PMN infiltrate was seen adjoining the cementum surface with evidence of cementum resorption. They also reported in some cases deposition of new cementum over cementum showing cellular changes although less frequently than resorption. The pattern of cemental change was different to areas of periodontal infarction, where the spread of degenerative changes in the cementocytes was from the periodontal ligament surface to the dentinocemental junction. Erausquin and Muruzabel (1967) felt the thickness of the dentine was important, as no necrotic changes were seen when the dentine was more than 100 microns thick.

Prolonged exposures of cementum to a microbial environment occurs in periodontal disease. Chemical and structural changes in cementum have been associated with this exposure. One of the pronounced structural changes is the presence of "pathologic granules" (Bass 1951, Benson 1963, Armitage and Christie 1973a,b, Bigarre and Yardin 1977). The pathologic granules consist of highly refractile granule like areas in both cellular and acellular cementum, seen in decalcified frozen sections (Armitage and Christie 1973a). Armitage and Christie (1973b) proposed that the granules might be sites where unmineralized collagen fibrils have been destroyed. Bigarre and Yardin (1977) demonstrated histochemically that these granules contain lipids. They stained frozen sections of decalcified periodontally diseased teeth by Sudan black and Schultz-Hershberger methods, which would indicate if the cementum had been penetrated by exogenous substances of bacterial or salivary origin. Benson (1963) found granules in all except two of the fifty six specimens studied. He found the sections no longer had the stained granules after the sections had been treated with alcohol. These cementum granules were associated with exposed root surfaces to the oral environment, and it was proposed that they were the result of action of microbial products on cementum (Armitage 1977). These granules have also been observed in unexposed cementum from carious teeth with infected root canals, while non carious teeth with normal pulpal tissue were devoid of this change (Armitage 1977).

Armitage et al (1983) then studied the cemental changes in the infected root canals to see if the changes were due to microorganisms within the canal or inflammatory products produced by inflamed pulpal tissue. Forty teeth, from twenty five patients with massive carious lesions and probable pulp exposures were used in the study. Twenty teeth with bacteria all along their canals, were grouped histologically as infected, while 20 teeth had no detectable bacteria present, but were inflamed. Pathological granules were observed in unexposed cementum of 35% of teeth in the infected group, but was not seen in any of the non infected, inflamed group. These pathological granules were not seen around the entire perimeter of the tooth but rather localized near the dentinocemental junction as in periodontal disease (Armitage and

Christie 1973a, Bigarre and Yardin 1977). Roots that had undergone resorption and new cementum had no granules visible. Selvig (1965) reported that an area rich in unmineralised collagen is found near the dentinocemental junction. Armitage and Christie (1973b) suggested that the granules occur at sites of collagen denaturation. Selvig (1966) reported changes in cementum where the collagen in cementum is dependent on metabolic interchange with surrounding tissues. He did not come to any conclusion as to the depth at which this exchange took place. Armitage and Christie (1973a) noted that the heaviest concentration of these granules was at the dentinocemental junction. Under the EM (Armitage and Christie 1973b) found these granules to appear as vacuoles, 15-25*u*m under the cemental surface, and up to 5-15*u*m, into dentine. Daly et al (1982) suggested the clinical significance of these pathologic granules could be derived from, or contain bacterial toxins, contributing to the pathogenicity of the cementum. They said the removal of these granules was required to regain clinical health.

2.3.4. Bacterial Penetration.

The work on the penetration of bacteria through dentine has been studied from two aspects. From the external surface, mainly enamel, to study bacteria in caries and under restorations, or from the internal pulpal surface, to confirm the scientific basis of endodontic therapy. Many studies are available to detail organisms being present within the root canal system, and a great deal of interest has been shown in their distribution. Vital organisms such as bacteria may not be dependent on simple permeation of tooth structure by filtration and osmotic pressures, but may act via their own motility and biochemical processes.

Jolly and Sullivan (1956) undertook histologic investigation of infected non vital teeth, and concluded that infection of the dentinal tubules was uncommon, and in the cases it did occur, the bacteria were seen in close proximity to the root canal. Shovelton (1964) undertook a histological study of 97 non vital teeth, extracted generally due to caries. Of these 97 teeth 79 were found to have infected pulp chambers or root canals, of these 61 teeth had bacteria penetrating to a greater or lesser extent in dentinal

tubules. The degree of invasion was varied in some cases bacteria entered the tubules of the predentine, but were prevented from penetrating the calcified dentine. Some teeth had bacteria penetrating up to halfway through the thickness of dentine, but in no cases did they reach the cementum. The chronically inflamed teeth appeared to have more bacterial invasion than the acutely inflamed teeth, probably due to a longer infection period.

There has been much concern in endodontics over organisms that have impregnated the dentine walls of the pulp as they may not all be removed by instrumentation, although little is known of their long term viability. Chirnside (1958) examined the radicular dentine of 50 extracted teeth with clinically infected pulps and disclosed the invasion of pulpal dentine wall by microorganisms in 31 teeth. He observed more bacilli penetrating dentine than cocci, and occasionally lysis of dentine associated with gram negative bacilli. Chirnside (1958) also inoculated a freshly extracted canine tooth with the organism *Serriata marcesens* and incubated it for 28 days to find organisms in the radicular dentine tubules. The work of Chirnside (1958) and Shovelton (1964) was not controlled, and from these studies the rate and extent of microbial invasion of organisms is difficult to interpret.

Akpata and Blechman (1982) prepared fourteen freshly extracted single rooted teeth with vital pulps. After standard endodontic access and instrumentation, then the teeth were sterilized by ethylene oxide. The teeth were then inoculated with a particular organism and incubated for 1, 2, or 3, weeks. They found the extent of bacterial invasion of root dentine was related to the incubation period. Only *Strep. sangius* of the organisms used invaded the cervical third of the root in one week. *Strep. faecalis* did not invade pulpal dentine until after the second week of incubation when the entire root length showed areas of bacterial invasion. *Strep. sangius* intensely infiltrated many tubules. The other organisms *Bacteriodes melanogenicus* and *Peptococcus asaccharolyticus* were not seen to invade tubules heavily, although the authors noted the *Bacteriodes melanogenicus* was not distinctively stained by the Brown and Brenn technique. They observed differences in invasiveness of the bacteria may be

partially due to their different growth rates. They found bacterial invasion most frequently in the cervical third of the root, as they felt this was because that portion of the root was nearest the access cavity, through which the organisms were inoculated. Also a consideration is the impermeability of the apical third of the root which is due to the commonly seen dentine sclerosis in this region (Vasiliades et al 1983a).

Louma et al (1984) looked at early fissure caries in rats using a enamel fracture technique, and found columns of organisms adherent to the crack surface in many preparations even though the surface appeared intact. Single organisms were sometimes seen in lacunae type depressions within the prismatic structure. At the DEJ, the microbes had spread along the enamel completely. Some of their SEM pictures suggested the entry point for organisms were developmental irregularities. Similar findings were seen by Seppa et al (1985), where they studied 23 human teeth with white spot lesions using a technique designed to remove the possibility of bacterial contamination. In 7 of the 23 specimens, bacteria were found beneath the enamel surface, including two specimens where the bacteria had penetrated to the DEJ. One specimen had bacteria penetrating the outer layer of dentine with the tubules filled with cocoid bacteria. In a few instances the bacteria appeared to lie in snug lacunae, as reported by Brannstrom et al (1980), suggesting that some bacteria may be able to destroy directly the apatite structure. The early invasion of enamel caries by bacteria has been also demonstrated by Hurst et al (1954), Brannstrom et al (1977,1980). The ability of organisms to penetrate the enamel prisms is important, where the inorganic material is a greater barrier than the dentinal tubules.

Brannstrom et al (1977) looked at experimental caries in human teeth implanted into dentures. These teeth were obviously non vital and the authors concluded that there was no indication that the dentinal fluid was important in providing nutrients to bacteria for the progression of dentinal caries. They found bacteria in both enamel and dentine at an early stage. The bacteria were seen to predominately attack peritubular dentine and the DEJ in dentine, with the demineralisation in enamel and dentine being

slightly in advance of the bacterial invasion. This study was of considerable length, up to 3.5 years, but no indication of the depth of bacterial penetration given.

Incipient carious lesions were examined by SEM techniques to demonstrate the invasion of microorganisms without cavitation by Brannstrom et al (1980). Microorganisms penetrated fairly deeply into these lesions sometimes reaching the DEJ before cavitation occurred, and the gap between the enamel and the dentine was occasionally filled with microorganisms. The authors found it difficult to find a correlation between the appearance of the enamel and the depth of penetration and degree of destruction. Microorganisms were frequently found under white spot lesions even though no visible cavitation had occurred, and there was irregular destruction near the DEJ rather than immediately beneath the enamel surface in some cases. Brannstrom et al (1980) confirmed previous observations of microbes in resorption lacunae (Brannstrom et al 1977). The observations suggested that bacteria had penetrated into the dentine prior to the enamel surface having cavitation. In addition the dentine facing the DEJ was heavily demineralised, with the peritubular dentine completely removed, and the tubule lumen filled in some places with bacteria.

The invasive capacity of *Capnocytophagia gingivalis* was studied in *in vitro* human dentine by Adriaens et al (1982). This organism is known to have gliding motility, and was isolated from human periodontal disease. After 144 hours the organism had spread over the entire dentinal surface and formed a dense layer. In the longitudinal sections 74% of the tubules contained microorganisms. The *Cap. gingivalis* was found deep in dentine in 67% of specimens with the mean depth of penetration being 55*u*m. below the surface. The organisms were seen in small groups, or isolated, giving evidence of their gliding motility (Ariaens et al 1982). Continuous rows of organisms are indicative of passive ingrowth, such as is seen with *Strep. mutans*. The ability of *Cap. gingivalis* to provide a 'piggyback' transport for other organisms has been demonstrated by To et al (1978) and Socransky et al (1978). This would result in more rapid penetration of dentine by cariogenic organisms. Not all organisms are transported by *Capnocytophagia*, as the cell to cell interaction seems dependent on a lactose reversible

coagulation with streptococci and Actinomycetes (Kolerbrander and Hurst-Calderone 1981). The observation of resorption lacunae surrounding *Cap. gingivalis* (Adriaens et al 1982) suggest a significant role in the carious process in root caries.

Williams and Goldman (1985) studied the penetration of the smear layer by *Proteus vulgaris* in freshly extracted teeth, after preparing cavities in the coronal dentine leaving approximately 1mm. thick dentine. One group had the smear layer removed by rinsing with EDTA and Sodium hypochlorite, and a control group were inoculated with the organism. The results found where the smear layer had been removed all teeth show penetration by 48 hours of *Prot. vulgaris* in the pulpal side of the dentine. In contrast, the group with the intact smear layer had 50% show bacteria penetration after 48 hours, but in all teeth the organism penetrated by 96 hours. This indicated the smear layer was not a barrier to the growth of this highly motile organism, but it did delay penetration.

Using one millimetre thick dentine discs, Michelich et al (1980) studied the penetration of *Strep. mutans* through dentine under growth and filtration pressure, in etched and non etched dentine. In five days bacteria were able to grow through the dentine discs of acid etched dentine, although not all tubules contained bacteria. In contrast there was no growth through the unetched dentine or control chambers. The filtration rate in the discs was decreased after bacterial growth had occurred, indicating bacteria lodged in the dentinal tubules, and reduced the functional radii of the dentine by getting trapped in irregular constrictions in the tubules. Under filtration pressure of 240 cm Hg. bacteria were seen on the pulpal side of the acid etched discs in 20 minutes. In unetched dentine no bacterial penetration was seen using the same filtration pressure. However the dentine disc appeared to offer considerable resistance to bacterial movement in etched discs in response to hydraulic forces, as the concentration on the pulpal side was only 0.2% of the occlusal side. The filtration pressures used were said to be within the realm of forces created during mastication and could aid the penetration of bacteria across dentine *in vivo*. Comparing the five days for bacteria to

grow through acid etched disc compared to the twenty minutes under filtration pressure, such pressures could aid bacterial penetration.

Other authors have been able to demonstrate bacterial penetration in the *in vivo* studies by other microorganisms (Olgart et al 1974, Lundy and Stanley 1969), even though there was the movement of dentinal fluid to contend with. The dentinal tubules are said to be 2-3*u*m in diameter at the pulpal side, a size much larger than the bacteria one would expect some penetration of organisms in these tubules, although Pashley et al (1978a) indicated the functional diameter of the tubules is much less than this.

Meryon et al (1986) found *Strep. faecalis* penetrating 0.5*u*m micropore filter and unable to penetrate a filter with a pore size of 0.45*u*m, reflecting the diameter of this organism said to be 0.5-1.0*u*m. They found in human and ferret dentine discs with intact smear layers *Strep. faecalis* and *E. coli* were unable to penetrate. Removal of the smear layer allowed rapid penetration of motile organisms *E. coli* and *Pseudomonas aeruginosa* and slower penetration of *Strep. faecalis*. The diffusion of nutrient broth through dentine accelerated the permeation of dentine (Meryon et al 1986). *Pseudomonas aeruginosa* were able to digest dentine, and seen to remove the smear layer after 96 hours. This was confirmed by the increased concentration of hydroxyproline in the medium due to the collagenase produced by this organism.

Tortenson et al (1982), and Browne et al (1983) found the amount of inflammation beneath restorative materials correlated with the presence of bacteria as a result of microperculation of bacteria around the filling, or may be entrapped at the time of placement of the restoration. In contrast Wennberg et al (1983) did not find a correlation with the bacterial numbers and pulpal inflammation. Diamond and Carrel (1984) stated that bacteria present could not penetrate the smear layer present at cut surfaces. Little penetration of dentinal tubules was seen under unetched cavities by bacteria in *in vivo* experiments (Paterson and Watts 1981). This suggests that any inflammatory response must be elicited by substances released from bacteria rather the bacteria themselves.

Lundy and Stanley (1969) attempted to correlate pulpal histopathology with clinical symptoms in teeth experimentally irritated, and class V cavities prepared and left exposed to the oral environment. Brown and Brenn staining did not reveal bacteria on the floor of the cavities until the second day. In the extended observation group bacterial penetration ranged from 0.04mm. after 25 days to 3.0mm. after 240 days, but bacteria were only found in the pulp in one specimen which had an instrumental exposure. Chirnside's (1961) study on non vital dentine found the bacteria were able to invade to the pulp cavity, via exposed dentinal tubules, in three weeks. This indicates the presence of a vital pulp with odontoblast processes within the dentinal tubule proffers some protective role in bacterial invasion.

Vojinovic et al (1973) prepared cavities, and treated them with citric acid before restoring them with composite restoration. A previous study had demonstrated a space between the resin filling and tooth of up to 20 microns and this space was commonly filled with bacteria, but microorganisms were seen in only a few tubules (Brannstrom and Nyborg 1971). The use of citric acid was seen to increase the bacterial penetration of the tubules due to the opening of the tubules blocked by the smear layer (Vojinovic et al 1973). All 46 cavities had a thick layer of bacteria covering the walls of the cavity, but the etched cavities had numerous tubules containing bacteria, while unetched cavities were rarely invaded. Brannstrom and Nyborg (1973) believed a bactericidal solution should be used after cavity preparation to eliminate bacteria from the cavity, and a liner place to stop regrowth of the microorganisms on dentine.

Qvist (1975) studied the penetration of bacteria in intact premolars of children to be removed for orthodontic reasons. They prepared cavities and filled them with one of several filling materials, Silicap, Sevriton, Simplified or Addent XV. Nineteen teeth were extracted after two weeks, and the remaining thirty after six months. At two weeks 21 of 38 cavities had a reduction in odontoblasts. In 19 of the cavities the inflammation was described as slight, moderate in seven cases, and severe under one restoration. Bacteria were seen in the cavities of eighteen of the restored teeth, and of these seven had bacterial penetration into dentine, the average depth of which was 150 microns. At

six months 46 of 60 cavities had a reduction in the number of odontoblasts. The inflammation recorded after six months was less than that at two weeks, as half the cavities were free from inflammation, 22 registered as having slight inflammation and nine scored as moderate. No necrotic tissue was seen in any of the pulps. Bacteria were present on the walls of 55 of the 60 cavities, and the mean thickness had increased compared to the two week period. In 20 cases bacteria were present in the dentinal tubules as well, with a average depth of penetration of 310 microns.

Mjor (1974) using 45 premolars extracted for orthodontic reasons removed enamel and part of the dentine. These flat surfaces were left exposed to the oral cavity for periods of up to 101 days. A bacterial plaque always covered the surfaces exposed to the oral cavity for some time. Bacteria were usually demonstrated in the dentinal tubules just underneath the plaque. Bacteria could only rarely be demonstrated in the tubules at some distance from the facet, and they were never seen reaching the pulp. Mjor (1974) reported only minor pulp reactions in the pulpal tissue under these areas of exposed.

Histological sections of experimentally inserted restorations were stained by the Brown and Brenn technique, and no bacteria were found at the dentine restoration interface, or in dentine if zinc oxide eugenol or calcium hydroxide were used as lining materials (Mjor 1977). A few bacteria were seen under unlined amalgam and lined silicate restorations. The author suggested that bacterial growth under fillings is not a problem if the fillings are adequately lined. Bacteria at the tooth filling interface have been considered a potential danger to the pulp (Brannstrom and Nyborg 1973), but the presence of stained bacteria gives no indication of their pathogenicity or viability (Mjor 1977).

Tziafas and Kolokuris (1987) looked at the effect of pulpal inflammation induced by thermal irritation, on bacterial penetration of dentine in class V cavities. Half of these cavities were acid etched, and all were left open to the oral cavity for 3-7 days. All etched teeth, and only half of the non etched teeth, nine teeth, exhibited slight bacterial

invasion of dentinal tubules. The effect of thermally induced pulpal inflammation had no effect on the histopathological findings.

The results of Vojinovic et al (1973), Brannstrom and Nordenvall (1978), Brannstrom and Johnson (1974), Olgart et al (1974), Michelich et al (1980) indicated that bacteria can readily penetrate acid etched dentine but not unetched dentinal tubules. Pashley et al (1978c) have reported that the smear layer produces an 86% increase in the resistance to fluid movement, but allows permeation of large molecules such as albumin. Brannstrom and Nyborg (1973, 1974) have shown bacteria to survive in the smear layer and multiply and produce toxins that are harmful to the pulp. Several studies have demonstrated these toxic properties of bacteria to the pulp (Mjor and Tronstad 1972, Bergenholtz and Lindhe 1975, Bergenholtz 1981, Bergenholtz et al 1982, Bergenholtz and Warfvinge 1982).

2.3.4.a. Bacterial Products.

Since the bacteria are rarely seen to penetrate to the pulp in vital tissue then the cause of changes within the pulpal tissue is the result of diffusion of some toxin into the pulp chamber eliciting an inflammatory response. Bacteria present in the dentine may cause or contribute to pulpal reaction (Reeves and Stanley 1966, Tronstad and Langeland 1971). Brannstrom and Nyborg (1973) indicated that the bacteria present under fillings constitute the major danger to the health of the pulp under restorations. Langeland and Langeland (1968) investigated the pulp response to dental caries have revealed that the dental pulp often had signs of inflammation before bacteria have come into contact with the pulp chamber. Brannstrom and Nyborg (1971, 1972) and Hansen and Bruun (1971) also indicated that dentine under restorations was permeable to toxins produced by bacteria in the space between fillings and dentine. The etiology of pulpal inflammation in superficial caries was the result of metabolites and breakdown products of bacteria, and disintegration products of odontoblastic processes initiating a pulpal response (Langeland 1987).

Brannstrom and Nyborg (1973) stated the development of inflammatory lesions in the dental pulp following restorative procedures is related to bacterial contamination.

The cutting of tooth structure exposes the dentinal tubules which Brannstrom (1981) refers to as 'highways' for bacteria and their products to penetrate. Dentine itself acts as a semi-permeable barrier (Pashley 1985), and therefore molecular size may be a determining factor in the permeation of molecules (Pashley et al 1977). Dentine may through either steric restriction or adsorption, allow only a small portion of the entire cascade of agents released from contaminating bacteria to actually affect the pulp.

Mjor and Tronstad (1972) designed a study model for inducing pulpitis in monkeys by preparing class V cavities and lining these with carious human dentine, and then sealing the cavity with amalgam, or left opened to the oral environment, or filled with gutta percha. After 8 days the animals were killed, and the teeth examined histologically. Those teeth with carious dentine and amalgam had localized severe pulp reaction, and gutta percha gave a slight reaction. The cavities left open to the environment had a varied response. They felt this supported Langeland (1957) concepts that the likely substances from infected dentine are the main causative agents.

Mjor (1977b) prepared class V cavities in monkey teeth, and then placed human carious dentine in these cavities with several filling materials, gutta percha, amalgam, calcium hydroxide, and zinc oxide eugenol cement. Bacteria could regularly be demonstrated in cavities where the carious human dentine had remained in the cavities for 82 days. Bacteria were seen only rarely in any of the other times. Only one tooth sealed with amalgam demonstrated slight penetration of bacteria into the dentinal tubules at 82 days. Teeth filled with carious dentine and gutta percha for 82 days could not demonstrate bacteria with any certainty. Cavities filled with zinc oxide eugenol or calcium hydroxide cement did not demonstrate bacteria. Mjor felt the findings supported the concept of vital dentine being resistant to infection, and did not support the suggestion by Brannstrom and Nyborg (1973) that all cavities are permanently infected by bacteria. The bacteriocidal effect of zinc oxide eugenol cement needs to be considered in these experiments, and it's ability to seal cavities.

Lervik and Mjor (1977) described the pulpal reaction of the above experiment (Mjor 1977b). Severe reactions were seen to the carious dentine during the initial 3-5

days. After a week the development of irregular dentine was noted, and this was followed by a gradual resolution of the pulp inflammation, although 3 teeth did become necrotic. Mjor (1977b) believed the subsequent healing indicated the tissue changes removed the inflammatory products of the bacteria which had diffused through to the pulp.

Mjor and Tronstad (1974) induced pulpal inflammation in the dental pulps of monkeys by preparing class V cavities and placing carious dentine in these for 8 days, and then treating the lesion by conventional procedures. They found the pulp reactions, including localized abscess formations, may heal if the agent producing the inflammatory response was removed, and the cavities restored in a conventional manner. These marked improvements were seen within 8 days after the agents inducing the inflammation were removed. They found secondary dentine formation the most striking feature of healing.

Brannstrom and Nyborg (1973) found the smear layer contained bacteria which could survive, and produce toxins beneath composite resin restorations, which diffused to the pulp and caused inflammation. Vojinovic et al (1973) removed the smear layer from the cavity, which resulted in the massive bacterial invasion of most dentinal tubules. Premolar teeth destined for extraction for orthodontic reasons were prepared for an inlay (Brannstrom and Nyborg 1977). The base of the cavity was cleaned by the use of a fluoride containing, microbiocidal, surface-active solution which removed the smear layer, but left plugs within the dentinal tubules. These inlays were cemented with either zinc phosphate or epoxylite CBA. Brannstrom and Nyborg (1977) noted that no bacteria were seen under any of the inlays cemented with zinc phosphate in the study, and they had only with minimal pulpal inflammation. This contrasted with the previous study where severe reactions were seen in the pulp underneath cemented inlays which were found to have a layer of bacteria. Brannstrom (1981) felt that a diffusion of toxins from a layer of bacteria on the cavity walls was the main cause of pulpal injury beneath restorative materials, rather than toxic effects of the materials themselves.

Brannstrom and Lind (1965) studied the pulpal response to early dental caries in 74 premolars removed for orthodontic reasons with superficial caries on proximal surfaces. Of 56 teeth demonstrating either only a white spot and no cavitation, or white spot with slight cavitation 33 teeth had changes with the pulpal tissue under the carious area. The most commonly seen changes seen were to the odontoblast layer, and accumulation of inflammatory cells, indicating a pulpal response very early to the carious lesion.

Using 40 teeth, class V cavities were prepared just above the gingival margin, and the effect of soluble plaque factors on the inflammatory reactions of the dental pulps of monkeys were studied (Bergenholtz and Lindhe 1975). Twenty test teeth had an extract of human dental plaque applied every 5 minutes for eight hours, while control teeth were treated in an identical manner using Ringer's solution. The animals were sacrificed after either 10 or 32 hours. Two hours prior to sacrifice the animals were injected with colloidal carbon. Histologic examination of the pulp tissue found the pulpal reaction to topical application of dental plaque extract was characterized by an increased carbon retention, indicating vascular exudation and enhanced rate of emigration of neutrophils and monocytes. The control teeth with few exceptions had healthy pulp tissue when examined.

Bergenholtz (1977) investigated the capacity of substances produced by bacteria isolated from human dental plaque to induce inflammatory reactions in the dental pulp of monkeys. Class V cavities were prepared in 94 teeth, 47 test and 47 control teeth, in six adult monkeys. Extracellular bacterial components were filtered from cultures, and intracellular material was obtained from disintergrated cells of cultured plaque bacteria. These were applied topically to the base of the cavity, and then sealed in place with a teflon plate and zinc oxide eugenol cement. Controls had either saline, or the culture media applied, and were sealed with zinc oxide eugenol cement. The animals were sacrificed after 32 hours, and the pulps examined histologically. Teeth treated with either extracellular or intracellular material had heavy accumulation of polymorphonuclear leukocytes subjacent to the prepared cavities, and abscesses were

regularly seen. The odontoblasts were destroyed in these cases, but in some instances portions of this cell layer seemed to be pushed away from the dentine. The reactions seen in this experiment were more severe than those seen by Bergenholtz and Lindhe (1975), because the concentration of bacterial substances was much higher than that from the pooled plaque extract. These reactions developed independent of differences in the thickness of the remaining dentine. The controls showed damage to the odontoblasts, but little or no neutrophil infiltration.

Using four adult monkeys class V cavities were prepared in the permanent teeth under sterile conditions and filter papers placed on the floor of the cavity prior to filling with either amalgam, composite resin, gutta-percha, silicate cement, or zinc oxide eugenol (Bergenholtz et al 1982). After the observation period of either 2-3 weeks, or 8 weeks, the filling was removed under sterile technique, and the filter paper disks were removed and cultured on Trypticase Soy Agar and the number of colony forming units determined, and the tooth was then analyzed histologically. They found bacterial growth beneath 44% of the test samples of all restorative materials. Bergenholtz et al (1982) identified gram positive and gram negative cocci and rods. Black pigmented Bacteriodes and *F. nucleatum* were identified in 5 and 12 samples respectively.

Mejare et al (1979) recovered bacteria from the floor of composite filled cavity and found large numbers of gram negative organisms, saying the material was similar to aging dental plaque. Bergenholtz et al (1982) using the Brown and Brenn technique found the gram positive bacteria stained adequately, while gram negative bacteria did not stain effectively. Others have mentioned the problems associated with bacterial staining (Mjor 1977a, Mjor and Tronstad 1974). Bergenholtz et al (1982) noted the growth of bacteria was dependent on the type of restorative material used, as all silicate cement and eight week amalgam exhibited bacterial growth. The other restorative materials had less bacteria cultured from the recovered filter paper, and no bacteria were cultured from under the zinc oxide eugenol cement.

Bergenholtz et al (1982) found in their histological examination that the zinc oxide eugenol restoration had significantly less inflammation, in contrast to the silicate

restoration which all had varying degrees of inflammation. Amalgam restored teeth had more severe responses at 8 weeks than 2-3 weeks. The authors also noted a few test cavities treated with EDTA which removed the smear layer had extensive bacterial invasion of the dentinal tubules, and occasionally the dental pulp. They found a significant relationship between the inflammatory cell response seen in the dental pulp, and the presence of bacteria in the recovered filter paper disks. Reparative dentine seen at eight weeks was associated with inflammatory cell infiltration.

Warfvinge et al (1985) assessed the pulpal response to bacterial cell wall material from several microorganisms, and compared the pulp inflammatory change with that of wound chambers. The LPS was collected from *Bacteriodes oralis*, *Veillonella parvula*, and cell wall material of *Lactobacillus casei*, and these were placed in class V cavities under a teflon plate and sealed with zinc oxide eugenol cement, as described by Bergenholtz (1977). Pulp samples of monkeys exposed to the bacterial materials had an intense polymorphonuclear leukocytes infiltration. The test materials at 72 hours had a small infiltration of mononuclear leukocytes as well as polymorphonuclear leukocytes. Warfvinge et al (1985) concluded that high molecular weight complexes of cell walls of both gram positive and negative oral bacteria are capable of inducing inflammation in the underlying pulp. These higher molecular weight complexes tested were seen to be less potent in inducing inflammation than the crude mixture of bacterial products released during growth and disintergration used by Bergenholtz (1977). Warfvinge et al (1985) found the responses to the bacterial and control substances were similar in the monkey tooth cavity experiment and the wound chamber in the rat, which they felt indicated that freshly cut dentine does not function as a barrier to the passage of high molecular weight cell wall complexes. The molecular weight of the cell wall components were considerable larger than those materials tested by Pashley et al (1977).

Warfvinge and Bergenholtz (1986) placed intra and extracellular products in Class V cavities in human and monkey dentine in aseptic conditions similar to Bergenholtz et al (1982). The experiment was to compare initial and late pulp tissue reaction. The

study was designed in one set of teeth to leave the bacterial material in the cavities for the entire experimental period, 4, 10 and 30 days, while the other group had the bacterial challenge stopped after 32 hours by removal and substituting with zinc oxide eugenol cement. After 32 hours all the human and monkey teeth had acute inflammatory changes. The polymorphonuclear leukocytes infiltrate was confined mainly to the pulp adjacent to the cut tubules. There was a severe response in 8 of 9 human teeth and 10 of 16 monkey teeth where microabscesses had often developed, and these lesions had inflammatory cells present deeper within the pulp tissue. The majority of the pulps had moderate to severe infiltrates of polymorphonuclear leukocytes after four days irrespective of whether the bacterial material had been removed or left in the cavity. After 10 days, less teeth had severe inflammation than the 4 day specimens in both experimental procedures. The authors also noted that the inflammatory cell infiltrate was now dominated by mononuclear leukocytes. After 30 days the majority of human and monkey teeth demonstrated repair by reparative dentine, and the lack of inflammatory cell infiltrate. The repair was more prominent in the teeth that had the bacterial challenge removed after 32 hours. The authors found that 8 of 40 monkey teeth, and none of the human teeth developed pulpal necrosis, and three monkey teeth and 2 human teeth still had severe inflammation present after 30 days. Healing and repair appeared to be the prominent feature after 10 and 30 days. This data appears to be in agreement with the studies of Lundy and Stanley (1969), Mjor and Tronstad (1974), and Lervik and Mjor (1977).

Following the placement lyophilized sonicates of pure cultures of three different dental plaque bacteria, *Actinomyces viscosus, Streptococcus mitis*, and *Actinobacillus actinomycetemcomitans* in the base of class five cavities. Bergenholtz and Warfvinge (1982) studied their capacity to induce leukocyte migration in monkey dental pulps. The materials were applied to the dentine for eight hours, and histological examination demonstrated *Actinomyces viscosus* and *Actinobacillus actinomycetemcomitans* consistently induced infiltration of polymorhonuclear leukocytes in one to several layers underneath the test cavities, while *Streptococcus mitis* seemed less potent as 50% of the

teeth had no reaction recorded. The authors used Cobra Venom Factor to inactivate complement, but this did not appear to affect the severity of the leukocyte responses.

There has been recent interest on the influence of bacteria on the pulp response which has led to a revision of the properties of the dental filling materials with several authors. Patterson and Watts (1981) suggested bacterial contamination may occur in the pulp by progress from carious lesions, during cavity preparation and the insertion of restorations, or as a result of continuous leakage at the margin of the restoration throughout its life. Amalgam restorations placed at least five years previously and graded clinically as satisfactory were demonstrated to have margins readily permeable to isotopes (Baumgartner et al 1963). Brannstrom and Soremark (1962) demonstrated significantly reduced marginal leakage and dentine penetration to Na^{22} following the application of a polystyrene based varnish. A recommendation of two coats of varnish was made by Brannstrom and Nyborg (1973) as a single coat was not effective when the teeth were subjected to thermal cycling and they believed a single coat to be inadequate.

Brannstrom and Nyborg (1972) found inflammation beneath composite resin fillings, and suggested this was as a result of the bacteria that had penetrated along the margins to the floor of the cavity. Wennberg et al (1983) noted a tendency for greater pulpal reaction to light cured composite resin at 78 days compared to 7 days, even though the toxicity of the material following curing was seen to decrease in cytotoxicity studies. However, they could not find a correlation between the inflammatory status of the pulp and the presence of bacteria on the cavity floor, but the Brown and Brenn stain technique is known not to stain all bacteria (Mjor 1977a). Wennberg et al (1983) found that there was very little correlation between the results attained from the pulp study and the implantation study. Qvist (1975) in a study of three different filling materials, silicate, composite, and non composite resins, thought it was possible that the pulpal reactions to all three materials could have been ascribed to the bacteria under the filling materials, a belief shared by Brannstrom (1981).

Browne et al (1983) studied the prevalence of bacterial contamination at the interface of material and cavity wall in cavities filled with a range of materials, and correlated their presence with the extent of inflammation observed in human and ferret dentine. The data from ferret material indicated a strong positive correlation between pulpal inflammation and the extent of bacterial microleakage. In 16 of the 21 teeth with evidence of bacteria but no pulpal inflammation the bacteria were only present on the cavity walls, with only five having bacteria demonstrated on the cavity floor. The authors thought the species of bacteria present were also important. Browne et al (1983) found the correlation between the human teeth and inflammation not to be as strong. In 10 of the 56 teeth that had no inflammation, ten had bacteria present, five with bacteria on the floor of the cavity and five with bacteria on the walls. In comparison between the human and ferret teeth the thickness of the human dentine was substantially greater, which would influence the pulpal inflammation due to diffusion of microbial products.

Microleakage is the term used to describe the penetration of oral fluids and microorganisms along the restorative material/cavity wall interface. It appears the size of the space and the consequential leakage varies from material to material, and also depend upon the method of placement. Most microleakage work has been done using tracer molecules which are evaluated along the material cavity/wall interface or demonstrating biologic consequences of this movement. Comprehensive reviews of this work can be found elsewhere as it is beyond the requirements for this study (Going 1979, Bauer and Henson 1984, and Shortfall 1982). It is important to remember though that microleakage to tracer molecules does not necessarily imply bacterial microleakage will also occur *in vivo*. An example of this is the tracer studies that have shown zinc oxide eugenol microleakage to be greater than zinc phosphate (Grieve and Jones 1981), but microorganisms are rarely found around zinc oxide eugenol because of its prolonged anti-bacterial effect (Tobias et al 1985).

Brannstrom and his coworkers have carried out many investigations over the last twenty years investigating pulpal inflammation under restorations. The results indicate that the main cause of the pulpal inflammation seen beneath these amalgam, composite

resin, silicate fillings, and underneath cemented inlays with zinc phosphate or polycarboxylate cements is due to bacterial irritation (Brannstrom 1981). The interpretation of the pulpal response under cavities in experimental studies is complicated by bacterial microleakage under these restorative materials (Watts and Patterson 1983, and Browne et al 1983). Demonstrating the presence of bacteria on cavity walls and floors is a problem. The histological methods used have problems as all bacteria are not stained by the Brown and Brenn technique (Mjor 1974, 1977a). Browne and Tobias (1986) in their review of microleakage noted prior to the last twenty years, few studies of the pulpal response beneath cavities filled with experimental materials had indicated a search for the presence of bacteria under the filling materials. They also concluded that bacterial microleakage complicated the interpretation of pulpal responses beneath cavities filled with a variety of experimental materials.

The microspace at the material cavity/wall interface arises shortly after the insertion of the material, due to volume changes in the material during the setting process, and because the materials do not adhere to the cavity wall (Browne and Tobias 1986). Qvist (1980) felt the marginal deterioration of the margin during exposure to the oral environment would allow entry to bacteria beneath the restoration. Qvist (1983) found greater bacterial microleakage around filled cavities in functional occlusion compared to unopposed teeth.

Bacteria were filtered from the base of class five cavities in rhesus monkeys and quantified (Bergenholtz et al 1982). Mejare et al (1979) found the bacteria under restorations was similar to that of aging dental plaque. Bergenholtz et al (1982) found bacteria under composite, amalgam, silicate, and gutta percha restorations over an eight week period, but no bacteria under zinc oxide eugenol cement, and the histology of the dental pulp correlated to these microbiological findings. Present evidence suggests therefore that none of the current dental materials provide a perfect seal with the cavity wall except for glass ionomer cements which adhere to the cavity walls to some degree (Patterson and Watts 1981). The fluid microleakage that results is accompanied by bacterial ingrowth unless the filling material has persistent antibacterial properties, like

zinc oxide eugenol cement (Browne and Tobias 1986). The evidence presented suggests the pulpal changes seen under restorations may be largely due to the presence of bacteria penetrating and growing between the cavity wall and floor.

Following the preparation of dentine by rotary instruments, and most other cutting methods, dentine is covered by a smear layer of microcrystalline debris (Diamond and Carrel 1984). The presence of a smear layer provides a physical barrier to bacterial penetration, and prevents diffusion of a wide range of substances *in vitro* (Pashley et al 1981b, Michelich et al 1980). Pashley (1985) and Brannstrom and Astrom (1972) concluded the outflow of dentinal fluid onto the cut surface of vital dentine under the hydrostatic pressure of the pulp was prevented *in vivo* by the smear layer. The effectiveness of this barrier is questioned as the application of bacterial products to freshly cut dentine cause pulp inflammation (Bergenholtz 1977) and this is supported *in vitro* by Pashley (1985). It is the penetration of bacterial toxins through dentine that is the mechanism by which bacteria induce pulpal inflammation, particularly in the initial stage after restoration placement. Bacteria may be found in the dentinal tubules, but more frequently in cavities which have had the smear layer removed (Vojinovic et al 1973). Following the removal of the smear layer greater pulpal inflammation has been seen beneath cavities compared to the same procedure without smear layer removal (Vojinovic et al 1973, Stanley et al 1975, Eriksen 1974).

Macko et al (1978) looked at the effect of phosphoric acid on the dentine and the pulpal response. Human premolars had class V cavities prepared, then 50% phosphoric acid was applied for one minute, the cavity was washed, and restored with zinc oxide eugenol cement. The experimental periods were 30 minutes and 150 days. With a remaining dentine thickness of 1.8 to 3.5 mm, the acid treated teeth demonstrated a moderate pulpal response at both time periods, while no pathologic changes were seen in the control teeth. After 30 minutes the experimental group had displacement of odontolast nuclei into the dentine tubules, while after 150 days healing was seen by the laying down of irritation dentine. In no cases experimental or control were bacteria seen on the cavity floor or in dentinal tubules. Goto and Jordan (1973) found no

harmful effect of 50% phosphoric acid placed in deep class V cavities in dog teeth. Brannstrom and Nyborg (1977) found acid from zinc phosphate cement did not cause any appreciable inflammation of the dental pulp irrespective of the thickness of the dentine on the cavity floor.

The smear layer is considered to be of some benefit to the pulp by reducing the permeability of the dentine following cavity preparation (Cotton 1984). The smear layer interferes with the adhesion of many restorative and lining materials (Diamond and Carrel 1984), and is considered to harbor bacteria and their products (Brannstrom 1981). In particular, composite restorations require a clean dentine surface to aid their mechanical bonding to the tooth surface. This conflict between the needs of the restorative material, and the health of the pulp means there are many differing opinions as to whether the smear layer should be removed or not. A technique proposed by Brannstrom and Johnson (1974) used a microbicidal fluoride solution with the ability to remove the smear layer from the peritubular dentine while still leaving the dentinal tubules plugged with dentinal debris, preventing bacterial penetration. Warfvinge and Bergenholtz (1986) have demonstrated that the pulpal tissue of humans and monkeys has the ability to repair after 10 and 30 days were a finite challenge was made by sealing bacterial material under teflon discs and zinc oxide eugenol cement. This may be explained by two mechanisms; either the bacterial agents in the cavity were unable to sustain an inflammatory response in the dental pulp due to the removal of the irritant material by the initial migration of leukocytes, or there was a decrease in the permeability as described by Pashley et al (1983a, 1983b, 1984a) due to the deposition of plasma protein complexes within the tubules, preventing the bacterial toxins from permeating to the dental pulp.

2.3.4.b. Root Caries.

These lesions are frequently seen as areas of softened cementum and dentine at or near the cervical regions of the teeth with exposed cementum. The lesions are not sharply localized, and may involve much of the surface of the tooth. Bacteria were seen to extend apically from the cervical lesion along the exposed cementum, and an early

penetration of filamentous bacteria into the dentine was seen generally following the dentinal tubules (Jordan and Hammond 1971). They concluded the dense mat of filament forming bacteria attached to the cemental surfaces appears to be intimately associated with the destructive attack on the cementum, with penetration of the cementum and invasion of dentine as the infection progresses. They identified the organisms as *Rothia dentocariosa, Actinomyces viscoses, Actinomyces naeslundi, Actinomyces odontolyticus* and *Actinomyces eriksonii* in association with these lesions. In their review of root caries Hazen et al (1973), agreed filamentous forms were associated with root caries, and contrasted this with coccal forms and enamel caries. A review by Jordan and Sumney (1973) indicate Actinomyces to be associated with cervical caries in experimental animals as well as gram positive rod organism suggested by Dick and Shaw (1966). These confirm the morphological evidence of the association of filamentous organisms with root caries.

Westbrook et al (1974) found most root caries lesions on proximal surfaces, and 70% of these had dentine tubule destruction histologically. They confirmed the occurrence of a densely mineralised surface layer and hypermineralised dentinal tubules beneath the lesion. They also noted there was cementum invasion by microorganisms prior to necessary cavitation, but associated with altered optical density. When carious destruction of the cementum was noted there was superficial invasion of the dentinal tubules which became more widespread as the lesion developed. Hypermineralised areas were seen within the dentinal tubules deep to the active lesions which they believed to reflect a protective reaction to the caries.

Animal studies have stimulated the awareness of root surface caries, being seen in animals both with and without enamel caries (Hazen et al 1973). They noted however that the root surface must be exposed to the oral environment prior to root caries developing. Jordan and Sumney (1973), indicated many clinicians feelings by saying that from an operative point of view this type of lesion is very difficult to restore. Problems such as extension beyond axial boundaries of teeth, and extension below the gingival margin are frequently encountered. Restorations in this area are often

sensitive to thermal changes, also the proximity to the pulp may cause inflammatory changes and recurrent decay has frequently been reported (Jordan and Sumney 1973, Hazen et al 1973). These lesions in light of leakage of restorations, with bacterial penetration around the margins of root restorations, and with the floor of the cavity close to the pulp, potentially pose a very serious threat to the health of the pulp.

