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The Effect of Depth on the Permeability and Ultrastructure of Human Coronal Dentin in Primary Molars as Compared to Premolars in Vitro

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THE EFFECT OF DEPTH ON THE PERMEABILITY AND ULTRASTRUCTURE OF HUMAN CORONAL DENTIN IN PRIMARY MOLARS AS COMPARED TO PREMOLARS *IN VITRO*

BY
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To my daughter
Aspasia

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INTRODUCTION

The permeability of dentin has been a subject of interest during the last three decades. Most of the previous research has been carried out on the coronal or radicular dentin of human permanent teeth (Anderson and Ronning, 1962; Linden and Brännström, 1967; Brännström, Linden, and Åstrom, 1967; Reeder et al., 1978; Pashley, Livingston, and Greenhill, 1978; Pashley, Stewart, and Galloway, 1984; Pashley, Andringa, and Derkson, 1987; Fogel, Marshall, and Pashley, 1988). However, no studies have been performed on primary teeth.

Many factors govern fluid movement through dentin. Among the most important factors are the number and diameter of the dentinal tubules, and the dentin thickness. According to the hydrodynamic theory of dentin sensitivity, fluid movement in the dentinal tubules results in the mechanical stimulation of mechanoreceptors in the inner dentin or peripheral pulp to cause pain. The proposed mechanism of action of certain dentin desensitizing agents is to obstruct the orifices of the dentinal tubules in the area of sensitivity. Pashley and co-workers (Greenhill and Pashley, 1981; Pashley et al., 1984; Pashley and Galloway, 1985; Pashley and Depew, 1986) and other researchers have shown that potassium oxalate greatly reduces the coronal dentin permeability of permanent teeth by forming crystals that block the tubule orifices. The ability of oxalates to also reduce microleakage of amalgam restorations, when used as cavity liners *in*

vitro (Sandoval, Cooley, and Barnwell, 1989), and to decrease hypersensitivity *in vivo* (Muzzin and Johnson, 1989) is of particular interest in the field of Restorative Dentistry.

The purpose of this study is to investigate the permeability of coronal dentin at various depths in human primary molars. This study will:

1) Measure the hydraulic conductance of the coronal dentin of primary and permanent teeth after sequential sectioning parallel to the long axis of each tooth and at cross-section to the dentinal tubules, from the dentinoenamel junction to the pulp.

2) Measure the effect of smear layer and potassium oxalate treatment on the hydraulic conductance of primary and permanent teeth.

3) Compare the hydraulic conductance of primary molars with permanent premolars.

4) Measure the density and diameter of the dentinal tubules of the primary molars at different depths from the dentinoenamel junction to the pulp utilizing the scanning electron microscope.

5) Correlate the hydraulic conductance to the overall morphology of the dentin in primary molars.

CHAPTER I

LITERATURE REVIEW

Introduction

Credit for originating the name "dentin" is given to Purkinye, the Slavic microanatomist/physiologist (Loevy and Kovitz, 1988). In his book, published in 1836, he described the tubular nature of dentin.

Dentin is produced from the secretory products of the odontoblast cells and their processes. The ectomesenchymal (neural crest) cells of the dental papilla induce the inner enamel epithelium to become preameloblasts during early tooth formation. These preameloblasts then induce odontoblasts to differentiate from the outer papilla cells. The odontoblasts produce an organic matrix that becomes mineralized to form dentin (Avery, 1987).

Dentin constitutes the bulk of the tooth and serves as a sensory and protective covering for the connective tissue of the pulp organ. It is characterized by the presence of a multitude of closely packed dentinal tubules that traverse its entire thickness and make dentin permeable. The dentinal tubules contain the cytoplasmic extensions of the odontoblasts that once formed the tissue and now maintain it. The cell bodies of the odontoblasts are aligned along the inner aspect

of dentin, where they also form the peripheral boundary of the dental pulp (Ten Cate, 1989).

Throughout the life of the tooth, there is an inseparable relationship of the pulp with dentin, developmentally, topographically, and functionally (Mjör, 1985). This relationship has been described as the "pulpo-dentinal complex" (Kuttler, 1959; Stanley et al., 1983).

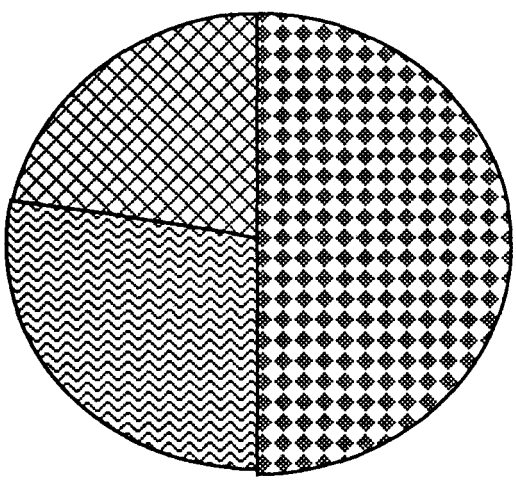
The structure, function, and composition of dentin are inextricably intertwined. First, the composition and structural features of primary dentin will be presented. Then, the dentinal fluid and dentin permeability will be discussed, followed by a brief review of the knowledge on smear layer, perhaps the most interesting feature of dentin recently discovered.

I. Composition of dentin

G.V. Black was one of the first American investigators who attempted to determine the components of teeth (Black, 1895).

The general chemical composition of dentin resembles bone (Avery, 1986). There are three major constituents: water, organic, and inorganic components. Figure 1 shows these components expressed as percentage of volume and weight.

Figure 1. Constituents of Human Dentin by Volume Percent and Weight Percent (after Linde, 1984)

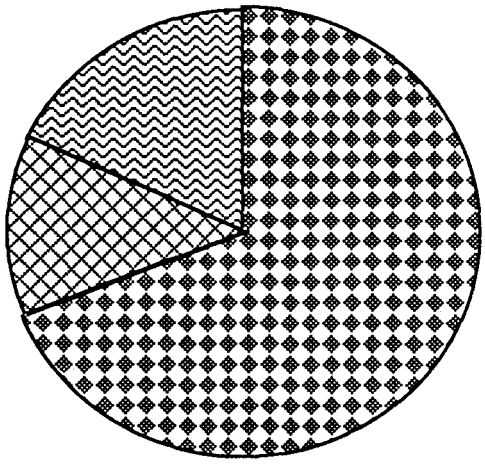


A. Volume %

Inorganic 50%I

Water 28%

Organic 22%



B. Weight %

Inorganic 69%

Water 8-16%

Organic 18%

Due to the difference in the specific gravity of the inorganic material (3.0), the organic components (1.6), and water (1.0), the volume distribution of the various fractions differs from that of the weight distribution (Linde, 1984).

The composition of enamel and dentin differ so much that it is essential to separate them before analysis. Several methods have been utilized for that:

a) Mechanical methods consist of either chipping off the enamel from the underlying dentin or grinding the enamel away with a bur. These methods do not yield pure enamel and dentin, are laborious, and it is not easy to collect the ground material quantitavely because some of it may be lost as fine dust which escapes into the air (Jenkins, 1978).

b) Flotation methods (Manly and Hodge, 1939). The whole tooth is ground to a fine powder in a device that prevents loss of particles escaping into the air. Powdered tooth is poured into a pointed tube open at the lower end, which is fitted into a centrifuge tube containing a liquid whose density is 2.70. A suitable liquid is 91% bromoform and 9% acetone. The whole mixture is centrifuged. The enamel (density 2.9-3.0) sinks into the outer tube and the dentin and cementum (density 2.14 and 2.03 respectively) float aside the inner tube. The dentin and cementum, and enamel are then washed free from bromoform by acetone. Dentin and cementum may be separated

by treating the mixture as above with a fluid of density 2.07 but this is rarely done as the composition of cementum is very similar to that of dentin except the ash content is lower (Neides et al., 1972). The purity of the samples obtained by the above method is 97-99% and can be improved by several repetitions of the process with fluids of slightly different densities. The bromoform-acetone partially removes lipids and therefore the use of an aqueous solution of cadmium tungstoborate (82% w/w) has been proposed instead (Prout and Shutt, 1970). The latter has a density of 2.70 also and does not alter the organic matrix but contaminates the inorganic fraction with cadmium (Prout and Shutt, 1970). For certain types of analysis therefore, mechanical methods of separation may be preferable.

The direct estimation of moisture content in teeth is difficult. When studying data on this content it is important to consider:

a) The extent of dehydration of the tooth after removal from the intraoral environment (Burnett and Zenewitz, 1958).

b) The water loss during separation of enamel and dentin by any of the methods mentioned above (Jenkins, 1978).

c) The analytical method used. Analyses have been carried out on moist, dry or ashed material (Jenkins, 1978).

Water in dentin occurs in two forms: one, loosely bound, probably to the

organic constituents and another, more tightly bound to the hydration shell of the apatite crystals or in interstices between crystals (Komrska, 1972; Jenkins, 1978). Water is also present within the dentinal tubules, and in the odontoblastic cytoplasm (Mjör, 1985).

Most estimates of moisture have been made "by difference", i.e. subtracting the percentage of organic matter from the percentage loss of weight during ashing. Considerable variation has been reported in the moisture content of calcified tissues of human teeth. Fetal teeth have been reported to contain 6.81% moisture (Le Ferre, Ball and Hodge, 1937). Other investigators (Bird et al., 1940) found deciduous dentin to contain 11.1% moisture as compared to the moisture content of permanent teeth which was found to be 13.2%. In another study, the moisture content of dentin, from freshly extracted permanent teeth heated *in vacuo* at the highest temperature used (197° C for 23hrs), was found to be 12% (Burnett and Zenewitz, 1958).

The organic phase of dentin in which the mineral crystals are embedded is often referred to as the organic matrix, thereby emphasizing its function as a molecular and structural framework or mold for the inorganic component (Linde, 1984). The average organic content of dentin is considered to be 18% by weight (Linde, 1984). The organic matter may be removed from dentin by two methods:

a) Dry ashing, which breaks down the organic matter by heating the dry tissue, altering at the same time the inorganic constituents and

changing the crystal form (Jenkins, 1978)

b) Wet ashing which removes the organic matter by boiling the tissue with ethyleneglycol and KOH, extracting also some of the calcium and phosphorus (Jenkins, 1978).

Approximately 92% of the organic matrix or 18% of the total weight of dentin is collagen (Eastoe, 1969; Jenkins, 1978). Collagen consists of an insoluble, fibrillar material that is constructed of many highly elongated, thread-like molecules cross-linked together (Linde, 1984). It makes up about one third of body protein and consists of 33% glycine, 12% alanine, 12% proline, 9% hydroxyproline - an amino acid unique to collagen (Levine, 1971) - while the remaining one third is made up of 14 amino acids including the rare hydroxylysine (6%) (Jenkins, 1978). Collagen from developing dentin was found to have a composition close to that in the fully developed tissue but it showed a 3-4% relative deficiency in glycine, alanine, and hydroxyproline with slightly increased quantities of valine, leucines, aromatics, serine, threonine, methionine, and histidine (Eastoe, 1963). This suggests that the preparation from developing teeth contained, in addition to collagen, protein material perhaps associated with the predentin or odontoblast processes (Eastoe, 1963).

For a number of years researchers have recognized that the amino acid composition of dentin collagen was the same as that of skin and other soft tissues (Piez and Likins, 1960). The only significant difference appeared to be in the

hydroxylysine content; dentin collagen contains two to three times as much hydroxylysine (Butler, 1973). However, the total content of lysine plus hydroxylysine is constant (Linde, 1984). The increased hydroxylysine content may contribute to the overall stability by influencing the type or extent of cross-linking (Mechanic, Gallop and Tanzer, 1971). The demineralized dentin matrix shows relatively large, cross-banded collagen fibrils ranging from 20 to 50 nm in diameter. The diameter appears to increase through predentin (Jessen, 1967; Johannessen and Bang, 1972) suggesting that there is a continued deposition of collagen molecules in predentin prior to the mineralization process and transformation of predentin to dentin.

Dentin collagen is essentially only Type I (Linde, 1984); minor amounts of the Type I trimer are present (Munksgaard et al., 1978). Small amounts of Type V collagen may also be present (Bornstein and Sage, 1980). Type III collagen and fibronectin are absent (Bergenholtz et al., 1985). There are some indications that the detailed pattern of cross-linking in bone and dentin collagen may be different. This suggests that certain structural differences in the collagens, reflected in the cross-linking pattern, may be related to different physiologic functions (Kuboki and Mechanic, 1982).

Studies of the distribution of collagen within dentin, using hydroxyproline as an indicator of collagen content, have shown that the concentration of collagen increases from the pulp to the DEJ, with the greatest increase in the outer 1/3 of dentin. This concentration corresponds to the region of mantle dentin and the

presence of Von Korff fibers (Levine, 1971), and coincides with a decrease in mineralization near the DEJ (Amprino and Camanni, 1956; Mjör, 1972). Collagen is located mainly intertubularly, with only scanty amounts in the peritubular matrix and in the "organic periodontoblastic space" (Frank, 1966).

If the collagen of dentin matrix is removed by digestion with collagenase the presence of about 20 minor components (the non-collagenous matrix) can be detected equal to about 9% of the total matrix. The non-collagenous macromolecules of dentin, all anionic ("acidic") in character, may be grouped into the following classes: acidic glycoproteins, γ-carboxygentamate containing proteins, phosphoproteins, proteoglycans, and serum proteins. Identification and characterization of the different dentin non-collagenous proteins mainly represent the investigations of the last decade and it is assumed that they play an important role in the calcification process of dentin, exerting their effects in intimate functional relationships with the collagen of the tissue (Linde, 1984).

Citrate in dentin amounts to about 0.9% but its function is not yet understood (Leaver, 1969). Lipids account for 0.2 to 0.3% of the dry weight or about 1.7% of the organic mass (Odutuga and Prout, 1974). Phospholipids, free cholesterol, cholesterol esters, and triacylglycerols comprise 90 to 95% of the total lipids, with free fatty acids, mono-, and diacylglycerols accounting for much of the remainder (Dirksen, 1963; Linde 1984). Evidence exists that lipids are involved in odontoblastic membranes, and production of prostaglandins (Linde,

1984) as well as in the *de novo* induction of hydroxyapatite formation (Vogel and Boyan-Salyers, 1976; Boskey and Posner 1976, 1977). In carious dentin lipids seem to undergo various degrees of degradation (Dirksen, 1963).

The greatest portion of the dentin, by volume or weight, is the inorganic fraction. Dentin is only 40-50% as mineralized as enamel (Jenkins, 1978; Avery, 1986). The mineral composition varies with the type of tooth (Jenkins, 1978), location within the tooth (Mjör, 1972), age (Nalbandian, Gonzales and Sognnaes, 1960), caries presence (Bergman, 1959), attrition (Erickson, 1965), oral exposure of dentin (Mjör, 1967a), and application of certain filling materials (Mjör, 1967b). The main constituent is the crystalline form of calcium phosphate, known as apatite, having the general formula $Ca_{10}(PO_4)_6X_2$. When an OH^- group is substituted for the X in the molecule, the compound is known as hydroxyapatite (HA), a mineral found in wide distribution in all hard biologic tissues (Elliot, 1973).

The three-dimensional arrangement of the constituent ion in well crystallized HA has been delineated by X-ray and neutron diffraction (Elliot 1973; Young, 1975). The *unit cell* contents of HA can be written $Ca_{10}(PO_4)(OH)_2$ (Linde, 1984). Bone and dentin apatites are distinct from enamel and high temperature synthetic apatites, not only in their submicroscopic crystal size but also in their higher structural distortion and less perfect chemical stoichiometry. It is not understood why enamel crystals are so much larger than those in dentin; it is possible that the

larger crystals provide a harder, more shock-resistant external dental tissue (Linde, 1984). In mature dentin there is a distribution in the size of of apatite crystals. The crystals have been reported to be needle-like or plate-like, with the average long dimension in the range of 350Å and the small dimension in the 50 to 60 Å range; the largest crystal dimension observed is always under 1,000 Å (Frank, 1980).

The $Ca:PO_4$ ratio of synthetic apatite is 2.15 (Jenkins, 1978). The $Ca:PO_4$ ratios of biological apatites are not fixed and substitutions are possible which change the ratio (Elliot, 1973). The ratio in dentin is approximately 2.0 (Jenkins, 1978) and in HA 1.67 (Linde, 1984). Possible explanations for the low $Ca:PO_4$ ratio of the biological apatites include: a) absence of some calcium ions b) substitution of calcium ions by sodium, magnesium, hydronium (H_3O^+) or other ions and c) common presence of a calcium phosphate compound in mineralized tissues, known as octacalcium phosphate, which has a ratio of 1.33 and is a transient constituent of hard tissue (Jenkins, 1978).

The carbonate in dentin, like bone, is of the order of 3 to 4%, expressed as CO_3, but the position of carbonate ions is controversial (Jenkins, 1978). Experiments on human osteoporotic bone lead to the assumption that lowering the CO_3 content of dentin would increase its chemical stability and thereby its resistance to caries, for CO_3 inclusion in apatite lowers its stability (Linde, 1984).

In addition to the apatite, there are many trace inorganic constituents of

dentin mineral, which show a nonuniform distribution (Jenkins, 1978). Over 45 elements have been found in trace amounts but it is not clear whether all of these are structurally substituted or absorbed on crystal surfaces (Wetherell and Robinson, 1973). Certain ions ingested in food and drink are specific apatite seekers which substitute in the structure. Examples of these are Cl^-, F^-, Mg^{2+}, Sr^{2+}, Fe^{2+}, Zn^{2+}, and Pb^{2+} (Linde, 1984). The biological significance of the trace elements, other than fluoride, remains speculative.

II. Morphology of Dentin

The dentin found in Mammalia appears essentially similar in structure when examined in longitudinal sections. It is termed "orthodentine" and it is characterized by tubules running parallel to each other (Miles, 1967; Linde, 1984).

There are three main structural units of dentin; they are the peritubular dentin (PTD), the intertubular dentin (ITD), and the dentinal tubule (Bradford, 1958).Much of the preceding general discussion regarding dentin composition applies to ITD, which is located between the zones of PTD and constitutes the main part of dentin.

PTD is defined as the area immediately surrounding each dentinal tubule (Avery, 1987). It is readily apparent when undemineralized ground sections of dentin cut at right angle to the tubules are examined under the light microscope. It has also been demonstrated by electron microscopy and soft X-ray analysis.

PTD has been refered to as "afibrous dentin" (Miles, 1967), "secondary dentin" (Martens, Bradford, and Frank, 1959), "tertiary dentin" (Kuttler, 1959), or "translucent dentin" (Blake, 1958; Harcourt, 1964). The term peritubular dentin, most widely used today, is considered to be anatomically incorrect by some analysts because this dentin forms within the dentinal tubule and not around it, thus narrowing the lumen of the tubule, and should therefore more accurately be referred to as intratubular dentin (Ten Cate, 1989).

PTD has not been found nearer to the pulp than about 60-100μm from the dentin/predentin border. Its presence could not be demonstrated in the integlobular dentin in newly erupted teeth (Blake, 1958; Symons, 1961; Mjör, 1966). The thickness of the PTD seems to be variable and generally it appears to be thinner at the surface region and thicker circumpulpally (Symons, 1961; Takuma and Eda, 1961). It is more obvious in coronal dentin than in radicular dentin (Takuma and Eda, 1961).

The organic base of the peritubular matrix appears to be composed of a structureless filamentous substance, chemically rich in acid mucopolysaccharides, that is especially dense at the rim of the dentinal tubules (Takuma and Eda, 1961). The presence of collagen has been demonstrated in PTD but it is less prevalent that the surrounding ITD and forms fine fibers continuous with those of the intertubular matrix, arranged circumferentially around the tubule (Takuma, 1960b; Takuma and Eda, 1961).

Mature PTD is regarded to be highly mineralized (Bradford, 1958; Takuma, 1960b; Mjör, 1972) with variations in mineral density. PTD is 9% more mineralized than ITD (Ten Cate, 1989). In transverse sections of the dentinal tubules, the presence of different zones of PTD has been noted (Blake, 1958; Takuma and Eda, 1961; Mjör, 1972). A two layer PTD has been described; the matrix seemed to be divided into a highly mineralized outer layer and a less mineralized inner layer (Takuma and Eda, 1961). Longitudinally, variations in calcification have also been noted (Bradford, 1958; Takuma and Eda, 1961; Miller, Eick, and Neiders, 1971; Mjör, 1972).

Controversy exists regarding the development of PTD. Most investigators believe that it is developmental (Takuma and Eda, 1961; Frank and Nalbandian, 1963; Mjör, 1972) while others feel that it is acquired (Bradford, 1958; Harcourt, 1964). The consensus at present considers the origin and development of PTD to be a continuum. Initially, the ITD and PTD assume a similar extent of mineralization but in later stages the PTD becomes more calcified than the ITD (Takuma, 1960b; Takuma and Eda, 1961; Frank and Nalbandian, 1963). After eruption, the dentinal tubule is subject to further physiologic calcification (sclerosis), involving the deposition of apatite (Nalbandiam, Gonzales and Sognnaes, 1960).

The highly mineralized PTD is acid labile and demineralizes more rapidly than does the intertubular matrix (Selvig, 1968); strong acid treatment may destroy it completely, resulting in enlargement of the dentinal tubule (Gwinnett, 1973; Lee

et al., 1973; Brännström and Johnson, 1974). This fact may have clinical implications in dental bonding.

The most striking feature of dentin is probably the dentinal tubules. The dentinal tubules are small, canal-like spaces within the dentin, filled with tissue fluid and occupied for part or all of their length by odontoblast processes (Ten Cate, 1989). They form early in dentinogenesis; during matrix formation the odontoblasts migrate pulpalward from the basal lamina leaving behind a single extension, and the several processes present during the initial stage joint into one which becomes enclosed in a tubule. As the matrix formation continues, the odontoblast process lengthens, as does the dentinal tubule (Avery, 1986).

The course of the dentinal tubules follows a gentle curve in the crown, less so in the root, where it resembles an S in shape (Avery, 1986). These S-shaped curvatures are least pronounced in the cervical third of the root and beneath incisal edges and cusps, where they may run a straight course (Ten Cate, 1989). They are the result of the crowding of the odontoblasts as they move towards the pulp (Ten Cate, 1989), which also results in a convergence of the tubules upon the pulp chamber (Pashley, 1984).

The dentinal tubules have lateral branches throughout dentin, which are termed "canalicular microtubules" (Avery, 1986), "auxiliary canals" (Kennedy, Teuscher, and Fosdick, 1953), "peritubular branches" (Boyde and Lester, 1967), or "lateral branch canals" (Kaye and Herold, 1966). These canaliculi originate at

right angles to the main tubules and enter other tubules or end in the intertubular dentin (Avery, 1986). The canaliculi have been reported to contain the lateral branches of the odontoblast processes (Kaye and Herold, 1966).

The dentinal tubules allow for a direct communication between the dentin and pulp, and as result of this pathway various stimuli, irritants, or therapeutic agents applied locally to the exposed dentin may also reach the pulp. The rate of transport and subsequent effect of such agents are dependent on the number and size of the dentinal tubules (Forssell-Ahlberg, Brännström, and Edwall, 1975).

In many early studies, the number and diameter of the dentinal tubules has been measured on decalcified dentin using light microscopy. During decalcification in acid, the acid labile peritubular dentin is dissolved to a large extent (Isokawa, Toda, and Kubota, 1970; Gwinnet, 1973; Lee et al. 1973; Brännström and Johnson, 1974). Therefore the tubule diameter measured on decalcified sections is an overestimate of the actual diameter.