2.3.5. Periodontal Disease.

It is generally believed that the degree of periodontal breakdown increases with age. There are also accompanying changes in the periodontal tissues with age. The composition of the plaque, and the reaction of the periodontium to the presence of plaque changes also (Van der Velden 1984). Below the level of the junctional epithelium, the thickness of the cementum increases with age over the entire root surface. Zander and Hurzeler (1958) found the cementum thickness tripled between the age of twenty and seventy years, with the increase being was most pronounced in the apical and middle thirds of the roots, and smallest in the coronal third. The permeability of the cementum has been found to decrease with age (Stones 1934). A review of the role of endotoxin, and its penetration of cementum is found below.

The exposed cementum in the periodontal pocket or above the gingival margin could play an important role in affecting the pulp tissue. Studies have indicated an increased mineral content in the surface layer of cementum (Selvig and Zander 1962, Selvig 1969, Furseth and Johanson 1970). Selvig and Zander (1962) reported the adsorption of calcium from saliva up to a depth of 50μm, but in most instances the width of the zone of increased calcification was considerably less. Selvig and Hals (1977) used electron probe and electron microscopy to characterize exposed cementum. The radiopaque zone had an elevated calcium and phosphorus content, with a 7-10 % increase, very high fluoride, and elevated sulphur content compared to unexposed cementum. Electron microscopy showed increased crystal size and improved crystallinity in the hypercalcified layer, and the decalcified sections indicated an alteration of the organic matrix. The added mineral content in the surface layer of non-carious cementum apparently results in the displacement of water, rather than of

organic substances because none of the specimens demonstrated a reduction in the sulphur content. The clinical implication of this increase in mineralisation of the surface of the cementum is an increased resistance to decay. The depth of penetration of the ions in this study was at a maximum of 50*u*m, and it would be unlikely the relatively larger organic molecules such as endotoxin would penetrate cementum to a greater depth (Selvig and Hals 1977).

Garcia (1987) recently reported the presence of bacteria in the cementum below the level of the junctional epithelium, indicating early bacterial invasion of cementum may occur in periodontal disease. Adriaens et al (1988) have reported widespread invasion of cementum and dentine in periodontally diseased teeth.

2.3.5.a. Periodontally Involved Cementum.

As periodontal disease progresses, the cementum covering the root surfaces becomes exposed as the connective tissue fibres degenerate, the pocket epithelium migrates along the surface and subsequently breaks down to expose the periodontally involved cementum. The management of periodontal disease has long called for the root planing of the involved cementum. Daly et al (1979) suggested that there is confusion as to what is to be achieved clinically by root planing, due to a rationale based on clinical impressions.

Hartzell (1911) advocated the removal of necrotic cementum which was thought to be a porous and infected surface layer of cementum. This belief persisted into the 1950's when calculus became the main cause of periodontal disease. Root planing was said to be necessary to ensure elimination of calculus from the surface of the tooth. An impression of the root surfaces being "softened" developed (Riffle 1956), and these softened surfaces were believed to contribute to the chronic periodontal disease. Thus a rationale was formed for the removal of the periodontally involved cementum. In addition to this Chace (1961) explained that an additional benefit was gained by making the surface hard and smooth, to facilitate plaque removal by the patient. The work of experimental gingivitis in man (Loe et al 1965, Thelaide et al 1966) produced the acceptance of bacterial plaque as the major etiologic factor in gingival inflammation.

Green and Ramfiord (1966) suggested root planing to remove plaque deposits to produce a smooth root surface to further inhibit plaque accumulation.

Interest then focused on the pathogenic factors of periodontal inflammation being dependent on toxic products from plaque which may impregnate the cementum (Nabers 1970). The cytotoxic effects of periodontally involved cementum may be due to cementum bound endotoxin from the cell wall of gram-negative bacteria (Hatfield and Baumhammers 1971). *In vitro* work suggested the presence of bacterial endotoxin within periodontally involved cementum, and therefore the removal of this cementum bound endotoxin was required to regain health (Aleo et al 1974, 1975, Jones and O'Leary 1978, Daly et al 1982). Electron microscopic findings suggested that the involved cementum had a hypermineralised surface layer (Furseth 1971). Garrett (1977) recommended the removal of this layer in order to assist new attachment procedures. Throughout this century, it has been deemed necessary to remove some or all of the cementum during the treatment of periodontal disease for various beliefs in order to regain periodontal health. A brief look will now review the changes in cementum in periodontal disease.

Many investigators have suggested periodontally involved cementum is softer than uninvolved cementum (Riffle 1956, Schaffer 1956, Chace 1961, Grant et al 1988, Carranza 1984). This softening was said to be the result of proteolysis of the embedded Sharpey's fibres by bacterial products Carranza (1984). The amount of root planing required to remove this softened component is not clear. Schaffer (1956) found the removal of all cementum resulted in the hard smooth desired surface. However despite all the suggestions of the cementum becoming softened in periodontal disease, no experimental evidence supports this, unless the cementum has also become carious. A hypermineralised surface layer was detected by electron microscopy (Yameda 1968, Furseth 1971). Tests by Rautiola and Craig (1961) and Warren et al (1964) found no significant differences in hardness of periodontally involved and non involved cementum.

Periodontally involved cementum is generally believed to have an irregular surface, facilitating the accumulation and retention of plaque and calculus. Herting (1967) described a rough pebbled surface of cementum using the electron microscope, covered by bacteria and their products. Waerhaug (1978) suggested the persistence of subgingival plaque in areas of cemental surface resorption (Sottasanti and Garrett 1975) permitted continued periodontal destruction. The concept of producing a smooth root surface harks back to the concept of Chace (1961), that this impedes plaque colonization and facilitates plaque removal. This must be questioned as Rosenburg and Ash (1974) found no difference in plaque accumulation on root surfaces on those smooth and root planed, compared to those not planed. Other studies have found no difference in the healing of periodontally involved root surfaces after various treatments, ultrasonics versus hand instruments (Thornton and Garnick 1982, Badersten et al 1984, Loos et al 1987), or whether treated with citric acid to expose collagen fibres (Polson and Proye 1983), or with no treatment of the cementum at all except the use of detergents (Blomof et al 1987).

The inorganic component of involved cementum has been widely examined with the belief that the mineral content of exposed cementum is increased (Selvig and Zander 1962, Selvig and Hals 1977). This was explained by Selvig and Zander (1962) as penetration of mineral ions from the crevicular fluid or the saliva, the latter being unlikely as saliva does not enter pockets (Baumhammers and Stallard 1966) unless the root is exposed due to recession. This is supported also by the work of Pitcher et al (1980) in the penetration of dyes into periodontal pockets. This hypermineralisation is confined to the surface layer of cementum and is 10-20 μm in width (Selvig and Zander 1962, Yameda 1968, Furseth 1971, Selvig 1969). This hypermineralisation has not been seen in unaffected cementum.

Hatfield and Baumhammers (1971) demonstrated cementum to be toxic to cell cultures in antibiotic medium. Aleo et al (1974, 1975) autoclaved cementum from periodontally diseased roots and found it to be toxic to fibroblasts, suggesting a cytotoxic substance within cementum and implicated endotoxin. Phenol water was used

to remove endotoxin from the cementum and positively identified by a *Limulus lysate* assay. The Limulus assay is known to be a sensitive test for endotoxin, and was thought not to give false positives with other bacterial toxins (Rojas-Corona et al 1969). It is also known to react positively to substances such as ribonucleic acid, proteins, and gram positive cell wall fractions which are also found in plaque (Elin and Wolf 1973, Wildfeur et al 1974), and the debate is whether the extraction procedure allowed these to be collected also. These studies should then conclude that the periodontally involved cementum contains Limulus positive substances rather than endotoxin only. Jones and O'Leary (1978) found root planing significantly lowered levels of Limulus positive substances from cementum.

Daly et al (1982) assessed histologically substances in periodontally involved cementum. Using stains for polysaccharide he found the stain penetrated approximately 3-7 microns into the cemental surface. Lipid granules were characterized by oil-red stain and seen ten microns beneath the cemental surface. However the staining for microbial deposits with hematoxylin and eosin, and gram staining, demonstrated these were present at the CDJ, and in cemental defects as well as penetrating up to twelve microns into cementum. Nakib et al (1982) soaked teeth in endotoxin for up to 12 weeks, and found endotoxin binds to the surface of the root cementum of periodontally healthy or diseased teeth, but did not penetrate the cementum and that this binding to the surface was relatively weak. In no circumstances could the penetration of endotoxin be seen into dentine. These authors suggested the role of endotoxin in cementum may have been over exaggerated.

Adelson et al (1980) found that autoclaving could eliminate the toxic effects of periodontally diseased roots on fibroblasts. Morris (1975) thought a thermolabile inhibitory factor was present but undetermined, suggesting inflamogens other than endotoxin were present. Eide et al (1983 ,1984) found endotoxins within a surface coating originating from inflammatory exudate on periodontally involved roots using electron microscopy. Wirthin and Hancock (1981, 1982) treated diseased roots surfaces

of monkeys by soaking with sodium deoxycholate and the plasma fraction Cohn IV after scaling with no attempt to remove cementum which allowed the lesion to heal.

Using a immunohistochemical technique Hughes and Smales (1986) looked at ground sections of 14 human teeth extracted because of periodontal reasons. Using an ELISA technique, LPS was detected on the cementum of periodontally involved root surfaces extending to within 1mm of the previous level of the connective tissue attachment. They found LPS only on the surface of cementum, and on no occassion was LPS seen penetrating into sub-surface cementum.

A further study using EM immunohistochemistry to locate endotoxin found most of the LPS was associated with bacteria, and calculus which persisted on the root surface following scaling (Hughes et al 1988). Smaller amounts of endotoxin were detected on the cuticle and surface cementum. They did not detect endotoxin in the sub-surface of cementum. They postulated the LPS seen was due to LPS associated with bacteria and calculus left in resorption lacunae in cementum following scaling.

Nyman et al (1986) simply polished the root surfaces only with rubber cups and points, with polishing paste in beagle dogs with similar results in healing to root planing. Blomof et al (1987) were able to get healing in dogs by treating the periodontally involved surfaces of teeth, with detergents and citric acid, which produced a better result than the scaled surfaces.

A study was conducted of the viability and distribution of bacteria within root dentine and pulp of periodontally diseased, caries-free teeth. (Adriaens et al 1988a). They detected bacterial growth in 83% of the dentine from periodontally diseased teeth, and 59% had bacteria present in their cultured pulp. This suggests the roots of periodontally diseased teeth have bacterial penetration, and they could act as bacterial reservoirs for recolonization of the root surface. The high levels of bacteria in the outer dentinal layers were explained by local invasion of subgingival bacteria. Further investigation by light and scanning electron microscopy of bacterial invasion were reported (Adriaens et al 1988b), where bacterial invasion of cementum was routinely observed, and some teeth had bacteria located in dentinal tubules. No bacteria were

seen in the portion of the root located apically to the epithelial attachment. At the base of the gingival pocket, bacteria were found in spaces between remnants of Sharpey's fibres and their point of insertion into cementum. From these studies the authors indicated the positive result to *Limulus* amoebocyte lysate assay test may have been the result of activation by components of whole gram positive and negative bacteria present in the root cementum, and because the penetration seen these will not be removed by mechanical means alone.

2.3.5.b. Endodontic-Periodontic Relationships.

While cementum is considered to be a barrier to the effect of dental plaque (Stones 1934), it may not always be present on the root surface. The dental pulp has been shown to be vulnerable to the effects of microbial, chemical, or mechanical irritants on attrited surfaces (Tronstad and Langeland 1971, Haugen and Mjor 1975). These reports would suggest the thickness of the dentine is insufficient to protect the pulp. Therefore in cases where the root is not covered by cementum as may be the case in periodontal disease, and following periodontal therapy, microbial products may have access to the pulp (Langeland et al 1974, Bergenholtz and Lindhe 1978, Harvey and Zander 1959, Muller and Zander 1960).

A number of histological studies have shown periodontal disease to affect the pulp with a wide range of changes being sited. Lang and McConnell (1920) reported calcification of areas within the pulp. Cahn (1927) found all teeth with "pyorrhea" had abnormal pulpal tissue. Rubach and Mitchell (1965) looked at the incidence of accessory and lateral canals in periodontally involved teeth and found significant number of inflamed and necrotic pulps. Seltzer et al (1963) found evidence of retrograde pulpitis, and degenerative changes. Smukler and Tagger (1976) in a study of vital root resection in periodontal disease found the amputated root to have degenerative changes of calcification and fibrosis. Mandi (1972) and Kipoti et al (1984) suggested there was contamination of pulps by bacteria from the surrounding chronic periodontal disease. Cultural studies of the bacterial flora in necrotic pulp tissue

suggested that the bacteria present resemble those isolated from subgingival plaque samples (Adriaen et al 1988a).

Bender and Seltzer (1972), Seltzer and Bender (1984), Langeland et al (1974) have demonstrated the presence of inflammatory cells or necrosis of the pulp adjacent to accessory canals and apical foramina which have connections to periodontal pockets. Langeland et al (1974) in their study of sixty teeth with various levels of periodontal disease found pathologic changes in the pulpal tissue, but the pulp did not succumb as long as the main canal was intact. Dentinal tubules are permeable to bacterial products, and these bacterial products have been shown to invoke an inflammatory response within the pulp (Bergenholtz and Lindhe 1975, Mjor and Tronstad 1972, Brannstrom and Nyborg 1971).

In a study of sixteen teeth, the microflora from root canals and periodontal pockets of teeth with advanced periodontal disease were examined to determine the predominant cultivable microflora from the root canals, and compared this to the flora with that from the adjacent periodontal pocket (Kipoti et al 1984). All teeth in the survey had pocket depths greater than or equal to 7 mm, intact clinical crowns, and lack of periapical lesions. Six of the sixteen teeth were non vital, and cultures of microorganisms were made from the root canals and pockets around these teeth. This study demonstrated the microorganisms present in the root canal of caries-free teeth with advanced periodontitis generally resembled those found in the adjacent periodontal pockets. The authors thought the likely source of the non-carious root canal infections was the adjacent periodontal pocket.

Torabinejad and Kiger (1985) presented a case report from a patient having full mouth clearance with varying degrees of attachment loss due to periodontal disease. The teeth were evaluated clinically and all were said to respond to vitality testing within normal limits. Histologically no evidence of pulpal pathosis was found. The teeth were scored on the Russell's index (Russell 1956) according to their average amount of attachment loss. Two groups were categorized, the teeth with a score of 6, indicating moderate periodontitis and teeth with a score of 8, indicating severe periodontitis. The

histologic examination of these teeth found them to be within normal limits, however most of them had foci of calcifications inside pulp chambers or root canals.

Simring and Goldberg (1964) reported on 109 cases where the tooth had failed to respond to periodontal therapy, and instituted endodontic therapy on the rationale the still active lesions were due retrograde periodontitis. They reported 12 failures after treatment, where success was indicated by reduction of inflammation and cessation of pus from the gingival margin. The possible mechanism of communication between the periodontal tissues and the pulp were listed by Simring and Goldberg (1964) as lateral canals, dentinal tubules, the periodontal membrane, the alveolar bone, apical foramen, and dentine permeability.

Seltzer et al (1963) histologically examined 85 teeth with periodontal lesions. The teeth were clinically tested and subjective symptoms recorded prior to extraction. They found a large number of teeth had lateral and accessory canals particularly in the furcation regions of molars. The dental pulps of only 6% of the teeth were found to be uninvolved, while atrophic pulps were found in 27% of the teeth. In 49% the pulps were inflamed, and 18% of the teeth had totally necrotic pulps. They concluded periodontal lesions had a degenerative effect on the pulp of involved teeth. Thirty two periodontally teeth were subsequently examined which had no evidence of caries or restorative procedures. In this group 37% had signs of pulp atrophy, 37% had inflammatory changes, while 9% were necrotic. They felt pulps subjected to combined irritation of operative procedures, caries, and periodontal irritants had a greater incidence of inflammatory reactions within the pulp than those subjected to operative procedures alone. They also found the pulp lesions had an effect on the severity of periodontal lesions, via spread along lateral and accessory foramen especially in molars.

Bender and Seltzer (1972) further studied the histological effect of periodontal disease on the pulp by studying 178 human, dog, and monkey teeth. They found lateral and accessory canals in large numbers especially in molar teeth, with granulation tissue emanating from the inflamed pulpal tissue. Inflammation of the periodontal ligament in inflamed and necrotic pulps was a common finding. They also found evidence of

periodontal disease influencing the state of the pulp tissue, producing degeneration and inflammation of the pulp. An increased incidence of pulp degeneration was seen in teeth subjected to a combination of irritants, caries, restorations, or both, and periodontal disease. The authors proposed an new syndrome from their work, called the pulpodontic-periodontic syndrome.

The endo-perio problem still poses a perplexing problem as to which lesion came first. Pulpal disease could initiate or maintain periodontal disease, however opposite to this long-standing periodontal disease may allow bacteria or their products to invade the pulp. Hiatt (1977) thought that periodontal disease may progress to the root apex and invade the dental pulp via the apical foramen, or bacteria or their products could invade the pulp through hypocalcified cementum, empty Sharpey fibre spaces, and accessory canals.

There is still some controversy with these concepts, and there is also ample literature to support the opposite view. The reason for this disagreement regarding the effect of periodontal disease on the pulp is the difficulty in getting access to proper control material, in which the pathologic pulp tissue alterations unrelated to periodontal destruction can be studied. Mazur and Massler (1964) found no correlation between the amount of exposed root due to periodontal disease and changes in the pulp. Czarnicki and Schilder (1979) examined histologically the pulps of teeth with severe periodontal disease and found no correlation with the dental pulp status. In a study of the periodontal syndrome in rice rats, no pulpal pathology was seen (Hattler et al 1977). Chacker (1974) could not find any role for the severity of periodontal disease, or the extent of the plaque accumulation and the status of the dental pulp. Chacker (1974) thought there were two mechanisms for bacteria to gain entry to the pulp, either by exposed lateral canals allowing direct ingress of the bacteria into the pulp, or the exposure of the dentinal tubules due to instrumentation, or direct microbial action on the cementum.

Vertucci and Williams (1974) after finding 46% of first molars had furcation canals, concluded that an isolated periodontal lesion in the furcation area of a posterior

tooth without periodontal destruction elsewhere in the mouth is most likely to be of pulpal origin. Bergenholtz and Lindhe (1978) induced periodontal lesions in monkeys finding that the destructive periodontitis was not always associated with pulpal alterations. They did notice however that the areas of the pulp subjacent to the root surfaces exposed to the oral environment due to periodontal destruction did have "mild" changes indicated by localized secondary dentine formation and/or inflammatory cell infiltrates.

Sinai and Soltanoff (1973) induced periodontal disease in 75 rats by opening the contact between the first and second maxillary molars by cutting the distal surface of the first molar, so the pulpal response in the second molar to periodontal disease could be evaluated. Only 44 of the animals were able to be used for the histological assessment. Nine teeth showed definite pulpal changes, characterized by internal resorption and deposition of irregular dentine with cellular inclusions and partial pulp necrosis. Eleven teeth were said to have possible pulpal changes characterized by interstitial hemorrhage.

In a second part of the study Sinai and Soltanoff (1973) looked at the effect of pulpal involvement on the periodontal structures by exposing the pulp of the maxillary first molar with a round high speed bur. Of sixteen specimens obtained for evaluation eleven had changes in the periodontal structures, ten showed inflammatory change and one exhibited ankylosis in the furcation area. They concluded that the pulp affected the periodontal structures more frequently than the periodontium affected the pulp. The changes in the periodontal ligament occurred as early as one and a half weeks after pulpal exposure, changes in the pulp due to periodontal lesions occurred not only less frequently, but also later. The change in the periodontal structures was invariably inflammatory in nature, while the changes in the pulps due to periodontal lesions were more resorptive, or proliferative in nature. The authors felt the transmission of pathologic change from the pulp to the periodontal structures could be due to the presence of accessory canals or due to the permeability of the chamber floor or root surface.

Winter and Kramer (1972) induced pulpal lesions in the deciduous teeth of monkeys, and observed the direction of spread of the resulting inflammatory lesions. They observed a preferential direction of spread toward the furcation, but these observations were not quantified. Walton and Garnick (1986) induced periapical lesions in the permanent molars of monkeys by exposing the pulp chamber to the oral cavity for one week and then sealing the cavity with amalgam. The experimental period was seven months with all teeth showing evidence of periapical lesions. In the 34 experimental teeth, the primary direction of spread was toward the furcation, in 53% of the periapical lesions. The next most frequent was either toward the mesial or distal, 32% of lesions, and the other directions, buccal, lingual, or apical were 15 %. They felt the pattern of spread of the lesions followed the path of least resistance and the marrow spaces being less resistant than the periodontal ligament, thus the frequent appearance of lesions in the furcation.

Hirsch et al (1987) proposed the condition of localized juvenile periodontitis may have been misdiagnosed previously as the pulp status of the involved teeth appears to have been overlooked. Their hypothesis was that the lesions seen in this disease could clinically, and radiographically be interpretated as those of primary endodontic lesions which have subsequently drained via the periodontal membrane. The localized nature of the disease could be explained by this hypothesis, in a otherwise healthy mouth, by the first molars being the most caries prone teeth in the mouth and the central incisors by bruxism during the mixed dentition stage or trauma during childhood accidents. Further support for this hypothesis was found in an extensive anthropological investigation of skulls to study periodontal disease and tooth loss (Clarke et al 1986). The data from the skulls was overwhelming in that little severe periodontal disease was seen, rather the common finding was deep infrabony lesions penetrating to the apex of the teeth, which was the cause of tooth loss.

A study of severe localised lesions in 153 teeth from 90 subjects was conducted with full periodontal and endodontic assessment of the teeth (Hirsch et al 1989). They found 55% of the teeth had no recoverable tissue within their root canal systems. One

or more necrotic canals were seen in 12.4% teeth, and 22.2% had only a small piece of tissue recoverable from the apex. This indicated 138 teeth of 153 in the study had some endodontic disease. Seventy-seven teeth responded normally to pulp testing, but 52% of these had no recoverable tissue from the pulp. They found severe alveolar lesions were a more accurate predictor of pulp pathosis than endodontic testing.

Relatively little has been written on the effect of root planing on the dental pulp. Scaling and root planing are standard treatment procedures for periodontal disease. Bergenholtz and Lindhe (1978) induced periodontitis in adult monkeys by placement of ligatures around the necks of the 92 permanent teeth over a period of 5-7 months. A number of these teeth were subsequently treated by scaling, followed by plaque accumulation for 2, 10, and 30 days on the freshly planed surfaces. The study demonstrated that scaling and root planing had been effective in removing most of the cementum from the treated teeth, and plaque had accumulated along the exposed dentine surface. Bergenholtz and Lindhe (1978) found 31% of the teeth demonstrated secondary dentine formation, and 32% had inflammatory cell infiltrates, but this was the same frequency as the roots with periodontitis alone. Bergenholtz and Lindhe (1978) made the comment that the pulp tissue alterations may not be only dependent on the degree of periodontal disease, but also on the duration of the disease process. The lack of pulpal changes under the freshly exposed dentine did not confer with the authors previous work of bacterial products in class V cavities (Bergenholtz and Lindhe 1975). However the thickness of the dentine wall after root planing was much thicker than the cavity experiments. The significant presence of dentine sclerosis in tooth roots could also significantly reduce the permeability of dentine to bacterial products (Vasiliadis et al 1983b).

Nilveus and Selvig (1983) root planed the mandibular and maxillary incisors of beagle dogs after surgical exposure. After 15 weeks reparative dentine had formed, but the procedure did not cause inflammatory changes in the dental pulp. Changes were seen irrespective of whether the root planed surface was exposed to the oral cavity, covered by junctional epithelium, or covered by new cementum and connective tissue

reattachment. The deeper the root planing the greater the thickness of the reparative dentine layer.

Hattler and Listgarten (1984) root planed the mesial surface of first molars of Sprague-Dawley rats, and the animals were then sacrificed at time intervals up to one year after the root planing procedure. Their results were 32 of 35 animals demonstrating microscopically detectable secondary dentine formation, but no other histological changes were detected. The thickness of the reparative dentine increased particularly in the first three months.

Prichard (1983) reported that periodontal treatment in the form of surgery, scaling and root planing may lead to pulpitis due to the opening of the dentinal tubules following the removal of the cementum. A textbook on endodontics (Simon 1984) states that periodontal procedures may lead to endodontic involvement. The potentially harmful effect of root planing by exposure of dentinal tubules to the oral environment was studied in premolar teeth (Wong et al 1989). They analyzed teeth both by histology and SEM to see the short term endodontic effects. Three of the teeth were found to have chronic pulpitis. Scanning electron microscopy demonstrated bacterial penetration in the outer 300μm of the dentine. Teeth with dentine sclerosis did not have bacterial penetration.

In a study on healthy beagle dogs, mucoperiosteal flaps were raised and the mandibular and maxillary teeth subjected to root planing, one side was subsequently exposed to citric acid, pH 1, for three minutes prior to replacement of the flaps (Nilveus and Selvig 1983). Sections were obtained at one and 15 weeks. The one week specimens had a reduced width to the predentine on the instrumented surface but no cellular changes within the pulp. After 15 weeks varying amounts of reparative dentine had formed in all specimens and although the acid treated tended to have more reparative dentine this was not significant. In a similar study on premolar teeth in beagle dogs with specimens collected at 1, 7, 14, 28, 56 days, no histologic evidence of pulpal inflammatory change was seen in any specimen (Yeung and Clarke 1983). In both of these studies the dogs had healthy periodontal structures.

Conversely, citric acid applied to the canine teeth of cats and observed over 4, 21, 83 days resulted in 28% of the teeth becoming abscessed or totally necrotic at 21 and 83 day observation periods (Ryan et al 1984). The effect of the citric acid leads to a significantly greater pulpal inflammation than the surgery alone. Four teeth exposed to citric acid which were relatively uninflamed had reparative dentine formation. The authors were able to demonstrate bacteria in the dentinal tubules of the acid treated teeth only. Ryan et al (1984) felt the necrosis seen in this study was most likely due to the conditioning of the dentine to allow for the penetration of bacterial products to the feline pulp, as indicated by Vojinovic et al (1973) in the work on acid etching of cavities. This was further supported by the response after 4 days being similar to the surgery alone (Ryan et al 1984). The different response seen in dogs and cats may be due to permeability differences between the two animals, as dogs are known to have narrower dentinal tubules than man, while cat tubule diameters are approximately equal to man.

2.3.6. Accessory Canals.

It is through tooth structure that irritants have to penetrate to pass from internal to external environments, or vice versa, to affect the opposing tissue. In the light of the evidence of sclerosis of dentinal tubules, and the presence of cementum, the importance of accessory or lateral canals must be considered. The literature supports the presence of substantial anatomical communications between the pulp and periodontium. The clinical significance of these accessory canals is perplexing as in endodontic therapy they are rarely filled, but the root canal therapy may still be successful. Several authors have reported an interrelationship of the periodontium and the dental pulp which indicated inflammatory products and, or toxic products can pass between these two tissues, via accessory canals (Rubach and Mitchell 1965, Bender and Seltzer 1972, Simring and Goldberg 1964, Ross 1972, Gutmann 1978). The question still not clearly answered is what are the consequences of these communications for the transmission of physiological, pathological, and therapeutic conditions between the pulpal and periodontal tissues.

From the beginning of the twentieth century attempts have been made to demonstrate the root canal system including its apices. Russell and Kramer (1956), Kramer (1960), and Saunders (1966) utilized perfusion techniques to demonstrate the intricate vascular architecture of the dental pulps. Their results showed in the bifurcation and trifurcation area, large vessels running through the radicular dentine to supplement supply to one canal. The authors reported in some cases these furcation vessels appeared to be of greater importance than the one at the apical foramen. Frequently one large and one small vessel as a pair were seen interconnecting the pulp and periodontium in other areas of the root. This evidence supports the presence of interradicular canals which would allow communication between the pulpal and periodontal tissues.

Extracted pulpally involved teeth have been examined histologically and demonstrated a high incidence of accessory canals especially in the furcation regions of molars (Barrett 1925, Rubach and Mitchell 1965, Seltzer et al 1977, Seltzer et al 1967, Winter and Kramer 1965). Associated with these communications interradicular pathology was noted in some of these teeth. Dye studies on deciduous molars (Moss et al 1965, Winter 1962) allowed estimation of the frequency of patent accessory canal in the pulpal floor to be 20-23 %.

Scanning electron microscopic studies were conducted by Koenigs et al (1974). These studies confirmed the presence of accessory canals in large numbers of the furcation areas of permanent molars. Koenigs et al (1974) found the size ranged from 4 to 250 microns in diameter, and indicated there were more accessory canals, and these were larger in size, in the maxillary molars compared to the mandibular molars. Burch and Hulen (1974) in a microscopic study of 195 maxillary and mandibular molars revealed that as many as 76% of the molars studied exhibited multiple foramina in the furcation region. The maximum number of openings was ten in one maxillary molar, but on average maxillary molars had 2.51 foramina per furcation, while mandibular molars had 2.14 foramina. These topographical studies do not indicate patency of canals as they were not seen to communicate with the other surface. Some of these

canals visualized in the root surface penetrate cementum only or they may curve to emerge back on the root surface. Burch and Hulen (1974) felt that even if canals do not reach the pulp they may still create problems in the treatment of periodontal furcation lesions, as once the furcation becomes accessible to bacterial plaque it would collect within these foramina and act as a nidus for an inflammatory response.

Rubach and Mitchell (1965) in a histologic study of periodontally involved teeth found accessory canals in 33 of 74 teeth, or 45% of the specimens. The majority of these canals were ramifications in the apical portion of the tooth. Eight of these canals were located more coronally, and five were shown to be in communication with a periodontal pocket. This provided evidence of direct communication between the pulp and the periodontal pocket. Kirkham (1975) studied 100 permanent human teeth that were indicated for extraction because of periodontal disease, and radiographic, and clinical records were taken. The teeth were then subjected to an injection dye procedure to study the presence of accessory canals, particularly those within the periodontal pocket. Twenty three teeth had one or more accessory canals. Kirkham found 8.7% of teeth had accessory canals located within periodontal defects. Rubach and Mitchell (1965) also said that this histological evidence was only from two of the four surfaces of the tooth due to the mechanism of tooth preparation for microscopy.

De Deus (1975) found the most frequent ramification to be the apical delta. He felt lateral canals extend from the main canal to the periodontal ligament more frequently in the body or middle part of the root compared to other regions. The study was extensive and 1,140 teeth were investigated with 27% demonstrated lateral or accessory canals. The molars and premolars showed the greatest variety, and the incisors the least accessory canals. The accessory canals were most frequently found in the apical third of the root, 17%, 8.8% in the middle third, and 1.6% in the coronal third. This study found only 2.3 % of furcation teeth demonstrated lateral canals, and no canals were found coming from the pulpal chamber.

Vertucci and Williams (1974) looked at 100 mandibular first molars using a radio-opaque dye, and found 46% of the teeth exhibited patent lateral canals in the furcation

region in three distinct patterns. A single lateral canal extended from the floor of the chamber to the interradicular region of the tooth in 13% of the specimens. In 23% of the specimens a lateral canal extended from the coronal third of a major root to the furcation region, most commonly from the distal root. Ten percent of the teeth exhibited both lateral and furcation canals, and in half of these specimens the lateral canal merged with the furcation canal before reaching the furcation. They felt it was possible inflammatory products can cause damage in the furcation region prior to reaching the periapical tissues.

Lowman and Burke (1973) used a vacuum technique to draw a radiopaque dye through 46 maxillary and mandibular molars, and found 59% of these teeth had patent accessory canals in the coronal and middle thirds of the root structure. Lowman and Burke (1973) had root planed the surfaces of these teeth prior to injecting the dye to simulate the clinical situation of periodontal therapy. In their preliminary work on teeth which had not been root planed the incidence of patent accessory canals was not as high. They concluded that some occluded canals are made patent by root planing, and established a direct communication between the pulp, the periodontium and the oral cavity. Alternatively the root planing may have exposed patent dentine tubules to carry the dye to the external surface.

Gutmann (1978) used 102 extracted permanent molar teeth with intact crowns and roots for a dye penetration study under vacuum, after sealing the apices with wax. The data indicated 29 teeth exhibited 43 patent accessory canals in the furcation region, with 29.4% of mandibular molars and 27.4% of maxillary molars exhibited patent accessory canals. This included an area of up to 4mm. down the internal surface of the root of the teeth. The time taken for penetration of the dye under vacuum of 525mm.Hg. was 30 to 120 seconds. The teeth were held under vacuum for up to 15 minutes and dye penetration was seen through the dentinal tubules, and in areas where the cementum was missing the dye penetrated to the surface. This study did not histologically examine the root surfaces and therefore the presence or absence of cementum in the permeated areas was not ascertained positively.

Perlich et al (1981) studied 62 human maxillary and mandibular molars using scanning electron microscopy. The teeth were sectioned 1.5mm. apical to the external furcation area and 5mm coronal to the cementoenamel junction to allow visual access to both the internal and external surfaces in the furcation region. Accessory canals were found on the pulpal floor in two maxillary and three mandibular teeth of 62 specimens (8%), of which four of these teeth had multiple foramina, which accounted for the total of 17. The authors also saw a few further openings on the pulpal floor between the mesiobuccal and mesiolingual canals of the mesial root. The external furcation surface showed 64% of specimens had accessory foramen, 22 mandibular and 18 maxillary. The teeth collected did not have any acute pulpal problems, had few restorations, and were not carious. Presumably they were extracted for generally prosthetic reasons, and hence from an older age group, where calcification of accessory foramen and the pulpal floor may have reduced the number of openings seen on the pulpal floor.

Hess et al (1983) looked at 27 permanent human teeth, two as controls and the others with various pulpal pathology or endodontic treatments, and examined the foramina with scanning electron microscope. The apical foramina of the root canals had a diameter of 200 to 250 microns, while the accessory foramina are usually smaller, 60 to 90 microns. The diameter of these canals are known to be important in determining the rate of permeation of compounds (Abbott et al 1988). They frequently observed varying degrees of closure by mineralising membranous structures which reduced the diameter of the foramen, and could lead to closure. This response was seen in inflamed pulps as well as endodontically treated pulps. Often one canal would show significant closure of the foramen, while another close by had no change. In cases of complete pulp degeneration and apical disease, granulomatous inflammation was seen around the foramina, which could involve all the foramina in that tooth, or granulomatous changes may be localized to only one of the foramina in the apical delta. Hess et al (1983) noted the reaction in the periodontal tissues was of a granulomatous nature causing root resorption, while chronic inflammation of the pulp was associated

with the laying down of calcified material, partially closing or totally closing the foramina.

Sinai and Soltanoff (1973) recorded similar differences in the reactions of pulpal and periodontal disease in their study of transmission of pathologic changes through between the pulp and periodontal tissues. Harrington (1979) said although there is little doubt that inflammation can, and does occur from the pulp to the periodontium, and from the periodontal pocket to the pulp, the exact incidence is unknown, and it is virtually impossible to locate lateral and accessory canals clinically. Weine (1984) felt these small accessory canals to be of little significance, because of their high incidence and the impossibility of cleaning and filling these canals, if they were of key significance in endodontic therapy then many treated cases would fail. The possibility exists that a similar inflammatory reaction may occur in the periodontal ligament adjacent to a lateral canal as occurs at the apex of a tooth with pulpal disease (Simon 1984).

There may be accessory canals at any point along the root or neck of a tooth which provides a connection between the pulp and periodontal tissues. These aberrant openings are said to be caused by a localized failure in the formation of Hertwig's Sheath (Scott and Symons 1982, Trowbridge 1984). This results in a lack of odontoblast formation so that the pulp remains in contact with the periodontal tissues. A gap in the Hertwig's Sheath may have been produced by a persistent blood vessel reaching the pulp, resulting in the formation of an accessory canal (Scott and Symons 1982).

CHAPTER 3. MATERIALS AND METHODS.

3.1. Introduction.

There has been some concern over the conventional theory of periodontal disease as more evidence comes to light which indicates the pathogenesis of the disease as presently known may be incorrect. A hypothesis proposed indicates severe angular bone loss around isolated teeth within the dentition is the result of pulpal disease (Clarke et al 1986). The belief is that either a necrotic pulp, or one which has pathologic changes due to inflammation, produces changes within the periodontal ligament. Inherent in this concept is that the tooth structure is permeable to various inflammatory mediators and irritants to produce the resultant pathologic change in the periodontal tissues. The problem presented is whether the tooth structure is permeable to these compounds, as they would have to penetrate dentine and cementum to affect the periodontal tissues.

It was decided to find data on the permeability of the tooth structure by undertaking a study of two parts. There is evidence of pulpal disease and periodontal disease being associated in both histologic studies of teeth affected by periodontal disease (Seltzer et al 1963, Bender and Seltzer 1972, Seltzer and Bender 1984), and anthropological data (Clarke et al 1986). The first part of the study was to identify the tooth root permeability of various root areas by the use of radiolabelled water. Then experiments were carried out to identify the size of the radiolabelled compounds which could penetrate dentine in an effort to find a size restriction. The final part of the study was to identify the areas through which the permeation occurred, and this was aided by the use of the electron microprobe analysis of root surface areas to the cobaltous ion.

3.2. Model Development.

The proposed studies required a model for testing the rate and areas of permeability of tooth roots. The initial experimentation was performed using a 1% solution of Trypan Blue dye in a group of 10 molar teeth to visualize the area of

penetration. The third molars were collected from the Adelaide Dental Hospital Oral Surgery Department and stored in PBS. A standard endodontic access cavity was prepared in the tooth with a tungsten carbide Jet 330 bur and the pulp tissue removed with a barbed broach.

The teeth were sealed into 20ml plastic vials by cutting the lid to fit the crown of the tooth, which was then sealed in place by using sticky wax and nail varnish. The apices of all teeth were air dried for 60 seconds, sealed with a coat of sticky wax and nail varnish (Gutmann 1978).

The dye used was a freshly prepared 1% gravitated solution of Trypan Blue. The chambers of the teeth were filled with the dye solution which was spun down the canal using a spiral root filler. A cotton pellet was placed in the chamber and the occlusal cavity was sealed with red modeling wax and sticky wax.

Ten milliliters of PBS was placed in the 20 ml vials, after secure closure the vials were inverted, and kept at room temperature for the duration of the experiment. During the recording of the Trypan Blue dye permeation of the tooth the vials were uprighted to remove the cap containing the teeth from the vial to allow visual assessment.

Sampling procedure was carried out at the following times, 0, 1, 2, 4, 8 hours, 1, 2, 4, and 8 days when the experiment was stopped. The teeth were visually assessed for dye penetration, supplemented by photographic record to allow later comparison of the teeth. A Nikon camera with a Nikon medical lens was used with 64 ASA Ektachrome film (Kodak) to record the tooth surface at all observation times, using a standard 1x magnification (Fig. 3.1.), and supplementing this with 3x magnification of areas (Fig.3.2.) which seemed to demonstrate interesting patterns of penetration by the trypan blue dye.

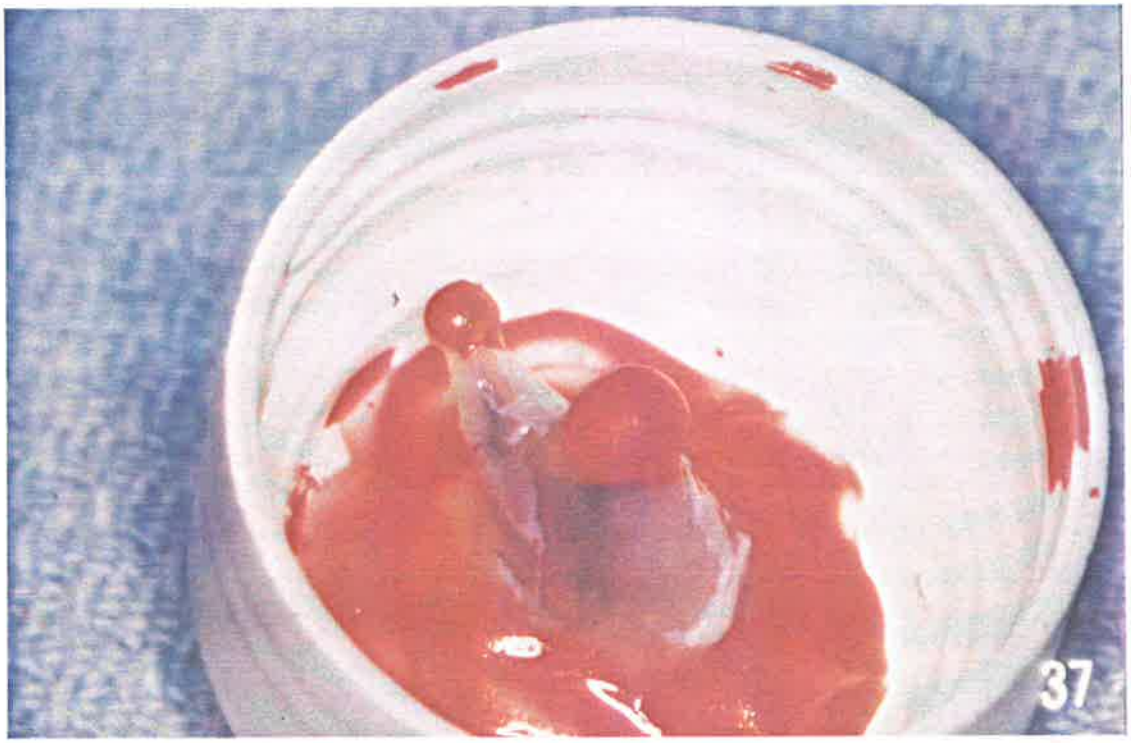

Figure 3.1. Trypan Blue dye Penetration on Tooth Mag. 1x.

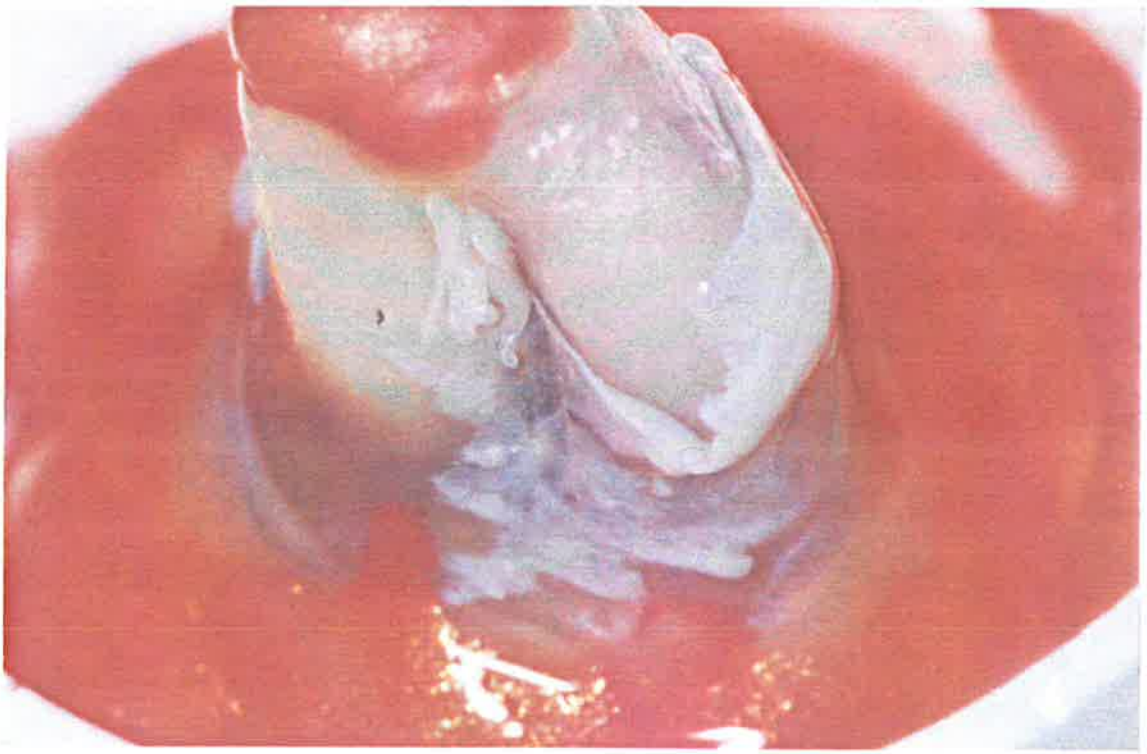

Figure 3.2. Trypan Blue dye Penetration of Tooth Mag. 3x.

After eight days the experiment was stopped by flushing the remaining dye from the pulp chamber using 20 ml of distilled water in a syringe. The teeth were then incubated in a low temperature oven (37^{o} C) for two days, after which the teeth were sectioned using a diamond saw to visualize in longitudinal section the penetration of the teeth (Fig.3.3.).

Figure 3.3. Sectioned Tooth Indicating Dye Penetration.

Further evidence of the penetration of the dye was given by the use of a UV spectrophotometer (Lambda 5 UV/VIS, Perkins-Elmer Corporation, Instrument Division, Norwalk, CT, U.S.A.) which demonstrated a peak of trypan blue in the bathing solution. The model tested indicated the dye did penetrate the tooth structure, and indeed there seemed to be variations in the site of permeability of the teeth. Under similar experimental conditions an assessment could be made of the areas of penetration, and a decision was made to proceed with this model to permeability experiments with the tritiated molecules, and electron microprobe analysis.

3.3. Tritium Labelled Permeability Studies.

These experiments were conducted in two parts, initially by using tritiated water to assess the permeability of differing aspects of tooth roots. Secondly an attempt was made to determine the limitations of the permeability of the root by molecular weight using tritium labelled water, glucose, and dextran.

3.3.1. Area Permeability Studies.

3.3.1.a. Collection of Teeth for the Study.

The initial study used first and second molars collected from the South Australian Dental Hospital, Department of Oral Surgery, by house dentists. The teeth were required to have a definitive furcation region, and that the root to be intact. Coronal caries was acceptable providing the entire crown was not destroyed, as assessed after removal of the carious dentine, but teeth with any sign of caries involving either the cemental surface, or the dentine undermining the cementum were rejected. Restored teeth were accepted only when the restoration did not involve the cemental enamel junction, or more than 50% of the tooth surface. These two conditions meant that the experimental teeth had a variable amount of secondary dentine formation affecting the permeability of the tooth crowns, due to restorations, caries and age. Individual tooth permeability has been noted to vary greatly between teeth in a study of young intact third molars (Hume 1985). No clinical exposure of the cementum in the furcation region was permitted. Any damage to the root during extraction precluded the tooth from the study. Calculus on the tooth root was non-existent or minimal for the tooth to be included in the study, and was not removed. The periodontal condition of the teeth was required to be sound, as exposed cementum becomes hypermineralised.

The teeth were extracted under local anaesthesia using forceps, where the operator was requested to keep the forceps on the crown whenever possible. At the time of extraction the patients age, sex, condition of the tooth, and reason for extraction were recorded.

Difficulty was experienced in gathering enough suitable material with the above criteria for first and second molars. To ensure the required numbers of teeth could be

collected, the decision was made to include third molars due to their availability. The third molars used were required to have the same criteria except they were restoration free, and were non-carious, or had only early caries present. In addition these teeth did not necessarily have a furcation present, as commonly the root anatomy is insufficiently formed to have well defined furcation architecture.

3.3.1.b. Tooth Preparation.

Immediately following extraction the teeth were wiped with a piece of gauze to remove excess blood from the surface of the tooth before being placed into a solution of Phosphate buffered saline, and processed within 24 hours. The periodontal ligament was left intact, as the soft tissue on the outer surface of the root was not a barrier relative to the root structure. Any attempt to remove the connective tissue remnants from the surface would have altered the permeability characteristics of the cementum. The epithelial cuff surrounding the CEJ was removed with a sharp scalpel without disturbing the cementum.

In the first 24 hours after extraction, the dental pulp tissue was removed. Using a high speed handpiece and tungsten carbide Jet 330 bur, with water spray, the enamel and dentine were cut to remove the roof of the chamber. Special care was taken to ensure that the floor of the furcation region was not mechanically traumatised at any time. The pulp tissue was removed by using a fine barbed roach (Nervnadeln CC-cord, Vereinigte Dentalwerke, KG, Munchen, Germany), and the undercut areas of the access cavity removed with a slow speed handpiece and No. 4 round bur. The standard endodontic access cavity was enlarged to allow for an adequate volume of fluid in the chamber and cavity to act as a reservoir for the tritiated water. The chamber was irrigated with PBS to remove any debris, and the teeth were then stored in PBS until used in the experiment. No other preparation of the canal was carried out. The phosphate buffered saline is thought to prevent lysis of cells for approximately 48 hours. This procedure is thought to leave the odontoblast cells intact against the dentine (Hume 1985).

3.3.1.c. Experimental Model.

The model used for this technique was a modification of Hume's model for testing permeability of crown dentine (Hume 1985). The teeth were fitted into the cap of a 15ml scintillation vial which was cut to fit the crown of the tooth. The crown was placed in the cap with the root facing the inside of the vial, and secured in place with sticky wax from both the external and internal aspects (Fig. 3.4.).

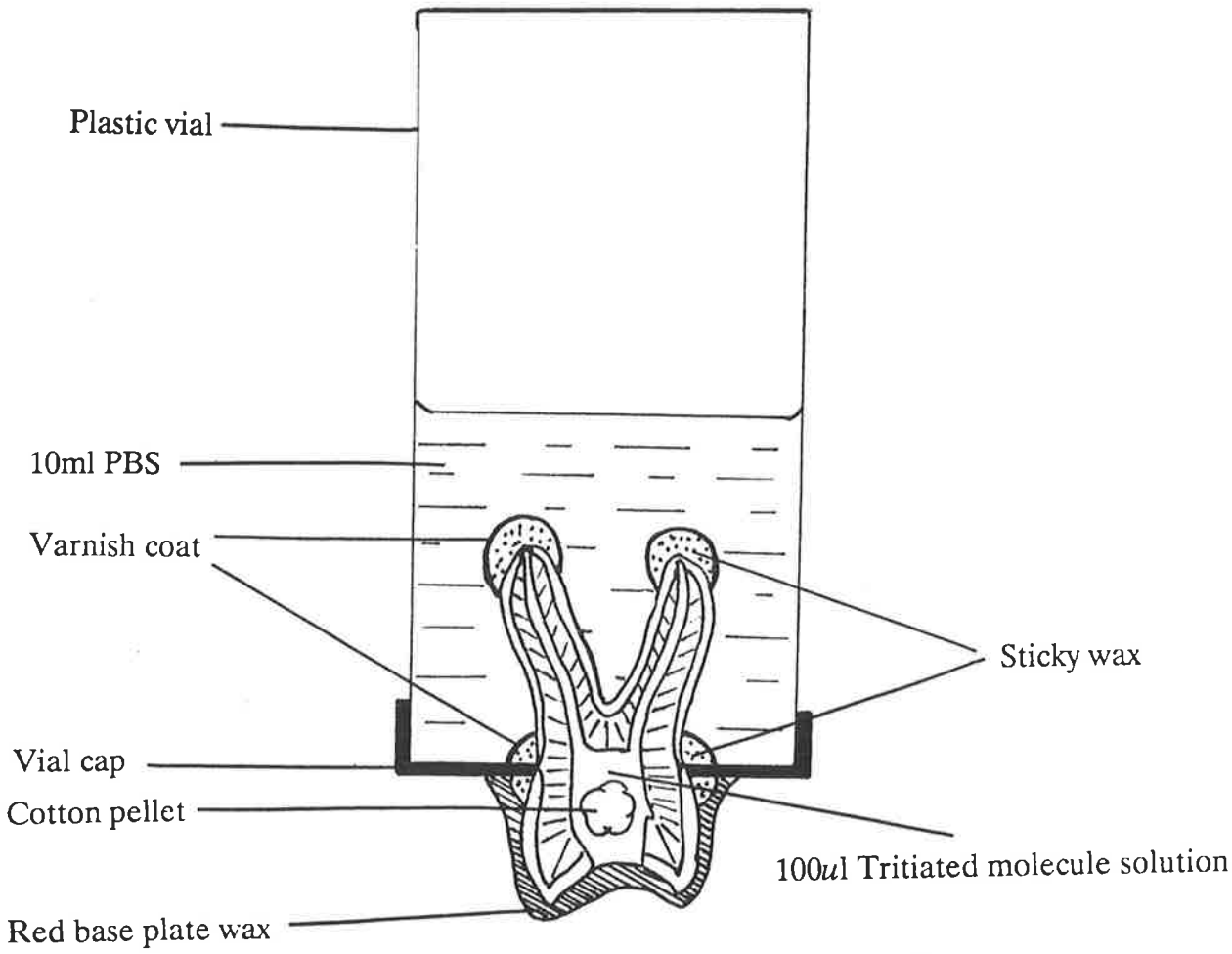

Figure 3.4. Diagram of Root Permeability Experimental Model.

Preliminary experimentation was conducted to find a suitable sealing material for the root surface, without any preparation of the tooth surface. These included modeling

wax, sticky wax, nail varnish, a combination of wax and nail varnish, silicone sealant, and glass ionomer cement. The best seal over a one month period was provided by the sticky wax and nail varnish combination. Tritium labelled water penetrated the modeling wax easily, as this wax did not adhere to the tooth structure. Nail varnish alone had a good initial seal, but was found to allow ready permeation of tritium after one to two days. Glass ionomer cement also appeared to provide a good seal initially, but faltered after approximately two days. The problem with the glass ionomer cement was to keep it free from moisture contamination for 24 hours, to prevent crazing. The cement was protected by a coat of varnish as suggested by the manufacturers, but Earl et al (1985) found these varnishes were ineffective in keeping the glass ionomer free of moisture. In addition the inner surface would have had ready permeation of phosphate buffering solution and allowed crazing to occur. The teeth could not be dried without affecting the permeability studies.

The molecule chosen for this permeability experiment was tritium labelled water. Following the sealing of the tooth with wax and varnish in the required areas in accordance with the assigned treatment group, 100*u*l of $^{3}H_2O$ was placed in the tooth cavity, along with a cotton pellet. During experimental design 1*u*Ci/ml Tritium labelled water was used, but later the concentration of tritium was increased to 250*u*Ci/ml because of the low counts obtained.

The entire crown surface of the tooth which protruded through the vial cap was then sealed with sticky wax and pink modeling wax. Ten milliliters of PBS was placed in the scintillation vial. The container was then inverted to bath the root in PBS, and stored in that fashion between measurements (Fig 3.4).

The vials were stored at room temperature for the duration of the experiments, because in the early stages of the experimental model it became obvious the wax seals were inadequate with temperature cycling. The initial concept during experimental design was to store teeth at the physiologic temperature of 37^{o}C. Other temperatures were also tried including cooling to 5^{o}C, but the best seal was at room temperature which was used during placement of the wax. Any further temperature change would

induce dimensional changes in the wax and induce leakage. The room in which the vials were kept was air-conditioned and maintained at a regulated temperature between 20-25^{o}C.

Sampling of the fluid in the vials took place at 1, 2, 4, 8 hours, 1, 2, 4, 8, and 24 days as in previous permeability experiments in this laboratory (Hume 1985, Abbott et al 1988). Each time a 100*u*l sample was taken from the vial, and not replaced. The total volume removed during the study was 0.9ml, and it was felt this was not significant to the total volume. The sample was then placed in a 5ml scintillation vial, and 3ml of scintillation fluid (see Table 3.1) added. The sample was mixed gently, and then counted in a liquid scintillation spectrometer, a Beckman Model LS2800 by Beckman, Fullerton, California U.S.A.

Table 3.1. Prescription for Scintillation Cocktail.

500 ml Triton X-100	(Ajax Chemicals, Sydney, NSW)
7.5 gm 2,5 diphenyloxazole	(Ajax Chemicals, Sydney, NSW)
250 mg 1,4 -di[2-(5-phenyloxazolyl)] benzine	(Koch-Light Laboratories, Colnbrook, England)
in 1 litre of Toluene	(Ajax Chemicals, Sydney, NSW)

3.3.1.d. Areas of Interest.

Each tooth was assigned to one of five treatment groups: furcation open, furcation closed, full wax of the root, apical wax only, or no wax at all. This was an attempt to see any differences in permeation between the furcation area of the teeth, and the other areas of the root were measured.

I) The furcation open group had the entire root surface sealed with wax and varnish except for the furcation region. The furcation region was defined as the area involving the division between the roots which extended down the inner aspects of the root surface two to three millimetres from the root division (Fig. 3.5). This allowed estimation of diffusion through the furcation region.

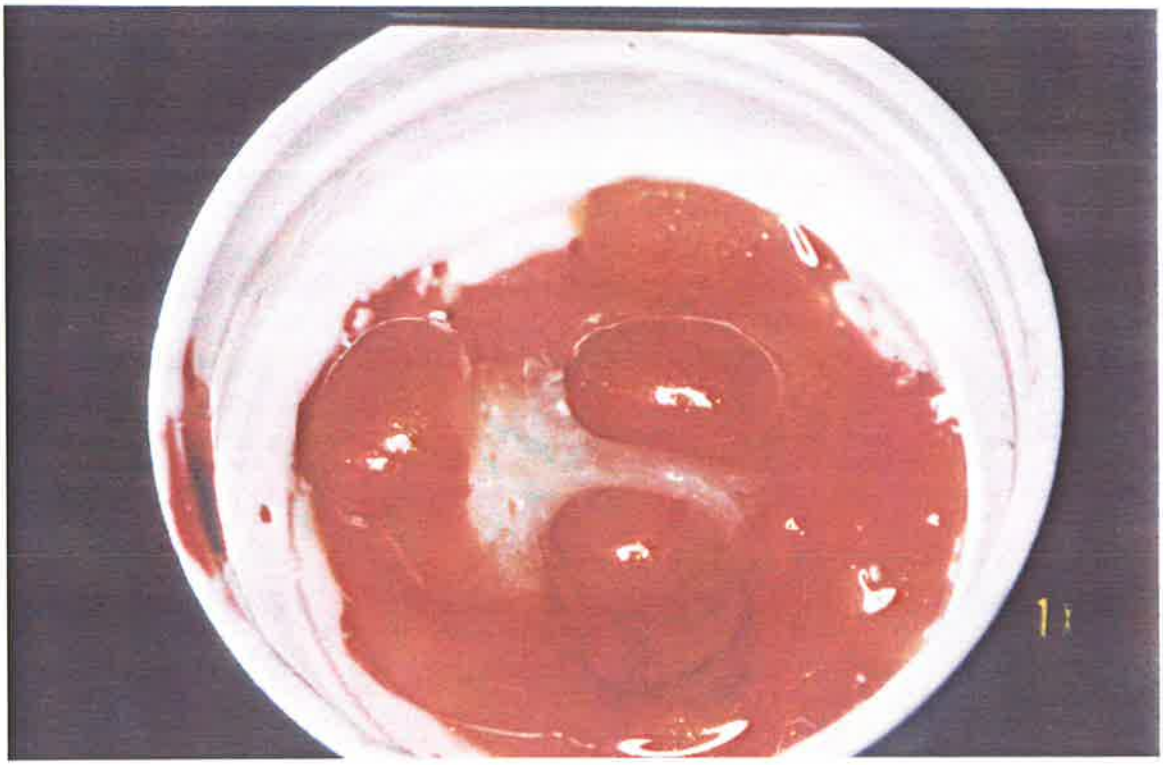

Figure 3.5 Tooth from the Furcation Open Group.

II) The furcation closed group had the furcation and the apices sealed with wax and varnish, while the rest of the root surface was left open to allow diffusion (Fig. 3.6).

III) A third group had the entire root surface sealed with a wax coat and varnish to act as a control group, and assess the effectiveness of the seal.

IV) This group had only the apices of the root sealed with wax and varnish to prevent leakage through the apices. The apex of the tooth was defined as the tip of the root and the surrounding 2-3 millimetres.

V) This group consisted of a small number of teeth which had no wax at all on their root surfaces.

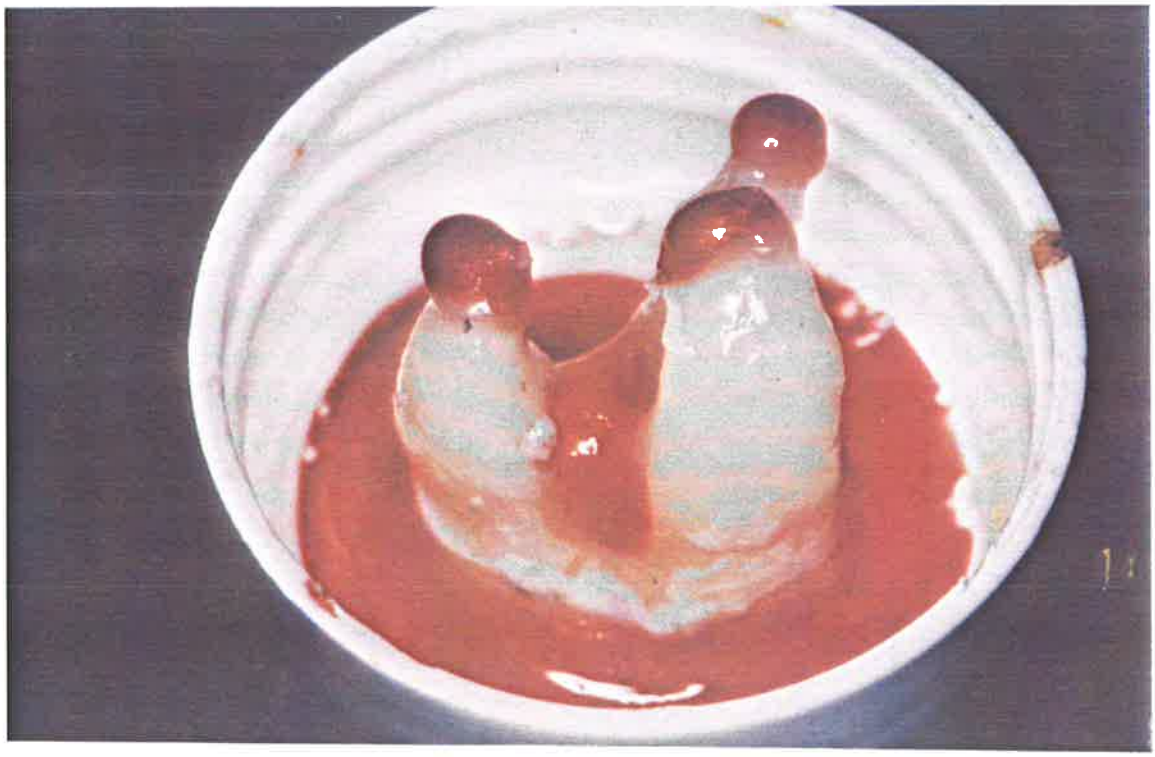

Figure 3.6 Tooth from the Furcation Closed Group.

3.3.1.e. Surface Area Diffusion.

At the completion of the measurements for the diffusion of tritium, the total area available for diffusion was measured to enable the rate of diffusion per square millimetre to be calculated. There was a clear difference in the size of the surface area available for diffusion between the groups. This was considered because it may have hidden differences between the experimental groups. Therefore a further analysis was done to take into account the surface of the root left exposed for permeation of the tritiated water. During analysis the time allowed for diffusion was also incorporated into the equation. The teeth with a small area free for permeation would by the very nature of the experiment, with a given volume and concentration for diffusion, have a larger diffusion rate per square millimetre as the tooth approached equilibrium in comparison to a tooth with a larger area for diffusion.

The teeth prior to the calculation of surface area were soaked in distilled water for a period of two weeks with the water being changed every two days. This allowed for

the tritium within the tooth structure to diffuse into the water, and reduced the remaining radiation level in the teeth to background levels avoiding any contamination of other equipment during the measurement process. The teeth were then allowed to dry in air to ensure an accurate impression of the exposed tooth surface available for diffusion was obtained.

The problem of reducing a three dimensional surface area into two dimensions was solved by using a polysilane impression material. An injectable material, GC Exaflex (G-C Dental Industrial Corporation, Tokyo, Japan), type 1 low viscosity, was mixed as per instructions, and spread approximating a uniform thickness of 1-2mm over the tooth surface. The material was kept thin to allow easy working, but with sufficient thickness to prevent tearing. After the polysilane impression material had set it was peeled off the tooth. The surface area available for diffusion was determined by the obvious visual boundary with the wax seal, and the material was trimmed back to this area using a sharp scalpel. The material remaining represented the surface area exposed for diffusion. Calculation of the diffusion area of this three dimensional material was changed into a two dimensional record of the area by two methods. The silicon material was flattened between two glass slabs, ensuring no folding of material occurred during this procedure, and then placed onto an X-ray viewer. Tracing paper was used to outline the silicon material. A computer surface area programme was used to calculate the area traced by placing the tracing paper on a Hewitt-Packard 9874A Digitizer (Hewitt Packard Corporation U.S.A.), and fed into a Hewitt Packard 9815A computer which tabulated the surface area. Each of the root surface replicas were analyzed on at least three occasions so that there was agreement between the surface area measurement to within one square millimetre, or the area measurement was repeated until three similar measurements were obtained.

As a precaution the measurements were checked by making contact prints of the silicon root surface outlines whilst flattened between glass sheets. These contact prints were also analyzed by the same digital process described for the tracing sheets. There were no significant differences between the two methods in surface areas calculated.

During the experiment several of the silicon replicas were measured at two separate occasions to measure for any experimental error, but no significant differences were found.

3.3.1.f. Treatment of Data.

The analysis was performed in two ways. Firstly simply statistical calculations were made to produce a mean and standard deviation for each measurement time in each group, in a similar way to the experiments conducted by Hume (1985), and Abbott et al (1988). Comparison between the groups was performed after consulting with Mr. P. Leppard (Statistician, Adelaide University). The comparative statistics were performed on the Adelaide University computing system, Digital Equipment Corporation, VAX/VMS Version V4.6. The statistical software programme used was the BMDP Statistical Software package (1988, BMDP Statistical Software Inc., Los Angeles, Ca. U.S.A.).

Analysis of the surface area model for tritiated water diffusion was performed using a repeated measures model with fixed effects for treatment and a linear within tooth effect for time. Analysis of the effect for each of the treatment groups was performed using the extended repeated measures programme 5V from the BMDP Statistical Software package as the data was unbalanced because of missing values. Analysis of the effect of sex, age and tooth were performed using the same package.

The analysis of the effect of the control group, furcation open, and furcation closed groups per unit area were performed using a general mixed model of analysis for repeated measures, by using the 3V programme of the BMDP Statistical Software package.

3.3.2. Molecular Weight Studies.

Experimentation was performed to identify the size of molecules able to permeate the dentinal and cemental structures using the extracted third molars. The compounds used in this part of the study were tritium labelled water, glucose and dextran.

3.3.2.a. Material Collected.

Third molars were collected from the Adelaide Dental Hospital Oral Surgery Department, and participating Oral Surgeons in private practice. The teeth were extracted for impaction reasons from young healthy patients, which had no sign of caries, restorations, or periodontal disease, and had not received any identifiable trauma to the root surface during extraction. This material was studied because it was more readily available than first and second molars. The extracted teeth were placed in PBS solution immediately after extraction. The teeth were collected from the oral surgeons on the same day.

3.3.2.b. Tooth Preparation.

The preparation was similar to that described above (3.3.1.b.). The third molar had an endodontic access cavity in the crown cut with a high speed tungsten carbide Jet 330 bur with water coolant to expose the roof of the pulp chamber. This was removed along with the undercuts to the access cavity with a slow speed No. 4 round bur. The pulp tissue was removed with a barbed broach (Nervnadeln CC-cord, Vereinigte Dentalwerke, KG, Munchen, Germany), and the chamber flushed with sterile PBS to remove all debris. The external root surface had the epithelial cuff removed with a sharp scalpel. To remove the surface bacteria from the root, and to sterilise the root canal system the teeth were bathed in 70% alcohol for 30 minutes, before being stored in sterile PBS until use.

3.3.2.c. Experimental Model.

The model used is described in 3.3.1.e. Following the disinfection procedures described in 3.3.2.d.(iv) the teeth were stored in sterile PBS. At the time of set-up for the experiment the teeth were again placed in 70% alcohol immediately prior to placing the teeth in the vial cap. During this final stage of preparation an aseptic technique was used keeping the preparation area clean, only new materials were used, and the operator wore sterile gloves and mask in an effort to keep bacterial contamination to a minimum.

The crown was fitted into the lid of a 15ml scintillation vial, and was sealed on both sides of the vial cap with sticky wax. The teeth received one of two treatments for this study, either full coverage of sticky wax and vanish, or wax and varnish on the apices only. The tooth chamber and cavity had 100*u*l of the tritiated compound to be used placed in it, and a cotton pellet. The entire crown of the tooth external to the vial cap was covered and sealed with wax. Into the scintillation vial 10ml of PBS was placed, the cap secured, and the vial inverted (Fig 3.4).

The teeth were stored at room temperature, and sampling of the bathing fluid occurred at 1, 2, 4, 8 hours, 1, 2, 4, 8, 16, 24, 32, and 40 days as previously used (Hume 1985, Abbott et al 1988). Each time 50*u*l was sampled and not replaced. This was a smaller sample than used by Hume (1985) due to the increased number of samples taken. The collected sample was place in a 5ml scintillation vial, and 3ml of scintillation cocktail added. This was gently mixed, and place in a liquid scintillation spectrometer (Beckman Model LS2800) to ascertain the presence of the tritium labelled compound.

These teeth used to test the permeability of glucose and dextran were recycled to test tritium labelled water. To decrease the background counts to insignificant levels the teeth were placed into sterile distilled water for a period of three weeks, during which time the water was changed every three days. At the end of the three week period the distilled water was sampled, the scintillation cocktail added, and the activity assessed. All the teeth at that time demonstrated negligible counts, and were equivalent to background only. The teeth were then soaked in 70% alcohol for one hour prior to the teeth being resealed into the scintillation vial caps with sticky wax and nail varnish. The roots were then given a fresh wax covering of the apical region only or full wax covering prior to 100ul of 250*u*Ci/ml $^{3}H_2O$ being placed into the root canal system, and sealed with modeling wax. The teeth were sampled at the same times as for glucose and dextran molecules.

3.3.2.d. Tritiated Labels Used.

This system was used to test the permeability of the root surface to various compounds of varying molecular size in an experiment to test if there was any size limit

to the permeability of tooth roots. The compounds used were tritium labelled water, glucose, and dextran molecules. The molecular weight of the glucose was 180.2 (New England Reactor products TRA 382 Batch 43), as an aqueous solution, while the molecular weight the dextran was 70,000 (Amerscham Lot No 1780-139, Amerscham Aust. Pty. Ltd., Nth. Ryde, N.S.W.), as freeze dried solid.

3.3.2.d.(i). Tritiated Glucose.

Glucose is a compound which is central to the production of energy within cells, with the production of ATP. The chemical formula for glucose is $C_6H_{12}0_6$ (MW 180.2). The tritium labelled glucose was purchased from New England Reactor Products, Du Pont (Aust.) Ltd. Sydney N.S.W.

The batch supplied by the New England Reactor Products contained 250 uCi of ^{3}H-Glucose in 0.25 ml of sterile solution. This contained 0.0068 mg of the labelled compound. The moles of molecules present were calculated by the following equation:

$$\text{No of Moles} = \frac{\text{weight present}}{\text{molecular weight}} \quad \frac{6.8 \times 10^{-6}}{180.2}$$

$$= 3.77 \times 10^{-8} \text{ moles}$$

The molar concentration was calculated by dividing this number by the volume of the solution. Therefore the molar concentration in the batch supplied 1.51×10^{-4} was diluted to 6ml with Phosphate Buffered Saline. The molar concentration was again calculated by division of the number of moles present in the batch supplied by the final volume:

$$= \frac{3.77 \times 10^{-8}}{6.0 \times 10^{-3}}$$

$$= 6.28 \times 10^{-6} \text{ mol}$$

The batch at the time of delivery had 250 uCi of activity in the 0.25 ml solution. This was diluted to 6ml with PBS solution. The final activity then is calculated by the following equation:

$$= \frac{\text{total activity}}{\text{final volume}}$$

$$= \frac{250\ u\text{Ci}}{6\ \text{ml}}$$

$$= 41.67\ u\text{Ci/ml}$$

100 ul of the 41.67 uCi/ml ^{3}H-Glucose was placed inside each tooth. The total activity of the 100 ul added to the tooth was calculated in C.P.M. by adding 100 ul of ^{3}H-Glucose to the scintillation cocktail. This was done five times and the mean calculated at the commencement of each study to allow for decay with time and variations in the experimental procedure, and is shown (Table 3.2). A percentage as calculated by dividing the CPM value of the bathing solution by the total activity of the labelled compound to allow comparison between experimental groups.

Table 3.2. Mean Values for 100ul Tritiated Glucose.

Experiment.	Counts Per Minute
Root perm.	1,022,678.99
Crown perm.A	1,584,651.24
Crown perm.B	1,015,659.42
Crown perm.C	1,010,039.90

3.3.2.d.(ii). Tritiated Dextran.

Dextran is a linear molecule produced by bacteria growing on sucrose substrates, containing a backbone of glucose units linked predominantly in an alpha-1,6 linkage. It is a storage polysaccharide of yeasts and bacteria, and has an empirical formula of $(C_6H_{10}O_5)_n$. The molecular weight of the tritiated Dextran used was 70,000. The material was supplied by Amerscham Nuclear Products Australia Pty. Ltd. as a freeze dried solid which contained 250uCi of ^{3}H-Dextran. The product was analyzed by Amerscham and found to contain 336 mCi/mg. From this the number of grams of ^{3}H-Dextran present in the sample was calculated:

$$\text{grams } ^{3}\text{H-Dextran} = \frac{\text{Activity}}{\text{Activity / gm}}$$

$$= \frac{250 \times 10^{-6}}{336 \times 10^{-3}}$$

$$= 0.74 \text{ mg}$$

The number of moles of molecules ^{3}H-Dextran present can then be calculated:

$$\text{moles of } ^{3}\text{H-Dextran} = \frac{\text{gm weight present}}{\text{molecular weight}}$$

$$= \frac{7.4 \times 10^{-4}}{70{,}000}$$

$$= 1.06 \times 10^{-8} \text{ moles}$$

This solid was added to 6ml of sterile PBS to allow for sufficient solution for experiments. This allowed the calculation of the final concentration of the ^{3}H-Dextran which was:

$$\text{final concentration} = \frac{\text{no of moles}}{\text{volume in litres}}$$

$$= \frac{1.06 \times 10^{-8}}{6 \times 10^{-3}}$$

$$= 1.76 \times 10^{-6} \text{ mol}$$

The activity of the solutions used in the experiment were calculated by dividing the total activity of the freeze dried solid supplied by the final volume of PBS:

$$\text{Final Activity} = \frac{250\ u\text{Ci}}{6 \text{ ml}}$$

$$= 41.67\ u\text{Ci/ml}$$

One hundred microlitres of 41.67 uCi/ml tritiated dextran was placed into the tooth cavity in each case. The total activity of the 100ul placed was calculated by micropipetting 100ul of ^{3}H-Dextran into a vial with the scintillation cocktail, mixed, and measured by the liquid spectrometer. This was done five times, and a mean calculated at the commencement of each study to allow for decay of the label, and variations in experimental procedure. This was used to calculate a percentage of the total label

present in the sampled bathing fluid and therefore allow comparison between experimental groups.

Table 3.3. Mean Values of 100*u*l Tritiated Dextran.

Experiment	Counts per Minute
Root perm.	874,391.83
Crown perm. A	1,245,250.09
Crown perm. B	871,480.90
Crown perm. C	869,444.40

3.3.2.d.(iii). Tritiated Water.

The batch of the tritium used in these experiments was unknown as the material was taken from stocks held by Dr. R. Hume for his crown dentine permeability studies testing effectiveness of the seal of restorative materials. The activity of the label used for all of the root permeability experiments was 250*u*Ci/ml. The crown dentine permeability experiments were initially performed with 250*u*Ci/ml label but the CPM were excessively high, which may have disguised some results. All crown dentine studies conducted used a concentration of either 50*u*Ci/ml or 1*u*Ci/ml tritiated water.

Table 3.4. Mean Values for 100*u*l Tritiated Water Label.

Experiment	Concentration	Counts per minute
Root perm.	250*u*Ci/ml	9,744,944.89
Crown perm.A	50*u*Ci/ml	735,427.10
Crown perm.B.	1*u*Ci/ml	24,356.14
Crown perm.C.	1*u*Ci/ml	14,720.48

To allow comparison between experiments the values for these tritiated water experiments were also expressed as a percentage of the total activity of the label. The total activity for the label was calculated at the beginning of each experiment by adding

100ul of the tritiated water to a scintillation vial with 3ml of scintillation cocktail and the CPM measurement from the liquid scintillation spectrometer recorded five times. This was used to calculate a mean activity of the label.

3.3.2.d.(iv). Stability of Labelled Molecules.

The molecules chosen were by necessity stable compounds in sterile solution, and did not show any tendency to disassociate. We were concerned that metabolism of the dextran and glucose into smaller molecules may occur, and affect the result. Both molecules are used by bacteria for the assembly of other macromolecules, or metabolized as part of their energy production cycle.

It was a major concern that bacteria contamination could affect the results in this experiment. Every effort was made to remove all bacteria before the tooth was used in the experiment, and to prevent the introduction of bacteria into the experimental vials. The key was to make the pulp chamber sterile and maintain the solutions sterile. Distilled water and PBS were autoclaved and kept sealed until use, the phosphate buffered saline solution had an antibiotic, Garamycin powder (Gentamicin Sulphate, Schering Corporation, Keniworth, N.J., U.S.A.), added to produce a solution containing 100ul/ml of gentamycin. This provided an effective bacteriocidal dose of the antimicrobial substance in the PBS. Cracking of the tooth structure occurs during autoclaving, and other sterilisation procedures, preventing the use of these techniques on the experimental teeth. The teeth were treated in a manner similar to the earlier experiment. In addition the root surface was lightly scrubbed with 70% alcohol to kill and remove bacteria from the root surface.

3.3.2.e. Effect of Cementum.

The effect of cementum on the diffusion of these compounds was examined by root planing one group. The teeth were at the time of pulp tissue removal, root planed to free the root of cementum, which was accomplished by giving each surface of the tooth fifty strokes with a sharp Gracey 1-2 curette. This has been shown to remove most of the cementum from the root surface (O'Leary and Kafrawy 1983). The teeth were then

prepared as described for the dextran and glucose experiment above. The same time intervals were used, and the results compared.

3.3.2.f. Coronal Permeability Experiments.

The final part of this study was to compare the permeability of root dentine and cementum, with that of coronal dentine. The coronal dentine permeability was assessed by a technique adopted from Hume (1985) which was used to assess the toxicity of restorative materials. Freshly extracted third molar teeth from young patients were collected and placed into sterile PBS solution as previously described.

3.3.2.f.(i). Tooth Preparation.

An aseptic technique to minimise the risk of bacteria contamination was carried out as previously described. To prepare the coronal dentine the soft tissue tags adherent to the tooth, which were remnants of the follicle were removed. The periodontal ligament was then removed by scraping the root surface with a Hollenback carver. The teeth were then placed into 70% alcohol for an hour prior to cutting a class I cavity using a high speed handpiece with a Jet 330 tungsten carbide bur with adequate water spray. An attempt was made to prepare the floor of the cavity as smooth and flat as possible. The crown was separated from the root of the tooth using the high speed Jet 330 tungsten carbide bur, and the two halves were pulled gently apart. Usually the dental pulp pulled away with the root leaving the crown clear. When this did not occur the pulp tissue was pulled free with a pair of college tweezers, taking care not to touch the pulp chamber surface. The thickness of the dentine from the floor of the cavity to the pulp chamber was measured using a pair of calipers (Manufactured by Dixon U.S.A.). The thickness was measured ten times over the various parts of the floor area, and the average taken for the thickness of the remaining dentine. The teeth were then stored in sterile PBS until used in the experiments.

3.3.2.f.(ii). Crown Dentine Permeability Model.

At the time of set up for the experiment the teeth were taken from the PBS and placed in 70% alcohol for an hour. The operator followed an aseptic technique described previously. The outer surface of the tooth was dried after removing it from

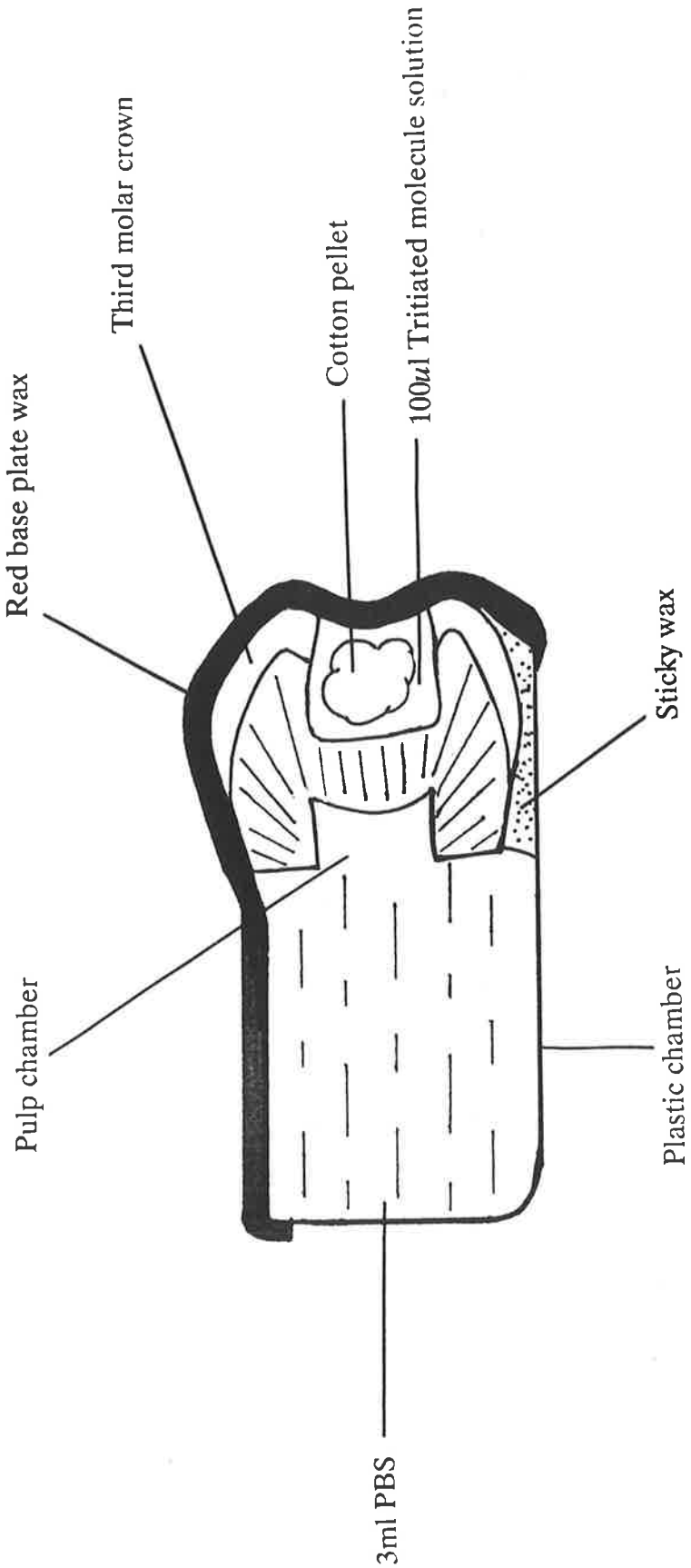

Figure 3.7. Crown Dentine Permeability Model.

the alcohol bath, and the tooth crown sealed into a plastic boat. Ten millilitre plastic vials were cut into halves to serve as boats for this experiment (Hume 1985). The crown was placed at the open end of the hemi-cylindrical chamber and fixed into position with sticky wax. The crown had a ring of sticky wax placed above the seal of the boat, which proved effective in preventing leakage. The boat was then sealed with modeling wax (Fig. 3.7). Three milliliters of sterile PBS containing 100ug/ml gentamycin was then injected into the boat through the wax roof. This was subsequently sealed with wax and checked for any leakage. Into the occlusal cavity 100*u*l of the test solution was placed along with a cotton pellet and this was sealed with red wax (Fig. 3.7). All samples were stored at room temperature. The sampling times were as described previously and 50*u*l samples were drawn from the boat by initially cutting a small hole in the wax seal of the boat, drawing the sample with a micropipette, and then sealing the wax boat with fresh wax.

3.3.2.g. Treatment of Data.

The analysis was performed in two ways. Firstly simply statistical calculations were made to produce a mean and standard deviation for each measurement time in each group, in a similar way to the experiments conducted by Hume (1985), and Abbott et al (1988). Comparison between the groups was performed after consulting with Mr. P. Leppard (Statistician Adelaide University). The comparative statistics were performed on the Adelaide University computing system, Digital Equipment Corporation, VAX/VMS Version V4.6. The statistical software programme used was the BMDP Statistical Software package (1988, BMDP Statistical Software Inc., Los Angeles, Ca. U.S.A.).

Analysis of the effect of time, the permeating compound, the tooth treatments, age, sex, and thickness of the coronal dentine were performed using a repeated measures model with fixed effects for treatments, and a linear within tooth effect for time. Analysis of the effect for each of the treatment groups was performed using the general mixed model analysis of variance programme 3V from the BMDP Statistical Software package.