Bradford (1955) reported that the tubule varies in diameter from 1-5μm and for the greater part of its length is 1.5μm in the calcified tissue; for decalcified dentine the tubules were found to have a diameter of 4μm for the greater part of their length. Ketterl (1961) using decalcified sections found the tubule diameter to be 4μm, one millimeter away from the pulp while the number of tubules was 64,000/mm^2. Close to the pulp the number was 70,000/mm^2 and near the enamel the numbers ranged from 9,000 to 24,000/mm^2.

Höhling (1966), examined undercalcified sections with light microscopy, and found tubules diameters ranging from 0.6 to 2.0 μm. Fromme and Riedel (1970) examined ultrathin sections of undecalcified coronal dentin with transmission electron microscopy (TEM). In young teeth the diameter of the dentinal tubules was found to be 1.7 μm peripherally and 1.8 μm near the pulp; the corresponding values for older teeth were 1.21 and 1.54 μm. Höhling (1966), and Fromme and Riedel (1970) did not measure the tubule density.

Tronstad (1973) studied the incisal area of the coronal dentin of intact anterior teeth by means of scanning and transmission electron microscopy (SEM, TEM). Without referring to any systematic investigation he noted that the number of dentinal tubules varied from about 7,000/mm^2 in the peripheral dentin up to 60,000/mm^2 near the pulp. The diameter of the tubules was about 2-3 μm. Near the dentinoenamel junction (DEJ), however, the tubules were much narrower with a diameter usually less than 0.5 μm. The great majority of the tubules were open regardless of age.

Garberoglio and Brännström (1976) used SEM to examine fractured coronal dentin of intact human permanent teeth in various age groups and at various distances from the pulp. Near the pulp the number of tubules per square millimeter was 45,000 and the diameter 2.5 μm. In the middle dentin, there were 29,500 tubules/mm^2 and the diameter was 1.2 μm. Peripherally the corresponding values were 20,000 tubules/mm^2 and 0.9 μm. To determine the difference in tubule

diameter and number between decalcified and undecalcified dentin, they examined a number of teeth both before and after decalcification. The decalcified dentin shrunk during dehydration *in vacuo* and thus gave a false value of the tubule diameter and number. The linear shrinkage of the decalcified dentin after dehydration *in vacuo* was 18 per cent. The number of tubules per unit area increased after decalcification and dehydration *in vacuo* by about 22-25 per cent. These researchers found no significant difference in tubule diameter and number between age groups but they observed large individual variations that were more prominent in older teeth. The mean value of the total tubule volume in coronal dentin was calculated to be 10 per cent of the whole dentin volume. This value near the pulp was 28 per cent and near the enamel 4 per cent.

The dentin/predentin junction of intact permanent teeth was studied in the SEM by Whittaker and Kneale (1979). The highest number of tubules was found in the coronal dentin, 42,000/mm^2, and the number decreased slowly along the length of the root canal and fell rapidly in the apical region to only 8,000/mm^2. The relationship of the number of tubules to the age of the individual was not strong and no relationship was seen between age and tubule diameters which were found to range between 0.1 and 1.5 μm.

Carrigan et al. (1984) in their SEM study examined the dentin of human maxillary central incisors. Undecalcified teeth from five age groups - 20 to 34, 35 to 44, 45 to 54, 55 to 79, and 80 and above - were examined in the crown and in

three areas of the root: cervical, middle, and apical. The mean tubule densities found in this study were: 44,243 tubules/mm^2 in coronal dentin, 42,360 tubules/mm^2 in cervical root dentin, 39,010 tubules/mm^2 in mid-root dentin, and 8,190 tubules/mm^2 in apical root dentin. Their results on coronal dentin are in close agreement with those of Garberoglio and Brännström (1976). However, Carrigan et al. (1984) did find an age related difference; they concluded that tubule numbers decreased significantly with age. They also suggested that these results may account for the marked sensitivity and increased bacterial penetration of coronal dentin when compared with the minimal bacterial and irritant penetration of apical dentin.

A possible explanation for the difference between the studies by Garberoglio and Brännström (1976), Whittaker and Kneale (1979), and Carrigan et al. (1984) as to the significance of age, is offered by the studies of Nalbandian, Gonzales, and Sognanes (1960) and of Bang and Ramm (1970). Nalbandian, Gonzales, and Sognanes (1960) found that sclerosis, as a dental aging characteristic, starts at the apical area and progresses crownward as the tooth matures. Bang and Ramm (1970) measured the extent of sclerotic dentin in permanent teeth from various age groups, to obtain a data base for age determination of unknown individuals (forensic investigation). They found that sclerosis begins around the age of 30 years at the apices of the teeth. The alteration is believed to be caused by a reduction in the diameter of the dentinal tubules probably caused by an increase

in intratubular calcification. Thereby, the difference in refractive index between intratubular organic and extratubular inorganic material is equalized with increasing translucency of the affected dentin. From this data it was shown that very few teeth develop sclerotic (translucent) dentin in the crowns prior to age sixty.

Since Garberoglio and Brännström (1976) only examined coronal dentin and the oldest age group studied was 40-60 years, it might be expected that no age-related differences between old and young teeth were found. Whittaker and Kneale (1979) measured tubule diameters in the apical areas where age changes should be the most marked. They found no apparent correlation between age and diameter and, surprised by these findings, they speculated that some tubules may remain unaffected by the formation of sclerotic dentin or that not all of the tubules are obliterated at the pulpal ends.

There is little agreement regarding these three characteristics of the tubule: 1) the extent of the odontoblast process, 2) the presence or absence of a membranous lining, and 3) the lack of or presence and size of a "peritubular space". The technical difficulties during tissue preparation for scanning electron microscopy or transmission electron microscopy are the reasons for the discrepancies between studies (Sigal, Aubin, and Ten Cate, 1985).

Dentin as a living tissue contains within its tubules the processes of the specialized cells that form it, the odontoblasts (Avery, 1986). Since 1856 when

Tomes described "fibrils" in the dentinal tubules it was assumed, on the basis of conventional histologic techniques, that the odontoblastic process occupied the entire length of the dentinal tubule (Ten Cate, 1989). This assumption was questioned some 25 years ago, leading to a number of investigations using many different techniques, in an effort to establish whether or not the process of the odontoblast occupied the entire length of the tubule to reach the DEJ.

Brännström and Garberoglio (1972) in a SEM study, found that the processes in the tubules extended a distance from the pulp to about one quarter of the total length of the tubule. In only a few tubules, the process was observed more than 0.4 mm from the pulp and none were seen beyond 0.7 mm from the pulp. Similar observations were made by Thomas and Payne (1983) who reported that odontoblast processes were limited to inner dentin in three different regions of the tooth. Thomas and Carella (1984), White et al. (1986), and Weber and Zaki (1986) have confirmed these findings using SEM and TEM.

The results of the afore-mentioned studies have been challenged by other investigators. Maniatopoulos and Smith (1983) showed that structures identified as odontoblastic processes were present in the inner, middle, and outer portions of dentin, using a different method for preparing the specimens for SEM than the previous researchers. La Fleche et al. (1985) offered a compromise position which states that the processes reach to the DEJ *in vivo* but retract to the inner third upon extraction or during exposure to fixatives.

Sigal, Aubin, and Ten Cate (1985) used an SEM/immunofluorescence technique with the light microscope in order to localize tubulin, actin, and vimentin, which are the major subunit proteins of microtubules, microfilaments, and intermediate fillaments. These three filamentous systems are the major components of the cytoskeleton in eukaryotic cells, and have not been shown to exist extracellularly in viable cells (Weber and Osborn, 1981). Therefore, the positive immunofluorescence labeling that Sigal, Aubin, and Ten Cate (1985) observed for all three of the cytoskeletal subunit proteins, which extended to the DEJ, supports the hypothesis that the odontoblast process are present at the DEJ in human molars. Furthermore Sigal and Chernecky (1988) demonstrated by SEM that the odontoblast process ends as a dilated sphere at or just below the dentinoenamel junction but they questioned whether the ending represents the actual *in vivo* morphology of the terminal end of the process.

The presence of a membrane lining within the tubule, known as "Neumann's sheath" has been reported (Kennedy, Teuscher and Fosdick 1953; Takuma and Eda, 1961). Thomas and Carella (1983, 1984), and Thomas (1984), using TEM and SEM identified an extracellular sheetlike structure surrounding the odontoblast process and extending from the predentin/dentin area to the DEJ. They believed this structure to be distinct from the cell membrane of the odontoblast process because it had a thickness of about 300 Å, which was approximately three times the thickness of a cell membrane. They referred to this structure as the

lamina limitans, a term introduced by Scherft (1972) to describe a structure lining the organic matrix of mineralized cartilage and bone. The susceptibility of the lamina limitans to hyaluronidase digestion indicated a high content of glycosaminoglycans, suggesting also that they are extracellular in nature. Weber and Zaki (1986) in SEM and TEM preparations were able to identify the collagenase-resistant inner sheaths of peritubular matrix, confirming the work of Thomas (1984), and Thomas and Carella (1983, 1984). White et al. (1986) could not identify the structures with TEM.

Another controversy is whether a space exists between the odontoblast and the tubule wall. If such a space is present, its contents have not been identified. The potential space has been termed "organic periodontoblastic space" (Frank, 1966), "periprocesseal area" (Brännström and Garberoglio 1972) or "peritubular matrix space" (Thomas, 1985).

Garberoglio and Brännström (1972) reported that the tubule is completely filled by the cell process in the predentin area only. Some investigators believe that the space is occupied by fine collagen fibers, oriented in a circumferential pattern, coursing between the membrane-like lining and the tubule wall (Kennedy, Tenscher, and Fasdick, 1953; Scott 1955; Brännström and Garberoglio, 1972). The implications for this space (if it exists), may be important; because it may serve as a route for the circulation of the dentinal fluid (Jenkins, 1978) and also, it may have a significant role in the elaboration of the PTD matrix (Frank, 1966).

The dentinal tubules are filled with the dentinal fluid or "dentin liquor™ (Frank, 1966). To analyze the composition of the dentinal fluid, several methods of collection have been devised. Expulsion by heating (Speter Von Krendenstein and Stüben, 1955), application of negative pressure to fractured dentin (Stevenson, 1965), and centrifugation (Coffey, Ingram, and Björndal, 1970) all have been used. The fluid obtained by centrifugation has been reported to coagulate upon exposure to heat and air (Coffey, Ingram, and Björndal, 1970). Analysis has been performed by electron microprobe (Coffen, Ingram, and Björndal, 1970) or flame photometry (Haljamae and Rocket, 1970). Contamination of the small samples (nanoliters) with cytoplasmic contents from cut or ruptured odontoblasts has been a concern (Coffey, Ingram, and Björndal, 1970).

Coffey, Ingram, and Björndal (1970) found the fluid to contain 3mEq/l of potassium, 150 mEq/l of sodium, and 100 mEq/l of chloride. These values are typical of intestitial fluid. Haljamae and Rocket (1970) reported the fluid to contain lower sodium and potassium levels and different free amino acids than blood or subcutaneuous fluid. Other investigators have likened dentinal fluid to cerebrospinal or synovial fluid (Berggren and Brännström, 1965). The variability of results is not surprising given the difficult methods of collection and small sample sizes.

Controversy exists about the source of dentinal fluid. Some investigators feel the evidence favors an extracellular transudate or filtrate from the subodontoblastic capillaries (Brännström and Åström, 1972; Tanaka, 1980; Thomas, 1985).

Others believe that the fluid is a combination of extracellular and intracellular constituents (Haljamae and Rocket, 1970) in which case the odontoblasts function as secretory cells to maintain electrochemical gradients. Radiotracers and SEM have been used to study the route of fluid from the pulp to the tubules. Tanaka (1980) using lanthanum as tracer, showed that fenestrations exist in the subodontoblastic capillary network. Micropinocytotic vesicles and fenestrae may participate in the trans-endothelial transport of the fluid from the subodontoblastic capillaries to the inter-odontoblast spaces to the predentin and finally to the periodontoblast spaces in the dentinal tubules (Tanaka, 1980). This author concluded that the dentinal fluid is a transudate from the terminal capillaries and circulates mainly within the dentinal tubules.