There was a combined treatment and chemical effect seen in the early analysis. An attempt was then made to gather more data on the effect of labelled chemical size on the rate of diffusion by comparing the diffusion of each of the treatment groups separately using the 3V programme. A similar analysis was performed for the treatment effect (normal, root planed and crown dentine) by comparing the effect of treatment on each chemical used separately.

3.4. Electron Microanalysis of Permeability.

Further information pertaining to the permeability of the tooth roots was sort in an effort to identify the region of penetration of the permeating molecules using electron microprobe analysis. A cobaltous salt was chosen as the marker as its resolution peak was distinctly clear from the elements likely to be found within the surrounding tooth structure. The electron microscope was the Phillips 505 scanning electron microscope, which was coupled to the Tracor Northern computer system 5500.

Taken literally microanalysis is the analysis of "very small" samples by various techniques available. When electrons of appropriate energy impinge on a sample, they cause the emission of X-rays whose energies and relative abundance depend upon the composition of the sample, and using this phenomenon one can analyze the elemental content. The technique used in this experiment was Energy Dispersive Spectrometer (EDS) microanalysis, in which the X-ray emissions are sorted electronically, in contrast to Wavelength Dispersive Spectrometer (WDS) which operates by the means of a diffraction crystal. The advantages of microanalysis are that it is relatively easy to perform, is sensitive to low concentrations, is non-destructive of the specimen, and sample preparation is minimal.

The main area of interest for microanalysis is the X-rays emitted. A large number of processes occur when an electron beam hits the specimen surface, resulting in signal which can be collected and interpreted (Fig. 3.8). The commonly considered signals are: i) Secondary Electron Imaging, ii) Backscatter Electron Imaging, iii) Cathodoluminescence Imaging, and iv) X-ray Collection.

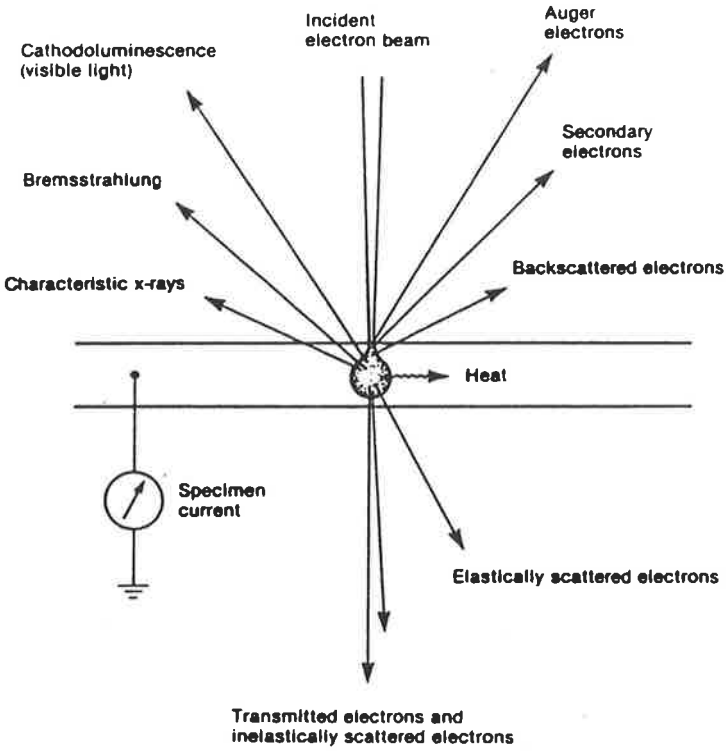

Fig. 3.8. Principal Interactions of the Electron Beam.

Schematic illustration of the principal results of the interaction of an electron beam with the specimen. As suggested by the figure, Auger and secondary electrons emerge from near the surface of sample, and elastically scattered electrons are typically scattered through larger angles than are inelastically scattered electrons.

X-ray generation occurs as a result of interactions between primary beam electrons and orbital electrons of the specimen atoms. An electron from the primary beam of the electron microscope can eject an electron from an inner shell of a sample atom. The resulting vacancy is then filled by an electron from a higher energy shell in the atom. In dropping to a state of lower energy, this vacancy filling electron must give up some of it's energy, which appears in the form of electromagnetic radiation. The energy emitted is exactly equal to the difference between the two electron shells, and because the energy difference between the inner and outer shells is fairly large, the radiation appears as X-rays. The interaction of the electron beam and the specimen results on the generation of X-radiation from the elements present. The energy of the radiation uniquely indicates the element from which it came, hence the name

characteristic emission. Each element will give rise to a number of spectral peaks, depending on its atomic number, and the accelerating voltage used.

All energy dispersive spectrometers have in common a solid state detector. The EDS system is comprised of Si(Li) detector, pre-amplifier, pulse processor and multichannel analyser. The Si(Li) detector chip is positioned behind a beryllium window which protects the detector from damage by accumulation of contaminates in the microscope. When an X-ray photon enters the detector the energy is dissipated principally through raising valence band Silicon electrons into the conduction band, thereby causing electrical conductivity. This produces a brief burst of current, which is amplified to produce a pulse suitable for counting. The magnitude of this current burst, or pulse, is proportional to the energy of the incident X-ray photon. Consequently the system can record not only the arrival of an X-ray photon, but also the energy of the photon.

3.4.1. Material Collected.

The material used in the study were third molars collected from oral surgery private practices on the day the tooth was extracted and returned to the laboratory. The third molar teeth used in this experiment were freshly extracted, non carious, periodontally sound, intact (undamaged during extraction) from young healthy patients mainly under the age of thirty years, which were removed because of impaction or non function. The teeth immediately following the extraction were rinsed in fresh saline and placed into the storing solution. Initially this was PBS, as in the previous permeability experiment, but due to the reaction between cobalt and free phosphates this was later changed to normal saline solution.

3.4.2. Permeating Element.

The element to be used for microanalysis was required to be above sodium (the lower end of the elemental analysis spectrum using the EDS system), and above calcium in atomic number to ensure the peak of the element was clear of the relatively common lower molecular weight elements. A relatively large element was also desirable to provide some test of the permeability of the tooth structure. Various heavy metal salts

were investigated for microanalysis, Lead acetate, Barium acetate, Ferrous chloride, Ferric chloride, and Cobaltous chloride. Ready availability of the salts, and toxicity levels were the determining factors, as it was envisaged allowance should be made for an *in vivo* experiment to be undertaken at a later date. Cobaltous chloride was chosen as 45.39% of it's weight was available as cobaltous ion, it was non poisonous, and had a deep red colour which gave an added bonus that the material could be followed visually. A 10% solution, which was stable, was prepared and used throughout the experiments.

3.4.3. Technique Development.

Thirty teeth were used in the initial trial study ranging in time from 8 hours to 15 days. At the completion of the experiment the occlusal cavity was reopened and all the remaining cobaltous chloride solution flushed from the pulp chamber and root canal system by using 20 ml of distilled water in a syringe. The teeth were air dried at room temperature for a week and then placed into a desiccating jar with silica gel for two weeks at $30^{o}C$.

A lilac precipitate was noticed on the surface of the tooth, inside the pulp chamber and the tooth colour changed to a similar colour. The cobaltous ion had reacted with the free phosphate ion of the PBS with the formation of insoluble cobaltous phosphate. Bound phosphate in the form of hydroxyapatite were not freely available to precipitate with the cobaltous ions. The PBS was replaced by 0.95% sodium chloride solution (isotonic saline) with no further experimental difficulties, although some phosphate dissolved from the tooth allowed the tooth to take on a lilac hue in some areas.

Phosphate buffered saline was used as a storage solution because it is isotonic with a buffered pH similar to *in vivo* tissue, and it preserves cells for an initial period of two days. As this experiment does not require the pulp chamber and root canal to have an intact odontoblast layer then isotonic saline was deemed a suitable bathing medium.

It was found that the remaining soft tissue of the periodontal ligament on the root surface was a problem in accurately imaging the root surface under the scanning electron microscope. A method was sought to remove the adherent remnants of the periodontal ligament without damaging the root surface cementum. Any mechanical

means of removing the soft tissue would result in some damage to the cementum surface, by either removing, or burnishing the cementum. This procedure may have had some effect on the permeability of the cementum, and have corrupted the experiment. Therefore a 5% solution of sodium hypochlorite was made up by diluting the Miltons concentrate solution, 10-13% available chlorine, (Milton Pharmaceutical Company, Villawood, NSW) with equal parts of distilled water (Table 3.5). Sodium Hypochlorite at 5% level has been shown to dissolve organic material and remove loose debris from the root canal during preparation for endodontic therapy (Trepagnier et al 1977). During preparation of root surfaces for SEM work Barber (1978) removed the soft tissue from the root surface by placing the teeth in 5% sodium hypochlorite for 24 hours.

Table 3.5. Concentration of Diluted Miltons Solution.

1% available chlorine is equivalent of 1% weight for weight
chlorine due to 1 chlorine atom to each NaOCl molecule
5% solution = 5.0g/100g = 5.0g/100ml solution

$$\text{chlorine g/ml} = 5 \times \text{MW} \frac{(\text{Na} + \text{Cl} + \text{O})}{\text{Cl}} = 5 \times \frac{74.5}{35.5} = 5.2 \text{ g/100ml NaOCl}$$

MW Na = 23
MW Cl = 35.5
MW O = 16

approx. % available Chlorine = % sodium hypochlorite

3.4.4. Tooth Preparation.

On returning to the laboratory the teeth were placed into fresh isotonic sterile saline bathing solution, and processed in the following manner. Any large tags of soft tissue were removed with a large scalpel blade, and then adherent bone was removed with tweezers or Howe pliers. The teeth were then rinsed in fresh saline and placed into a 5% solution of sodium hypochlorite for 24 hours, to remove the remaining soft tissue on the root surface. The teeth were removed, rinsed in distilled water, and then rinsed a further two times in fresh saline solution before being placed in a further solution of saline for storage until the teeth were used in the experiment.

A standard endodontic access cavity was prepared in the occlusal surface of the tooth to enter the pulp chamber with a Jet 330 tungsten carbide bur at high speed with adequate water spray, the cavity was refined with a slow speed round bur No.4 to remove any ledges. The pulp tissue was removed with a barbed broach, and the pulp chamber and root canal were flushed with the isotonic saline to remove debris.

3.4.5. Experimental Model.

The crowns of the teeth were fitted into a vial cap, and sealed with sticky wax and nail varnish, as previously described in 3.3.1.c. (Fig.3.4). The apex of the tooth was sealed by drying the tooth surface with a blast of air for 60 seconds, and sticky wax and nail varnish applied. Fifteen milliliters of normal saline solution were placed in the vial. The tooth had 100*u*l of 10% cobaltous chloride solution placed in the chamber, which was spun down the canals with a spiral root filler. The crown was sealed with wax as in previous permeability experiments (see 3.3.). The vial was securely closed and kept inverted during the experiment to bathe the tooth in saline, and righted only during measurement times.

The experiment was conducted over ten days, using 140 third molars. Sampling consisted of a photographic record at each of the prescribed times; 1, 2, 4, 8 hours, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 days. Ten teeth were removed from the experiment at each of the time periods listed. At that time a 2 times magnification was taken of the tooth surfaces with cobalt ion permeating to the root surface (Fig 3.9). The teeth were photographed with a Nikon camera fitted with a Nikon Medical lens, and Ektachrome 64 ASA colour transparency film.

At the completion of the experiment the teeth were uprighted, occlusal seal removed, the root canal system was flushed thoroughly with distilled water to remove any cobaltous chloride remaining inside the tooth. The teeth were then dried and prepared for SEM.

The electron microscope focuses an electron beam in a vacuum chamber on meticulously dried specimens. Dryness affects the stability of the electron beam current, and the electron beam current is directly proportional to the vacuum level. It

was required that no gas is given off by the sample and therefore the tooth must be dry. The teeth were prepared in a desiccating jar in air at 30^{o}C and finally at this temperature over silica gel under vacuum, which was pumped down daily for a period of two weeks. The temperature was kept low to keep the mobilization of the permeating cobaltous element at a low level and to reduce cracking of the cemental surface of the teeth.

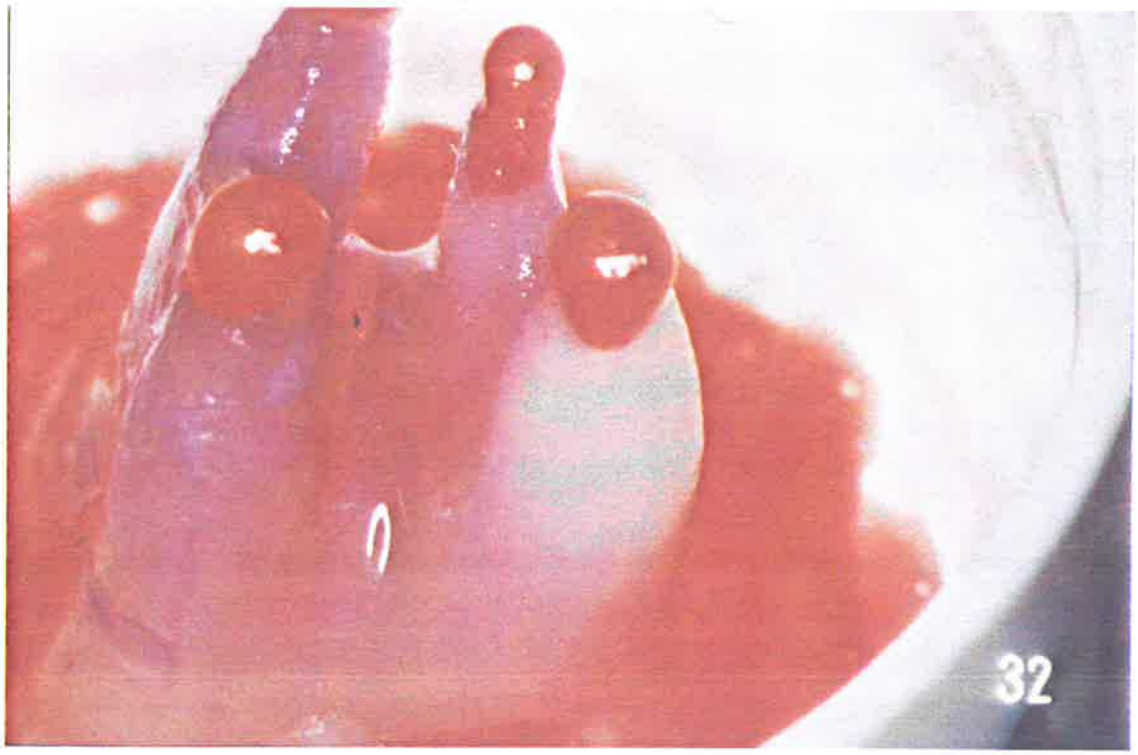

Figure 3.9. Cobalt Permeation on Day 5 Mag.2X.

The dried teeth were mounted onto Etec aluminium stubs using doubled sided adhesive tape. Conductivity between the stub and the specimen was secured by placing conductive carbon dag from the aluminium stub to the crown of the tooth. Carbon dag was used in preference to silver as it does not interfere with microanalysis. All the teeth were tagged for identification during electron microscopy by writing the identification letters on the crown in felt pen and by applying labels to the aluminium stubs. The

dried teeth were coated with a layer of carbon 10-20nm thick by arching of carbon rods in a high vacuum chamber. The teeth were then stored and maintained in a dry state in containers which had a base of silica gel, and sealed until examined under the SEM.

3.4.6. Areas of Interest.

For each tooth five areas were analyzed; the whole root surface, the furcation region if present, the cervical third, the middle third, and the apical third of the root. All teeth were viewed from the buccal or mesial aspect. The magnification for each of these views was predetermined from the initial experimentation and standardized enabling comparison of the results from various areas.

The whole tooth section comprised a view of the entire length of the root of the tooth from the CEJ to the apex. The magnification for this view was required to be between 10 to 14 times. This often left the background exposed which resulted in pronounced peaks of copper and aluminium from the SEM structures.

The cervical, middle, and apical third sections were formed by drawing an imaginary line across the root surface to divide the root surfaces into equal thirds from the CEJ to the root apex. The magnification of these views was a range of 28 to 32 times. The apical third also had distinct peaks of copper and aluminium present on occasions because of the tapering nature of the roots, which meant the apical third did not fill the whole screen.

The furcation region when present on the tooth was also assessed. A view at a magnification of 28 to 32 times was taken of this region. Without sectioning the roots great difficulty was experienced to manage to orientate the specimen so the roof of the furcation could subjected be to microanalysis. Assessment was made by using standardized views of the furcation entrance sites which comprised the two to three millimetres surrounding the opening of the furcation on the buccal or mesial aspect of the tooth.

In addition, the presence and location of accessory canals on the cemental surface were assessed. This was to localize the area from which the cobalt had permeated the tooth, and to assess whether there had been any leakage at the apical seal or through

accessory canals. The video image of the tooth, at increased magnification, was used to do a complete search of the exposed root surfaces for the presence of accessory canals. The location of these accessory canals and their approximate size was recorded on the recording sheet for each specimen (Appendix A).

3.4.7. Electron Microanalysis.

The start up procedure consisted of placing a standard in the Phillips 505 SEM chamber and the chamber pumped down via rotary and oil diffusion pumps. Once a vacuum at the level of 10^{-5} atomospheres had been obtained, a video image of the standard was obtained through the cathode ray tube of the microscope. Electrons were accelerated at 20 KV in the 15-30 KV range through an aperture of 50*u*m. To ensure constant beam the spot size control was set at 50nm, which controls the beam current. The Tracor Northern Computer 5500 was rebooted with the reference disk in drive A. The Flextran programme was loaded, and the region of interest file defining spectral peaks for elements sodium to uranium was loaded.

The copper standard was used to daily check the operating and standardization of the microanalysis programme. This standardization programme measures the energy spacing or the X-ray spectrum for the K-alpha and L-alpha shell characteristic X-ray energies of copper. The acceptable error for zero was +/- 2eV for the zero scale, and the acceptable error for gain was +/- 0.1 eV/channel. This procedure was carried out at the commencement of each session, and was within limits prescribed, which is essential to allow comparison of information collected on the specimens.

Following the placement of a specimen within the chamber, and the accomplishment of the required vacuum level, an image of the tooth was obtained on the cathode ray tube of the SEM. The specimen stage was rotated to give the best possible view of the specimen within the required parameters. The IPP programme of the Tracor Northern computer 5500 attached was utilized to gain a half screen image of the area to be examined.

To allow for comparison between specimens the Kilovoltage, count time, beam current, and the stage tilt were all kept constant. Some teeth were examined with an

older filament in place with a spot size of 100nm, because as the filament ages there is a decrease in electron emission. The working distance for the teeth roots was kept constant to allow comparison of specimens.

3.4.7.a. X-Ray Spectrum Analysis.

The procedure for each of the five views was the same. An X-ray elemental spectrum was taken for a time of 20 seconds from 0 to 10 keV. Peaks for all the elements present on the tooth surface were then identified and measured. A computer print out of the elemental graph as obtained by the adjacent printer (Figs.3.10 - 3.13). The actual value for the number of emissions for the elemental peaks of phosphorous, calcium, cobalt and germanium were enumerated from the computer programme. The value of the peaks, the assigned score for the X-ray spectrum and the X-ray image, were recorded on the form shown in Appendix A.

A scoring system allowed the calculation of whether the amount of cobalt was significant, by dividing the quantified cobalt peak by the background radiation. When the cobalt peak was more than twice that of the background cobalt it was considered to be present.

A scoring system devised of 0, 1, 2, 3 on the following system. A score of zero represents a level not significantly above background, with a peak to background ratio of less than two (Fig. 3.10). A score of one represented a peak to background ratio of greater than or equal to two and less than three (Fig. 3.11.). A score of two represented a peak to background ratio of greater than or equal to three and less than five (Fig. 3.12). A score of three represents a peak to background ratio of greater than or equal to five (Fig. 3.13).

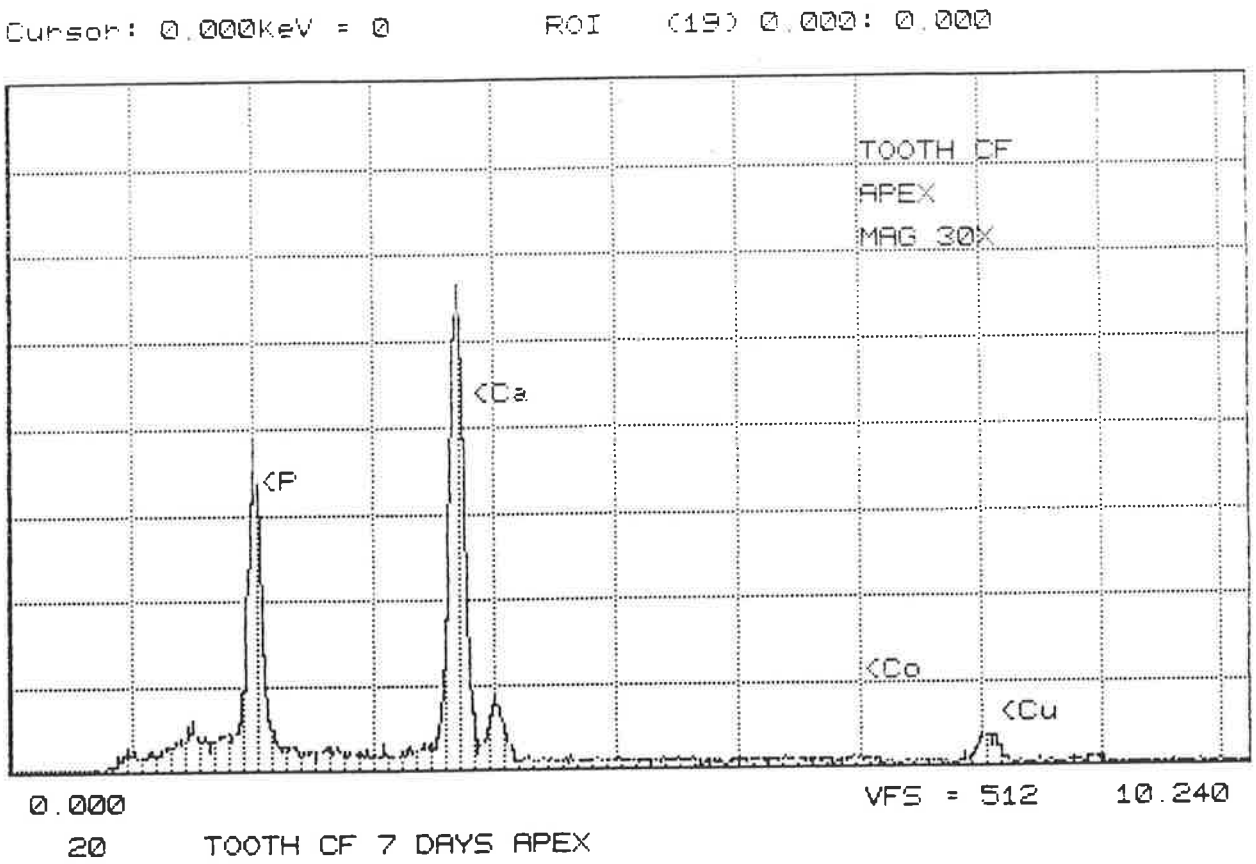

Figure 3.10. X-Ray Spectrum Representing a Score of Zero.

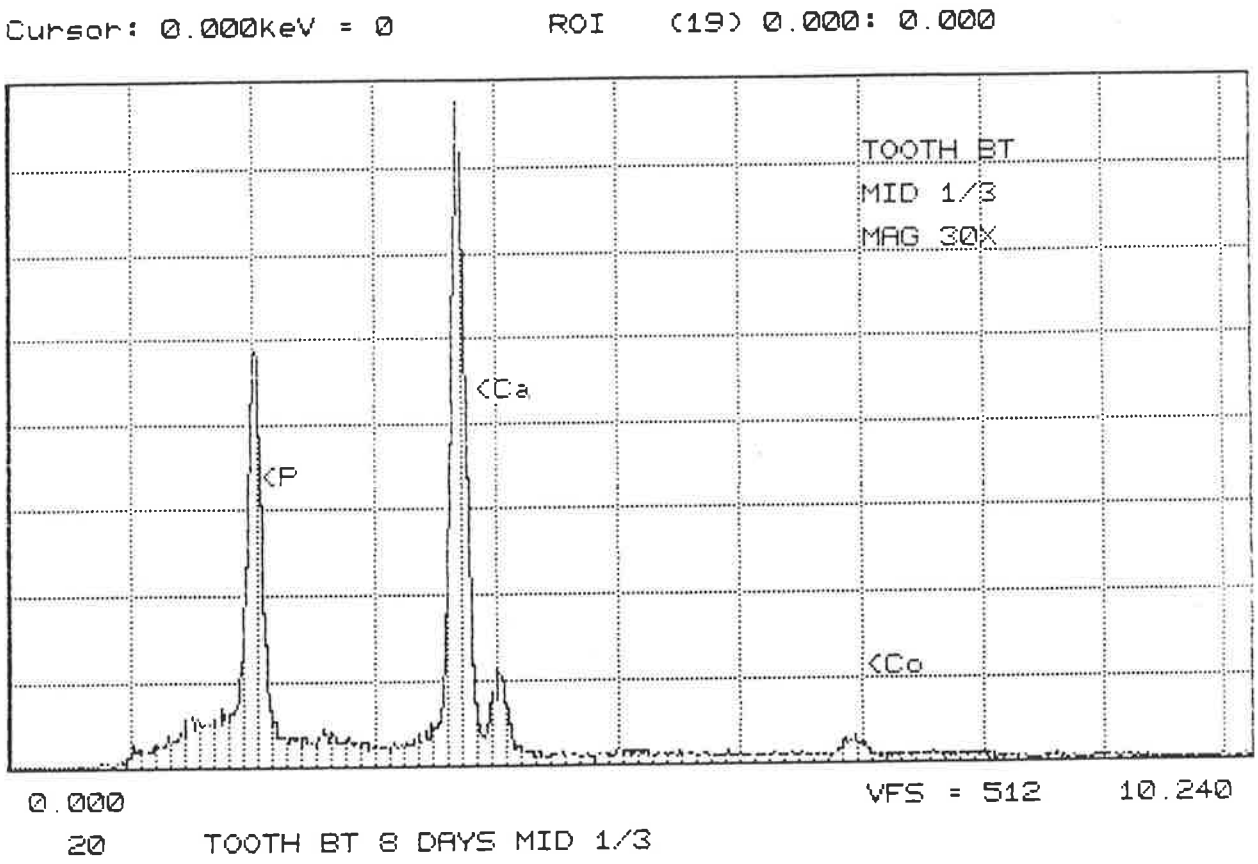

Figure 3.11. X-Ray Spectrum Representing a Score of One.

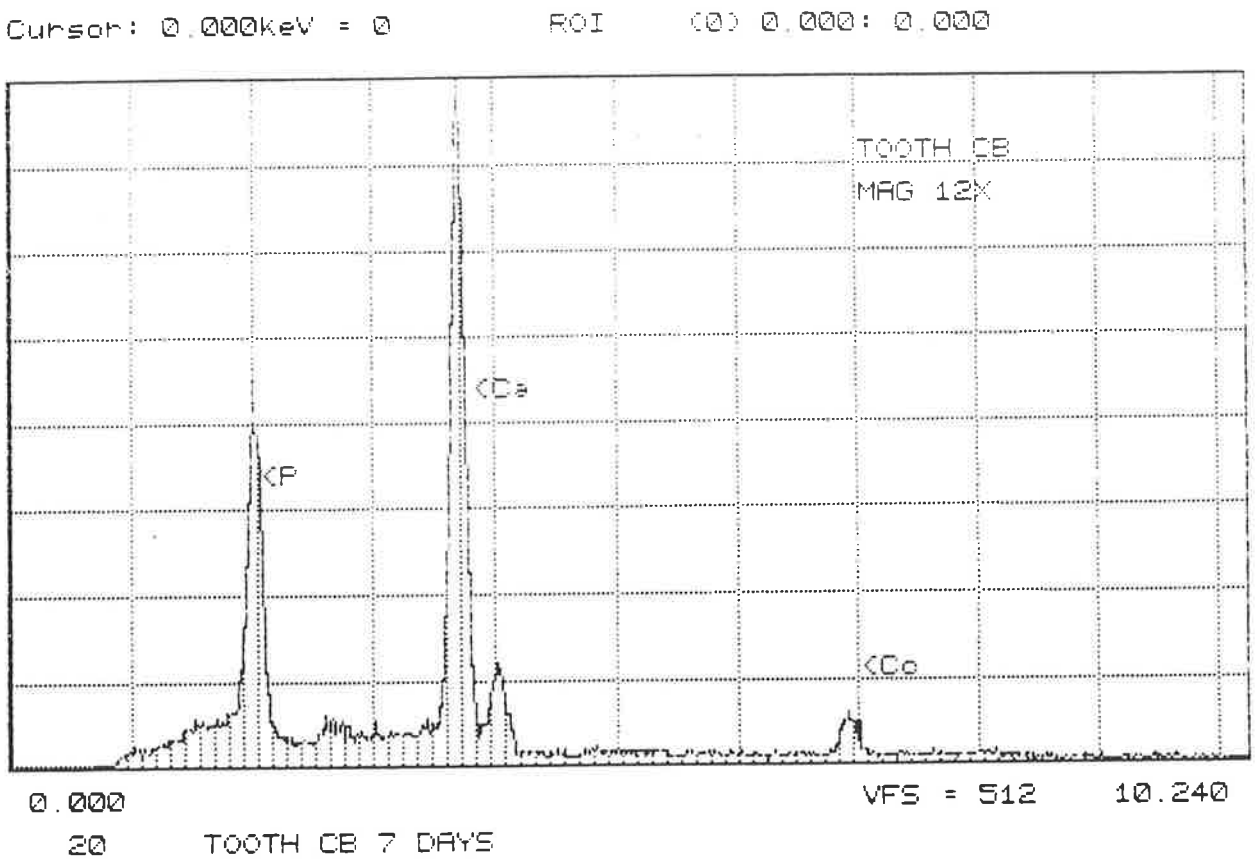

Figure 3.12. X-Ray Spectrum Representing a Score of Two.

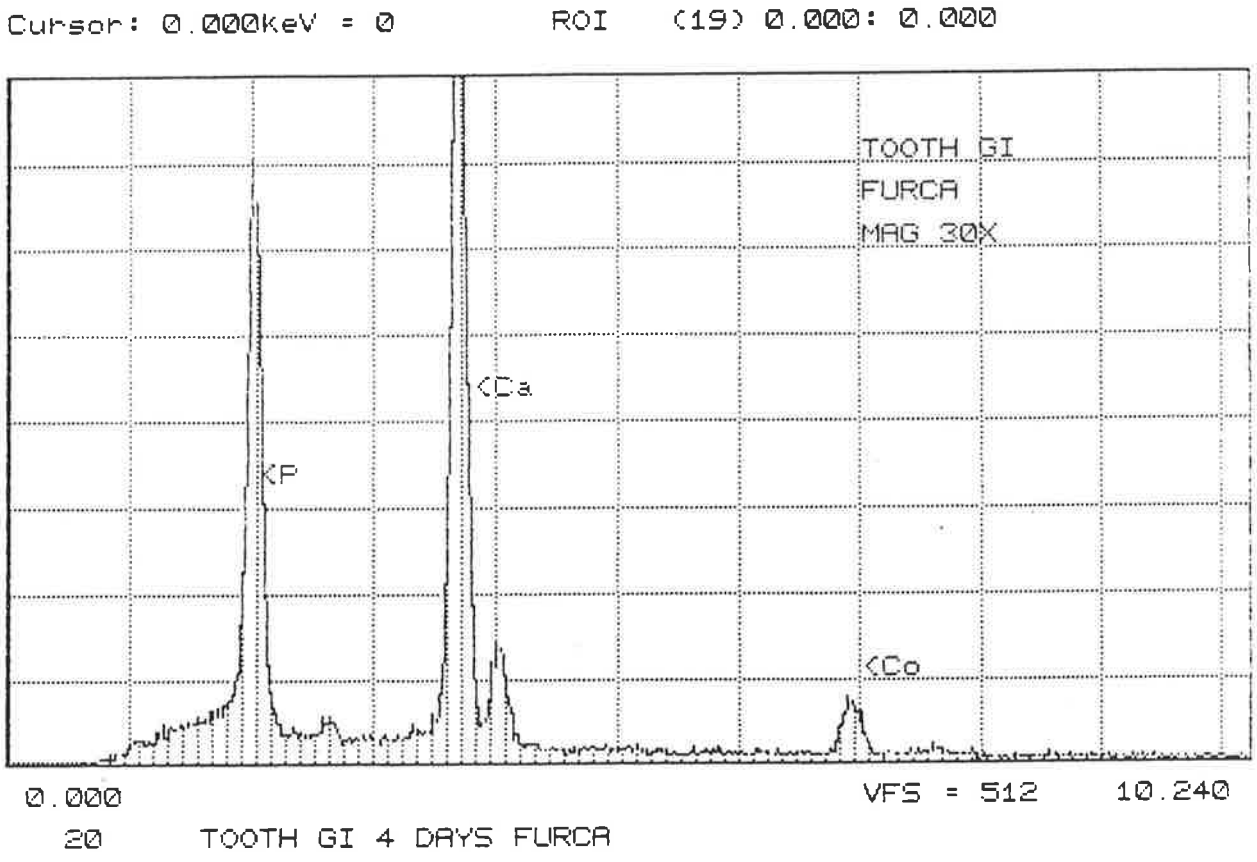

Figure 3.13. X-Ray Spectrum Representing a Score of Three.

3.4.7.b. X-Ray Imaging Analysis.

X-ray mapping has been a part of electron microanalysis almost since it's inception. The principal aim is to provide spatial information of the distribution of elements. Simply a pulse recorded by an X-ray detector is shown as a bright dot on the CRT display of the computer. Variation in the dot intensity then defines variations in the abundance of the element of interest. Several factors are involved.

The X-ray spatial resolution determines the lowest resolution between X-rays before smearing occurs. The level of the incident energy, and the mean sample density (mean atomic number) have an effect on the excited volume of the sample, and therefore limit the resolution. The resolution of the image process is inversely proportional to the density of the material being analyzed. The diameter of the excited volume about the electron beam can be calculated easily. Reed (1975) has given the expression:

$$\text{Resolution diameter} = 0.231 \frac{(Eo^{1.5} - Ec^{1.5})}{P}$$

Where Eo = incident electron energy
Ec = excitation energy for the element
P = density of the specimen

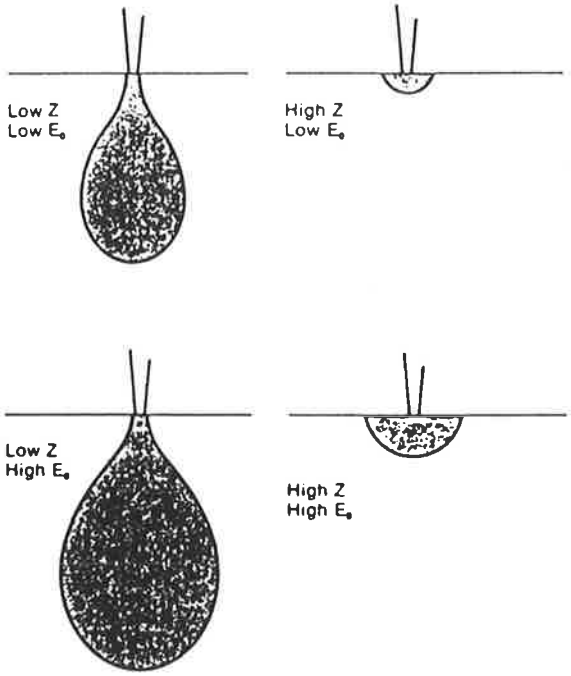

Fig. 3.14. Resolution Limits of X-Ray Image

Schematic depiction of the variation of the variation of the interaction volume shape with average sample atomic number (Z) and electron beam accelerating voltage (E_O).

Defocussing effects may be induced by the surface variation of the specimen such as the normal variations of tooth anatomy. The variation in the path length of the radiation in the sample results in differences in observed X-ray intensity not related to compositional changes.

The image data is converted to a digital signal where the system codes the signal to a range of 0-255, black to white respectively, giving 256 grey levels. These can be colour coded to give false colour images, and importantly it is a simple matter to deduce areas of cobalt phases where they give rise to different signal levels.

An image processing programme was used via an EDS system to produce X-ray image maps of the four elements; calcium, phosphorous, cobalt and germanium. The germanium was used to provide a map of background levels. Visualization of the cobalt map was improved by magnification of the image, which allowed a photographic record to be taken from the visual display unit of the computer, using a Nikon FM camera, a 50 mm macro lens, Ektachrome colour slide film (64 ASA), and exposure units of f5.6 and 1/2 second.

The maps were assigned a score of 0, 1, 2, or 3 in a similar fashion to those of the X-ray spectrum. The scoring system was as follows. A score of zero was given when there was no tooth outline, and there was less than 10 bright red spots (Fig. 3.15). A score of one was assigned when a faint outline of the tooth was seen, and greater than 10 and less than 50 bright red spots were seen (Fig. 3.16). A score of two was assigned when the tooth had a clear outline with more than 50 bright red spots, or 1 to 6 green spots were present (Fig.3.17). A score of three was assigned where the tooth had a distinct outline, and there were 6 or more green spots present (Fig.3.18).

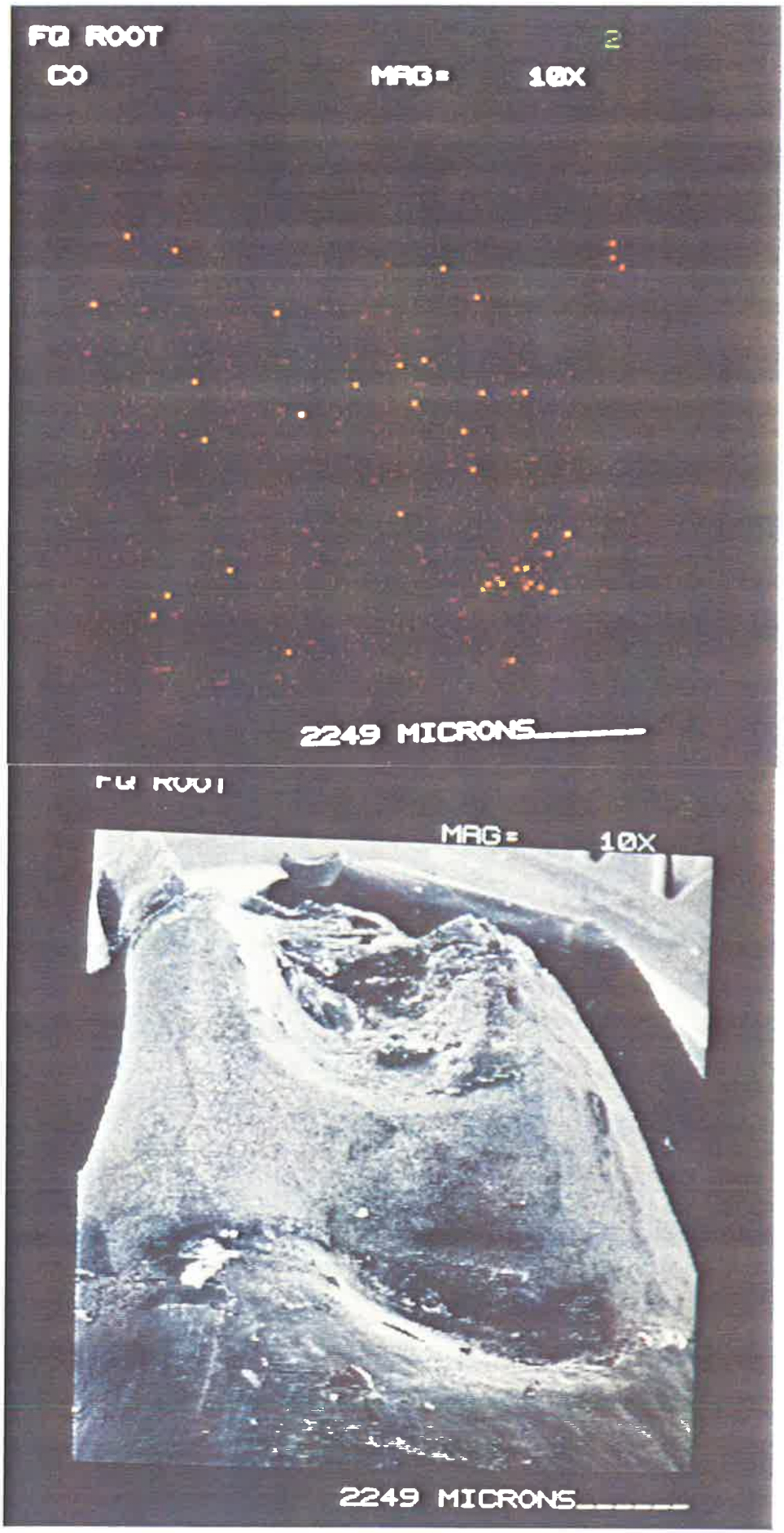

Figure 3.15. X-Ray Image Representing a Score of Zero (SEM Image top).