Nagi and Frank (1974) using ^{45}Ca, provided some evidence that intracellular pathways may exist. They suggested that the Golgi apparatus actively produces elongated, electron-dense, fluid-containing vesicles, which are discharged by pinocytosis from the odontoblastic process into the tubule. In these studies, the lanthanum tracer did not enter the dentin, but ^{45}Ca was detected within the dentin six hours later.

Tubular fluid appears to have many functions. During dentinogenesis it is presumed to transport the organic components for matrix formation and minerals for the active mineralization process (Nagi and Frank, 1974). It may facilitate calcium and metabolite transport at a slower rate throughout life (Nagi and Frank,

1974; Seltzen and Bender, 1975). It may act as a reservoir for aqueous solutes of medications or toxins (Linden and Brännström, 1973). The presence of some mineral and organic substances may provide the dentinal fluid with buffering capacity against toxic substances (Meryon, 1984). Outward movement of fluid may act to resist the invasion of bacteria into tubules (Olgart, Brännström, and Johnson, 1974). Finally, Brännström and co-workers (Brännström, 1962; Brännström, Linden, and Åström, 1967; Brännström and Åström, 1972), accumulated a great amount of clinical and laboratory evidence to show that movement of fluid within the tubules transduces surface stimuli by deformation of pulpal mechanoreceptors, which in turn cause pain according to the so-called hydrodynamic theory of dentin sensitivity.

III. Dentin Permeability

Several investigators have demonstrated fluid movement in dentin (Anderson and Ronning, 1962; Brännström, 1962, 1966; Brännström and Åström, 1964; Anderson, Matthews, and Garretta, 1967; Brännström, Linden, and Åström, 1967; Anderson and Matthews, 1967).

Anderson and co-workers (Anderson and Ronning, 1962; Anderson and Matthews, 1967) applied various hyperosmotic solutions, such as calcium chloride or sucrose, to exposed dentin *in vivo*, thus causing pain. They then demonstrated *in vitro* that these solutions produced fluid movement through dentin.

Brännström and co-workers applied various stimuli to exposed dentin and found that they caused fluid movement and pain. These stimuli included drilling (Brännström, Linden, and Johnson, 1968), impression taking (Brännström, Linden, and Johnson, 1968), a stream of air (Brännström, 1966; Brännström, Linden, and Johnson, 1968), heat (Brännström, 1966; Brännström, Linden, and Johnson, 1968), cold (Brännström, Linden, and Åström, 1967), dry absorbant paper (Brännström, 1966; Brännström and Åström, 1964), hydrostatic pressure (Brännström, Johnson, and Linden, 1969), positive pressure (Brännström, 1961), negative pressure (Brännström, 1966), and osmotic pressure (Brännström, 1962; Linden and Brännström, 1973). These experiments, both *in vivo* and *in vitro,* suggested that fluid shifts, in either direction through the tubules, caused deformation of the mechanoreceptors near the pulpal termination of the tubules, which then caused pain. This was termed "*the hydrodynamic theory of dentin sensitivity*" (Brännström and Åström, 1972) and is the most popular theory of dentin sensitivity today.

The fluid movement through the tubules is laminar, and little or no molecular sieving occurs (Reeder et al., 1978). The rate of flow through dentin has been quantified by several investigations, in either linear or volumetric units. Movement through the tubules has been measured at 4mm per second (Haljamae and Rocket, 1970; Brännström and Åström, 1972). The flow has been calculated to be about 0.6 microliters per square mm (Johnson, Olgart, and Brännström,

1973; Pashley, Nelson, and Pashley, 1981).

Permeability is defined as the movement of solutes through a semipermeable membrane (Reeder et al., 1978). The presence of dentinal tubules makes dentin a semipermeable membrane; intertubular dentin is thought to be impermeable. Although a poor one, dentin is also a protective barrier to the diffusion of noxious substances to the pulp (Pashley, 1985).

There are two mechanisms by which solute movement through tubules can occur. One is bulk fluid movement, known as filtration, and the other is diffusion (Merchant, Livingston, and Pashley, 1977).

Diffusion or diffusive transport is the physical process by which solutes travel through tubular fluid by moving down a concentration gradient. In diffusion the driving force is the chemical concentration gradient. The rate of transport of material by diffusion can be quantified by the Fick equation (Pashley, 1985):

$$J = D\,A\,\frac{dc}{dx} \qquad \textit{Equation 1}$$

where : J = solute flux

D = diffusion coeficient

A = diffusional surface area

dc/dx = change in concentration over a distance x

The most important variables that influence filtration or convective support across dentin are illustrated by considering the Poiseuille-Hagen equation (Pashley, 1985):

$$V = \frac{\pi \Delta P r^4}{8 \eta l} \qquad \textit{Equation 2}$$

where:

	V	=	volume flow
	ΔP	=	hydrostatic pressure differences across dentin
	η	=	viscosity of fluid
	r	=	radius of tubule
	l	=	length of tubule
	π	=	3.1415929 constant

Note that filtration varies with the fourth power of the radius rather than with the square of the radius (diffusional surface area) as in the case of diffusion. The driving energy for bulk fluid movement is either osmotic or hydrostatic pressure.

Hydraulic conductance (L_p) quantitates the ease with which bulk fluid movement occurs down a hydrostatic or osmotic pressure gradient. The measurement of hydraulic conductance of dentin provides a convenient quantitative measurement of dentin permeability (Pashley, 1985). Conductance (L_p) can be thought of as the inverse of resistance (Reeder et al., 1978). The formula for calculation of L_p is:

$$L_p = \frac{J_v}{A\ \Delta P\ t}$$ *Equation 3*

where: L_p = hydraulic conductance

J_v = fluid flow

A = surface area

ΔP = hydrostatic pressure gradient

t = time

The anatomic factors controlling diffusion are: The dentin surface area, dentin thickness, and proximity to the pulp. From equations 1 and 3 it can be seen that the calculation of hydraulic conductance and diffusion requires precise knowledge of both dentin surface area and thickness. The early investigations used cavity preparations in intact teeth to study permeability and these studies were mostly observational. The variability of enamel thickness, the uneven contour of the dentinoenamel junction, and variations in the pulp horn and pulp chamber morphology made the quantitation of dentin surface area and dentin thickness almost impossible.

Outhwaite, Livingston, and Pashley (1976) used dentin disks of known thickness and placed them in a split chamber device in which dentin surface area and hydrostatic pressure were controlled. They were able to quantitate the effects of changes in the surface area, thickness, temperature, and post-extraction time on

human coronal dentin permeability *in vitro*.

As the surface area of exposed coronal dentin increases, so does the filtration rate of fluid passing through it. This relationship is linear at a constant dentin thickness reflecting constant tubular density and constant tubule dimensions at a constant distance from the pulp. Increasing the available surface area increases diffusion as well. A ten-fold increase in dentin temperature can almost double its permeability to radioactive iodine; the post-extraction time has little effect on dentin permeability (Outhwaite, Livingston, and Pashley, 1976).

The rate at which fluid filters through dentin is very sensitive to the length of the tubules. As remaining dentin thickness decreases, filtration increases exponentially (Reeder et al., 1978). This has been attributed, in part, to the reduction in the frictional resistance of the shorter tubule walls. Diffusion is also inversely proportional to dentin thickness (Outhwaite, Livingston, and Pashley, 1976; Pashley, Livingston, and Outhwaite, 1977). As medicaments are placed closer to the pulp, the greater will be the diffusion of molecules across the remaining dentin, thus increasing pulpal reactions (Stanley, 1985). During diffusion, the concentration of substances is dissipated over distance. The concentration of bacterial products on the surface of thick overlying coronal dentin is much greater than that at the pulpal surface (Pashley, 1984).

The resistance to fluid movement through dentin in teeth with vital pulps can be thought of as the summation of a series of three resistances (Pashley,

Livingston, and Greenhill, 1978):

1. Surface resistance, due to the presence of debris, smear layer, partially or totally occluding the peripheral ends of the tubules.
2. Intratubular resistance, due to the mineralized fibrils, nodules, and internal irregularities within the tubules, and
3. Pulpal resistance, due to the presence of the odontoblast processes and cell bodies.

The contribution of each of these resistances to the total resistance across freshly extracted teeth was reported to be 86% at the surface, 6% in the tubule and 7% at the odontoblastic end.

It is of some interest that resistance to fluid movement varies, depending upon whether positive or negative pressures are applied. Negative pressures applied to dog dentin *in vivo* reduced L_p more than did positive filtration pressures (Pashley, Nelson, and Pashley, 1981).

From the Poiseuille-Hagen equation (Equation 2) it can be seen that small changes in tubule diameter by blockage or reduction in actual size produce large changes in the rate of fluid flow. This equation refers to the functional radius of the tubule, that is, the area within the tubule available for fluid movement. Michelich, Pashley, and Whitford (1978) compared the functional versus anatomical tubular radii in coronal dentin. These authors found that at a constant distance from the pulp, the functional radii varied from 5-40% of the anatomical

radii. This means that the dentinal tubules were 60-95% narrower than they appeared microscopically when partial tubule blockages out of view were taken into account. They concluded that microscopic measurements of the dimensions of open tubules provide only the maximum boundary of the functional radius.

The presence or absence of pulp tissue does not significantly affect the permeability of coronal dentin to radioactive isotopes in dogs (Pashley, Nelson, and Pashley, 1981) or in humans (Pashley, Livingston, and Outhwaite, 1977). When Pashley, Nelson, and Pashley (1981) compared *in vivo* versus *in vitro* dog dentin permeability of the same teeth, it was observed that the rates of isotope permeation were very similar. When the effect of post extraction time on the permeability of human coronal dentin was studied (Outhwaite, Livingston, and Pashley, 1976) no change was detected over a 3-4 week period, during which the odontoblast processes would have undergone autolysis. Therefore, the presence or absence of the odontoblast process seems to have no effect on dentin permeability.

The number and diameter of the dentinal tubules have an obvious effect on permeability. The tubules are less dense and narrower at the periphery, and become more dense and wider near the pulp (Garberoglio and Brännström, 1976). This anatomical fact suggests that superficial dentin is less permeable than deep dentin. This speculation has been confirmed experimentally in coronal dentin (Outhwaite, Livingston, and Pashley, 1976) and in radicular dentin of permanent teeth (Fogel, Marshall, and Pashley, 1988).

In sclerotic dentin in which intratubular crystals obstruct the tubules, permeability would presumably be less. The same situation may be true of dead tracts, (empty tubules), but these speculations have not been confirmed experimentally. On the advancing front of developing carious lesions in dentin, the tubules may become partially blocked by precipitated calcium phosphates, known as "caries crystals" (Daculsi et al., 1979). Carious dentin is much less permeable (Miller and Massler, 1962; Sarnat and Massler, 1965). This is a major reason why the carious process does not cause pain (Pashley, 1989).

There is considerable evidence to show that dentin permeability varies a great deal from one region of the tooth to another. The permeability of occlusal coronal dentin is highest over pulp horn areas and lowest in the center of the occlusal surface (Pashley, Andringa, and Derkson, 1987). SEM examination of the permeable and non-permeable areas revealed the presence of open tubules in both regions. While there were less than half as many tubules in the low permeable regions, there was no difference in their diameters (Pashley, Andringa, and Derkson, 1987). Even though nearly all the tubules in the low permeable area were open, the L_p was zero. Pashley (1989) speculated that there is more intratubular material in central dentin than over the pulp horns and he also suggested employing a combination of both functional and SEM techniques for permeability studies, rather than utilizing only one method which is more likely to lead to misinterpretations. If an MOD restoration is prepared, the proximal

boxes appear to be more permeable than the occlusal surfaces (Sturdevant and Pashley, 1989). Interestingly, dog molars *in vivo* have been shown to be more permeable than cuspids (Pashley, Nelson, and Pashley, 1981).

After removing all the cementum, radicular dentin is relatively impermeable. The L_p increases with decreasing dentin thickness but even the most permeable inner radicular dentin is still only 20% as permeable as coronal dentin (Fogel, Marshall, and Pashley, 1988).

The solute characteristics controlling diffusion across dentin are molecular size, charge, concentration, and solubility in either water or lipid (Pashley and Livingston, 1978). Small molecules may pass easily through dentin to the pulp and the systemic circulation. Very large molecules, which do not easily permeate, can cause water movement (osmosis) out of the dentinal tubules to an extent dependent on the concentration gradient (Pashley and Livingston, 1978).

When dentin is desiccated there is a decrease in fluid movement compared to rehydrated dentin (Polhagen and Brännström, 1971). Changes in dentin temperature have two important effects on the rate of fluid flow through dentinal tubules. First, the viscosity of dentinal fluid varies inversely with temperature; fluid flows more easily as it is heated. Second, as dentin is heated, the tubules become slightly larger due to the linear coefficient of dentinal expansion, reported to be 3.0×10^{-6} per °C (Söremark et al., 1973). Although the increase in tubular dimension is small, when the value is raised to the fourth power (Equation I-2), small changes

produce exponential and significant effects. Those temperature effects are additive and of similar magnitude, and both contribute to an increase rate of fluid flow (Pashley, Thomson, and Stewart, 1983).

Treating dentin disks with plasma has been reported to largely decrease the L_p (Pashley, Nelson, and Kepler, 1982). Fibrinogen, platelets and other large molecular weight components of plasma are considered to be capable of reducing L_p. The presence of red blood cells in dentinal tubules also results in a large reduction of fluid flow through dentin (Pashley, Nelson, and Williams, 1981). Saliva also contains macromolecules capable of absorbing to dentinal tubules and potentially capable of reducing L_p (Pashley, Nelson, and Kepler, 1982). Whole saliva also contains bacteria which can enter the tubules of exposed dentin (Olgart, Brännström, and Johnson, 1974; Michelich, Schuster, and Pashley, 1980) and reduce fluid flow. Clinical experience has shown that hypersensitivity of exposed dentin sometimes disappears spontaneously. The conditions discussed above may be responsible, in part, for this phenomenon (Pashley, Nelson, and Kepler, 1982)

Dentin's permeability to bacteria and bacterial products is of particular interest. Several investigators have shown that bacteria may penetrate dentinal tubules (Chirnside, 1961; Vojinovic, Nybora and Johnson, 1974; Michelich, Schuster, and Pashley, 1980). Olgart, Brännström and Johnson (1974) found that an outward flow of fluid in the dentinal tubules, due to intrapulpal pressure, may mechanically hinder bacterial growth into the tubules. However, the blocking of

the outer apertures of the dentinal tubules by the smear layer was found to be a more important obstruction to bacterial penetration (Michelich, Shuster, and Pashley, 1980; Olgart, Brännström, and Johnson, 1974). Removal of smear layer by acid-etching (Vojinovic, Nyborg, and Brännström, 1973; Olgart, Brännström, and Johnson, 1974) or allowing it to be washed away by saliva (Michelich, Schuster, and Pashley, 1980) resulted in the ingrowth of bacteria in the exposed dentinal tubules.

It should be noted that while the presence of a smear layer markedly inhibits bacterial penetration, it does permit fluid filtration and the penetration of bacterial products at reduced rates (Pashley, 1984). Bergenholtz and co-workers (Bergenholtz and Lindhe, 1975; Bergenholtz, 1977, 1981) placed bacterial products in contact with cut dentin surfaces and found pulpal inflammation in response. It was bacterial products diffusing through the dentin which initiated the pulpal inflammation.

A very critical variable affecting both fluid flow and diffusion is the nature of the dentin surface, that is, whether the surface is coated with a smear layer or not. The smear layer is a layer of cutting debris that is produced whenever dentin is cut by hand or rotary instruments. It is analogous to the "wet sawdust" and serves as a natural cavity liner (Pashley, Livingston, and Greenhill, 1978; Reeder et al., 1978) but it only reduces diffusion about 25-30% (Pashley, Livingston, and Greenhill, 1978; Dippel, Borggreven, and Hoppenbrouwers, 1984). Removal of

the smear layer increases diffusion across dentin to the same extent as a 25% reduction in thickness (Pashley, 1989). Another complication of removal of the smear layer is that it increases the L_p by 5-40 fold (Reeder et al., 1978; Pashley, Livingston, and Greenhill, 1978; Pashley, Michelich, and Kehl, 1981). Clinically, this removal results in increased sensitivity to osmotic, thermal and tactile stimuli (Johnson and Brännström, 1974).

The mechanism for dentin sensitivity is not fully understood; nor is the mechanism of the effect of desensitizing agents. Pashley and co-workers (Greenhill and Pashley, 1981; Pashley et al., 1984) studied several desensitizing agents that are used clinically, to evaluate their ability to reduce the permeability of discs of coronal dentin *in vitro*. They found that agents such as silver nitrate, barium chloride, and calcium hydroxide were effective. Agents containing oxalate as the active ingredient were proven to be far more effective than any of the other agents tested.

Pashley and co-workers found that crystalline agents had to: a) have crystals smaller than the dentinal tubules and b) form crystals within 1 to 2 minutes after application (Pashley, 1986). Oxalates out perform other agents in their ability to occlude dentinal tubules. In early studies, treatment of acid-etched discs with 3% monopotassium-monohydrogen oxalate for 2 minutes led to a significant increase in reflection coefficients for sucrose, haemoglobin, albumin and other solutes. The results of this study (Pashley, Livingston, and Whifford, 1979) indicate that

oxalate treatment of acid-etched dentin restores tubular occlusion to the level found in sanded (smear layer-covered) dentin.

Greenhill and Pashley (1981) tested the effects of various desensitizing agents on the L_p of dentin discs *in vitro*. Discs treated with 30% potassium oxalate showed the largest reduction in L_p (98.4%) recorded in the study. SEM examination revealed a homogeneous, moderately dense precipitate covering the dentin surface. The crystal size was approximately the size of etched dentinal tubules. Crystals were frequently observed partially occluding the tubules, thereby reducing their functional diameter. The authors speculated that oxalate salts react with the free ionized calcium that presumably exists in the dentinal fluid trapped in the smear layer, thus forming insoluble crystals of calcium oxalate.

An experimental dentrifice containing 2% potassium oxalate as the active ingredient reduced the L_p of dentin discs by 96% *in vitro* and was proven to be far more superior than commercially available desensitizing dentrifices (Pashley et al., 1984). When applied to exposed dentin of dogs *in vivo*, 30% potassium oxalate was found to greatly reduce or completely block the sensory response to dentinal stimulation (Hirvonen, Närhi, and Hakumäki, 1984).

Burnishing dentin surfaces *in vitro* with a NaF/Kaolin/glycerin paste was less effective than treating dentin with 3% monopotassium-monohydrogen oxalate without burnishing (Pashley, Leibach, and Horner, 1987). When oxalates were used *in vitro* as cavity liners under amalgam restorations they reduced the dentin

permeability by 98% and produced a significant reduction in microleakage (Pashley and Depew, 1986).

When an original smear layer was treated *in vitro* with 30% neutral dipotassium oxalate for 2 minutes followed in succesion by 3% monopotassium-monohydrogen oxalate, the dentin surface became covered with a mixture of large and small crystals which also resisted acid attack (Pashley and Galloway, 1985). When only 3% monopotassium-monohydrogen oxalate, a half-neutralized oxalic acid, was used to treat the smear layer, it removed the original smear layer and replaced it with a layer of very small crystals of insoluble calcium oxalate, which did not change their appearance when challenged with citric acid (Pashley and Galloway, 1985).

Chan and Jensen (1986) compared Scotchbond and oxalates for their ability to block hydrogen ion diffusion across very thin dentin discs. Their results indicate that the oxalates were superior to Scotchbond in that respect. Sandoval, Cooley, and Barnwell (1989) reported that Class V cavities, prepared half in enamel and half in dentin, showed the least microleakage when amalgam restorations were lined with oxalate solutions. They found oxalates to be superior to cavity varnishes in minimizing dye microleakage on the gingival floor of Class V cavities (a particularly difficult area to seal).

The effectiveness of oxalates in reducing cervical root dentin hypersensitivity ,due to previous periodontal surgery, gingival recession, or abrasion, was

studied in vivo, using a thermal test. Highly significant reductions of hypersensitivity were reported during the first four weeks after a single application of 30% dipotassium oxalate, 3% monopotassium monohydrogen oxalate, or both but the long-term effects of these agents was not studied (Hasson, 1987; Muzzin and Johnson, 1989).

IV. The Smear Layer

Throughout the previous discussion of dentin permeability, smear layer has been referred to several times. It is worthwhile to examine at this subject more closely since it occupies a unique position in clinical dentistry.

Prepared tooth surfaces were first studied in an attempt to improve the retentive ability of amalgam. The original method utilized was by topographic measurement with a profilometer and light microscopy (Charbeneau, Peyton, and Anthony, 1966), and the presence of an organic film was suspected. It was not until 1970 when the use of a scanning electron microscope, equipped with a microprobe, confirmed the presence of a layer of smeared cutting debris (Eick et al., 1970). Smear layers are formed on tooth surfaces whenever they are cut with hand or rotary instruments (Gwinnet, 1984). All three constituents of the tooth, enamel, dentin and cementum, produce smear layers when they are cut (Tao, Pashley, and Boyd, 1988).

Since dentin itself is not homogeneous, the smear layer created from it

reflects the composition of the underlying dentin. Under low magnification, the smear layer appears smooth and amorphous (Pashley, 1984) while under high magnification it appears globular (Tao, Pashley, and Boyd, 1988). TEM has shown the presence of a surface layer of degraded collagen and particulate matter, and an inner layer of denaturated collagen (Eick et al., 1987). Deeper dentin may be more organic because of the increased number of dentinal tubules and the greater probability of amputating odontoblasts, whose contents are subsequently incorporated into the smear layer (Pashley, 1984).

The thickness of the smear layer is 0.5 to 15 μm and it depends upon whether the dentin is cut dry or wet, the amount and composition of the irrigating solution used, the size and shape of the cavity or root canal, and the type of instrument used (Gieboe et al., 1980). Generally speaking, cutting without water spray and using coarse diamond burs generates thicker smear layers than cutting with a copious air-water spray and carbide fissure burs (Shortall, 1981). The cutting edge characteristics determine the size of the particles; fine grit abrasives and carbide burs leave a finer, denser smear layer than course grit abrasives (Pashley, 1989; Tao, Pashley, and Boyd, 1988).

The smear layer cannot be rinsed off or scrubbed away (Pashley et al., 1988). Water, hydrogen peroxide, benzalkonium chloride, EDTA and other agents have been used, attempting to remove it (Brännström, 1984; Brännström, Nordenvall and Glantz, 1980). The smear layer is not sensitive to temperature

(Pashley, Thomson, and Stewart, 1983).

As the debris is being smeared across the dentin surface, some particles enter the tubules to become "smear plugs". *In vivo*, the plugs are usually 1-10 μm long (Eick and Welch, 1986). The smear plugs are responsible in great part of the 86% reduction of dentin permeability of the smear layer (Pashley, Livingston, and Greenhill, 1978). While the plugs restrict bacterial entry into the tubules, they do permit permeation of bacterial products which can cause pulpal inflammation (Olgart, Brännström, and Johnson, 1974; Bergenholtz, 1977). From a restorative and sensitivity view point, it is desirable to retain the smear plugs *in situ* (Mjör, 1985).