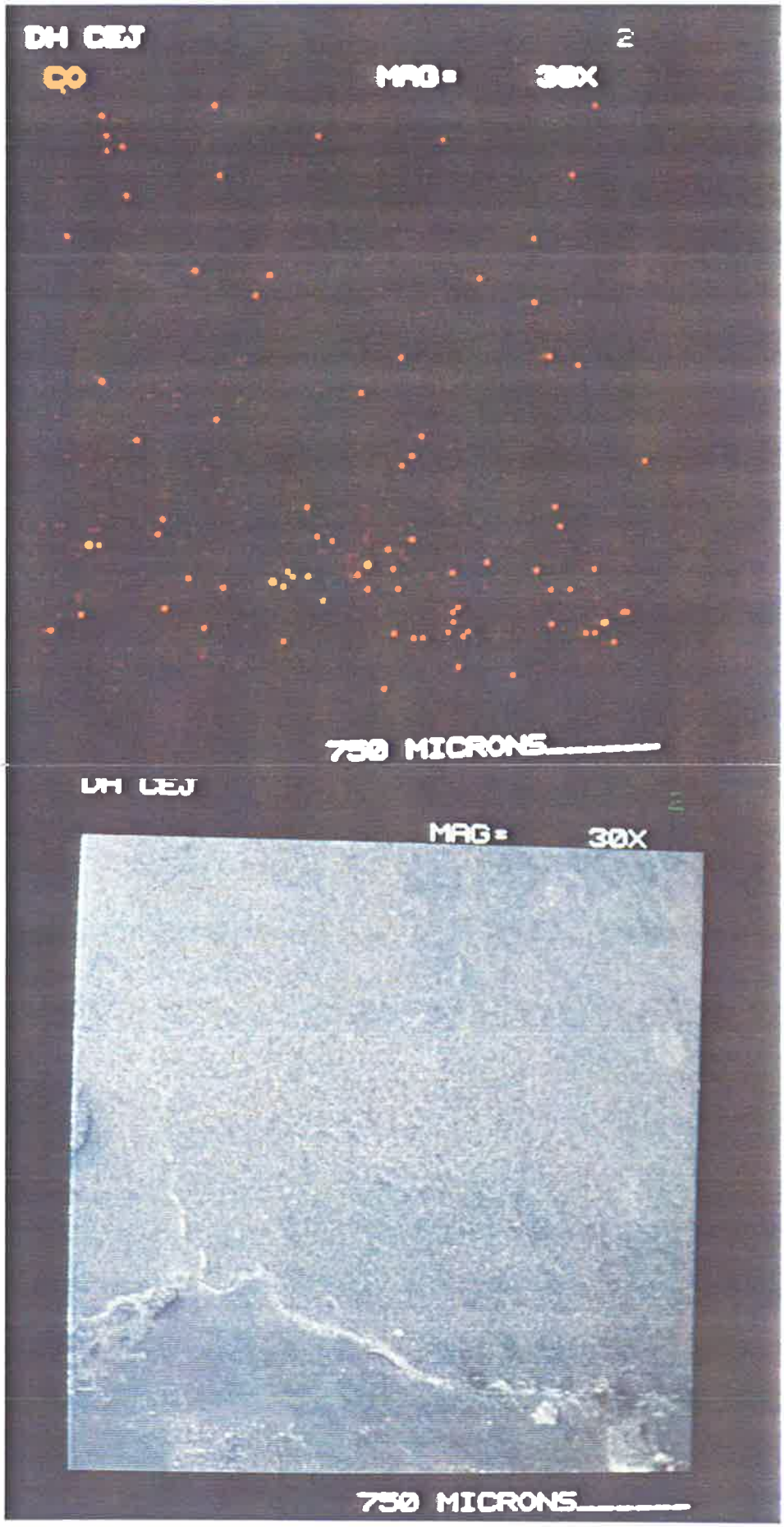

Figure 3.16. X-Ray Image Representing a Score of One (SEM Image top).

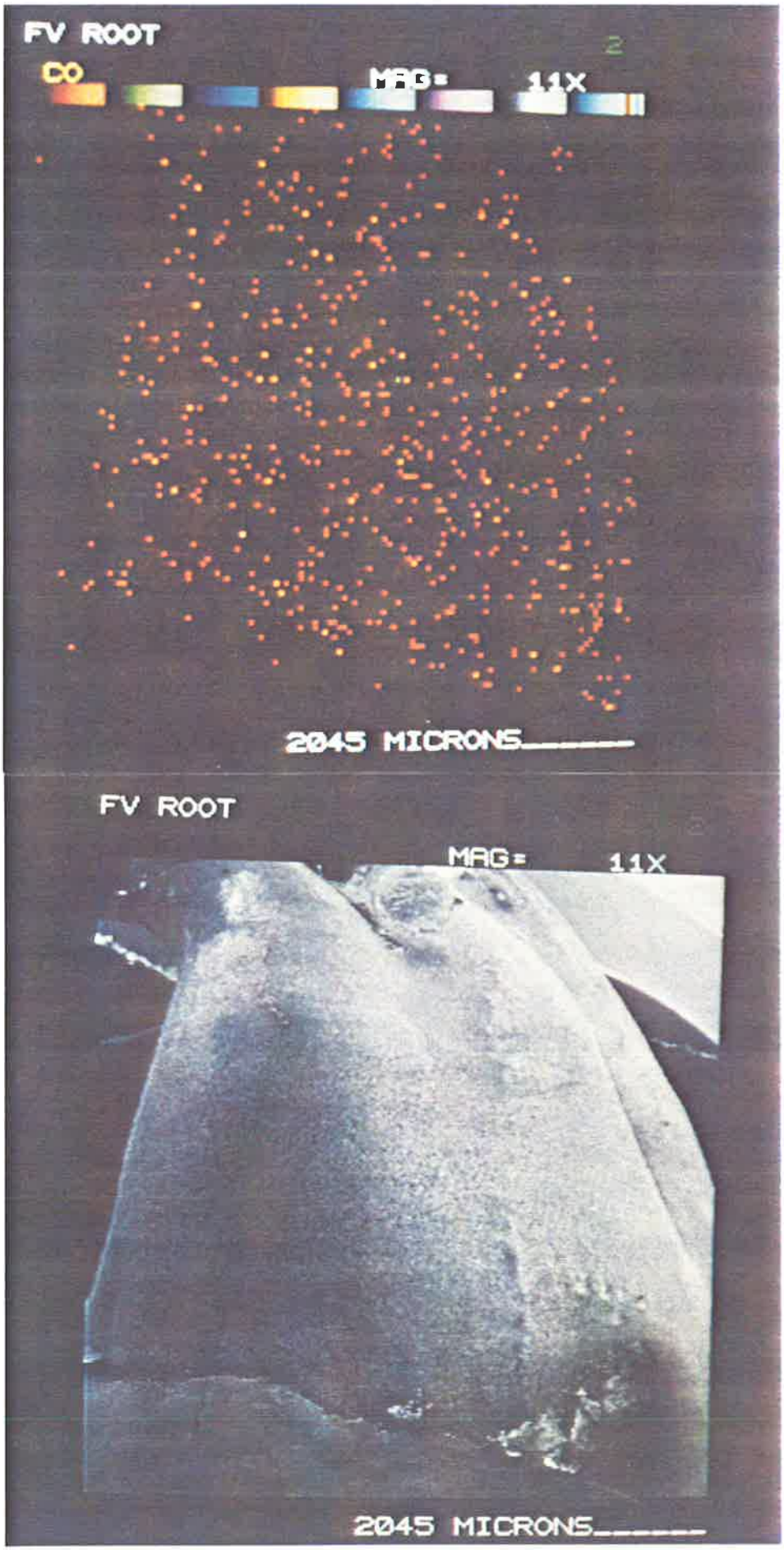

Figure 3.17. X-Ray Image Representing a Score of Two (SEM Image top).

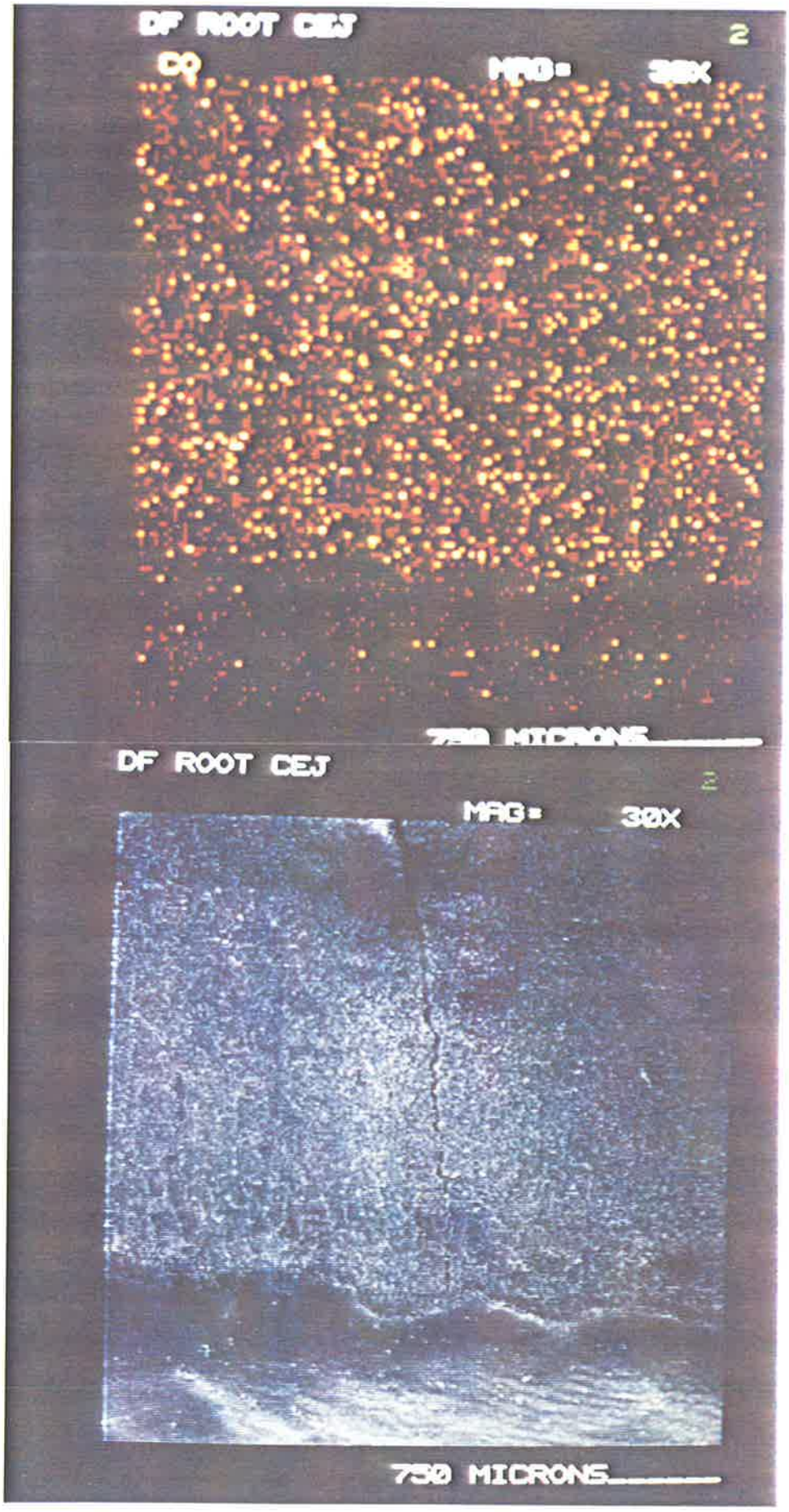

Figure 3.18. X-Ray Image Representing a Score of Three (SEM Image top).

The assessment for leakage was made on the pattern of cobalt detected on the root surface. Cobalt localized at the apex along with greater concentration of cobalt in the apical third was taken as evidence of leakage. This was particularly the case if the pattern on the root surface resembled that of the cobaltous salt flowing from the apex along the root surface. Leakage from the coronal region was established if there was evidence of a line of cobalt extending over the enamel surface past the coronal seal. Cobalt associated with an accessory canal was recorded as leakage and excluded from permeability data.

3.4.8. Treatment of Data.

The analysis was performed in two ways. Firstly simply statistical calculations were made to produce a mean and standard deviation for each measurement time in each group. Comparison between the areas of interest for the X-ray spectra and X-ray mapping were performed after consulting with Mr. P, Leppard (Statistician Adelaide University). The comparative statistics were performed on the Adelaide University computing system, Digital Equipment Corporation, VAX/VMS Version V4.6. The statistical software programme used was the BMDP Statistical Software package (1988, BMDP Statistical Software Inc., Los Angeles, Ca. U.S.A.).

Analysis of the time effect for each of the areas of interest was performed using a one way analysis of variance programme 1V from the BMDP Statistical Software package. Analysis of the effect of sex, and age were performed using the same package.

The analysis of the differences between the various areas of interest were performed using a one way analysis of variance with repeated measures in the programme 2V of the BMDP Statistical Software package. Comparison between the groups with, and without furcations, and accessory canals was performed using the same statistical programme.

CHAPTER 4. RESULTS OF MOLECULAR PERMEABILITY STUDIES.

4.1. Area Permeability Studies.

4.1.1. Material collected.

The material collected consisted of first, second and third molars, from the Oral Surgery clinic of the Adelaide Dental Hospital, and participating Oral Surgeons in private practice. A total of 60 teeth were used as described in Chapter 3, one tooth was discarded for technical reasons. The distribution of the extracted teeth is shown (Table 4.1). The mean age was 33.08 years, the range was from 13 to 69 years, collected from 31 males and 28 females. A total of 22 of the 59 teeth (38.98%) were impacted third molars. Upper third molars comprised the majority of teeth used, because they were less likely to suffer root surface damage during extraction.

Table 4.1. Table of the Teeth Used.

Molar Teeth		Number	Male	Female	mean age
Max.	6	8	8	0	31.88 +/- 13.85
	7	15	11	4	39.20 +/- 14.83
	8	16	5	11	29.38 +/- 13.61
Mand.	6	7	4	3	27.43 +/- 13.35
	7	7	1	6	36.43 +/- 14.70
	8	6	2	4	31.50 +/- 13.85
Total		59	31	28	33.08 +/- 16.17
Maxillary		39	24	15	
Mandibular		20	7	13	

Tritium (100ul of 250 uCi/ml) was placed within the prepared pulp chamber and sealed. In order to calculate the total amount of tritium available for diffusion, 100ul of the solution was added to 3ml of scintillation cocktail. This was repeated five times to calculate an average. The calculated mean CPM for 100ul of 250uCi/ml Tritium in this

experiment was 9,744,944.89. This allowed the CPM measured during the experiments to be calculated as percentages of the total amount of tritium available, which allowed comparison between experiments with varying radiolabels.

4.1.2. Full Wax - Control Results.

This group consisted of a group of 12 teeth, 10 from females and 2 from males (Table 4.2.). The mean age of this group was 35.17, with a standard deviation of 15.71 years, and with a range of 17 to 59 years.

Table 4.2. Material Used in Full Wax Experiment.

Molar Teeth	Number	Male	Female
Max. 6	-	-	-
7	1	-	1
8	6	-	6
Mand. 6	-	-	-
7	3	1	2
8	2	1	1
Total	12	2	10
Max. teeth	7	-	7
Mand. teeth	5	2	3

The teeth had 100*u*l sample withdrawn from the bathing PBS solution at the designated times 0, 1, 2, 4, 8 hours, 1, 2, 4, 8, 16, 24 days. The teeth were used to calculate a mean for each collection time which are set out (Table 4.3.). Two teeth, designated O and U, had persistently higher counts than the others, with their CPM being greater than five times any of the others in the group.

No significance tests were done on this group separately, but as can be seen later comparing all groups there was a significant increase with time. Table 4.3 shows a steady and significant rise in CPM registered with time.

Table 4.3. Results of Full Coverage Teeth.

Time	Counts Per Minute		
Hours	Mean	SD	Percentage
0	14.33	2.96	.0001
1	19.78	12.64	.0002
2	23.32	15.57	.0002
4	42.70	33.84	.0004
8	90.59	75.06	.0009
24	439.75	375.98	.0045
48	946.39	892.26	.0096
96	2635.93	3055.54	.0267
192	6240.27	6553.83	.0632
384	17868.24	20855.17	1.8006

4.1.3. Furca Open Group.

This group of teeth had the furcation region left free of wax and the remaining tooth root sealed with wax and varnish. Seventeen teeth comprised this group, 14 from males and 3 from females. The mean age of this group was 30.94 years with a standard deviation of 11.65, and a range of 15 to 56 years. The analysis of the teeth used is shown (Table 4.4.).

At the designated times 100*u*l of the bathing PBS solution was withdrawn and mixed with the scintillation cocktail to derive the CPM for each tooth. The means for each time, along with their standard deviations are shown (Table 4.5.). No separate significance test was conducted in the furca open group, but the later significance test found all groups to have a significant time effect, and as can be seen in Table 4.5 there is a significant increase in CPM with time.

Table 4.4. Material Used in Furca Open Experiment.

Teeth		Number	Male	Female
Max.	6	5	5	-
	7	7	7	-
	8	-	-	-
Mand.	6	3	2	1
	7	2	-	2
	8	-	-	-
Total		17	14	3
Max. teeth		12	12	-
Mand. teeth		5	2	3

Table 4.5. Results of the Furca Open Experiments.

Time	Counts Per Minute		
Hours	Mean	Std. Dev.	Percentage
0	13.00	3.38	.0001
1	51.54	67.45	.0005
2	119.39	149.36	.0012
4	274.86	342.21	.0028
8	988.86	1091.32	.0100
24	5537.35	4738.94	.0561
48	14200.61	8655.84	.1437
96	28809.95	13921.52	.2917
192	46383.51	15263.25	.4696
384	52864.61	21564.08	.5352
576	66384.20	10137.09	.6720

4.1.4. Furca Sealed Experiment.

This group consisted of fourteen teeth which had the furcation region sealed with wax and varnish, while the remaining root except for the root tip was left uncovered. These teeth were collected from 9 males and 5 females. The mean age of the group was 38.93 years, with a standard deviation of 13.52, and a range of 15 to 60 years. The distribution of teeth by type is shown (Table 4.6.).

Table 4.6. Material Used in Furca Sealed Experiment.

Tooth	Number	Male	Female
Max. 6	3	3	-
7	7	4	3
8	-	-	-
Mand. 6	3	2	1
7	1	-	1
8	-	-	-
Total	14	9	5
Max.	10	7	3
Mand.	4	2	2

The experimental procedure was the same as the furca open and full coverage experimental groups. At the designated times 100μl of bathing PBS solution was withdrawn and added to the scintillation cocktail, and the counts recorded. The resultant mean CPM and standard deviation of these are shown (Table 4.7.). No separate significance test was conducted in the furca closed group, but all groups were found to have a significant time effect, and as can be seen from Table 4.7 there is a significant increase in CPM with time.

Table 4.7. Results of Furca Sealed Experiments.

Time	Counts per minute		
Hours	mean	Std. Dev.	Percentage
0	20.33	18.40	.0002
1	95.25	136.68	.0010
2	212.14	233.38	.0021
4	529.86	450.87	.0054
8	2143.18	1557.58	.0217
24	10495.43	6245.34	.1062
48	17711.81	8722.96	.1793
96	29860.64	13685.14	.3023
192	42255.10	12636.71	.4278
384	53602.71	9167.40	.5426

4.1.5. Apical Wax Seal Only.

This experimental group consisted of twelve teeth with a mean age of 28.67 years, and a standard deviation of 16.63 years. The age ranged from 13 to 69 years, and 8 teeth were from females and 4 from males (Table 4.8.).

The counts per minute for these teeth were measured as in preceding experiments, but several samples were lost at 48 and 96 hours due to technical difficulties. Two measurements were recorded at 48 hours, and six measurements were made at 96 hours. Earlier experimentation had shown little change after day 8 using tritium indicating the diffusion was reaching equilibrium, no measurement was made after 8 days.

Table 4.8. Material Used for Apical Seal Experiment.

Teeth		Number	Male	Female
Max.	6	-	-	-
	7	1	-	1
	8	7	4	3
Mand.	6	1	-	1
	7	2	-	2
	8	1	-	1
Total		12	4	8
Max.		8	4	4
Mand.		4	-	4

Table 4.9. Results of Apical Seal Experiment.

Time	Counts per minute		
Hours	Mean	Std. Dev.	Percentage
0	11.86	3.41	.0001
1	61.50	41.72	.0006
2	310.70	212.14	.0031
4	1297.62	804.57	.0131
8	4528.17	2240.00	.0458
24	18125.97	6137.25	.1835
48	27450.32	4401.80	.2779
96	32666.21	13712.81	.3307
192	43318.00	9162.45	.4385

Two teeth (YC and YM) demonstrated scintillation counts that equated with a continuity between the pulp chamber and the external surfaces, and therefore were rejected. It was presumed that accessory canals were left unsealed at the conclusion of the sealing process. After rejection of the two results the calculated mean CPM were as

shown (Table 4.9.). This group was seen to have a significant time effect, and as can be seen from Table 4.9 there is an increase in CPM with time.

4.1.6. No Wax Seal Teeth - Open Apex.

This group of four teeth was a small negative control group, and is shown (Table 4.10.). The mean age of the group subjects was 28 years and the standard deviation was 11.34. The age range of the sample was 18 to 41.

Table 4.10. Material Used for No Wax Seal Experiment.

Teeth	Number	Male	Female
Max. 8	2	1	1
Mand. 8	2	1	1
Total	4	2	2

Table 4.11. Results of the Non Sealed Teeth.

Time	Counts per minute		
Hours	Mean	Std. Dev.	Percentage
0	11.83	1.53	.0001
1	1647.50	2194.80	.0167
2	2963.81	3358.05	.0300
4	4276.32	4939.52	.0433
8	5389.84	5685.59	.0546
24	7198.49	5282.65	.0729
48	12967.50	-	.1313
96	23295.55	-	.2358
192	54890.00	8631.50	.5557

Samples were taken from these teeth at the designated times, scintillation CPM were tabulated (Table 4.11.). Malfunction of the scintillation counter destroyed several

samples, and as a result only a single value was collected for this group at the 48, and 96 hour collection times. This group was too small for statistical analysis.

4.1.7. Comparison of Permeability of Root Areas.

Differences between the groups for diffusion rates was analyzed, and the Figure 4.1. indicates the mean values versus time for each of the five groups.

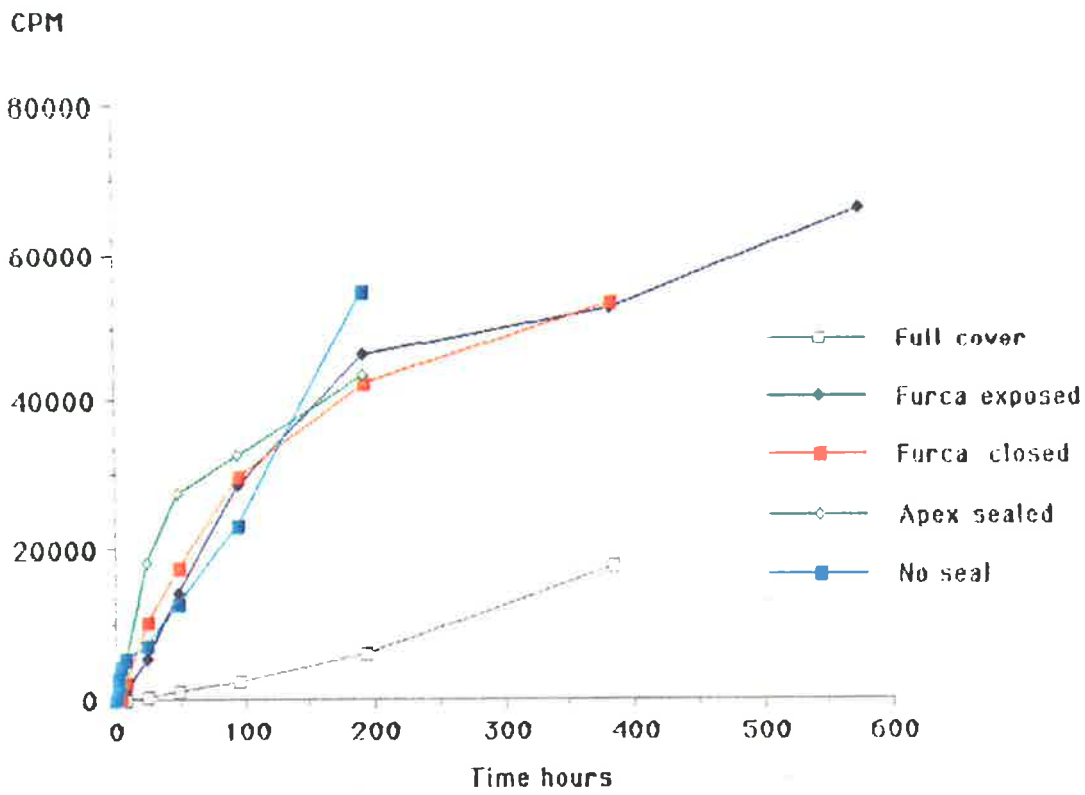

Figure 4.1. Mean Values of Root Regions Versus Time.

The statistical analysis of this material used the Adelaide University Computer and the BMDP 5V statistical software package. A comparison of the results from all five groups indicated that there was a time and treatment effect. The Chi square analysis with the four degrees of freedom indicated there was a treatment effect, and had a value of 26.34 ($P < .001$). Chi square analysis also indicated a time effect, with a value of 475.37 ($P < .001$).

However the results did not indicate any significant difference between the furcation closed and the furcation opened groups. The full wax coverage group were significantly less than with a two sided probability value of .0000 as shown (Table 4.12.). The Furca open and Furca closed group were shown to be similar with probability values of .1267 and .2061 respectfully. The Apex only group was seen as having a significantly higher count per minute result at the 0.5 percent level. Examination of the coefficients and standard errors for the two treatments Furca open and Furca closed indicated these two were not significantly different, and had a asymptotic t-value of .24.

Table 4.12. Results Table of 5V Analysis.

Parameter	Estimate	Asymptotic SE	Z	P-Value
Const.	4517.00	1587.12	2.846	.0044
Full wax	-11523.15	2711.28	-4.250	.0000
F. Closed	3102.55	2453.70	1.264	.2061
F. Open	3944.12	2582.73	1.527	.1267
Apex wax	8201.74	2930.78	2.798	.0051

Wald Tests of Significance of Fixed Effects and Covariates.

Test	DF	Chi-square	P-value
Treatment	4	26.34	.0000
Time	1	475.37	.0000

4.1.8. Diffusion per Unit Area Results.

Although the statistical analysis indicated there were no differences between the furca open and the furca closed groups, the area available for diffusion of the chemicals was substantially different. The surface area for diffusion was calculated for each tooth used in the study to calculate the mean area available able for diffusion for the following groups, furca open and furca closed, and the apical wax only group.

As described in Chapter 3 this three dimensional surface area was converted to two dimensional surface, and measured using computer techniques, and the results are shown (Table 4.13.). The reason the apical wax group had slightly less surface area than the furca closed group was this group contained some third molars which are generally smaller teeth, and have smaller root systems.

Table 4.13. Surface Area for Diffusion.

Area mm	Mean	Std.Dev.
Furca Open	62.41	14.54
Furca Closed	289.82	65.81
Apical Wax	255.73	58.35

The surface area measurement for each tooth was used to calculate the number of counts per minute per unit surface area, and these were then collated to calculate means for each time in each group.

4.1.8.a. Furca Open Teeth.

The calculated means and standard deviations are seen below (Table 4.14.) for each time in this group.

Table 4.14. Results for Furca Open per Unit Surface Area.

Time	Counts per min. / mm^2	
Hours	Mean	Std. Dev.
1	0.95	1.19
2	2.15	2.33
4	4.76	5.35
8	16.42	16.49
24	89.62	65.54
48	242.86	115.27
96	470.20	169.54
192	760.03	218.21
384	953.63	294.46

4.1.8.b. Furca Sealed Teeth.

The calculated means and standard deviations at the designated times are shown (Table 4.15).

Table 4.15 Results of Furca Sealed per Unit Surface Area.

Time	Counts per min./ mm^2	
Hours	Mean	Std.Dev.
1	0.33	0.47
2	0.72	0.80
4	1.79	1.48
8	7.18	4.77
24	35.04	17.85
48	62.12	22.36
96	111.31	28.22
192	147.13	35.88
384	190.90	41.98

4.1.8.c. Results of Apical Wax Only.

The calculated means and standard deviations at the designated times are shown (Table 4.16.). Technical difficulties with the scintillation counter resulted in small number of teeth for the 48 hour results.

Table 4.16. Results for Apical Wax Only per Unit Surface Area.

Time	Counts per min. / mm^2	
Hour	Mean	Std. Dev.
1	0.24	0.18
2	1.29	0.92
4	5.66	3.34
8	19.72	8.17
24	82.16	20.02
48	115.79	28.45
96	126.41	49.04
192	201.47	42.79

Analysis of the diffusion per unit area between the three groups was carried out using a BMDP 3V statistical software programme. This demonstrated there were significant differences between the three groups, at the 0.5% level. The Chi-square value with two degrees of freedom was 10.832, and the probability was 0.004. The furca closed and apex only wax groups were found to be equal over the 16 day experimental period. The furca open group was found to be significantly more than the other two. This indicates the furcation area was an important area of diffusion (Figure 4.2.).

Care is required with this analysis. The early times were the key regarding diffusion rates, while the total surface area available was the over-riding factor in the analysis at the end point when the experiments were reaching their equilibrium point. The three groups all had similar amount of tritiated water placed in them, but the furca

open group had significantly less surface area for diffusion, and therefore by physical laws would have a larger CPM/unit area at equilibrium.

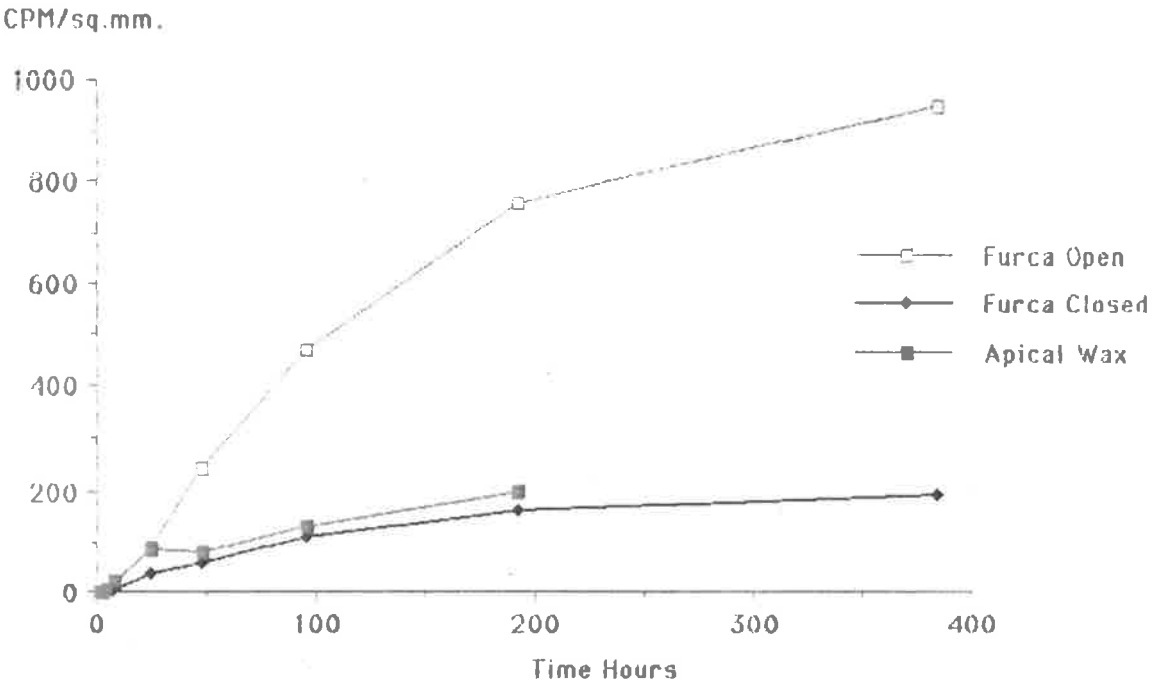

Figure 4.2. Mean Values of CPM/mm^2 Versus Time.

Similar significance tests were conducted after one and four days of the experiment. After one and four days there was a significant difference in three groups, both with probability of <0.001. The Chi-square was 17.274 and 17.476 for the one and four days respectively. As seen in the previous analysis the furca closed was significantly less than the furca open group, and equal to the apical wax groups. These findings

support the concept of the furcation being an important area for the diffusion of molecules.

4.1.9. Third Molar Teeth.

These teeth used were collected as part of the experiment on the permeability of root areas, and due to the lack of availability of the first and second molar material, and the greater availability of third molars included to make up sufficient numbers. The label used in these teeth was 100*u*l of 250*u*Ci/ml Tritium sealed in the pulp chamber. There were two experimental groups of teeth full wax coverage and apical wax seal only. Due to the lack of third molar teeth with definitive furcations this was not examined.

4.1.9.a. Full Wax - Control Teeth.

There were 7 third molars used as part of this experiment ranging in age from 18 to 55 years and a mean age of 25.71 years, with a standard deviation of 15.00. Six of the teeth were collected from females and one from a male. Six of the teeth were upper third molars.

Table 4.17. Material Used for Full Wax Third Molars.

Tooth	Number	Male	Female
Max. 8	6	-	6
Mand. 8	1	1	-

At the times assigned in the preceding tables 100*u*l samples were drawn from the bathing fluid and the counts per minute were ascertained. The resultant means and standard deviations at the designated times are shown (Table 4.18.).

Table 4.18. Results of Full Wax Third Molars.

Time	Counts per minute		
Hours	Mean	Std. Dev.	Percentage
0	14.21	3.15	.0001
1	15.14	3.68	.0001
2	17.79	5.83	.0002
4	28.79	11.80	.0003
8	70.57	48.65	.0007
24	404.79	354.71	.0041
48	800.14	726.24	.0081
96	2379.84	2159.91	.0186
192	4846.68	4317.26	.0373
384	8879.05	7263.35	.0899

4.1.9.b. Apical Wax Only.

This group consisted of eight teeth, and had a mean age of 23 years and a standard deviation of 5.18. The age of the teeth ranged from 18 to 34 years. Seven of the teeth were maxillary third molars, and four teeth were from males and four from females (Table 4.19.). These teeth were sampled as described above at the designated times, and the means of each time are shown (Table 4.20.).

Table 4.19. Material Used for Apex Wax Third Molars.

Tooth	Number	Male	Female
Max. 8	7	4	3
Mand. 8	1	-	1

Table 4.20. Results of Apical Wax Third Molars.

Time	Counts per minute		
Hours	Mean	Std.Dev.	Percentage
0	12.81	3.45	.0001
1	54.06	43.82	.0005
2	289.69	216.82	.0029
4	1256.38	758.72	.0127
8	4381.90	1887.90	.0444
24	18060.38	4267.76	.1828
48	27450.32	4401.80	.2779
96	25941.78	2524.52	.2626
192	46666.00	7798.61	.4724
384	-	-	-

These two groups were significantly different indicating the difference between the two types of treatment and supported the use of this model as a method of studying the effect of diffusion through tooth roots.

4.1.10. Comparison First and Second Molars to Third Molars.

This compared the full wax coverage and apical wax first and second molars to similarly treated third molars.

4.1.10.a. Full Waxed First and Second Molars.

This group consisted of four teeth, with a mean age of 43.75 years, standard deviation of 14.5 years, and ages ranged from 25 to 51 years. The material used is displayed (Table 4.21.). The counts per minute were calculated by collecting samples as previously described, and the means are shown (Table 4.22.).

Table 4.21. Material Used for Full Wax First and Second Molars.

Tooth		Number	Male	Female
Max.	6	-	-	-
	7	1	-	1
Mand.	6	-	-	-
	7	3	1	2
Total		4	1	3

Table 4.22. Results of Full Wax First and Second Molars.

Time	Counts per minute		
Hour	Mean	Std.Dev.	Percentage
0	14.75	3.18	.0001
1	30.50	18.52	.0003
2	36.63	21.06	.0004
4	63.13	53.20	.0006
8	106.38	109.09	.0011
24	501.00	519.47	.0051
48	1310.00	1255.50	.0133
96	4852.88	4144.82	.0491
192	11083.06	8424.60	.1122
384	32837.54	26947.55	.3324

4.1.10.b. Apical Wax Only First and Second Molars.

This group consisted of four teeth with a mean age of 40, and a standard deviation of 26.36 years. The ages ranged from 13 to 69 years. The teeth used are shown (Table 4.23.).

Table 4.23. Material Used for Apex Wax First and Second Molars.

Tooth	Number	Male	Female
Max. 6	-	-	-
7	1	-	1
Mand. 6	1	-	1
7	2	-	2
Total	4	-	4

Table 4.24 Results Apex Wax First and Second Molars.

Time	Counts per minute		
Hours	Mean	Std. Dev.	Percentage
0	10.38	2.53	.0001
1	91.50	7.07	.0009
2	394.75	241.48	.0040
4	1462.60	1314.65	.0148
8	5936.73	3422.15	.0601
24	20690.75	9816.24	.2095
48	-	-	-
96	42752.86	14537.32	.4328
192	54243.33	14537.32	.5491
384	-	-	-

The 100*u*l sample was collected at the designated times and the number of counts per minute were calculated via a scintillation method previously described, and used to calculate the means (Table 4.24.). No data was collected for the 384 hour collection time. Technical difficulties with the scintillation counting machine at 48 hours prevented collection of data for this time.

Comparison between the full wax coverage first and second molars and the third groups found the teeth had no effect on the permeability, and the groups were

comparable. Similar results were seen between the Apical wax groups. Figure 4.3. shows the apical wax first and second molars gave evidence of leaking at the 192 hour time. Figure 4.3. visually compares the diffusion of the material versus time using the calculated means.

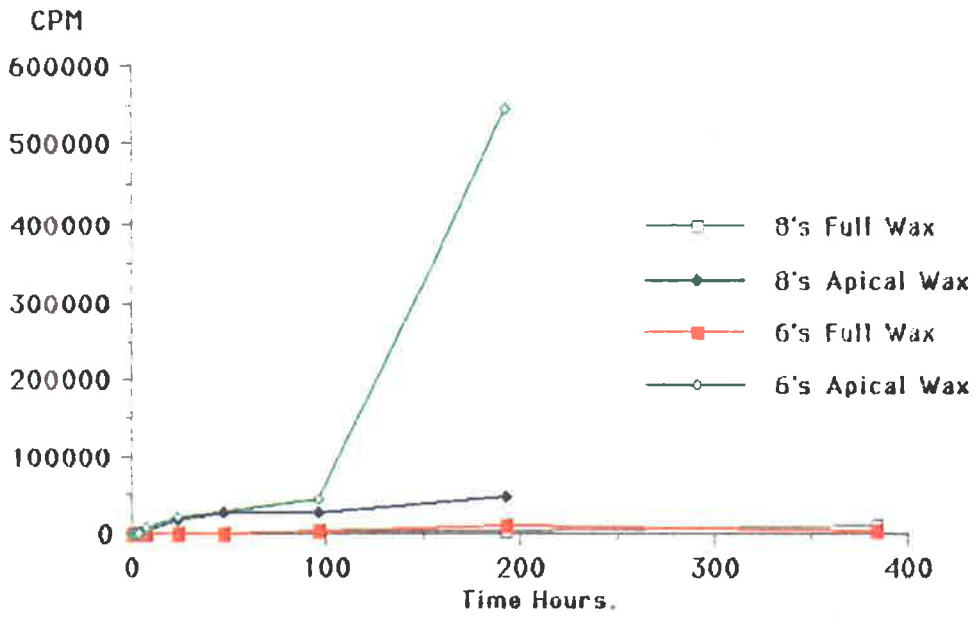

Figure 4.3. Comparison First and Second Molars to Third Molar Results.

4.2. Molecular Weight Studies.

This Experiment used third molars because of their ready availability and intact root surfaces to study different molecular weight molecules. A total of 69 teeth were used in this experiment, and all were used twice. In the first experiment either glucose or dextran was placed within the tooth, and following decontamination the teeth were treated with tritium. The mean age of the teeth collected was 22.81, with a standard

deviation of 5.89 years. The age range of the patients was from 16 to 53 years. The details of the material used in the molecular weight studies of the tooth root is shown (Table 4.25.)

Table 4.25. Material Used for Tooth Root MW Studies.

Tooth	Number	Male	Female
Max. 8	64	27	37
Mand. 8	8	-	8
Total	72	27	45

In addition to this it was decided to study the effect on crown dentine of the various molecular weight chemicals. The material used for crown dentine studies was that of third molar material collected from the same sources as indicated above. This group had no requirement for intact root structure, but required an intact crown, and subsequently had a larger proportion of lower third molars than the root dentine study. The mean age for the crown dentine group was 21.95, with a standard deviation of 2.90 years. The teeth were from patients of ages varying from 16 to 28 years. Details of the material used is shown (Table 4.26.).

These experiments were carried out using Tritiated compounds of water, dextran, and glucose. The study groups consisted of the following normal root surfaces, and root planed surfaces, with each group having experimental teeth (Apical Wax only) and controls (Full wax covering). An additional study was carried out on crown dentine. The preparation techniques were carried out as described in the Materials and Methods Chapter 3.

Table 4.26. Material Used for Crown Dentine MW Studies.

Tooth	Number	Male	Female
Max. 8	41	13	28
Mand. 8	32	17	15
Total	73	30	43

This study has the counts per minute provided by the scintillation spectrometer converted to percentages of the total amount of radio-label available to allow for direct comparison between the different molecular weight substances as the activity of the compounds used varied. The CPM represented by percentages are shown giving means and standard deviation for each compound.

4.2.1. H^3-Glucose Experiments.

4.2.1.a. Glucose Label Used.

Tritium labelled Glucose MW 180.2 was used as a diffusion compound to review the effect of increasing size of the molecule on tooth root diffusion. The batch supplied by the New England Reactor contained 250 *u*Ci of ^{3}H-Glucose in 0.25 ml of sterile solution. This was diluted to 6ml with PBS and the final activity of the label used was 41.7*u*Ci/ml.

The total activity of ^{3}H-Glucose added to the tooth was measured at each experiment by adding 100*u*l of tritiated glucose to scintillation cocktail and a mean calculated (Table 3.2). This allowed for decay with age and variation in experimental procedure. These values represent the total activity of the Glucose material added to the pulp chamber. The activity calculated for individual teeth was divided by this mean value of total activity to calculate a percentage. The percentage of the label compound that had diffused through the root can be compared to other compounds regardless of the initial activity of label.

4.2.1.b. Glucose Non Root Planed Third Molars.

The normal root surfaces of this group of 14 teeth were treated by alcohol wash, and had wax placed around their apices (Table 4.27.). The mean age was 23.21, with a standard deviation of 10.74 years, and a range of 18 to 53 years.

Table 4.27. Material Used Glucose Non Root Planed Third Molar Study.

Tooth	Number	Male	Female
Max. 8	11	4	7
Mand. 8	3	-	3
Total	14	4	10

Table 4.28. Results H^3-Glucose Third Molars - Non Root Planed.

Time	Counts per minute		Percentage of Label Used.	
Hours	Mean	Std. Dev.	Mean	Std. Dev.
0	-	-	-	-
1	42.75	36.10	.0042	.0035
2	57.46	35.20	.0056	.0034
4	73.29	49.00	.0072	.0048
8	133.86	77.10	.0131	.0075
24	648.32	338.24	.0634	.0331
48	1344.46	1636.58	.1315	.1600
96	2127.64	899.54	.2080	.0880
192	4362.97	1644.46	.4266	.1608
384	5708.28	1751.30	.5582	.1712
576	7350.75	2268.02	.7188	.2218
768	8748.45	2631.27	.8554	.2573
960	9152.72	2643.60	.8950	.2585

Data were collected from the teeth at the designated times used in the preceding experiments, but due to the larger molecular size and anticipated slower diffusion the collecting time was extended to 40 days. Each tooth had 100*u*l of the bathing solution withdrawn at the examination times, which was mixed with scintillation cocktail mixture to allow calculation of the activity of the fluid. The means for each time are recorded appear in Table 4.28. The mean activity of 100*u*l of tritiated-Glucose was 1,022,678.99, and was used to calculate the percentage of compound present at each observation time.