Clinically, etching dentin with 6% citric acid for 60 seconds removes all of the smear layer and smear plugs as does 15 seconds of etching with 37% phosphoric acid (Pashley, Michelich, and Kehl, 1981) which leaves the tubules open and available for increased retention and the surface collagen exposed for linkage with dental materials. Chelating agents, such as ethylenediamine tetra-acetic acid (EDTA), have also been used effectively for removal of the smear layer (Seidberg and Schilder, 1974). Solubility of the smear layer to oral acids has been reported (Bowen, 1978). The increase in surface area with the particles makes the smear layer more labile to acid dissolution (Pashley and Galloway, 1985) while collagenase producing bacteria are capable of dissolving the organic constituents. This enzymatic process may take 6-11 days *in vivo* (Olgart, Brännström, and

Johnson, 1974).

The *in vitro* cohesive strength of the smear layer is weak (Tao, Pashley, and Boyd, 1988). This has caused investigators to advocate removing it to expose the underlying dentin; a more stable substrate for bonding. Although stronger bonds may occur, this removal of smear layer can cause a 3-9 fold increase in dentin permeability. The removal of the original smear layer and its replacement with a newly formed precipitate, such as the calcium oxalate, may be beneficial (Pashley, 1984).

The majority of the studies have been conducted using occlusal, coronal dentin of human, permanent third molars. There have been no studies on the permeability of deciduous teeth.

CHAPTER II

MATERIALS AND METHODS

I. Tooth Preparation

Fifteen extracted, human posterior, noncarious primary teeth and ten premolars, obtained from the Department of Pediatric Dentistry of Loyola University Chicago, School of Dentistry, were collected. The teeth were placed immediately after extraction into vials containing phosphate buffered saline (Dulbecco's Phosphate Buffered Saline, Gibca Laboratories, Grand Island, NY) with 0.2% sodium azide, to inhibit microbial growth, and were stored at 4° C.

All the experiments were performed at the laboratory of the Department of Oral Biology-Physiology, School of Dentistry, Medical College of Georgia, Augusta, Georgia, under the direction of Dr. D.H. Pashley.

The occlusal surfaces of the teeth were cemented onto plastic cylinders by means of Epoxy cement (Cole-Parmer Instrument Co., Chicago, IL) and prepared by use of a low-speed diamond saw (Buehler Ltd., Isomet, 11-1180 slow-speed saw, Evanston, IL) with water coolant. The plastic cylinders were placed in a holding device attached to the saw and the roots of the teeth were removed perpendicular to the vertical axis of the tooth and approximately 1 mm apical to

the cementoenamel junction. The pulp tissue was removed with cotton forceps, avoiding contact with the walls of the coronal pulp chamber.

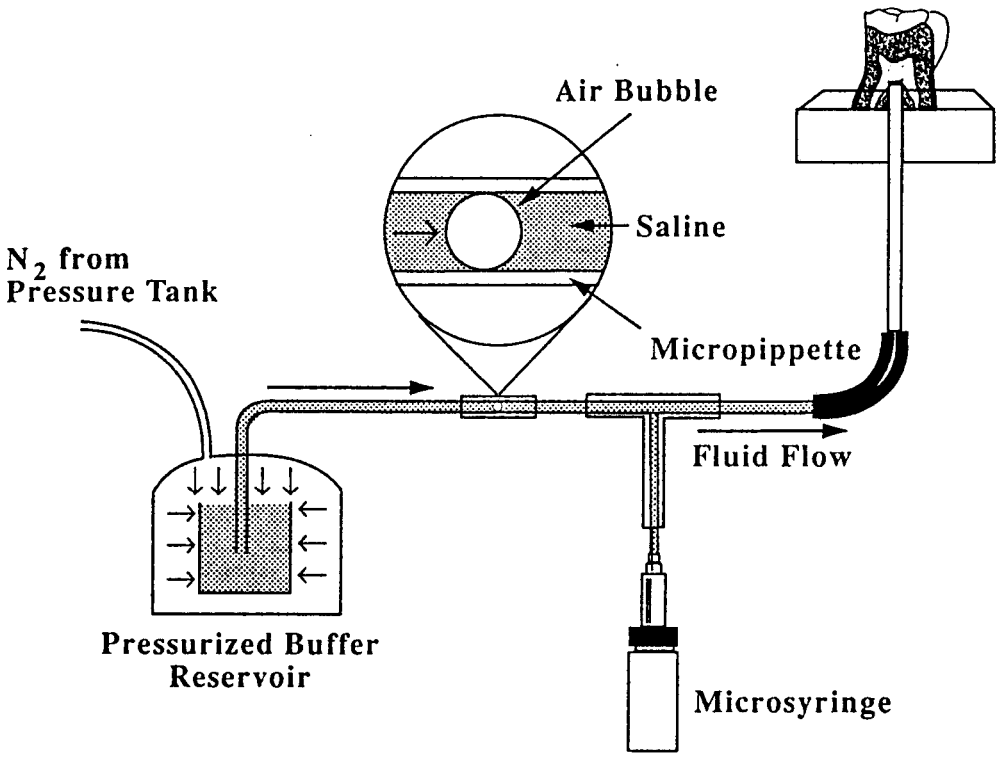
The crowns were removed from the plastic cylinders, and they were attached with fast-setting cyanoacrylate adhesive (Zapit, DVA, Yorba Linda, CA), to 2x2x0.7 cm pieces of Plexiglas containing 15 mm lengths of 18 gauge stainless steel tubing through their centers (Figure 2). The cyanoacrylate adhesive was applied around the cervix of the tooth to ensure a tight seal. This method served to connect the pulp chamber of the tooth with the apparatus used to measure dentin permeability and it ensured that the pulp chamber and dentin were always full of phosphate buffered saline, avoiding possible inclusion of unwanted air bubbles.

II. The L_p measuring Apparatus

The apparatus used is a Microsyringe-Micropipette-Pressure Reservoir System (Pashley and Galloway, 1985),(Figure 3). A stainless steel pressure cooker (Presto, National Presto Industries Inc.) was modified to a pressure reservoir system by placing an 18-gauge stainless steel tubing through the center of the top, extending approximately to the bottom of the lower chamber. The tube was then soldered in place. A small hole was drilled in the top of the cooker to receive a brass elbow to permit the system to be pressurized from a standard nitrogen tank via a pressure regulator and high pressure tubing. The pressure cooker was equipped with a safety valve rated at 25 psi (1750 cm H_2O) pressure and it

Figure 2. Primary Molar Crown Attached with Cyanoacrylate Adhesive to the plexiglass

Figure 3. The L_p Measuring Apparatus



was mounted in a wire cage as a safety precaution. The phosphate buffered saline was placed in the pressure cooker in a disposable polyethylene beaker with the 18-gauge stainless steel tubing extended into the beaker.

A 25μL glass micropipette (65 mm in length) was interposed between the pressure cooker and the teeth (Microcaps, Drummond Scentific Co., Broomall, PA) in which a 1-2 mm air bubble was inserted. A two ml Gilmont microsyringe (Gilmont, Great Neck, NY) was used to adjust the position of the air bubble in the micropipette, adjacent to a millimeter scale. Connections between the parts of the apparatus were performed using PE90 polyethylene tubing with an internal diameter of 1.14 mm (Clay Adams, Division of Becton, Dickinson and Co., Parsippany, NJ), and the tubing was kept full of phosphate buffered saline during the experiment.

Under pressure, the nitrogen gas within the cooker forced the phosphate buffered saline up the stainless steel tubing to the mounted crown, and into the pulp chamber, thereby permeating through the dentinal tubules onto the exposed dentin surface. The movement of the air bubble in the micropipette along the millimeter scale over a time interval permitted quantitation of the rate of fluid filtration through dentin (Equations 3, 4). Prior to starting reductions of dentin, the crowns were connected to the apparatus under pressure to ensure that no leakage occured.

III. Dentin Reductions

A groove was prepared with a diamond bur in the enamel on the lingual surface of the primary molars and premolars, which served as a reference mark to measure the buccolingual thickness of the crowns using a digital micrometer (Sylvae Ultra-Cal II, Fowler Co., Inc., Newton, MA). The total thickness of the teeth buccolingually was measured and recorded. The enamel on the buccal surface of the teeth was removed using a diamond bur on a highspeed handpiece under water spray. When all enamel was removed, and the first dentin could be seen, the thickness was measured and recorded again.

The buccal dentin was ground further until it was visually estimated that the dentin surface area was sufficient for permeability measurements. The buccolingual thickness was recorded and the crowns were connected to the apparatus to measure the permeability of the dentin covered with smear layer. Each permeability measurement was repeated four times. Two drops of 0.5M EDTA (pH 7.4) were then placed on the dentin surface for 2 minutes to remove the smear layer. The dentin surface was rinsed with distilled water for 5 seconds and four additional measurements were taken. Then, the dentinoenamel junction was traced on the tooth using a sharp, lead pencil, the crown was placed in a holding device and a photograph of the dentin surface area was taken under standardized magnification. A millimeter scale was included with each photograph to aid in the calibration of the electronic tracer, used to calculate the dentin surface area later, and as a

precautionary measure in case of accidental change in the camera magnification (Figure 4).

This procedure was followed by an additional grinding of approximately 0.4 mm of dentin from the labial surface, remeasurement of thickness, dentin permeability measurements with smear layer and after smear layer removal, and photographing of the dentin surface. This routine was carried out four to five times until it was visually estimated that the pulp chamber was close to being exposed. After measuring the permeability of the EDTA-treated dentin from the last reduction, the dentin surface area was treated for 2 minutes with 1 drop of Protect (3% monopotassium-monohydrogen oxalate; John O. Buttler Co., Chicago, IL) followed by 5 seconds of rinsing with distilled water. Then, the permeability was measured again, prior to taking the final photograph of each tooth. The teeth were removed from the Plexiglas and sectioned buccolingually in two pieces with a diamond disc. The minimum remaining dentin thickness was measured to the closest 0.01 mm using a machinist's micrometer.

Total dentin thickness was determined from the initial dentin thickness and minimum remaining dentin thickness. The dentin depth of each sequential step was then calculated and expressed in both millimeters and as a percent of the total dentin thickness. Photographs (5 inch x 7 inch prints) were made, and the dentoenamel junction which had been marked with the lead pencil, was traced with a movable cursor. The dentin surface area was then calculated in mm^2, utilizing

Figure 4. Dentin Reduction of a Premolar Crown. The Dentinoenamel Junction has been Traced with Lead Pencil

a computer program.

IV. Calculations

Hydraulic conductance (L_p) was calculated using this formula:

$$L_p = \frac{J_v}{A \Delta P t} \qquad \textit{Equation 3}$$

where : J_v = fluid flow in μL

A = dentin surface area in cm^2

ΔP = hydrostatic pressure gradient in cm H_2O

t = time in minutes

L_p = hydraulic conductance in [μL cm^{-2} min^{-1} cm H_2O^{-1}]

Of these variables, J_v, the fluid flow can be calculated from the equation:

$$J_v = B_m \frac{V_o}{L_e} \qquad \textit{Equation 4}$$

where : J_v = fluid flow in μL

B_m = air bubble movement in mm

v_o = volume of micropipette in μL

L_e = length of micropipette in mm

Due to variations in permeability between different teeth, results were expressed as percent changes of the hydraulic conductances of the EDTA treated surfaces, for each reduction. Thus, each tooth served as its own control.

V. Scanning Electron Microscopy (SEM)

SEM was performed on five intact primary molar crowns. The teeth for SEM were prepared in a manner similar to that for the permeability measurements. Three reductions of dentin were made for each tooth. At the end of each reduction, the diamond bur smear layer was replaced by a 320-grit aluminum oxide sandpaper smear layer (Figure 5). The exposed dentin surface was placed on the sandpaper and it was moved 10 cm along the paper for 10 times with finger pressure. Care was taken so that the tooth was constantly moving over clean, fresh, wet sandpaper. This procedure was utilized to create a smear layer more easily removed by sonication (Pashley, personal communication).