4.2.1.c. Glucose Root Planed Third Molars.

Fifteen third molars, with mean age of 21.4 years, a standard deviation of 2.64 years, and ages ranging from 18 to 25 years, had their root surfaces thoroughly root planed by hand instruments prior to commencing the experiment. The teeth used are described in Table 4.29.

Table 4.29. Material Used for Glucose Root Planed Third Molar Study.

Tooth	Number	Male	Female
Max. 8	14	6	8
Mand. 8	1	-	1
Total	15	6	9

These teeth were sampled for up to 40 days as previously described. The mean activity of the tritiated-Glucose used in this experiment was 1,022,678.99. The results are shown in counts pre minute and percentages (Table 4.30).

Table 4.30. Results of H^3-Glucose Root Planed Third Molars.

Time	Counts per minute		Percentage of Label Used	
Hours	mean	Std.Dev.	mean	Std.Dev.
0	-	-	-	-
1	29.70	12.69	.0029	.0012
2	28.17	14.27	.0028	.0014
4	40.17	39.33	.0039	.0038
8	118.37	105.55	.0116	.0103
24	907.63	660.87	.0888	.0646
48	1641.00	1062.37	.1605	.1039
96	783.60	417.67	.0766	.0408
192	2244.27	1045.61	.2195	.1022
384	3496.61	1603.66	.3419	.1568
576	4749.60	2217.40	.4644	.2168
768	5951.37	3094.31	.5819	.3026
960	5886.20	3083.86	.5756	.3015

4.2.1.d. Glucose Control Teeth - Full Wax.

This group consisted of seven teeth with a mean age of 28.43, and a standard deviation of 9.16 years. The ages ranged from 20 to 44 years and the breakdown of teeth used is shown in Table 4.31. All teeth had their entire root surface sealed with wax and varnish.

Table 4.31. Material used for Glucose Control Third Molar Study.

Tooth	Number	Male	Female
Max. 8	7	5	2
Mand.8	-	-	-
Total	7	5	2

Table 4.32. Results of H^3-Glucose Non-Root Planed Control Third Molars.

Time	Counts per minute		Percentage of Label Used.	
Hour	Mean	Std.Dev.	Mean	Std.Dev.
0	-	-	-	-
1	11.00	6.28	.0011	.0006
2	9.88	2.69	.0010	.0003
4	11.13	2.29	.0011	.0002
8	16.25	2.90	.0016	.0003
24	57.63	41.91	.0056	.0041
48	168.75	120.09	.0165	.0117
96	295.38	271.84	.0289	.0266
192	1673.13	1796.72	.1636	.1757
384	2664.95	1807.64	.2606	.1768
576	5061.22	4540.11	.4948	.4439
768	6266.16	5051.59	.6127	.4940
960	6710.83	5399.16	.6562	.5279

The teeth were assigned to two groups, three were placed in the root planed group, and four in the non-root planed group. The teeth were sampled as described previously at the designated times. The data were then used to calculate means and standard deviations which appear in Tables 4.32. and 4.33. The mean activity of 100ul of H^3-Glucose was 1,022,678.99 CPM. The means of all H^3-Glucose third molar root experiments are shown for comparison in Figure 4.4.

Table 4.33. Results of H^3-Glucose Root Planed Control Teeth.

Time	Counts Per Minute		Percentage of Label Used.	
Hours	Mean	Std.Dev.	Mean	Std. Dev.
0	-	-	-	-
1	15.17	7.49	.0015	.0007
2	11.33	0.76	.0011	.0001
4	8.83	1.89	.0009	.0002
8	9.67	1.04	.0009	.0001
24	10.17	1.53	.0010	.0001
48	13.67	6.01	.0013	.0006
96	17.00	8.23	.0017	.0008
192	30.83	17.55	.0030	.0017
384	66.00	59.35	.0065	.0058
576	109.17	99.49	.0107	.0097
758	161.33	150.35	.0158	.0147
960	178.67	153.76	.0175	.0150

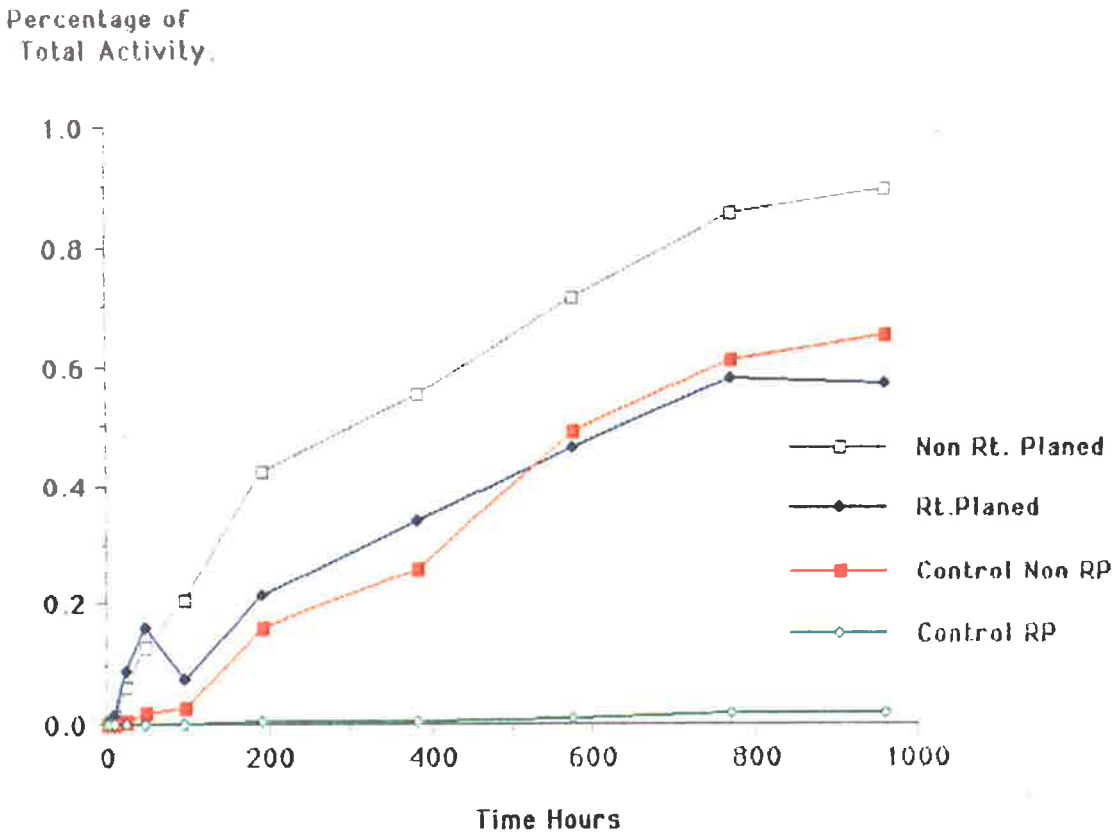

Figure 4.4. Mean Values of Glucose Root Permeability Versus Time.

4.2.1.e. Glucose Crown Dentine Studies.

This group used 25 third molar teeth with a mean age of 21.48, and a standard deviation of 1.96 years. The age ranged from 16 to 24 years, with 12 teeth from females and 13 teeth from males (Table 4.34.). This experimental groups represented the compilation of three separate experimental trials in crown dentine.

Table 4.34. Material Used for Glucose Crown Dentine Studies.

Tooth	Number	Male	Female
Max. 8	11	4	7
Mand. 8	14	9	5
Total	25	13	12

Fifty microliters was drawn from the 3ml bathing solution contained in the boat at the designated times used throughout the experiment. During experimentation two teeth, M and N, were removed from analysis because they had demonstrated obvious leakage as indicated by a reading of more than 2,000 at one hour. The results were combined to produce the Table 4.35. No readings at 96 hour time were taken due to a machine malfunction in experiment A.

Table 4.35. Results of H^3-Glucose Crown Dentine.

Time	Counts per Minute		Percentage of Label Used.	
Hours	Mean	Std.Dev.	Mean	Std.Dev.
0	-	-	-	-
1	226.18	249.50	.0157	.0151
2	767.71	789.19	.0523	.0475
4	2281.40	1726.64	.1656	.0973
8	3354.00	1368.87	.2497	.0677
24	11689.03	4909.93	.9549	.4109
48	24573.73	9415.66	2.0398	.7775
96	33603.43	12149.48	3.3205	1.2047
192	64330.42	33303.53	5.1074	1.7300
384	77405.41	45134.42	6.0226	2.2493
576	-	-	-	-
768	98108.83	62515.09	7.3688	3.0026
960	-	-	-	-

A graphic display is shown of the three separate experiments and their combined means indicating their similarity (Fig. 4.5). The results of the crown dentine studies clearly indicated that the crown dentine was more permeable than the root dentine even when differences in the size of bathing solutions was taken into account.

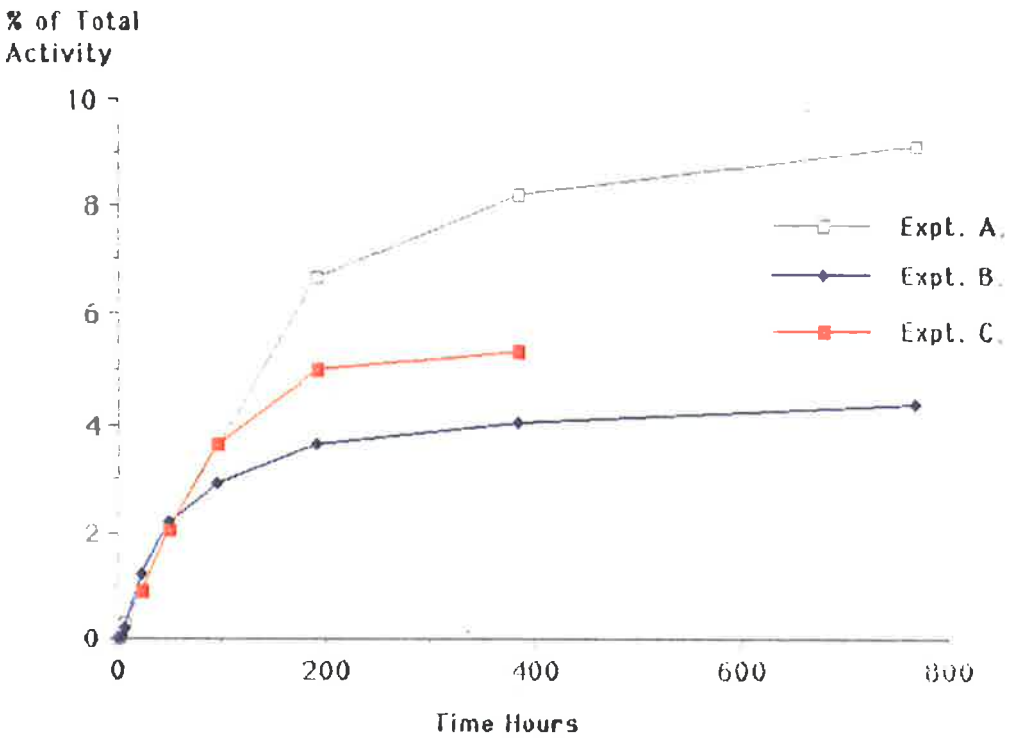

Figure 4.5. Mean Values of Glucose Crown Dentine Permeability against Time.

4.2.2. H^3-Dextran Experiments.

4.2.2.a. Dextran Label Used.

Tritium labelled dextran of MW 70,000 was used as a diffusion compound to test for the diffusion of medium size molecules. The compound was supplied as a freeze dried solid by Amerscham Nuclear Products which contained 250*u*Ci of ^{3}H-Dextran. The product was analyzed to contain 336 mCi/mg. One hundred microliters of 41.67*u*Ci/ml H^3-Dextran was placed inside the tooth in each case. The total activity of the 100*u*l added was calculated by micropipetting 100*u*l of H^3-Dextran into a vial with scintillation cocktail, mixed, and measured in the liquid scintillation spectrometer. This was done 5 times prior to the commencement of each study to allow for decay with time

of label, and variations in experimental procedure, and the mean calculated (see Table 3.3). Comparison between experiments required values to be expressed as a percentage by dividing the activity of the labelled Dextran in the bathing solution by the total activity.

4.2.2.b. Dextran Non Root Planed Third Molars.

The experimental group consisted of 16 teeth whose apices were sealed prior to placing 100*u*l H^3-Dextran inside the pulp canal systems. The age range was 16 to 29 years, with a mean age of 20.94 years, and a standard deviation of 4.06 years. Three teeth were from males and thirteen teeth were from females, and details are shown (Table 4.36).

Table 4.36.

Material Used for Dextran Third Molar Study - Non Root Planed.

Tooth	Number	Male	Female
Max. 8	14	3	11
Mand. 8	2	-	2
Total	16	3	13

At the designated times 100*u*l of bathing solution was collected, scintillation added, and the CPM calculated by the liquid scintillation spectrometer. These results were collated to calculate a mean and standard deviation for each measurement time, and are shown (Table 4.37). The results were also converted to percentages for comparison with other molecular weight tooth root permeability experiments (mean activity of H^3-Dextran 874,391.83 CPM).

Table 4.37.

Results H^3-Dextran Third Molars - Non-Root Planed.

Time	Counts per minute		Percentage of Label Used.	
Hours	Mean	Std.Dev.	Mean	Std.Dev.
0	-	-	-	-
1	67.66	98.31	.0077	.0112
2	61.90	85.86	.0071	.0098
4	70.88	85.94	.0081	.0098
8	97.46	93.76	.0111	.0107
24	495.63	327.79	.0567	.0375
48	862.72	481.76	.0987	.0551
96	284.74	175.81	.0326	.0201
192	1009.09	603.80	.1154	.0691
384	1552.84	929.47	.1776	.1063
576	1843.50	1095.59	.2108	.1253
768	2127.34	1225.89	.2433	.1402
960	2384.90	1355.36	.2727	.1550

4.2.2.c. Dextran Root Planed Third Molars.

This group consisted of 15 teeth, 8 from female and 7 from male patients. The age range was from 18 to 28 years, and the mean age was 22.93, with a standard deviation of 3.37 years. The details of the teeth used are shown (Table 4.38.).

Table 4.38. Material Used for Dextran Root Planed Third Molar Study.

Tooth	Number	Male	Female
Max. 8	13	7	6
Mand. 8	2	-	2
Total	15	7	8

Table 4.39. Results of H^3-Dextran Root Planed Third Molars.

Time	Counts per minute		Percentage of Label Used.	
Hours	Mean	Std.Dev.	Mean	Std.Dev.
0	-	-	-	-
1	27.97	40.88	.0032	.0047
2	31.13	44.75	.0036	.0051
4	51.67	73.77	.0059	.0084
8	111.57	115.02	.0128	.0132
24	511.43	494.76	.0585	.0566
48	1016.23	796.27	.1162	.0911
96	358.27	160.92	.0410	.0184
192	1419.20	750.14	.1623	.0858
384	1850.83	862.45	.2117	.0986
576	2169.70	1021.5	.2481	.1168
768	2491.92	1811.02	.2850	.2071
960	2556.01	1173.62	.2923	.1342

The data were collected as described previously, with means and standard deviations calculated for each measurement time, and are shown (Table 4.39.). The mean activity of H^3-Dextran used was 874,391.83 CPM, which was used to calculate the percentages.

4.2.2.d. Dextran Control Teeth Full Wax.

This group of 5 teeth had the entire root surface covered with wax and varnish. The age of the teeth ranged from 17 to 22 years, with a mean of 19.6, and a standard deviation of 2.5 years (Table 4.40.).

Table 4.40. Material Used for Dextran Control Third Molar Study.

Tooth	Number	Male	Female
Max. 8	5	2	3
Mand.8	-	-	-
Total	5	2	3

The results of these control teeth are shown in Table 4.41. and 4.42. for the root planed, and the non root planed group respectively. The sampling procedure was as described for the other Tritiated dextran and glucose experiments. The mean activity of 100*u*l H^3-Dextran was 874391.83 CPM, and was used to calculate the percentages shown. Figure 4.6. shows the means of all dextran root permeability experiments plotted versus time for comparison.

Table 4.41. Results of H^3-Dextran Non Root Planed Control Third Molars.

Time	Count per Minute		Percentage of Label Used.	
Hours	Mean	Std.Dev.	Mean	Std.Dev.
0	-	-	-	-
1	9.25	4.60	.0011	.0005
2	13.25	1.77	.0015	.0002
4	11.00	1.41	.0013	.0002
8	8.25	0.35	.0009	.0001
24	82.75	69.65	.0095	.0080
48	226.73	155.21	.0259	.0178
96	125.25	22.98	.0138	.0031
192	582.50	57.98	.0666	.0066
384	1281.25	132.58	.1465	.0152
578	1373.75	842.52	.1571	.0964
768	2441.50	259.51	.2792	.0297
960	2740.95	112.78	.3184	.0129

Table 4.42. Results H^3-Dextran Root Planed Control Third Molars.

Time	Counts per minute		Percentage of Label Used.	
Hours	Mean	Std.Dev.	Mean	Std.Dev.
0	-	-	-	-
1	12.00	5.07	.0014	.0006
2	11.17	2.57	.0013	.0003
4	12.00	1.80	.0014	.0002
8	9.67	2.02	.0011	.0002
24	17.67	4.73	.0020	.0005
48	10.83	2.82	.0012	.0002
96	14.83	2.31	.0017	.0003
192	18.50	4.44	.0021	.0005
384	80.00	94.00	.0091	.0108
578	89.83	110.07	.0103	.0126
768	127.83	158.41	.0146	.0181
960	133.50	166.33	.0153	.0190

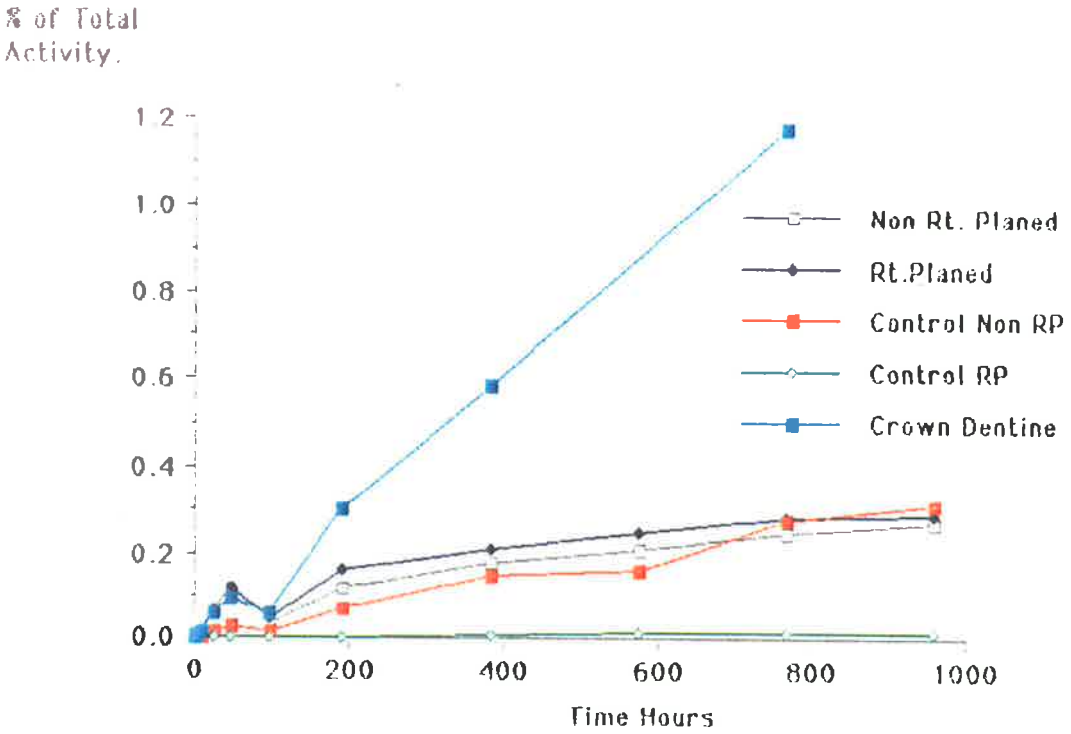

Figure 4.6. Mean Values of Dextran Root Permeability Versus Time.

4.2.2.e. Dextran Crown Dentine Studies.

A group of 24 third molar teeth were used in the dextran crown dentine experiments (Table 4.43.). The mean age of the group was 22.71, with a standard deviation of 3.24 years. The age ranged from 16 to 28 years.

Table 4.43. Material Used for Dextran Crown Dentine Studies.

Tooth	Number	Male	Female
Max. 8	15	6	9
Mand.8	9	5	4
Total	24	11	13

The study was conducted as three separate experiments. A machine malfunction resulted in no 96 hour time readings in Experiment A. The data were collected by taking 50ul samples from the surrounding bathing solution in the boat and added to 3ml of scintillant, and the CPM were collated for all three experiments. At each measurement time a mean and standard deviation were calculated (Table 4.44.). The counts per minute were changed to a percentage of the total activity of the 100ul H3-Dextran solution added to the occlusal cavity in the tooth. The activity varied even although all H3-Dextran solutions were 41.7uCi/ml the C.P.M. and shown previously (Table 3.3).

Table 4.44. Results H^3-Dextran Crown Dentine.

Time	Counts Per Minute		Percentage of Label Used.	
Hours	Mean	Std.Dev.	Mean	Std. Dev.
0	-	-	-	-
1	56.43	76.78	.0054	.0083
2	75.87	86.08	.0071	.0090
4	206.07	188.45	.0149	.0117
8	489.83	418.04	.0415	.0324
24	1930.18	2145.05	.1727	.1753
48	3638.20	3697.06	.3048	.3072
96	2563.35	2169.15	.1792	.1603
192	10848.87	8455.38	.9778	.6438
384	32437.36	34901.17	1.9094	1.4504
576	-	-	-	-
768	45719.47	36638.69	3.8233	2.7914

One tooth A2 had an unacceptable high count present after one hour, 1,000 C.P.M. or 0.75%, indicative of leakage between the tooth cavity and the bathing solution in the boat, probably by improper seal, and was removed from the results.

The results of the three experiments are shown in Fig. 4.7. along with the combined results. These clearly show the crown dentine is more permeable than the tooth roots even when the differences in the size of the bathing solutions is taken into account (Fig. 4.7.).

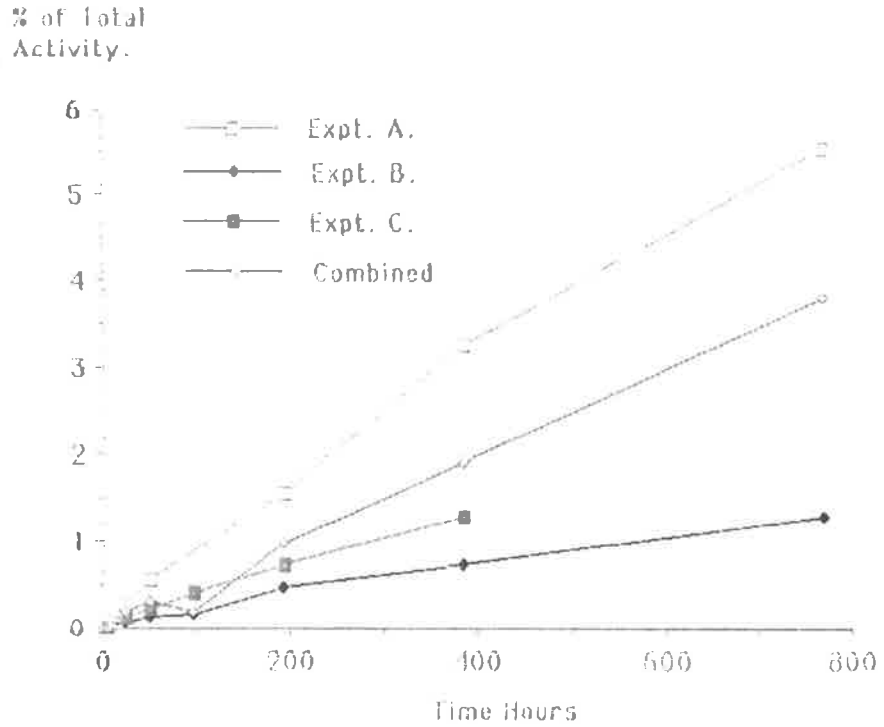

Figure 4.7. Mean Values of Dextran Crown Dentine Permeability Versus Time.

4.2.3. H^3-Water Experiments.

4.2.3.a. Tritium Material Used.

Tritiated water was used as a reference material for comparison of the heavier molecular weight compounds, glucose and dextran. The tritium was diluted with PBS as required to give the working *u*Ci/ml concentrations required. Table 3.4 gives the

concentrations of Tritium used during the third molar root and crown dentine studies, and their calculated activity at the time of experimentation.

The results were converted to percentages to provide for comparisons between the Tritiated water, Glucose, and Dextran materials, and to allow comparison between the various Tritium concentrations used in the root and the crown dentine studies.

4.2.3.b. Tritiated Water Non Root Planed Third Molars.

The material used in these experiments was derived from the dextran and glucose studies. Following completion of each tooth's participation in either the H^3-labelled glucose and dextran experiments they were decontaminated by vigorous washing with phosphate buffered saline before being recycled in the Tritium experiments. Measurement of the C.P.M. at time zero indicates that in each case the calculated level of activity of these teeth was below that of background levels. A total of 72 teeth were used, and a detailed breakdown of the teeth used is shown (Table 4.25.).

Thirty teeth were used for the non root planed study. The mean age was 22.33, with a standard deviation of 6.70 years, and a range of 16 to 53 years. Details of teeth used are shown (Table 4.45.).

Table 4.45. Material used Tritiated Water Non Root Planed Third Molar Study.

Tooth	Number	Male	Female
Max.8	25	7	18
Mand.8	5	-	5
Total	30	7	23

Scintillation cocktail was added to 100*u*l drawn from the bathing solution and used to calculate means and standard deviations at the experimental times. The concentration of Tritium used was 250*u*Ci/ml, and 100*u*l had a mean activity of 9,744,944.89 counts per minute. The means were also expressed as a percentage of total

activity for comparison to other permeability experiments. Initially all thirty teeth were collated into a single table to represent the effect of tritium (Table 4.46.). To allow comparison between the previous dextran and glucose groups the results of these experiments are shown (Table 4.47. and 4.48.). One tooth (WP) was removed from the tables because after one hour the result was greater than 2,000, or .0200%, indicating probable leakage of the tooth. The teeth used in the glucose and dextran studies had similar permeability (Table 4.47. and 4.48.).

Table 4.46. Results of All H^3-Water Non-Root Planed Third Molars.

Time	Counts Per Minute		Percentage of Label Used.	
Hours	Mean	Std.Dev.	Mean	Std. Dev.
0	13.95	4.21	.0001	.0000
1	81.43	88.73	.0008	.0009
2	386.22	398.88	.0040	.0041
4	1569.55	1071.43	.0161	.0110
8	5640.55	3459.09	.0579	.0355
24	19198.63	8979.71	.1970	.0921
48	31571.67	14400.62	.3240	.1448
96	42542.90	20482.81	.4365	.2102
192	49147.36	20787.49	.5043	.2133
384	49459.44	21229.50	.5075	.2179

Table 4.47. Results of H3-Water Non-Root Planed Third Molars (Glucose Gp.).

Time	Counts Per Minute		Percentage of Label Used.	
Hours	Mean	Std.Dev.	Mean	Std. Dev.
0	15.86	4.03	.0002	.0000
1	44.43	43.33	.0005	.0004
2	192.93	213.82	.0020	.0022
4	1168.39	882.58	.0120	.0091
8	3880.37	2121.37	.0398	.0218
24	14884.57	5106.59	.1527	.0524
48	25152.12	7782.54	.2581	.0799
96	35225.75	10827.70	.3615	.1111
192	42762.34	12186.77	.4388	.1251
384	43423.42	12603.28	.4456	.1299

Table 4.48. Results of H3-Water Non-Root Planed Third Molars (Dextran Gp.).

Time	Counts Per Minute		Percentage of Label Used.	
Hours	Mean	Std.Dev.	Mean	Std. Dev.
0	12.31	3.78	.0001	.00004
1	115.97	106.57	.0012	.0011
2	566.63	451.13	.0058	.0046
4	1943.96	1123.16	.0199	.0115
8	7283.38	3709.70	.0747	.0381
24	23225.08	10053.58	.2383	.1032
48	37563.26	16698.66	.3855	.1714
96	49372.25	25034.25	.5066	.2569
192	55106.71	25446.24	.5655	.2611
384	55093.04	26115.93	.5653	.2680

4.2.3.c. Tritiated Water Root Planed Third Molars.

The label used was 100*u*l of 250 *u*Ci/ml Tritium which had a mean activity of 9,744,944.89 counts per minute. This group consisted 30 teeth with a mean age of 22.17, and standard deviation 3.07 years, with a range of 18 to 28 years (Table 4.49.).

Table 4.49. Material used Tritiated Water Root Planed Third Molar Study.

Tooth	Number	Male	Female
Max. 8	27	13	14
Mand. 8	3	-	3
Total	30	13	17

Table 4.50. Results of All H^3-Water Root Planed Third Molars.

Time Counts Per Minute Percentage of Label Used.

Hours	Mean	Std.Dev.	Mean	Std. Dev.
0	12.85	3.61	.0001	.0000
1	116.85	197.88	.0012	.0020
2	547.98	643.30	.0056	.0066
4	2041.31	1482.73	.0209	.0152
8	6082.21	3299.60	.0624	.0339
24	18828.85	7110.24	.1932	.0730
48	31247.31	13757.31	.3207	.1412
96	44657.87	19200.43	.4583	.1970
192	50302.43	20323.37	.5162	.2086
384	52036.70	20254.73	.5340	.2078

Table 4.51. Results of H^3-Water Root Planed Third Molars (Glucose Gp.).

Time	Counts Per Minute		Percentage of Label Used.	
Hours	Mean	Std.Dev.	Mean	Std. Dev.
0	12.00	2.60	.0001	.0000
1	83.47	106.91	.0009	.0011
2	471.50	587.65	.0048	.0060
4	1890.47	1662.08	.0194	.0171
8	5467.33	3435.76	.0561	.0353
24	16368.35	6171.18	.1680	.0633
48	26107.17	7215.89	.2679	.0740
96	34388.73	6866.24	.3529	.0705
192	40706.88	6958.39	.4177	.0714
384	43193.61	7460.39	.4432	.0766

Table 4.52. Results of H^3-Water Root Planed Third Molars (Dextran Gp.).

Time	Counts Per Minute		Percentage of Label Used.	
Hours	Mean	Std.Dev.	Mean	Std. Dev.
0	13.07	4.26	.0001	.0000
1	150.27	259.42	.0015	.0027
2	624.47	706.65	.0064	.0073
4	2192.20	1320.10	.0225	.0135
8	6711.80	3147.33	.0689	.0323
24	21289.35	7325.68	.2185	.0752
48	37510.05	14806.57	.3849	.1519
96	54727.01	22344.78	.5616	.2293
192	59897.94	24695.27	.6147	.2498
384	60881.12	25030.98	.6247	.2569

All teeth were combined to calculate means and standard deviations at the designated times shown (Table 4.50.). The teeth used previously for glucose and dextran studies are also shown separately (Table 4.51. and 4.52.). The root planed teeth used in the glucose and dextran studies are shown to have similar permeabilities.

4.2.3.d. Tritium Control Teeth - Full Wax.

This group comprised a total of 12 teeth, with 6 belonging to each the non root planed and root planed groups. Details of the material used for each of the experiments are given in the Tables 4.53 and 4.54. In the non root planed group the age ranged from 22 to 44, with a mean of 29.17, and standard deviation of 9.72 years. The root planed group had an age range of 17 to 24 years, with a mean of 20.83 and standard deviation of 3.14 years.

Table 4.53.

Material Used Tritiated Water Non Root Planed Control Third Molar Study.

Tooth	Number	Male	Female
Max. 8	6	5	1
Mand.8	-	-	-
Total	6	5	1

Table 4.54.

Material Used Tritiated Water Root Planed Control Third Molar Study.

Tooth	Number	Male	Female
Max. 8	6	2	4
Mand.8	-		-
Total	6	2	4

The control non root planed and root planed teeth were sampled in a similar manner to the preceding experiments, and a mean and standard deviation were calculated at each time as shown (Table 4.55. and 4.56.). These were also converted to percentages using the known activity of the Tritium label (9,744,944.89 CPM). One tooth (XJ) in the non root planed control group leaked and was removed from the study.

Table 4.55. Results of H^3-Water Non Root Planed Control Third Molars.

Time	Counts Per Minute		Percentage of Label Used.	
Hours	Mean	Std.Dev.	Mean	Std. Dev.
0	12.67	4.25	.0001	.0000
1	16.30	0.45	.0002	.0000
2	22.10	15.89	.0002	.0002
4	26.50	10.93	.0003	.0001
8	70.20	53.23	.0007	.0005
24	554.60	422.49	.0057	.0043
48	1643.40	1212.26	.0169	.0124
96	4168.15	3190.17	.0428	.0327
192	10129.29	9618.58	.1039	.0757
384	20596.11	13764.83	.2114	.1413

The mean percentage values for all H^3-Water root studies is shown (Fig. 4.8) and very little difference was seen between the root planed and non root planed teeth.

Table 4.56. Results of H^3-Water Root Planed Control Third Molars.

Time	Counts Per Minute		Percentage of Label Used.	
Hours	Mean	Std.Dev.	Mean	Std. Dev.
0	10.58	1.36	.0001	.0000
1	16.33	2.40	.0002	.0000
2	19.58	9.68	.0002	.0001
4	27.67	18.99	.0003	.0002
8	60.67	75.04	.0006	.0008
24	277.68	352.95	.0028	.0036
48	927.42	848.31	.0095	.0087
96	3045.35	2172.87	.0313	.0223
192	7809.69	4991.74	.0801	.0512
384	17523.58	10771.04	.1798	.1105

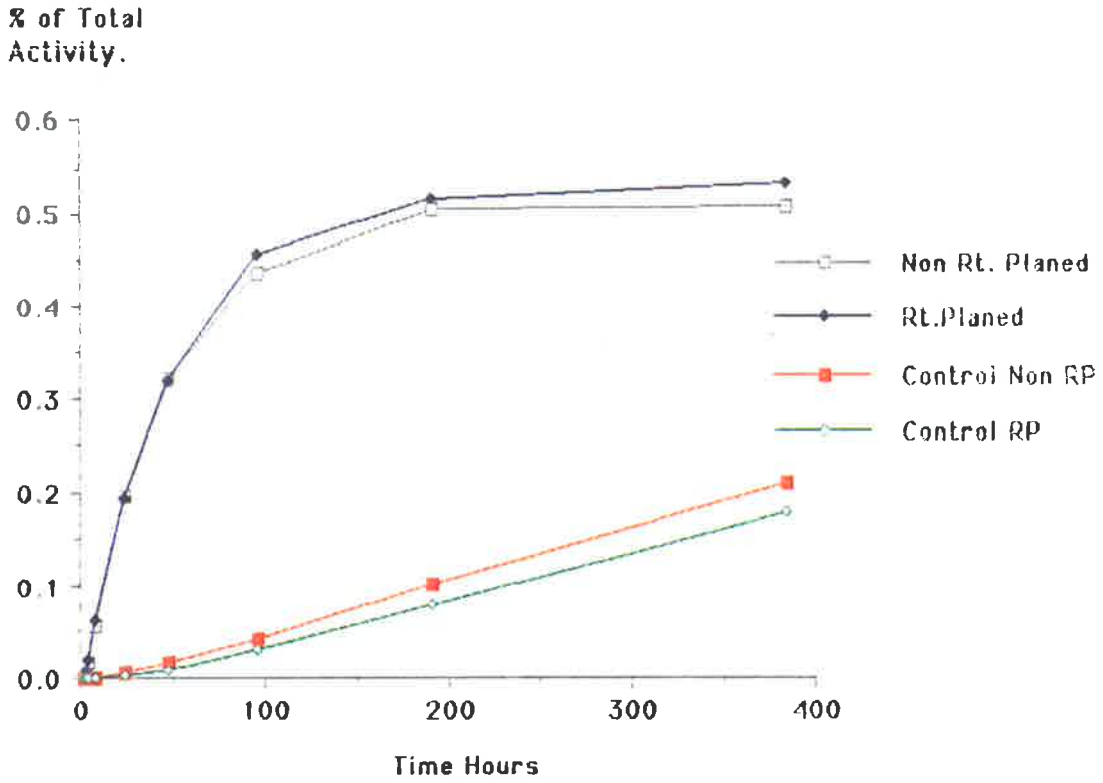

Figure 4.8. Mean Values of Tritium Root Permeability Versus Time.

4.2.3.e. Tritium Crown Dentine Studies.

A total of 24 teeth were used in this three part experiment (Table 4.57.). The mean age of the group of teeth was 22.83 with a standard deviation of 2.39 years. The age ranged from 20 to 28 years. The three parts of this experiment were conducted with varying concentrations of tritium placed in the occlusal cavity, and therefore had varying activity (see Table 3.4).

Table 4.57. Material Used Tritiated Water Crown Dentine Studies.

Tooth	Number	Male	Female
Max. 8	16	4	12
Mand.8	8	4	4
Total	24	8	16

The teeth were prepared as described in Chapter 3, at the designated times 50*u*l of bathing solution was removed from the pulpal side, and added to 3ml scintillation cocktail. The mean CPM and standard deviation for each collection time were calculated. Table 4.58. shows the results of all three parts of the crown dentine studies together, and since the activity of the Tritium varied the only meaningful result is the percentage of the total activity of the Tritium.

Table 4.58. Results of H^3-Water in Crown Dentine.

Time	Percentage of Label Used	
Hours	Mean	Std. Dev.
0	-	-
1	.5256	.7904
2	.7166	.8302
4	1.8110	1.3985
8	2.3185	1.2837
24	4.7755	1.4720
48	6.4395	1.7204
96	6.6757	1.4536
192	7.1364	2.3024
384	7.4503	2.2101
576	-	-
768	7.1921	2.4934

Results of the tritiated water three part study on crown dentine indicated that although the activity of Tritium used varied, the results stated in percentage of total activity were similar (Fig.4.9). As in previous crown dentine studies of glucose and dextran, the tritium studies indicated the crown dentine was more permeable than the tooth roots after the difference in bathing solutions was taken into consideration.

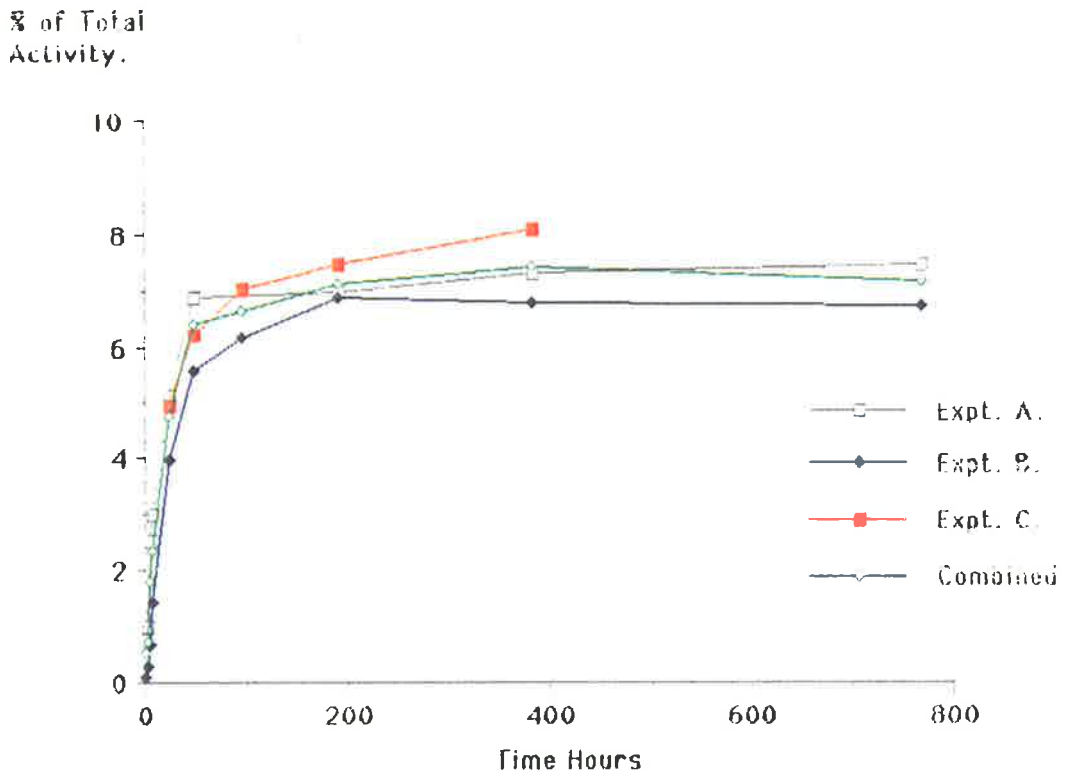

Figure 4.9. Mean Values of Tritium Crown Dentine Permeability Versus Time.

4.2.4. Comparison of Different MW Diffusion Rates.

Three chemicals with similar labels, but of different molecular weights, Tritium (20), Glucose (180.2), and Dextran (70,000) were used in third molar teeth, and their rates of diffusion were compared. Using the BMDP statistical software package a 3V general mixed model analysis of variance was conducted for molecular and treatment effects. This demonstrated there was a significant interaction between the treatment and chemical effects. This precluded further analysis of the differences between the control and test teeth groups, but further investigation of the pairwise t test tables indicated the control teeth were less than the test teeth. Analysis was then performed on the effects of MW on the various third molar treatment groups separately.

4.2.4.a. Results from the Non Root Planed Third Molars.

The BMDP 3V statistical software package was then used to analyse the chemical effect in the non root planed third molars. The Chi-square calculated with 2 degrees of freedom had a value of 18.430. This indicated there was a significant difference between the chemical groups with a probability of less than 0.001. from the pairwise t test tables it was evident that the tritium and glucose molecules had comparable permeability, and were significantly greater permeability than dextran. The mean permeabilities of the three molecular weights for the non root planed teeth are shown (Fig 4.10).