The physical method of sonication was used to remove the smear layer. Acid-etching is an effective chemical method of removing the smear layer but it also removes a part or all of the peritubular dentin (Gwinnett, 1973; Lee et al., 1973; Brännström and Johnson, 1974) to a depth of 5 - 10 μm below the etched

surface (Andriga, 1987). This effect distorts the diameter of the tubules to be measured. After forming the 320-grit sandpaper smear layer, each crown was placed in the cuphorn container of a powerful sonicator (Model 450 Sonifier/350 Watts, Branson Ultrasonics Corporation, Danbury, CT). During sonication, the cup was kept filled with ice and the tooth submerged in it. Pilot studies showed that sonication of each tooth for 10-15 minutes would result in removal of the smear layer to permit visualization and measurements of the dentinal tubules.

The specimens were air-dried overnight and vacuum coated with a 100 Å film of gold. The dentin surfaces were examined in a JEOL JSM-35cF scanning microscope with an accelerating voltage of 25 kV, at magnifications ranging from 480X to 6000X. This procedure was repeated three times for each of the five teeth. Three 480X and six 6,000X micrographs were taken for each of the fifteen surfaces examined. Figures 6-11 are representative photographs taken at different depths from the pulp. The cervical third of the tooth was the area most frequently examined. This region was found, visually, to be the most permeable during the permeability measurements. SEM examination of the most permeable area would permit better correlation between permeability and morphology. Tubule density was determined from SEM micrographs taken at 480X. The calculation was facilitated by covering each micrograph with tracing paper on which the tubules could be marked off as they were counted. Only the whole tubules shown in the micrograph were counted. Areas partly covered with smear layer remnants or

areas out of focus were excluded. Based on the magnification, the area used for the calculation of tubule density was measured in mm^2 and then the density was expressed in tubules/mm^2.

Tubule diameter was determined from SEM micrographs taken at 6,000X. A computer program was utilized to measure the diameter in μm to the closest 0.001. For each micrograph one tubule was selected for calibration. The computer was commanded to trace the edge of the tubule, based on the grayscale of the micrograph projected onto the monitor screen. The computer was able to calculate the minor axis of the elipse which best fitted to the traced tubule orifice. This measurement of the smallest diameter across the tubule orifice minimized the error caused by tubules fractured obliquely. The diameter of the rest of the tubules of the same micrograph were calculated without altering the grayscale. This calibration was repeated for each micrograph, adjusting the grayscale so that the traced edge of the tubule was the same as the edge that could be estimated visually.

VI. Statistical Methodology

The hydraulic conductance (L_p) data was stratified into the following groups with respect to the type of tooth and dentin surface:

1. Permanent teeth with smear layer.
2. Permanent teeth without smear layer.

3. Permanent teeth with oxalate.

4. Primary teeth with smear layer.

5. Primary teeth without smear layer.

6. Primary teeth with oxalate.

The above groups were considered at the following dentin depths:

i. 0-30% from the pulp-deep dentin.

ii. 30.1-60% from the pulp-intermediate dentin.

iii. 60.1-90% from the pulp-outer dentin.

iv. 90-100% from the pulp-superficial dentin.

A two-way analysis of variance (ANOVA) was performed ($p=0.05$) to test for the effect of the independent factors of tooth type and dentin surface, and dentin depth on hydraulic conductance (L_p). A one-way ANOVA (repeated measurement) was also used to test the difference of the L_p values between primary and permanent teeth with smear layer, without smear layer, and with oxalate treatment. Whenever there was a significant difference, the pairwise multiple comparison Scheffé test was used for each of the factors, with level of significance at $p=0.05$.

Correlation testing was used to evaluate graphically the effect of dentin depth on L_p for primary and permanent teeth with and without smear layer.

Figure 5. Typical Appearance of 320-grit Aluminum Oxide Sandpaper Smear Layer on the Dentin Surface of a Primary Molar

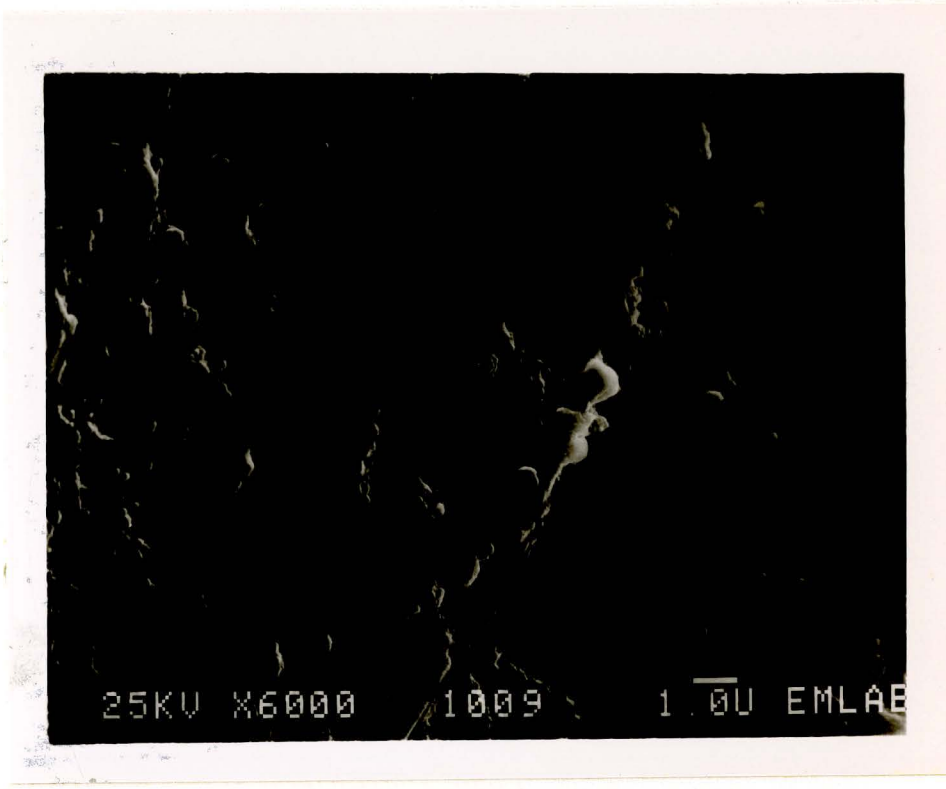


Figure 6. Scanning Electron Micrograph (6,000x) of Primary Molar Dentin at 14.8% (0.35 mm) Depth from the Pulp

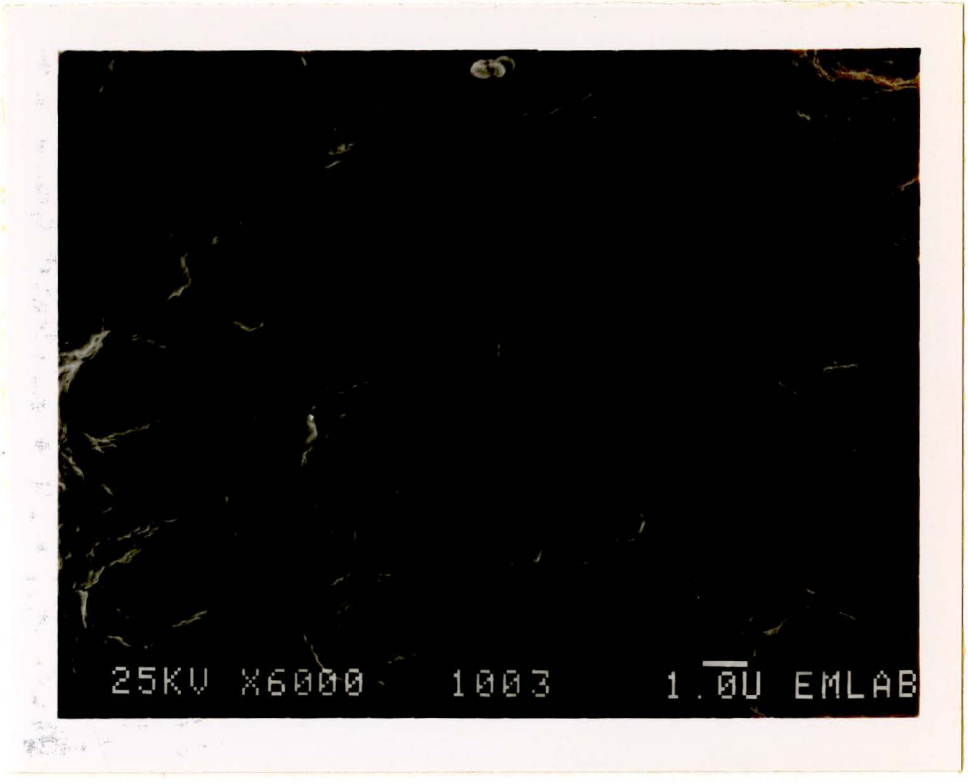


Figure 7. Scanning Electron Micrograph (6,000x) of Primary Molar Dentin at 56.5% (1.38 mm) Depth from the Pulp

Figure 8. Scanning Electron Micrograph (6,000x) of Primary Molar Dentin at 91.5% (1.83 mm) Depth from the Pulp. The arrow indicates a "smear plug"

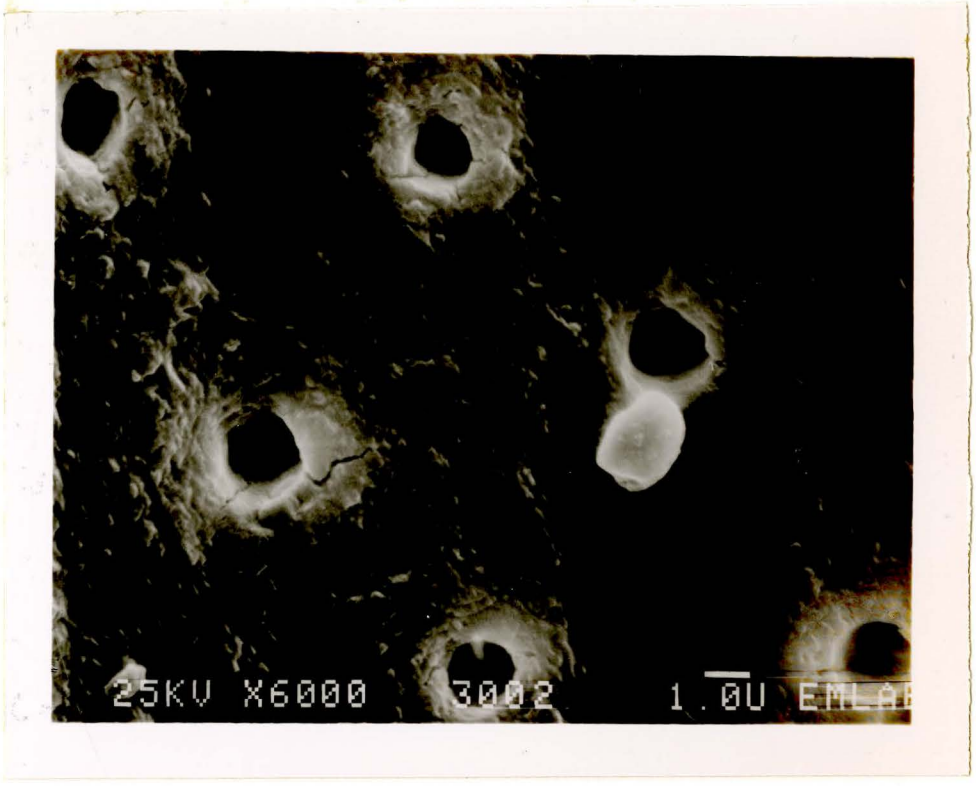


Figure 9. Scanning Electron Micrograph (480x) of Primary Molar Dentin at 14.8% (0.35 mm) Depth from the Pulp