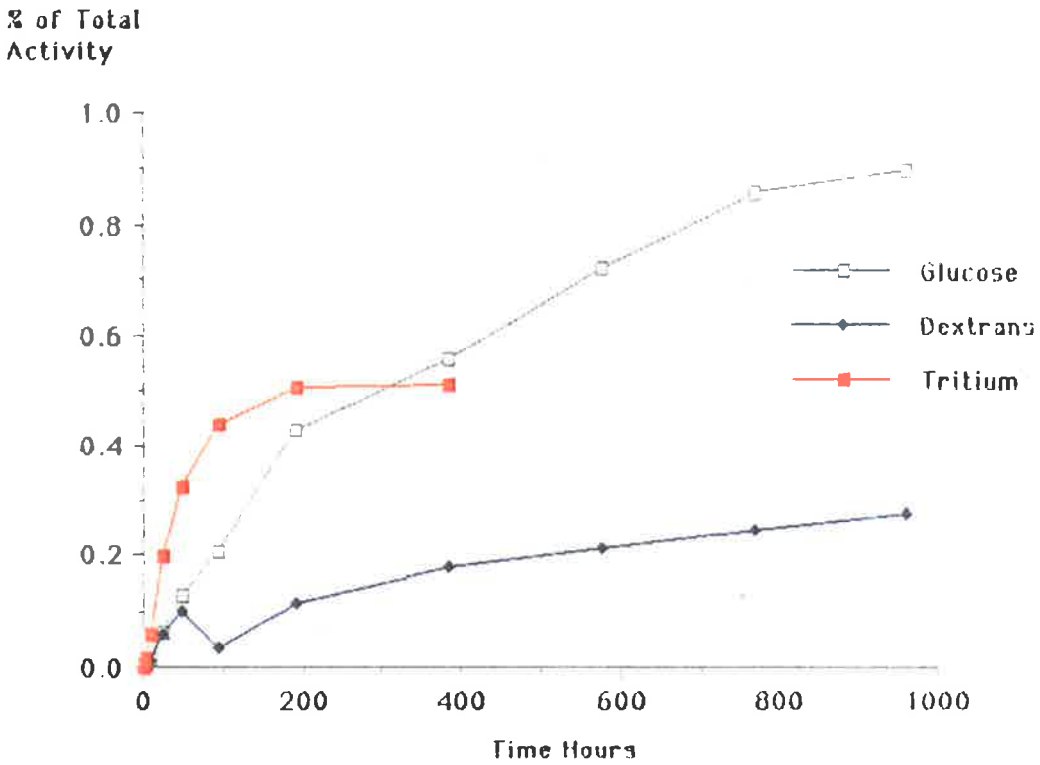

Figure 4.10. Mean Permeability Values of Non Root Planed Third Molars.

4.2.4.b. Results from the Root Planed Third Molars.

Statistical analysis using the BMDP 3V programme was carried out to analyze the effect of chemical MW on the permeability in the root planed third molars. The Chi-square calculated with 2 degrees of freedom had a value of 18.825. This indicated there was a significant difference in the permeability of the three different molecules with a probability of less than 0.001. From the pairwise t test tables it was seen that tritiated water had significantly greater permeability than glucose which was significantly greater than dextran. The mean permeability values of the three different MW compounds are shown (Fig. 4.11).

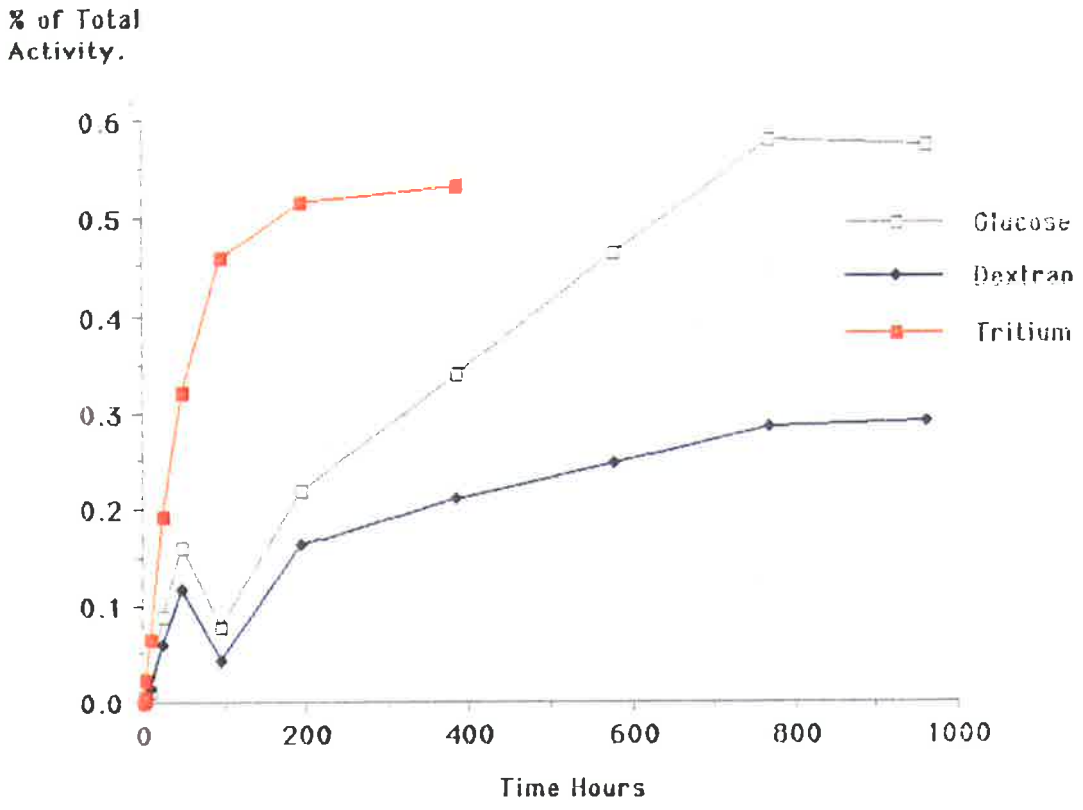

Figure 4.11. Mean Permeability Values of Root Planed Third Molars.

4.2.4.c. Results from the Crown Dentine Third Molar Studies.

The thickness of the crown sections was evaluated for differences in the thickness of the dentine between the cavity and the pulp side of the tooth. The overall mean dentine thickness of the teeth used in the crown dentine permeability studies was 1.8 mm. with a standard deviation of 0.56 mm. The mean crown dentine thickness for the different molecules in each experiment is shown (Table 4.59.). There was no significant differences between any of the experimental groups.

Table 4.59. Crown Dentine Thickness.

	Thickness in mm.		
Molecule	Expt.	Mean	Std. Dev.
Tritium	A.	2.21	0.37
	B.	1.90	0.37
	C.	1.05	0.28
	Combined	1.75	0.61
Glucose	A.	2.38	0.33
	B.	1.67	0.21
	C.	1.46	0.41
	Combined	1.88	0.54
Dextran	A.	2.16	0.33
	B.	1.93	0.21
	C.	1.18	0.37
	Combined	1.78	0.54

Statistical analysis using the BMDP 3V programme was used to analyze the effect of molecular weight on permeability in the crown dentine experiments using thickness as a covariate. The Chi-square calculated with 2 degrees of freedom had a value of 49.929. This indicated there was a significant difference in the permeability of the

different MW molecules with a probability of less than 0.001. From the pairwise t test tables the tritiated water was seen to be significantly more permeable than glucose which in turn was significantly more permeable than dextran. The mean permeability of the three MW molecules are plotted versus time (Fig. 4.12).

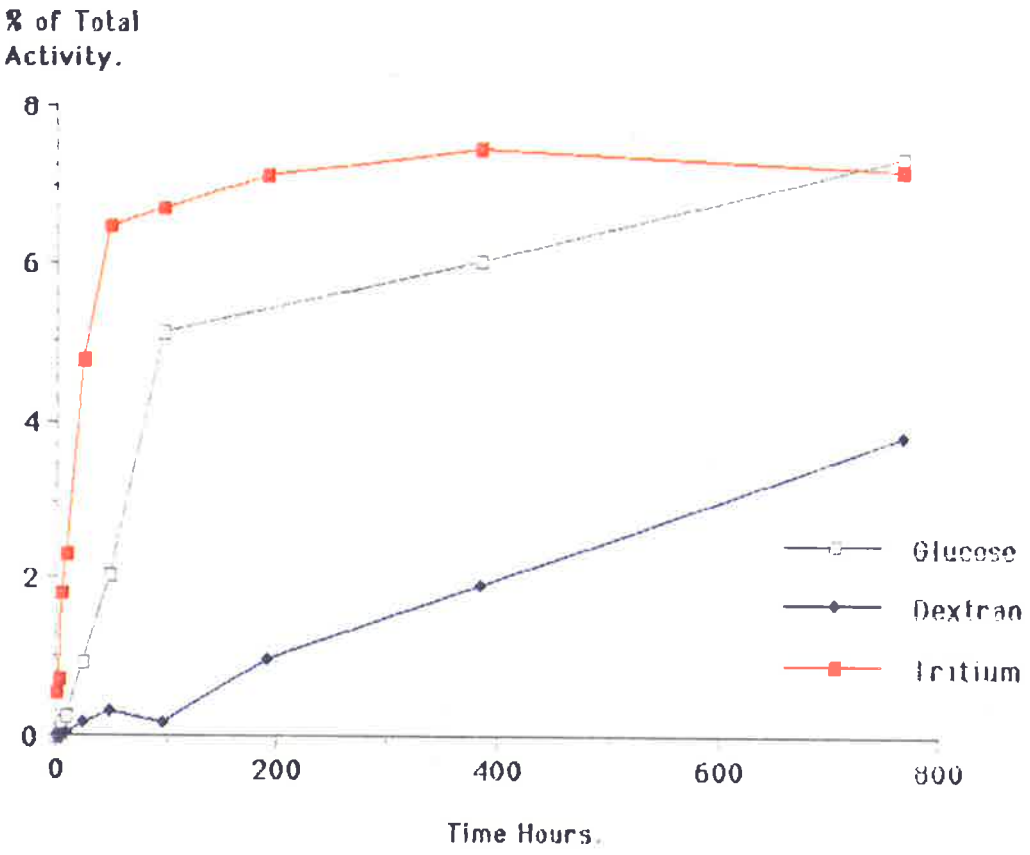

Figure 4.12. Mean Permeability Values of Third Molar Crown Dentine.

4.2.5. Comparison of Different Root Treatment Diffusion Rates.

Three different permeability barriers, root planed, non root planed tooth roots, and crown dentine, were used in this study. Using the BMDP 3V general mixed model analysis of variance was utilized to assess which barrier was the most and least permeable to each of the three molecules.

Two areas of caution in the analysis of the results should be considered. Firstly the experimental conditions were not identical between the root and crown permeability experiments. The bathing solution from the crown dentine comprised only 3ml compared to the root experiments which had 10ml. The 100*u*l sample in the root experiments represented 1.0% of the bathing solution, and the 50*u*l sample from the crown dentine experiments represent 1.67% of the bathing solution. The thickness of the root dentine represents an unknown quantity, although the total surface area available for diffusion is substantially larger for the tooth root group. The tooth root group have also two substances through which the compounds must diffuse, dentine and cementum. This may be partially overcome by analyzing the crown dentine to the root planed dentine.

4.2.5.a. Results of the Tritiated Water Molecule.

The BMDP 3V statistical soft ware package analyzed the treatment effect on the permeation of tritiated water in third molars. The Chi-square calculated with 2 degrees of freedom was 92.635. this indicated there was a significant difference between the tooth treatments with a probability of less than 0.001. From the pairwise t test it was evident that the root planed and non root planed teeth were similar, and both were significantly less than crown dentine permeability. The mean permeability of each of these treatment groups was plotted versus time (Fig. 4.13).

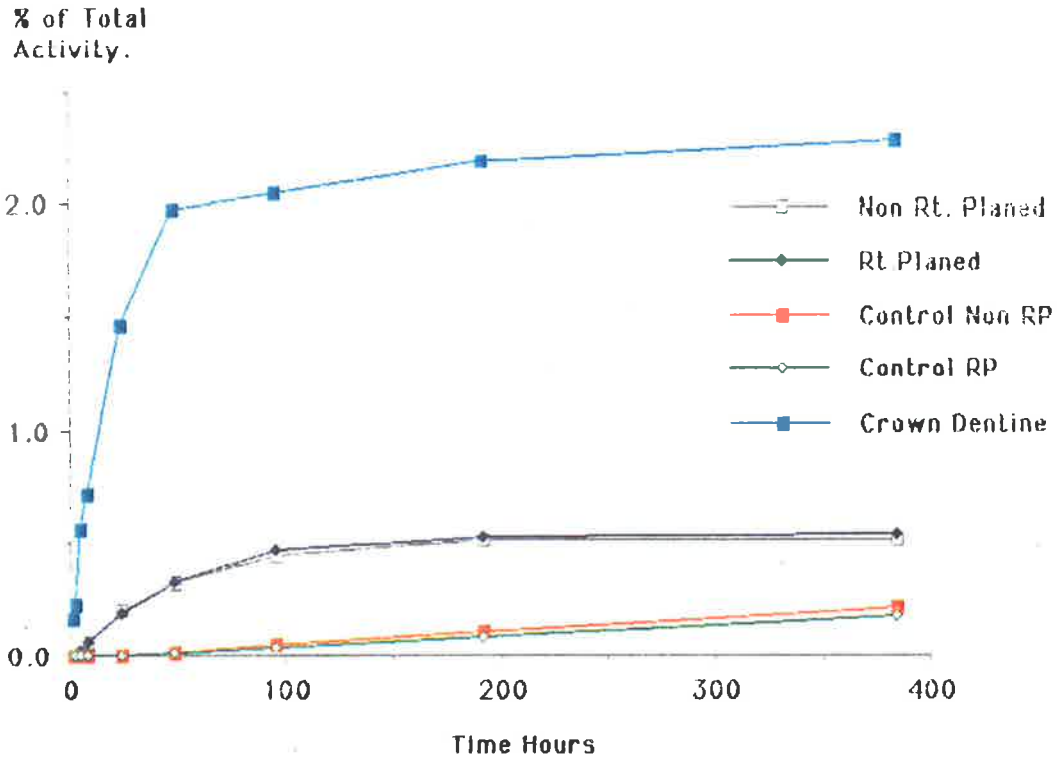

Figure 4.13. Mean Permeability Values of Tritiated Water.

4.2.5.b. Results of Tritiated Glucose Molecule.

Statistical analysis using the BMDP 3V programme was carried out to test the effect of various treatments on the permeability of glucose in third molars. The Chi-square calculated with 2 degrees of freedom was 67.107. This indicated there was a significant difference between the tooth treatments with a probability of less than 0.001. From the pairwise t test tables it was evident that the crown dentine was significantly more permeable than the non root planed group which was significantly greater than the root planed group. The permeability of each of these treatment groups for glucose was plotted versus time (Fig. 4.14).

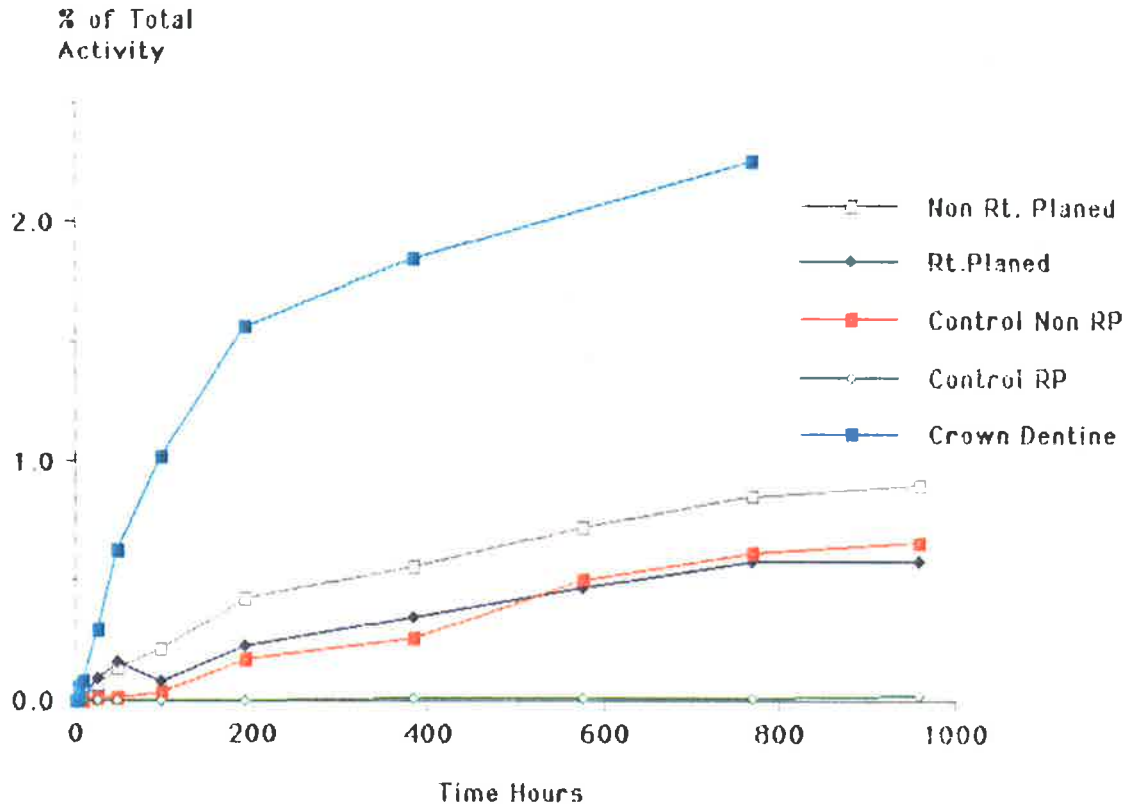

Figure 4.14. Mean Permeability Values of Tritiated Glucose.

4.2.5.c. Results of Tritiated Dextran Molecule.

Statistical analysis using the BMDP 3V programme was utilized to analyze the treatment effect on the permeability of dextran through third molars. The Chi-square calculated with 2 degrees of freedom was 11.617. This indicated there was a significant difference between the tooth treatments with a probability of less than 0.005 (0.003). From the pairwise t test tables it was evident that the root planed and non root planed groups had similar permeabilities to dextran, but were significantly less than the crown dentine. The mean permeability values of each of the treatment groups was plotted versus time (Fig. 4.15).

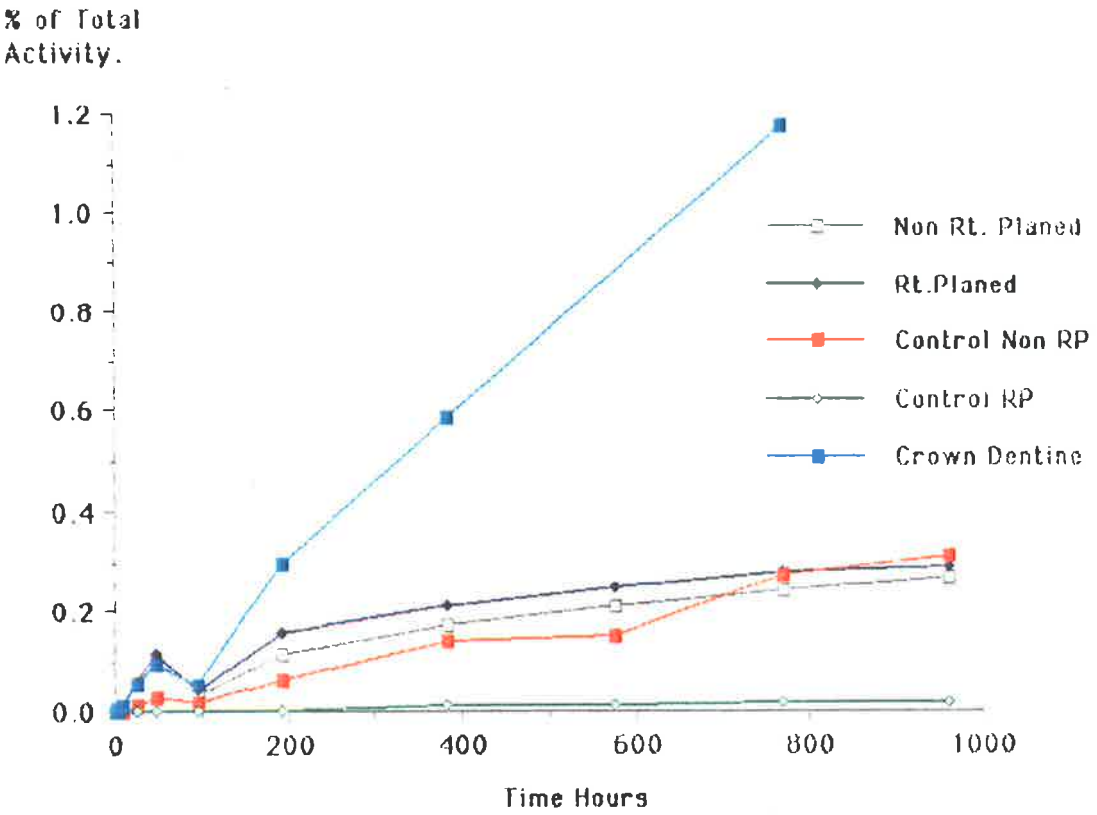

Figure 4.15. Mean Permeability Vales of Tritiated Dextran.

CHAPTER 5. RESULTS OF ELECTRON MICROSCOPIC PERMEABILITY STUDIES.

5.1. Material Collected.

Teeth were collected from the Adelaide Dental Hospital Oral Surgery Department and participating oral surgeons. While 144 third molars were considered suitable for inclusion in the experiment, only 140 third molars were required for use in this study (Table 5.1.). One tooth was lost due to technical difficulties, but was replaced to maintain numbers for statistical analysis. The mean age of patients was 21.34 years, with a standard deviation of 4.08, and a range of 16 to 34 years, and details are shown (Table 5.1.).

Table 5.1. Material Used for Electron Microscope Study.

Tooth	No.	Male	Female
Max. 8	101	35	66
Mand. 8	39	11	28
Total	140	46	94

Age of Patient from whom the Teeth were Collected.

Age Years	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	>30
Number	10	11	13	13	19	24	11	9	3	4	6	2	5	-	2	5

A ten percent solution of cobaltous chloride was placed in the prepared pulp chamber and sealed. The tooth roots were bathed with saline for periods of 1 hour to 10 days. At designated times the cobaltous ion was washed from the pulp chamber, and the teeth thoroughly dried at room temperature. The teeth were prepared for SEM where the roots were analysed by electron microanalysis, using X-ray spectrum and an image processing package, for the presence of cobalt on their surface.

5.2. X-Ray Spectrum Results.

The results are expressed in two ways, a ratio of cobalt to background radiation and a scoring system. Both methods required the cobaltous ion to be at least twice the background radiation in order for it to be considered significant. In the case of the ratio the measured cobalt level is divided by the background level to give a value for each tooth, and in addition the level of cobaltous ion was assigned a score of 0, 1, 2, or 3, as described in Chapter Three. The X-ray spectrum was analysed for the effect of time, and for the effect of tooth region to ascertain any variations in root area permeability.

5.2.1. Whole Tooth Surface Results.

All teeth from each group were analysed, mean and standard deviations were calculated for each measured time period (Table 5.2). The cobalt to background ratio (Co/BG) was required to be greater than 2 for the level of cobalt present to be considered significant. All 140 teeth examined had very similar background values for the whole tooth in a range of 6 to 12. This data supported comparison of tooth analysis when standardized electron microscope settings were used.

These data were then analyzed using the BMDP statistical programme with a one way analysis of variance for evaluation of the time effect. The data indicated there was a significant time effect ($P < .0001$) on the amount of cobalt showing up on the external tooth surface. There was an increase in cobaltous ion found on the external surface of the tooth with time, and significant cobaltous ion was present on the tooth surface at all times after 1 day. The T-test values indicated the one hour was not significantly different from the other measurement times until after 3 days. Significant differences at the 1 % level were seen at all times after 3 days except days 4, 8, and 10, which were significant at the 5 % level. The non arithmetic progression of means indicated differences in individual tooth permeability.

Table 5.2. X-Ray Spectrum Ratio Results for Whole Tooth.

Time	Co/BG	Cobalt (Co)		Background (BG)	
Hours	Mean	Mean	SD	Mean	SD
1	1.6580	14.9	5.65	8.9	0.74
2	1.4850	14.1	1.71	9.2	1.32
4	1.7460	15.7	4.97	8.9	1.10
8	1.3380	11.5	3.06	8.6	1.17
24	2.3300	22.2	9.98	9.1	1.85
48	2.1310	20.3	10.21	9.5	1.18
72	2.9460	24.3	15.49	8.6	1.26
96	2.6560	24.4	10.72	9.0	1.56
120	3.3130	29.9	6.74	9.6	0.97
144	3.0560	30.0	11.54	9.7	1.25
168	3.1470	29.3	12.34	9.3	0.82
192	2.8080	26.0	9.94	9.2	0.92
216	3.8880	40.8	15.24	10.4	1.17
240	2.6060	25.2	10.04	9.7	1.34

A trend towards larger mean scores occurred with an increase in time using the scoring system detailed in chapter three. The mean scores for each time, and standard deviations and the distribution of scores for each recorded time are shown (Table 5.3.). The mean scores assigned to the whole tooth group at each time are shown (Figure 5.1).

Table 5.3. X-Ray Spectrum Score Results for Whole Tooth.

Time			Scores			
Hours	Mean	Std.Dev.	0	1	2	3
1	0.4	0.70	7	2	1	-
2	0.1	0.32	9	1	-	-
4	0.2	0.42	8	2	-	-
8	0.1	0.32	9	1	-	-
24	0.9	0.74	3	5	2	-
48	0.7	0.82	5	3	2	-
72	1.3	0.95	2	4	3	1
96	1.2	0.92	3	2	5	-
120	1.7	0.48	-	3	7	-
144	1.3	0.67	1	5	4	-
168	1.4	0.70	1	4	5	-
192	1.0	0.67	2	6	2	-
216	1.7	0.82	1	2	6	1
240	1.2	0.63	1	6	3	-

5.2.2. Cervical Third of Root.

The 140 teeth were examined to calculate the mean ratio values and standard deviation of the cervical third of the root surfaces at each of the recording times (Table 5.4.). The Co/BG ratio greater than two indicated significance. Background levels of the X-ray spectrum were confined to a small range (6-12, with two exceptions 13 & 15), which supported comparison of X-ray spectra when a standardised experimental procedure was used.

The statistical analysis of the 140 teeth indicated there was a significant time effect in the CEJ root thirds ($P < .0001$). The mean values for each time period show a variation rather than a linear progression of the mean values which suggested that

variations in tooth permeability occurred within the time groups. The T-test values indicated the 1 hour mean was not significantly different from the other measurement times until day three. Significantly differences were noted for all times greater than three days, with the exception days 7, 8 and 10 which had probability values of 5%, 23%, and 1% respectively.

Table 5.4. X-Ray Spectrum Ratio Results for Cervical Third of Root.

Time	Co/BG	Cobalt		Background (BG)	
Hours	Mean	Mean	SD	Mean	SD
1	2.2090	21.1	9.56	9.4	0.97
2	2.0540	20.2	9.47	10.1	1.10
4	2.2300	21.8	7.42	9.9	1.85
8	1.4500	14.3	4.03	9.9	1.37
24	2.8150	28.9	12.29	10.1	1.10
48	2.3220	21.7	11.51	9.0	1.25
72	4.3350	43.0	19.62	9.7	1.34
96	3.7560	37.3	11.14	10.1	1.20
120	3.7290	36.4	11.63	9.7	1.06
144	3.9210	35.3	13.87	9.6	1.65
168	3.3110	32.4	10.93	9.8	0.79
192	2.8720	29.1	12.06	10.1	0.82
216	4.1330	41.7	15.43	10.0	1.41
240	3.5730	31.2	16.57	9.7	0.95

The score system described for the X-ray spectrum is fully tabulated (Table 5.5.), indicating the means, standard deviations, and distribution of scores for each recorded time. Figure 5.1 has the mean values at each measurement time plotted against time, and indicates a trend for an increase with time.

Table 5.5. X-Ray Spectrum Score Results for Cervical Third of Root.

Time			Scores			
Hours	Mean	Std.Dev.	0	1	2	3
1	0.8	0.79	4	4	2	-
2	0.7	0.82	5	3	2	-
4	0.7	0.67	4	5	1	-
8	0.2	0.42	8	2	-	-
24	1.4	0.84	2	2	6	-
48	0.8	0.79	4	4	2	-
72	2.1	1.10	1	2	2	5
96	1.5	0.71	1	3	6	-
120	2.0	0.67	-	2	6	2
144	1.6	0.84	1	3	5	1
168	1.5	0.71	1	3	6	-
192	1.3	0.82	2	3	5	-
216	1.9	0.88	1	1	6	2
240	1.6	0.84	1	3	5	1

5.2.3. Middle Third of Tooth Root.

All 140 teeth had the middle third region of the tooth root examined via the X-ray spectral analysis, with ten teeth in each of the measurement times. The mean ratio for Co/BG each time was calculated, and standard deviation and is shown (Table 5.6.). The background levels of the individual teeth were consistent, all values were between 7 and 12.

Table 5.6. X-Ray Spectrum Ratio Results for Middle Third of Root.

Time	Co/BG	Cobalt		Background (BG)	
Hours	Mean	Mean	SD	Mean	SD
1	1.6140	14.7	6.68	9.0	0.82
2	1.9130	17.0	13.19	9.0	0.94
4	1.5020	13.2	7.86	8.5	1.08
8	1.2770	11.8	4.05	9.3	1.25
24	2.5740	24.5	11.79	9.2	1.23
48	2.1760	19.4	9.50	8.9	0.99
72	4.0050	36.7	15.83	9.0	1.25
96	3.9430	37.3	13.52	9.5	0.53
120	3.6440	32.4	9.26	8.8	1.14
144	3.8900	35.9	14.76	9.2	1.03
168	3.7200	35.5	16.73	9.3	1.16
192	3.2950	32.9	16.00	9.9	0.88
216	4.4020	43.7	14.70	9.9	1.29
240	3.5090	33.5	15.39	9.6	1.26

Statistical analysis indicated that there was a significant increase in the mean value of cobalt on the surface of the tooth root with time ($P < .0001$). The mean values at each time indicated the Co/BG ratio was not a linear progression which can be attributed to the variations in tooth permeability between groups. The T-test results demonstrated that the value for one hour differed significantly ($P < 0.001$) from all values after 3 days.

The mean scores and standard deviations are shown (Table 5.7.) along with the distribution of scores for each time. The means were plotted against time (Fig. 5.1), and indicated a progressive increase in cobaltous ion on the root surface.

Table 5.7. X-Ray Spectrum Score Results for Middle Third of Root.

Time			Scores			
Hours	Mean	Std.Dev.	0	1	2	3
1	0.4	0.52	6	4	-	-
2	0.3	0.95	9	-	-	1
4	0.3	0.67	8	1	1	-
8	0.1	0.32	9	1	-	-
24	1.0	0.67	2	6	2	-
48	0.8	0.92	5	2	3	-
72	1.8	1.14	2	1	4	3
96	1.6	0.84	1	3	5	1
120	1.7	0.48	-	3	7	-
144	1.9	0.99	1	2	4	3
168	1.9	0.99	1	2	4	3
192	1.5	1.05	2	3	3	2
216	1.8	0.79	1	1	7	1
240	1.5	0.97	2	2	5	1

5.2.4. Apical Third of Root.

All 140 teeth, ten teeth in each time group, were examined via electron X-ray spectral analysis. The cobaltous ion to background ratio for each tooth was used to calculate a mean and standard deviation for each time (Table 5.8.). The background radiation readings were in a narrow range (6-12).

Table 5.8. X-Ray Spectrum Ratio Results for Apical Third of Root.

Time	Co/BG	Cobalt		Background (BG)	
Hours	Mean	Mean	SD	Mean	SD
1	1.6630	14.5	6.38	8.7	0.48
2	1.4720	13.7	3.59	9.3	0.67
4	1.6520	13.4	5.42	8.1	0.74
8	1.2990	12.3	4.69	9.5	1.35
24	2.0790	18.4	6.20	8.9	1.20
48	1.8370	17.1	8.20	9.2	0.92
72	2.7090	24.6	12.30	8.7	1.83
96	2.7500	26.3	9.50	9.5	0.97
120	2.9450	27.4	7.65	9.3	1.25
144	2.9850	27.4	12.54	9.0	0.94
168	3.3940	30.6	12.76	8.9	1.10
192	3.1240	30.5	14.77	9.7	1.06
216	3.4050	30.6	12.19	9.0	0.67
240	2.8800	27.2	11.93	9.4	1.58

Statistical analysis indicated that there was a time effect in the apical third ($P < .0001$). With time the mean ratio value was seen to rise from 1 hr to 9 days, and on the eight and tenth days there was a slight regression. The means in the Table 5.8. did not have a direct linear relationship, as seen in other root thirds suggesting that variability of tooth permeability was present. The T-test matrix indicated the value from 1 hour was significantly different from all values after 4 days at the 1% level, and days 3 and 4 were significant at the 5% level, with probabilities of 1.7% and 1.3% respectively.

Table 5.9. X-Ray Spectrum Score Results for Apical Third of Root.

Time			Scores			
Hours	Mean	Std.Dev.	0	1	2	3
1	0.4	0.52	6	4	-	-
2	0.1	0.32	9	1	-	-
4	0.3	0.67	8	1	1	-
8	0.1	0.32	9	1	-	-
24	0.5	0.71	6	3	1	-
48	0.6	0.70	5	4	1	-
72	1.3	0.95	3	1	6	-
96	1.3	0.82	2	3	5	-
120	1.6	0.70	1	2	7	-
144	1.1	0.88	3	3	4	-
168	1.4	0.84	1	5	3	1
192	1.4	0.97	2	3	4	1
216	1.6	0.97	2	1	6	1
240	1.2	0.92	3	2	5	-

The means, standard deviations, and the distribution of scores for each time using the scoring system are shown (Table 5.9.). The means were plotted against time, and indicated that there was a progressive increase in cobaltous ion on the root surface (Fig. 5.1).

5.2.5. Comparison of Root Third Permeability.

A statistical analysis was carried out to see if there was any difference between the permeability of the root thirds to the cobaltous ion. The means for each time period for the various root thirds were compared, and a significant time effect was found (P. <0.0001). The analysis demonstrated a significant section effect for the cervical,

middle, and apical thirds (P. < 0.0001), and a significant combined time and section effect (P. <0.001). This was due to a non uniform relationship throughout the experiment between the root thirds as seen in Table 5.10.

Table 5.10. X-Ray Spectrum Scores Comparison of Root Thirds.

Time Hours	Cervical Mean	Middle Mean	Apical Mean
1	2.2090	1.6140	1.6630
2	2.0540	1.9130	1.4720
4	2.2300	1.5020	1.6520
8	1.4500	1.2270	1.2990
24	2.8150	2.5740	2.0790
48	2.3220	2.1760	1.8370
72	4.3350	4.0050	2.7090
96	3.7560	3.9430	2.7500
120	3.7290	3.6440	2.9450
144	3.9210	3.8900	2.9850
168	3.3110	3.7200	3.3940
192	2.8720	3.2950	3.1240
216	4.1330	4.4020	3.4050
240	3.5730	3.5090	2.8800

The cervical and middle thirds had means which were approximately the same for 11 of the 14 time periods. The apical section had significantly less cobalt present on it's surface compared to the other two sections. The cervical region had the largest mean

followed by the middle section, and then the apical section of the root, in 10 of 14 measured time periods (Fig. 5.1).

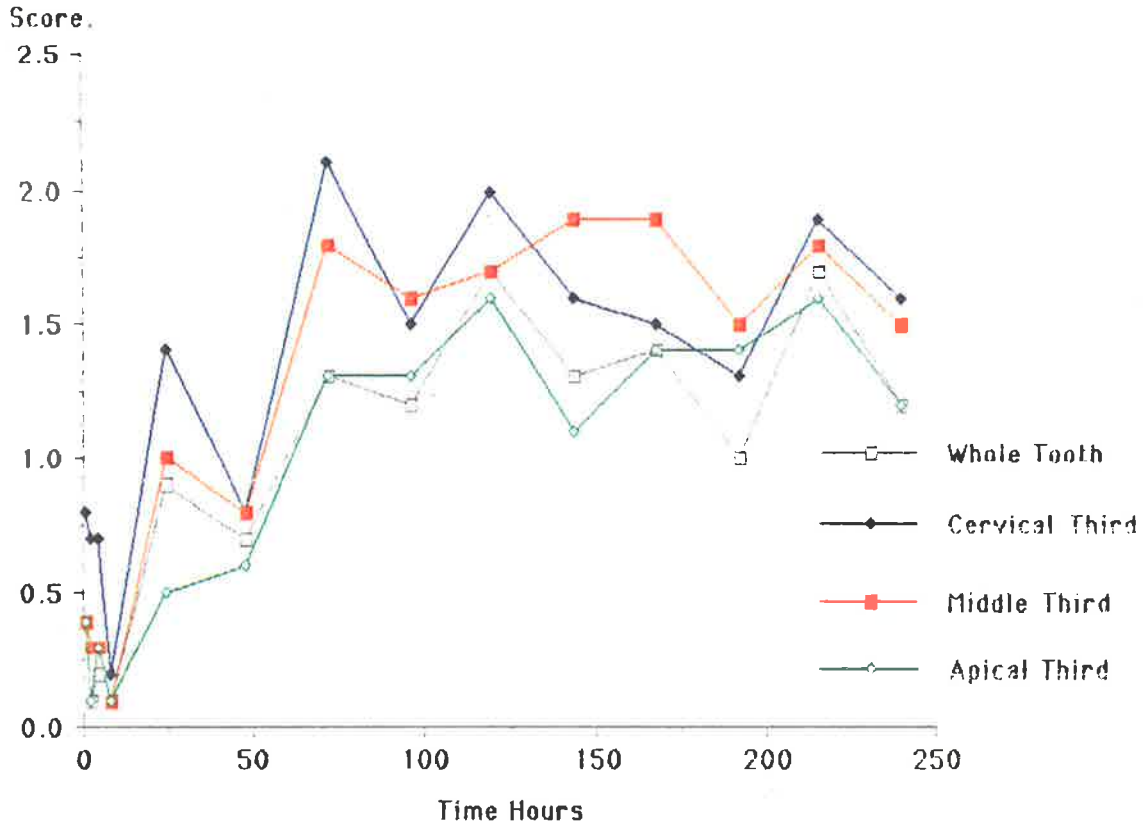

Figure 5.1. Comparison of X-Ray Spectrum Scores for Root Areas Versus Time.

5.2.6. Comparison of the Calculated Ratio and the Scoring System.

The two measuring techniques were compared by placing them in a cross table, the pattern displayed is the ideal complimentary pattern made up by a diagonal from top left to bottom right corner indicating an identical match (Table 5.11.). This was to be expected as they both utilized the same sample, and the scoring techniques complimented each other.

Table 5.11. Comparison of X-Ray Spectrum Scoring Methods.

		Co/BG ratio			
Scoring		Ratio<2	2< Ratio <3	3<Ratio<5	5<Ratio
System	0	193	-	-	-
	1	-	141	-	-
	2	-	-	188	-
	3	-	-	-	38

5.3. X-Ray Image Analysis Results.

X-ray mapping with the principal aim of providing spatial information of the distribution of elements has been a part of electron microanalysis for many years. A pulse recorded by an X-ray detector is shown as a bright dot on the display monitor of the microscope. The image analysis programme was set to detect calcium, phosphorus, cobalt and geranium on the tooth surface. Calcium and phosphorous were used as positive controls, geranium was a negative control representing background values. Analysis of the image required an empirical formula designed by the operator unlike the X-ray spectrum where a mathematical formula was used to assign scores. The teeth were analysed to test for any time effect, and regional differences similar to the X-ray spectrum.

5.3.1. Whole Tooth Surface Results.

All 140 teeth were scored for the X-ray image of cobalt produced. These were then collated into means and standard deviations for each time group (Table 5.12.). Included in the table are the details of the distribution of scores within each time group. The scores shown in Table 5.12. are for cobalt only.

Table 5.12. Image Analysis Scores for the Whole Tooth.

Time			Scores			
Hours	Mean	Std.Dev.	0	1	2	3
1	0.5	0.53	5	5	-	-
2	0.5	0.53	5	5	-	-
4	0.4	0.52	6	4	-	-
8	0.2	0.42	8	2	-	-
24	1.1	0.74	2	5	3	-
48	0.7	0.82	5	3	2	-
72	1.5	0.97	1	5	2	2
96	1.2	0.92	3	2	5	-
120	1.7	0.48	-	3	7	-
144	1.3	0.67	1	5	4	-
168	1.5	0.71	1	3	6	-
192	1.2	0.79	2	4	4	-
216	1.6	0.70	1	2	7	-
240	1.2	0.63	1	6	3	-

Analysis of this statistically was not performed due to the empirical nature of the scoring system. The scoring system did demonstrate a trend towards an increase in the mean score with time.

5.3.2. Results of Image Analysis for the Cervical Third.

All 140 teeth were scored for the cervical third X-ray image they produced. A mean and standard deviation for cobalt were calculated for each time group, and details of the distribution of scores in each group are included (Table 5.13.). There was no clear linear increase in the score with time due to the day 3 result which had the highest score overall (Table 5.13.). If this is ignored there was a trend for an increased score with time.

Table 5.13. Image Analysis Scores for Cervical Third of Root.

Time Hours	Mean	Std.Dev.	Scores 0	1	2	3
1	0.7	0.67	4	5	1	-
2	1.1	0.88	2	6	1	1
4	1.3	0.67	1	5	4	-
8	0.5	0.53	5	5	-	-
24	1.4	0.84	2	2	6	-
48	1.0	0.94	4	2	4	-
72	2.2	0.91	-	3	2	5
96	1.5	0.71	1	3	6	-
120	1.8	0.42	-	2	8	-
144	1.7	0.95	1	3	4	2
168	1.5	0.71	1	3	6	-
192	1.2	0.79	2	4	4	-
216	1.8	0.79	1	1	7	1
240	1.6	0.84	1	3	5	1

5.3.3. Results of Image Analysis of the Middle Third.

The X-ray image of the middle third of the root was scored, and a mean and standard deviation for the ten teeth in each time group was calculated (Table 5.14.). Details of the distribution for each time are shown (Table 5.14.). There was a graduation towards a higher mean score with time in the middle third indicating an increase in the permeation of cobalt with time.

Table 5.14. Image Analysis Scores for Middle Third of Root.

Time			Scores			
Hours	Mean	Std.Dev.	0	1	2	3
1	0.5	0.53	5	5	-	-
2	0.4	0.97	8	1	-	1
4	0.3	0.67	8	1	1	-
8	0.1	0.32	9	1	-	-
24	1.1	0.57	1	7	2	-
48	0.7	0.82	5	3	2	-
72	1.7	1.06	2	1	5	2
96	1.6	0.84	1	3	5	1
120	1.8	0.42	-	2	8	-
144	1.8	0.79	-	4	4	2
168	1.6	0.84	1	3	5	1
192	1.3	0.82	2	3	5	-
216	1.9	0.88	1	1	6	2
240	1.5	0.97	2	2	5	1

5.3.4. Results of Image Analysis of the Apical Third.

The X-ray image produced of the apical third of the root was scored. A mean and standard deviation was calculated for the ten teeth in each time group (Table 5.15.). Details of the distribution for each time are shown (Table 5.15.). There was a graduation towards a higher mean score with time in the middle third indicating an increase in permeation of cobalt with time.

Table 5.15. Image Analysis Scores for Apical Third of Root.

Time			Scores			
Hours	Mean	Std.Dev.	0	1	2	3
1	0.6	0.84	6	2	2	-
2	0.2	0.42	8	2	-	-
4	0.3	0.67	8	1	1	-
8	0.1	0.32	9	1	-	-
24	0.8	0.79	4	4	2	-
48	0.7	0.82	5	3	2	-
72	1.4	0.97	3	-	7	-
96	1.3	0.67	1	5	4	-
120	1.5	0.71	1	3	6	-
144	1.3	1.06	3	2	4	1
168	1.4	0.70	1	4	5	-
192	1.4	0.84	2	2	6	-
216	1.5	0.85	2	1	7	-
240	1.3	0.95	3	1	6	-

5.3.5. Comparison of Image Scores for All Root Thirds.

A comparison of the mean scores for each of the root thirds and the whole root at each measurement time can be seen (Fig. 5.2), which depicts the mean scores against time. No statistical analysis of the data was carried out, but there was an observable trend for the mean scores for the cervical and middle thirds of the teeth to be higher than that of the apical third, or the whole tooth. The cervical and middle third scores at each time were seen to be similar.

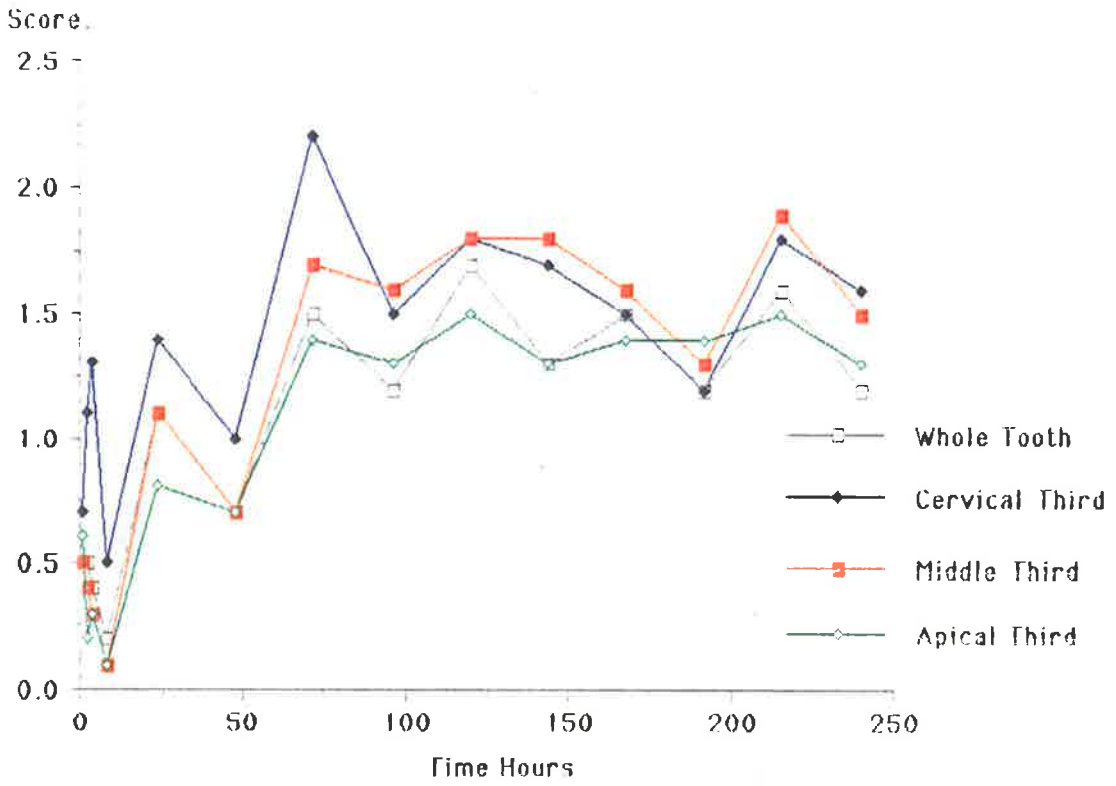

Figure 5.2. Comparison of X-Ray Image Scores for Root Areas Versus Time.

5.3.6. Comparison of the X-Ray Spectrum and X-Ray Image Scoring Methods.

The differences between the two measuring techniques used in this experiment was tested (Table 5.16.). Statistical analysis was not considered appropriate because of the empirical nature in which the score for the X-ray image analysis was calculated. A strong correlation between the two scoring systems existed, 82.86 percent. There was a tendency for the image analysis score to be higher than the X-ray spectrum score for the lower scores, and lower for the highest X-ray spectrum scores. This suggested the image analysis was not as a sensitive scoring system as that of the X-ray spectrum. The image analysis scoring system showed a tendency to overestimate the low scores which were considered to be background only by the spectrum scoring method, and underestimated the higher levels.

Table 5.16.

Comparison between the X-Ray Image Analysis and X-Ray Spectrum Scores.

		X-Ray Spectrum Scores			
		0	1	2	3
X-Ray	0	165	3	-	-
Image	1	31	121	18	-
Scores	2	-	15	162	22
	3	-	-	7	16

Table 5.17. Number of Teeth with Furcations.

Time Gp.	Teeth With Furcations		
Hours	Number	Mand.8	Max.8
1	2	1	1
2	7	4	3
4	3	1	2
8	5	2	3
24	2	-	2
48	5	2	3
72	4	1	3
96	5	3	2
120	8	4	4
144	7	5	2
168	4	1	3
192	6	5	1
216	5	3	2
240	8	6	2
Total	71	38	33

5.4. Furcations.

Some third molar teeth had a discernible furcation present. Seventy one of the molar teeth had recognizable furcations (50.71%), and these were broken down into the various time groups, and whether they were from maxillary or mandibular teeth (Table 5.17.). Furcations were more prevalent in the mandible, 97.44% of mandibular third molars, all but one of teeth used. The maxilla had a prevalence of 32.67% third molar teeth with furcations.

Table 5.18. X-Ray Spectrum Ratio of Furcation Regions.

Time	Co/BG	Cobalt		Background (BG)	
Hours	Mean	Mean	SD	Mean	SD
1	1.17	10.5	2.12	9.00	0.0
2	1.44	13.14	3.24	9.29	0.95
4	1.00	8.50	2.12	8.50	0.16
8	1.09	10.20	1.30	9.40	0.55
24	2.00	21.00	21.21	9.00	4.24
48	2.65	26.15	17.23	9.75	1.61
72	3.70	43.50	45.32	10.00	3.46
96	3.29	33.40	18.57	9.80	1.48
120	3.98	34.38	9.72	8.63	0.74
144	4.34	39.57	10.81	9.14	1.21
168	2.94	26.25	10.75	9.25	0.96
192	3.14	29.50	13.38	9.33	1.51
216	4.59	44.60	12.26	9.80	1.30
240	3.82	35.38	15.01	9.13	0.99

An X-ray spectral analysis was carried out on the furcation region of these teeth, and from this a Co/BG ratio was calculated for each tooth. These were then used to

calculate a mean and standard deviation for each of the time groups shown (Table 5.18.).

The numbers of teeth with furcations in each time group varied, and combined with the individual tooth permeability lead to variation of the Co/BG ratio with time. There appears to be a progression with time towards a larger ratio indicating a time effect is also seen in this region, and was confirmed by statistics. The variation in the ratio corresponds to the dips and rises seen in the other tooth areas, and may be due to individual tooth permeability variation. Figure 5.3 shows the mean furcation ratios were plotted against time on the same plot as the cervical, middle thirds and the whole tooth (Fig 5.1.).

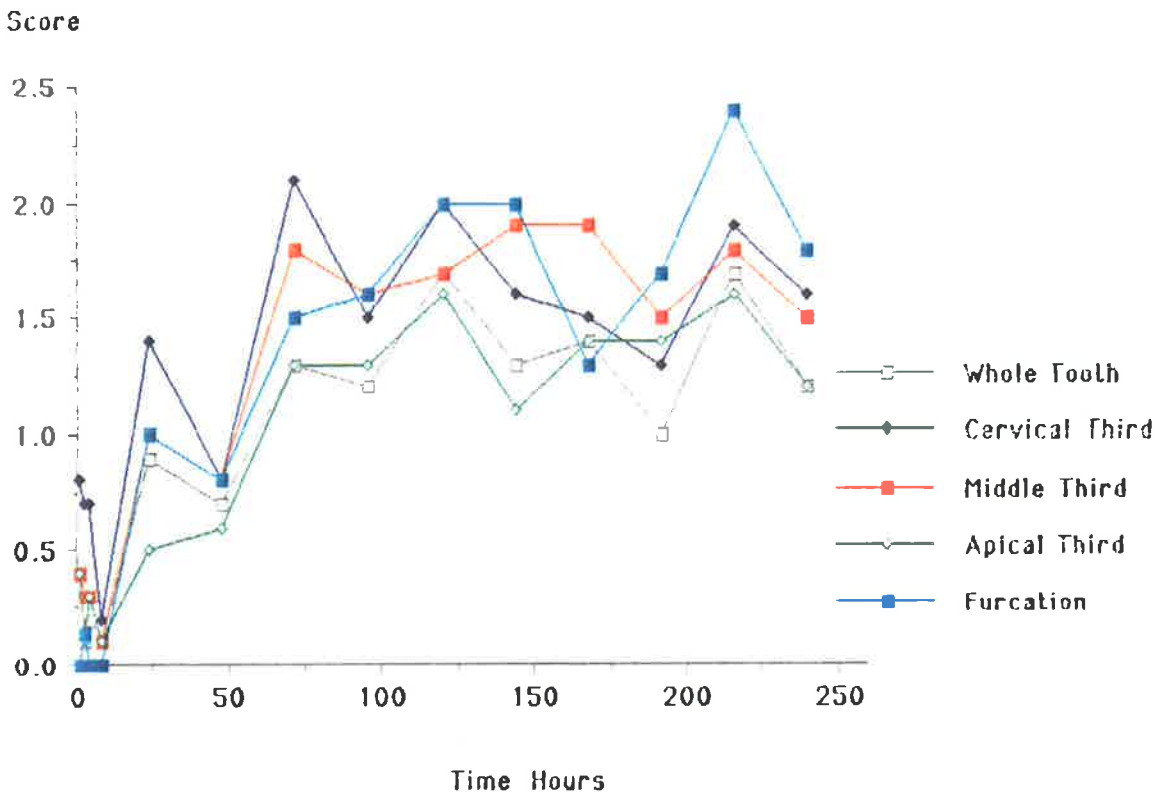

Figure 5.3. Comparison of X-Ray Spectrum scores of Furcations, and Root Areas Versus Time.

A statistical analysis was done to compare the teeth with furcations to those without, and calculate any differences in the permeability of the cervical, middle, and apical thirds of the root and the whole root. The analysis was done by the 2V BMDP statistical software programme. The means for each group are shown (Table 5.19.). The presence or absence of the furcation was not found to be significant in the X-ray spectral score for the teeth with a probability of 0.3085. A trend was seen for the teeth with furcations to have a higher mean for all tooth sections.

Table 5.19. Analysis of Effect of Furcation Presence.

	Without Furca		With Furca	
Tooth Section	Mean	S.D.	Mean	S.D.
Cervical Third	2.90	1.28	3.20	1.64
Middle Third	2.89	1.57	3.03	1.61
Apical Third	2.34	1.16	2.54	1.16
Whole Root	2.40	1.09	2.62	1.14

5.5. Accessory Canals.

The surface of the root was examined visually under the scanning electron microscope for the presence of accessory canals on a maximum of two root surfaces. Accessory canals were observed on 92 teeth, or 67.71% of the teeth examined. These were divided into maxillary and mandibular teeth with accessory canals, 68.31% and 58.97% respectively. A total of 347 accessory canals were noted in these teeth, and their distribution, including the number of canals per tooth are shown (Table 5.20.).

Table 5.20. Incidence of Accessory Canals

Time	Teeth		Total	No.of Canals per Tooth						
Hours	Max.	Mand.	Canals	1	2	3	4	5	6	>6
1	4	2	20	1	2	-	1	1	1	-
2	4	1	17	2	1	-	-	-	1	1
4	6	2	35	3	2	-	1	-	-	2
8	3	4	18	3	1	1	-	2	-	-
24	5	-	16	2	-	1	1	-	-	1
48	5	2	23	2	2	2	-	-	-	1
72	4	1	31	-	2	1	1	-	-	1
96	6	2	26	3	1	2	-	1	-	1
120	5	3	28	3	-	1	1	2	-	1
144	6	1	14	3	1	3	-	-	-	-
168	6	2	38	1	1	1	2	1	1	1
192	5	-	17	1	1	2	-	-	-	1
216	5	2	41	-	1	-	2	3	-	1
240	5	1	23	2	1	1	-	-	-	2
Totals	69	23	347	26	16	15	9	10	3	13

The area where these canals were located was also recorded, whether in the furcation, or cervical, middle or apical thirds. One canal did not have it's location recorded and was left out of Table 5.21. Fifty of the 71 teeth with furcations, 70.42%, had accessory canals present in the furcation region, but owing to the techniques used here this may have been an under estimation. The furcation regions were found to harbour 28.32% of the accessory canals found. As was expected the apical third of the root accounted for 53.76% of the accessory canals seen, with 13.29% and 4.62% for the middle and cervical thirds respectively. The full distribution of the canals is displayed (Table 5.21.).

Table 5.21. Location of Accessory Canals.

Time	Location of Furca			
Hours	Furca	Cervical	Middle	Apical
1	2	1	3	14
2	2	-	3	12
4	12	-	6	17
8	4	-	1	13
24	-	-	1	15
48	4	-	6	13
72	4	2	9	16
96	10	-	-	15
120	15	2	-	11
144	7	2	3	2
168	8	1	11	18
192	7	1	-	9
216	12	6	2	21
240	11	1	1	10
Total	98	16	46	186

In addition the size of the accessory canals was also recorded (Table 5.22.). Most of the accessory canals were less than 0.1mm in diameter, 88.76% . Five of the six accessory canals identified as greater than 0.2mm and less than 0.5 were located in the furcation regions, and all three accessory canals greater than 0.3mm were located in the furcation.

Table 5.22. Size of Accessory Canals.

Time	Size in millimetres			
Hours	<0.1	<0.2	<0.3	<0.5
1	18	1	1	-
2	16	-	1	-
4	34	1	-	-
8	18	-	-	-
24	16	-	-	-
48	21	2	-	-
72	31	-	-	-
96	23	3	-	-
120	20	8	-	-
144	7	6	-	1
168	32	6	-	-
192	14	3	-	-
216	39	2	-	-
240	19	1	1	2
Totals	308	33	3	3

The teeth with accessory canals and those without were separated into two groups and statistically analysed for any differences in permeability. The calculated means and standard deviations for each of the four sections, cervical, middle, apical thirds, and whole root are shown (Table 5.23.). The presence of accessory canals was not found to be a significant factor in permeability of the tooth roots to the cobaltous ion, probability of 0.1298. There was a trend for the teeth with accessory canals to have greater permeability than those without accessory canals, as was seen with greater mean values for each section.

Table 5.23. Analysis of Effect of Accessory Canal Presence.

	Without Acc. Canal		With Acc. Canal	
Tooth Section	Mean	S.D.	Mean	S.D.
Cervical Third	2.88	1.44	3.15	1.49
Middle Third	2.74	1.53	3.07	1.61
Apical Third	2.19	1.05	2.56	1.20
Whole Root	2.26	1.07	2.63	1.14

CHAPTER 6. DISCUSSION.

The physiological unity of dentine and pulp is well accepted, but the closeness of the relationship between the pulp and the adjacent periodontal ligament has received less attention, although they too are related to each other via dentine and cementum. Chronic inflammation is a common sequela of caries and its restoration, and therefore a common presence in pulp tissue. Inflamogens are known to exist as a result of chronic inflammation and their ability to influence external tissues of the periodontal ligament is dependent upon the permeability of the root dentine and cementum.

Endo-perio lesions are described and experimentally verified (Seltzer et al 1963, 1967, Winter and Kramer 1972, Sinai and Soltanoff 1973, Clarke et al 1986,). Root permeability to tetracycline has been described (Abbott et al 1988). The results presented in chapters 4 and 5 present evidence supporting the concept that these structures are permeable.

6.1. Experimental Model.

The experimental model proved to be an appropriate system to evaluate root permeability. Teeth with obvious leakage were identified by the sharp early rise in CPM. A total of eight teeth, two from the full wax group, two from the apical seal group, and four from the molecular weight studies leaked and were removed from the study. The leakage may have resulted from technique failure, lack of wax adaptation, or to large patent accessory canals. Other teeth within experimental groups had noticeably higher CPM, and may also have had minor leakage, but where there was doubt they were retained. It is possible that all specimens leaked, but differences were seen between the various groups which could not be statistically assigned to chance.

6.2. Area Permeability Studies.

Tritiated labelled water (MW 20) has a molecular diameter of 0.197 nm (Renkin 1954). Tooth roots were permeable to this molecule. Using an arbitrary measure, that

10 times background indicated significance, tritiated water was seen to permeate the tooth root after two hours. The levelling off of the time versus CPM graph was indicative of equilibration. The equilibration was estimated to be approximated between 8 to 16 days (Figure 4.1).

The reason for conducting the tritiated water diffusion experiments was to compare between the various root areas, furca open, furca closed, and apical wax only. Initial comparison of the total CPM revealed no differences between these three groups (Figure 4.1). The furca open group had a significantly smaller surface area available for diffusion than the other two groups. The surface area for diffusion was used as a covariate in analysis, and the groups were analyzed for CPM/mm^2. The furca open group permeability was found to be significantly greater than the other two throughout the 8 day experimental period. When the CPM/mm^2 were analyzed after one and two days the furca open group was confirmed as more permeable than the other groups.

6.2.1. Furcation Permeability.

This increased furcation permeability to the tritiated water may have been due to the molecule permeating dentine and cementum, or it could be the result of patent furcation canals. Root dentine tubules are heavily sclerosed (Vasaliadas et al 1983a), and covered by acellular cementum that could result in non-permeability of the tissue. Accessory canals may provide a pathway for irritants to communicate between the internal and external surfaces of the root.

Accessory canals were found in 70.42 % of the furcations in third molars in the current SEM experiment, five of these were between 200 and 500 microns. Without removing the teeth and sectioning them, it was not always possible to orientate the electron beam of the electron microscope to get a good visual image of the entire furcation region. The number of accessory canals reported was probably underestimated.

Large accessory canals have been reported in the furcation region (Russell and Kramer 1956, Kramer 1960, Saunders 1966). Topographical studies do not assess patency of accessory canals, but have suggested the presence of accessory canals over a

wide range: from 2.3 % (De Deus 1975) to 76% (Burch and Hulen 1974). In a topographical study of the pulpal floor and external root surface the presence of accessory canals was 8 % and 64 % respectively (Perlich et al 1981). Patent accessory canals were estimated in early studies in 20 to 23% of furcations (Moss et al 1965, Winter 1962). More recent studies using dyes have found furcations to have accessory canals in 46 % of first molars (Vertucci and Williams 1974), and 67.4 % of molars (Gutmann 1978), suggesting they were of frequent occurrence.

Patent furcation canals should be significantly more permeable than dentine, and provide for rapid permeation of the tritium labelled compound. The radius of a narrow tube determines the rate of flow (Poiseuille's Law).

The reason for the greater permeability of the furcation region can not be concluded from this study, but suggests that the area represents a means of communication between the internal and external tissues of the tooth. This finding should be evaluated in relation to the work of Winter and Kramer (1972), and Walton and Garnick (1986) where pulpal lesions in molar teeth of monkeys were seen to spread early to the furcation region. They suggested this was due to the spread of periapical lesions into the furcation region from pulpal irritation, but permeation of inflamogens in the pulp chamber to the furcation periodontal ligament could induce the inflammatory changes recorded. The literature reviewed and the present study indicated the presence or absence of a furcation on a tooth may have a pronounced effect on the permeability of teeth. The implications are widespread in both periodontal and endodontic disease.

6.3. Comparison between Molar Material.

The control fully waxed teeth were separated into two groups, first and second molars, and third molars, for comparison. The two groups were similar until the fourth day, when the control first and second molar group had a pronounced increase in CPM counts. This suggested that the wax and varnish seal was failing, and the CPM were distinctly greater than the third molar group. An explanation of this may have been

sealing difficulties of the complex root anatomy of the first and second molars. In the group where only the apex was sealed, the results of both the third molar and first and second molar groups were comparable. The increased age of the first and second molar teeth did not appear to reduce the permeability, a finding contrary to that of Linden (1968). Changes observed with increasing age include secondary dentine formation due to attrition, caries, restorations and sclerosis within dentine tubules. The sample sizes in this case were small and no assessment of secondary dentine, or sclerosis was made.

6.4. Molecular Weight Studies.

The tritiated water studies were repeated in this group with similar findings, significant permeation after two hours and equilibration between 8 and 16 days. The glucose studies indicated the tritiated glucose could be readily identified in the bathing solution after eight hours. The equilibration point appeared to be around forty days (Figure 4.4,). Glucose has a molecular weight of 180.2, and a molecular diameter of 0.357nm (Kim et al 1971). This model demonstrated these teeth were permeable to small molecules. The concentration of glucose used in this experiment was 6.28×10^{-6} mol. as the driving force for the permeability experiments.

The dextran studies indicated the tritiated dextran was readily seen in the external bathing fluid after 24 hours, and no equilibration point was identified before the end of the forty day observation period (Fig 4.6). The results supported the hypothesis that the teeth are permeable to tritiated dextran. The dextran used had a molecular weight of 70,000 a long chain linear molecule, and a diameter of 4nm (Kim et al 1971). The concentration of dextran used was 1.76×10^{-6} mol.

Diffusion of compounds can be represented as the flux of fluid through a material from the Fick Equation (Pashley 1985).

$$\text{Flux} = D_S \, A \, (dC/dX)$$

D_S = Diffusion Coefficient A = Diffusion surface area

dC = Concentration of solute dX = dentine thickness.

The concentration of a solute to permeate the tooth structure is directly proportional to the flux of the solute through the tooth root. This has importance in drawing conclusions regarding the physiologic importance of the permeability of the tooth roots to molecules tested. Most biologic compounds are found in small concentration (nanomolar), if a large concentration was required to drive the solute through the tooth root it would be unlikely to have clinical significance. The present findings support the work of Pashley and Livingston (1978), which indicated an increase in the size and the molecular weight of the molecule resulted in decreased diffusion rates on these molecules.

The experimental model was the same as that developed for the area studies. Parallel control studies were conducted with full wax coverage of third molars for each of the molecular weight experiments. There were significant differences between the control group and the corresponding test group for each chemical tested. This evidence supports the finding that the teeth were permeable to tritiated glucose and dextran as well as radiolabelled water.

These studies indicated medium sized molecules of 180.2 and 70,000 molecular weight were capable of permeating the tooth roots. The linear molecular form of dextran may have been an advantage for diffusion through the tooth root. A spherical molecule of similar molecular weight may have been restricted. Molecular dimensions were more important than molecular weight or charge in determining the rate of permeation through dentine (Pashley et al 1977). A large number of biologically important molecules are within this range. The biologic significance of these findings remains speculative, because the concentrations of the glucose and dextran used were many times greater than the physiological concentrations of the local hormones found in tissues, micromolar compared to nanomolar amounts.

However, *in vivo* experiments in dogs have shown little or no molecular sieving of protein diffusion through crown dentine (Pashley et al 1981d). The pulpal interstitial fluid was essentially the same as that collected from the dentine surface. The major diffusion barrier to protein was the capillary endothelium, or its basement membrane

(Pashley et al 1981d). Fibronectin is a high molecular weight protein (340,000) and slowly permeated dentine (Haldi and Wynn 1963). Okamura et al (1979) reported plasma proteins adsorbed to dentine tubules in carious dentine and in dentinal fluid. These experiments indicate that very large molecules penetrated dentinal tubules, and if there was any sieving effect this was via the enamel, cementum, or altered dentine, sclerotic or tertiary.

This study indicated the cementum was not a barrier to diffusion and is supported by the work of Fogel et al (1988). Dextran (70,000 MW) was able to penetrate tooth roots with time. The potential exists for small to medium molecular weight inflamogens to pass from the dental pulp to the periodontal ligament, and vice versa. Small concentration gradients are known to have important physiologic effects, for example chemotaxis of phagocytes, and local hormone effects of prostaglandins, and complement (for review see McHugh 1990a,b). Diffusion of inflamogens in low concentrations may illicit an inflammatory response on the opposing root surface, and has histologic support (Kipoti et al 1984, Seltzer et al 1963, Bender and Seltzer 1972, Sinai and Soltanoff 1973).

6.5. Root Planing Effect.

In these experiments cementum was removed with hand instruments as described by O'Leary and Kafrawy (1983), most of the cementum was removed with fifty strokes of a gracey curette. No histologic assessment was made of the root surface to determine whether there was complete removal of cementum. The root planing was performed to evaluate the effect of cementum removal on tooth root permeability.

The effect of root planing was studied with tritiated labelled water, glucose, and dextran molecules. The results indicated there was no significant difference between the root planed and the non root planed third molars. The experiment using glucose had a trend for the non root planed teeth to be more permeable than the root planed group, an unexpected finding.

Cementum has previously been considered to be a major diffusion barrier on the tooth surface (Wach et al 1955, Marshall et al 1960). Cementum permeability has not been assessed by adequate experimentation, due to the difficulty in isolating the cementum intact from other components of the tooth root. Opinions have been expressed relating to the permeability of cementum without any investigation (Lindhe 1984, Andreasen 1985). The role of intracanal medicaments in endodontic therapy and their diffusion through dentine have been investigated, but have ignored the cementum in their conclusions (Avny et al 1973). Others have produced evidence of limited permeability of cementum to a range of compounds (Abbott et al 1988).

The limited barrier effect of cementum seen may result from a smear layer being produced by hand instrumentation in root planing. All dentine cutting procedures resulted in smear layer production (Eicke et al 1970). This substantially reduced the open diameters of the underlying dentine tubules, and has been indicated to reduce the fluid movement across the dentine surface (Stevenson 1965, Johnson et al 1973, Pashley et al 1978c, 1981b, Reeder et al 1978, Outhwaite et al 1976). No attempt was made to remove any smear layer produced by hand instrumentation, and no histological assessment of the presence or absence of a smear layer was conducted. The smear layer was unchallenged by chemical attack from bacterial source due to the aseptic technique, and no chemicals were applied to the root, allowing the smear layer to remain intact.

The smear layer of the dentine surface following the removal of cementum had a similar effect to cementum on the permeability of tooth roots. In the glucose experiment the smear layer produced appeared to be a greater barrier than cementum although this was not statistically significant. Further experimentation to establish the difference in permeability between teeth with smear layer present and removed, and with intact cementum would be useful.

6.6. Coronal Dentine Compared to Root Dentine.

The crown dentine was significantly more permeable than the tooth roots to all radiolabelled chemicals, even when the difference in volume of the bathing solutions in

different experiments was considered. Several points may help to explain these findings: firstly there have been several reports of decreased permeability of root dentine compared to crown dentine (Wach et al 1955, Marshall et al 1960, Hampson and Atkinson 1964, Vasiliadas et al 1983a,b, Fogel et al 1988). An increased incidence of dentine sclerosis in the root systems of the teeth has been noted to begin at a young age and progress through life (Vasiliadas et al 1983b). More sclerosis was found in the apical portions of dentine, and Marshall et al (1960) found that permeability decreased from the cervix to the apex of the root. The blocking of dentinal tubules would constitute a significant increase in the resistance of the dentine tubules to diffusion of molecules through them.

The thickness of the crown dentine was known, mean value 1.8mm, and no significant differences in the thickness between experimental groups was found. The thickness of the root dentine was unknown. The thickness of dentine is less in the apical regions where dentine sclerosis is most prevalent, and the areas close to the CEJ could be thicker than the crown dentine studies. Changes in dentine thickness lead to an exponential change in hydraulic conductance (Reeder et al 1978). The area around the CEJ comprises the majority of the area available to permeation of compounds. The surface area available for diffusion of material in the tooth root was substantially larger than a class one cavity, but not measured.

The number of dentine tubules, and their diameters was decreased in the root compared to the coronal dentine (Fogel et al 1988). This suggested that the root dentine would be less permeable than the coronal dentine, and was substantiated (Fogel et al 1988). Their study ignored the effect of cementum, and although reduced in amount the root dentine was permeable.

The amount of sclerosis and therefore narrowing of the root dentine tubule diameters, or blockage was unknown. In addition the teeth roots had the presence of cementum, or a smear layer on the external surface (root planed teeth), which reduced the rate of permeation of radiolabelled compounds.

6.7. Electron Microanalysis.

The experimental model used during electron microanalysis was the same as the radiolabelled permeability studies. Electron microanalysis is an effective tool for identifying micro-quantities of elements either within sections of structures or on their surface. The accuracy varies with the machine and method used. The EDS system used via the Phillips 505 SEM and Tracor Northern 5500 remains an accurate method of assessment for the presence of elements in structures, and is capable of locating the presence or absence of cobalt on the root surface.

Measurement of the permeation of the cobaltous ion in the microanalysis was made via scoring X-ray spectra of the root surface, and a score for the X-ray image analysis. Statistical analysis of the image analysis was not performed because of the empirical nature of the scoring system. The cervical third was shown to have reached detectable cobalt on the root surface, at two hours, prior to the other two thirds, middle and apical, 1 day and 3 days respectively. There was a strong correlation between the two scoring systems (Table 5.16). There was a tendency for the image analysis score to be higher than the X-ray spectra at the lower detection range, and lower than the X-ray spectrum at the higher detection range. This indicated the image analysis was not as sensitive a scoring system as that of the X-ray spectrum, but gave graphic visualization of cobalt permeation of the root.

6.8. X-Ray Spectra Results.

When the whole root surface was analyzed statistically it was shown that there was an increase in the X-ray spectrum value for cobalt with time, supporting the finding that the tooth was permeable to the cobaltous ion, and that diffusion was a factor of time, and when each third of the root was analyzed similar results were seen.

Cobalt was clearly detected on the external surface of the tooth after one day. The cobaltous ion has a molecular weight of 58 and a diameter of 0.074 nm, and required a comparable time to 70,000 MW dextran, diameter 4 nm (Kim et al 1971). Cobalt was present in solution as a doubly positive charged ion. The effect of ionic

charge according to Pashley and Livingston (1978) was not as important as molecular size. However fluoride and chlorhexidine which bind to dentine had substantially reduced permeability (Pashley et al 1977). Cobaltous ion forms an insoluble lilac precipitate with free phosphate ions. A lilac colour was seen in the root system, which suggested the cobaltous ion had become bound to free phosphate ion in dentine. The apparent slower diffusion of cobalt across the tooth than radiolabelled studies can be explained by binding of cobaltous ion, and the difference in method of measurement used.

Statistical analysis between the root thirds indicated the cervical and middle thirds were significantly different to the apical third. The data suggested a trend for the cervical third to be more permeable than the other two thirds. At 10 of 14 data collection times the cervical third means were greater than the middle third, which were more than the apical third at all times. The cervical third had mean spectral ratios which were significant for cobalt at all times except at eight hours. The middle thirds had significant spectral ratios for cobalt from one day onwards. The apical third spectral ratios indicated cobalt present on the external surface at 1 day, but not at 2 days, and all times after this.

The means for each root third were not a steady progression with time due to individual variations in tooth permeability within groups. The X-ray image scoring system although not analyzed statistically had means which supported the spectral findings of cobalt permeability with the cervical third being greater than the middle, which was greater than the apical third.

The data collected show that the roots were permeable to cobalt, and suggest the cervical third was more permeable than the other thirds of the root. There may be several reasons for this including the presence of furcations, the thickness of cementum, the level of dentinal sclerosis, and the absence of cementum at the CEJ in some teeth.

Half of the teeth used had furcations present on the tooth root, which were located in the cervical and middle thirds. The presence of a furcation may have resulted in increased permeability via the associated accessory canals. Although a trend was

seen in the statistical analysis no significant difference between the teeth with and without furcations was seen.

The electron microanalysis observed a trend for the furcated teeth to be more permeable in all sections, as the cervical, middle, and apical thirds had larger means than the non-furcated teeth. The furcation region was usually located in the cervical third of the tooth, but this was not reflected by a significantly greater cervical third permeability. The furcation region tended to have the highest mean values for X-ray spectra of all tooth sections (Fig 5.3). These findings support those from the radiolabelled studies.

The cementum in the cervical third is the thinnest seen on the root surface, and often only of an acellular type, 20 to 50 microns thick (Sicher and Bhaskar 1972). This contrasts to the apical regions where the thickness may be 200 microns or more, and consists of both acellular and cellular types of cementum. Dentine sclerosis was prevalent in the apical regions of the tooth (Vasiliadas et al 1983b). The combination of thicker cementum, and dentine sclerosis could be the reason the cobaltous ion was seen to permeate first the cervical third of the root.

It has been estimated ten percent of the population have a gap between the where the enamel finishes, and where the cementum commences on the root surface (Sicher and Bhaskar 1972, Schroeder and Scherle 1988), a number of the teeth used in this study did not have cementum covering dentine in the cervical third, and dentine was the only barrier to cobalt diffusion.

Cervical third accessory canals have not been a common finding (Rubach and Mitchell 1965, De Deus 1975), and a finding of the present SEM study. They were unlikely to be the reason for greater cervical third permeability observed.

Evidence from the SEM was used to discount the possibility of the present observation being due to leakage. The teeth may have leaked from either the apical seal, or the tooth crown and vial cap seal. There was a reduced possibility of leakage, because of the distinct smooth surfaces which were easily dried with air, which ensured better wax adaptation than in the radiolabelled experiment. If leakage had occurred

from the enamel side under the coronal wax seal (refer to model set up Fig 3.4) then a trace of cobalt should have been seen on the enamel surface. One tooth only had cobalt located on it's enamel surface, in an anatomical concavity, which may have been due to the crown wax seal being imperfect.

Failure of the wax seal at the apex would have been expected to produce a different picture. In this case the apical third of the tooth root was expected to have a greater cobalt to background ratio than the other two thirds. This was found in 14 teeth. The apical third having a larger X-ray spectral score was not conclusive of leakage, as cobalt may have penetrated an accessory canal commonly found in this region (De deus 1975), and the present SEM study. Alternatively leakage of the apical seal may appear as a fanning out over the root surface from the point of failed wax adaptation, which would have been located by the image analysis. One tooth had a pattern which fitted this category.

An explanation that the X-ray spectral permeability results seen were the result of cobalt having leaked from the apical region, and then settled over the root surface, was not supported by the X-ray image analysis, as the exposed enamel would also have had cobalt covering its surface. The images obtained always, with one exception, had a clearly demarcated CEJ region.

6.9. Comparison of X-Ray Spectra and X-Ray Image Scoring.

There was a tendency for the image analysis score to be higher than the X-ray spectrum score for the lower scores, and lower for the highest X-ray spectrum scores. This suggested the image analysis was not as sensitive a scoring system as that of the X-ray spectrum. The image analysis scoring system demonstrated a tendency to overestimate the low scores which were considered to be background only by the spectrum scoring method, and underestimated the higher levels.

6.10. Accessory Canals.

The presence of patent accessory canals could have an important effect on the permeability of the tooth because of it's larger radius than dentine tubules (Poiseuille equation). Assessment for the presence of accessory canals was only made on two surfaces of the teeth due to the method of fixing the teeth to the electron microscope stubs. In spite of this 67.7% of teeth were seen to have a total of 347 accessory canals. This indicated teeth with accessory canals commonly had more than one.

Whether the canals located in the present topograhical study were patent was not examined. The incidence of accessory canals has been most likely underestimated in the present study because only two of the four surfaces were examined. The teeth examined in this study were third molars, and represent the highest incidence of accessory canals recorded in literature, while other studies cited used a range of teeth from incisors to molars. Molar teeth have been shown to have more accessory canals than incisors and premolars (De Deus 1975), due to their complex root morphology and root canal systems.

Apart from furcations the most common site for accessory canals was the apical third of the tooth root followed by the middle and cervical thirds, 53.7%, 13.3%, and 4.6% respectively. This was in agreement with all other studies cited previously.

Most of the accessory canals seen (88.7 %) were less than 100 microns in diameter. Of the six recorded between 200 and 500 microns, five were located in the furcation region. These represent very large canals, and if patent would be considered a highway for communication between the pulp chamber and the periodontal ligament. Koenigs et al (1974) reported the range of size of accessory canals to be 4 to 250 microns. Hess et al (1983) reported the accessory canals to be small of 60-80 microns in diameter in contrast to the apical foramina which were 200-250 microns. The present study indicates some of the accessory canals seen in the furcation region were as large as apical foramina, and therefore would be a major site of interaction between the internal and external environments of the tooth. Some of the canals seen would be expected to be patent, and these teeth could present a difficult case for endodontic

therapy, and would allow spread of inflammation from dental pulp to the periodontal tissues, and vice versa. It could be envisaged that these teeth could have endodontic problems presenting as an abscess in the furcation region, or chronic lesion could be a differential diagnostic problem for furcal bone loss.

6.11. Further Experimentation.

Using Tritiated water further experimentation to examine the importance of the dentinal and cemental structures in comparison to the apical foramen in providing communication between the pulp and the external root surface would be valuable. This could be done with an equal number of teeth with no wax on any part of the root, and compared to a group with their entire surface of the root sealed with wax and varnish except for their apices. This would complete the variations possible in the Tritiated water experiments, and would provide important information on the permeability of the root structure, dentine and cementum to water, compared to the apical canal.

Other sealing methods may need to be explored, particularly to support the later collection times, using some of the modern adhesives. The effect of removing the ligament from the tooth surface by the use of solutions such as sodium hypochlorite, could be examined, as well as the effect of first drying the tooth surface. Ten percent of teeth with porosity evident on their root surfaces after sodium hypochlorite treatment may have had altered permeability to cobalt, and were spread throughout the experimental groups, and they did not have permeability values grossly different from other teeth in their time group. The significance of this finding requires further investigation. Total dehydration of the tooth is known to induce crazing and cracking of the cementum surface, and experimental design would require a compromise not to alter the permeability of the root, and get maximal adhesion without crazing of the root surface.

Alternative methods of isolating desired root areas for study could be considered by sectioning tooth roots. Dentine discs from crowns have been used by Pashley and co-workers (Outhwaite et al 1976). A more sophisticated model may be designed from this

experimental system, however root anatomy prevents the cutting of flat dentine discs with tubules basically at right angles to the surface. Isolation of intact cementum from the other tooth structures for study is most difficult, and this problem remains unsolved.

CONCLUSIONS

1. There is a close relationship between the pulp and periodontal ligament as well as between pulp and dentine.

2. The root structure is permeable (dentine and cementum) to molecules with a molecular weight of at least 70,000.

3. Inflamogens have a lower molecular weight than 70,000, and the potential exists for pulpal-periodontal irritation by permeation through tooth structure.

4. The furcation region of molar teeth is more permeable than the remaining root structure, which has implications in the spread of endo-perio lesions.

5. The cervical third tended to be the most permeable root third, at a point where cementum is thinnest or absent from the root.

6. The SEM study found accessory canals to be a common finding in third molar teeth.

7. The importance of these pathways in clinical dentistry should be considered.

Appendix A.

TOOTH _____ EXPOSURE TIME _____

SPOT SIZE _____

WHOLE TOOTH

Stage tilt _____

MAG (10±2) _____

	0	1	2	3
Xray spectrum				
Xray mapping				

FURCATION (if present) M B D L

Stage tilt _____

MAG (25±5) _____

	0	1	2	3
Xray spectrum				
Xray mapping				

CEJ Root surface M B D L

Stage tilt _____

MAG (25±5) _____

	0	1	2	3
Xray spectrum				
Xray mapping				

ROOT SURFACE (middle third)

Root M D MB DB P

Surface M B D L

Stage tilt _____

MAG (25±5) _____

	0	1	2	3
Xray spectrum				
Xray mapping				

ROOT SURFACE (apical third)

Root	M	D	MB	DB	P
Surface	M	B	D	L	

Stage tilt _____

MAG (25±5) _____

	0	1	2	3
Xray spectrum				
Xray mapping				

NOTES

Kept on disk? _____ Disk _____ File No. _____

Photographs _____

Accessory canals number _____

location _____

Did it leak? _____

Areas of interest _____

Appendix B.

Abbreviations Used:

A.a.	*Actinobacillus actinomycetemcommitans*
Å	Angstrom Unit (10^{-10} metres)
Ag	Silver
^{14}C	Carbon 14 isotope
^{14}C-Urea	Carbon 14 labelled Urea
^{o}C	degrees Celsius
Ca	Calcium
Ca^{++}	Calcium ion
^{45}Ca	Calcium 45 isotpoe
CEJ	Cementoenamel Junction
$^{36}Cl^{-}$	Chloride ion with chlorine 36 isotope
CDJ	Cementodentinal Junction
CPM	Counts Per Minute
cm	Centimetres (10^{-3} metres)
CRT	Cathode Ray Tube
DEJ	Dentinoenamel Junction
ELISA	Enzyme-Linked Immunosorbent Assay
EM	Electron Microscope
eV	Electron Volts (1.602×10^{-19}J)
GAG	Glycoaminoglycan
$H^{32}PO_4^{-}$	Hypophosphate ion with Phosphorus 32 isotope
^{3}H	Tritium Hydrogen isotope
$^{3}H_2O$	Trtium labelled water
^{3}H-Eugenol	Tritiated Eugenol
Hg	Mercury

^{131}I	Iodide 131 isotope
K^+	Potassium ion
KV	Kilovolts (10^3 volts)
LM	Light Microscopy
LPS	Lipopolysaccharide
mm	Millimetre (10^{-3} metres)
MW	Molecular Weight
Na	Sodium
Na^+	Sodium Ion
nm	Nanometres (10^{-9} metres)
^{32}P	Phosphorus 32 isotope
PO_3^-	Phosphite ion
PBS	Phosphate Buffered Saline
$^{86}Rb+$	Rubidium 86 isotope ion
rpm	Revolutions per minute
^{35}S	Sulphur 35 isotope
SEM	Scanning Electron Microscope
$^{99m}TcO_4^-$	Pertechnate ion isotope
TEM	Transmission Electron Microscope
*u*m	Micrometres (10^{-6}) metres
*u*Ci	micro Curie
Zn	Zinc
ZNOE	Zinc Oxide Eugenol

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