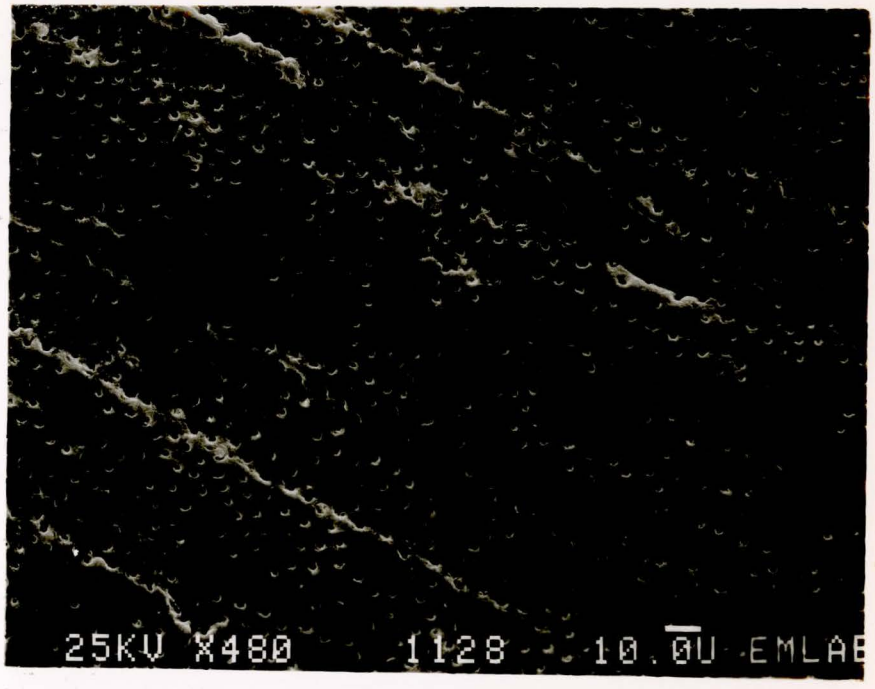


Figure 10. Scanning Electron Micrograph (480x) of Primary Molar Dentin at 43.9% (1.04 mm) Depth from the Pulp

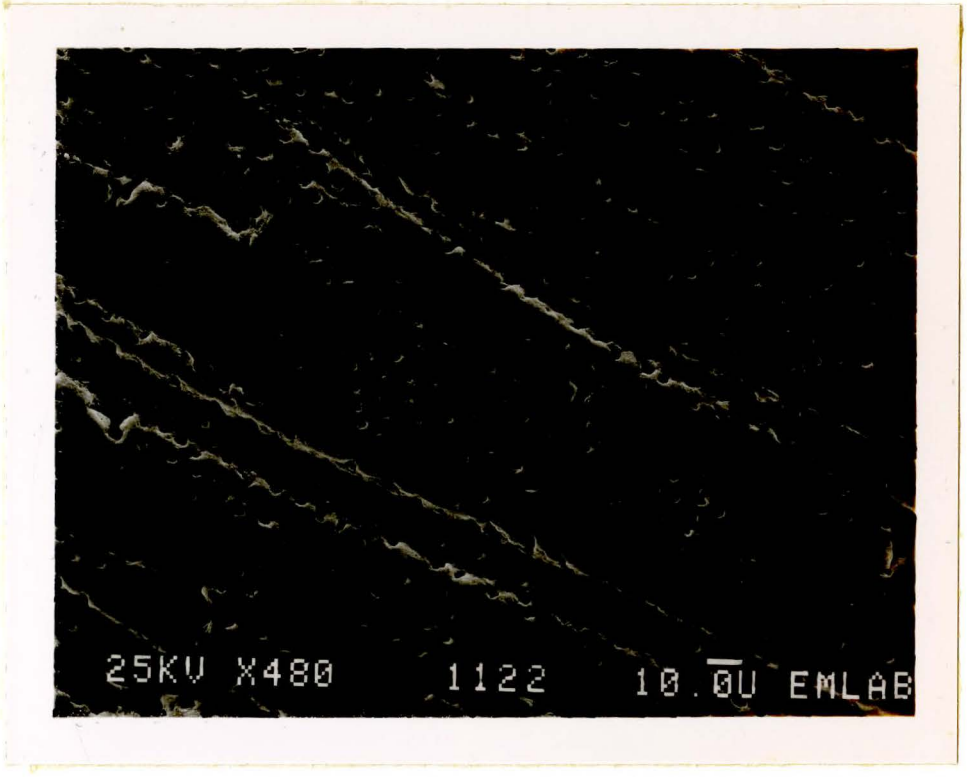
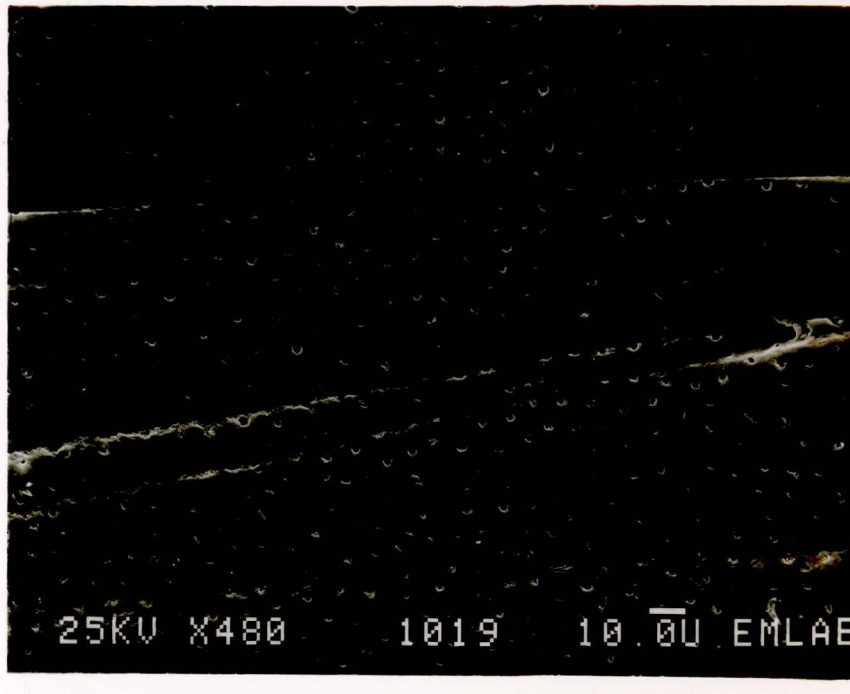


Figure 11. Scanning Electron Micrograph (480x) of Primary Molar Dentin at 91.5% (1.83 mm) Depth from the Pulp.



CHAPTER III

RESULTS

A summary of the number of specimens, L_p mean values, and standard deviations for the investigated surface treatments, (with, without smear layer, and with oxalate) are presented in Tables 1 and 2 of the Appendix, for primary and permanent teeth respectively. Tables 3-10 and 14-17 of the Appendix present all the L_p data collected during the experimental part of the research. L_p mean values $\pm$ standard deviation (SD) at four different dentin depths from the pulp are illustrated in Figure 12.

I. The effect of dentin depth and tooth-surface treatment

Two way ANOVA was performed to test the effect of dentin depth, and tooth-surface factors on the L_p of primary and permanent teeth (Table 11). The analysis revealed a significant effect of tooth-surface by dentin depth interaction ($p=0.042$).

Pairwise multiple comparison Scheffé test at $a=0.05$ indicated that statistically significant differences exist for:

i. Dentin depth factor. The significant difference exists between deep

dentin with L_p mean value ± SD of 0.01 ± 0.001 versus superficial (0.001 ± 0.001) (p=0.0111), and outer dentin (0.003 ± 0.006) (p=0.0030). There was no statistical difference between deep and intermediate dentin. (Table 12).

ii. Tooth/surface factor. The significant difference is shown mainly between the high L_p of permanent dentin without smear layer *versus* primary dentin with smear layer (p=0.001), permanent dentin with smear layer (p=0.0001) and primary dentin without smear layer (p=0.033). Permanent dentin without smear layer at deep depth showed the highest mean L_p values ± SD of (0.021±0.016), (Tables 1,2,13 and Figure 12).

The Scheffé test for overall comparison of all pairs of means at a=0.05 indicated that the significant difference is mainly caused by the high L_p, mean value ± SD of (0.02 ± 0.016), of the deep permanent dentin without smear layer *versus* each of the following:

i. Intermediate (30.1-60%) permanent dentin with smear layer, (0.001 ± 0.001), (p=0.018).

ii. Outer (60.1-90%) primary dentin with smear layer, (0.001 ± 0.001), (p=0.014).

iii. Intermediate (30.1-60%) primary dentin with smear layer (0.001 ± 0.002), (p=0.039),(Tables 1,2).

Correlation test was used to evaluate the effect of dentin depth on L_p for primary and permanent teeth with and without smear layer at a=0.05:

a) For primary teeth the results indicated that there is no significant linear relationship between L_p with smear layer and dentin depth (p=0.0966), (Figure 13). After removal of the smear layer there is a significant linear relationship between L_p and dentin depth, (p=0.0001), (Figure 14).

b) For permanent teeth the results are similar. No significant linear relationship exists between L_p with smear layer and dentin depth (p=0.9795), (Figure 15), but after the removal of smear layer the relationship of L_p and dentin depth becomes significantly linear (p=0.0006), (Figure 16).

II. The effect of potassium oxalate treatment

One-way ANOVA was used to test the difference of the L_p values of primary and permanent teeth with, without smear layer, and with potassium oxalate treatment in deep and intermediate dentin.

a) Deep dentin (0-30% from the pulp).

For primary teeth, the one-way ANOVA indicated that a significant difference exists between dentin surface treatments (p=0.0013), as shown in Table 19. Pairwise multiple comparison Scheffé test indicated that the difference was

shown between the dentin without smear layer with L_p mean values ± SD of 0.01 ± 0.002 *versus* dentin with smear layer (0.001 ± 0.0002), and dentin treated with oxalate (0.001 ± 0.002) (Table 20).

For permanent teeth the results of one-way ANOVA are similar (Table 21); a significant difference exists between dentin surface treatments ($p=0.0057$). Pairwise multiple comparison Scheffé test indicated that the difference occured between dentin without smear layer with L_p mean values ± SD of 0.02 ± 0.016 *versus* dentin with smear layer (0.001 ± 0.002) and dentin treated with oxalate (0.001 ± 0.002) (Table 22).

b) Intermediate dentin (30.1-60% from the pulp)

The one-way ANOVA for both primary (Table 23) and permanent teeth (Table 24) indicates that no significant difference exists between treatments.

The effect of the oxalate on the L_p of deep and intermediate dentin is illustrated in Figures 17 and 18 respectively.

III. Tubule density and diameter

Tables 25,26, and 27 of the Appendix present the data collected when 5 primary molars were studied under the SEM for tubule density and diameter.

a) Deep dentin (0-30% from the pulp)

The tubule density of deep dentin was (26,391 ± 6,605) tubules/mm^2 and ranged from 18,816 to 36,650 tubules/mm^2. The tubule diameter was (1.286 ±

0.104) μm and ranged from 1.13 to 1.387 μm.

b) Intermediate dentin (30.1-60% from the pulp)

The tubule density of intermediate dentin was (20,433 $\pm$ 2,568) tubules/mm^2 and ranged from 16,794 - 22,782 tubules/mm^2. The tubule diameter was (1.096 $\pm$ 0.089) μm and ranged from 1.004 to 1.198 μm.

c) Outer dentin (60.1-90% from the pulp)

The tubule density of outer dentin was (18,075 $\pm$ 2,415) tubules/mm^2 and ranged from 15,266 to 21,132 tubules/mm^2. The tubule diameter was (1.075 $\pm$ 0.125) μm and ranged from 0.894 to 1.173 μm.

d) Superficial dentin (90.1-100% from the pulp)

The tubule density of superficial dentin was (17,433 $\pm$ 137) tubules/mm^2 and ranged from 17,335 to 17,530 tubules/mm^2. The tubule diameter was (0.963 $\pm$ 0.033) μm and ranged from 0.94 to 0.986 μm.

The effects of depth on tubule density and diameter are graphically presented in Figures 19 and 20 respectively.

Figure 12. The Effect of the Interaction of the Type of Tooth and Dentin Surface, and Dentin Depth on Hydraulic Conductance

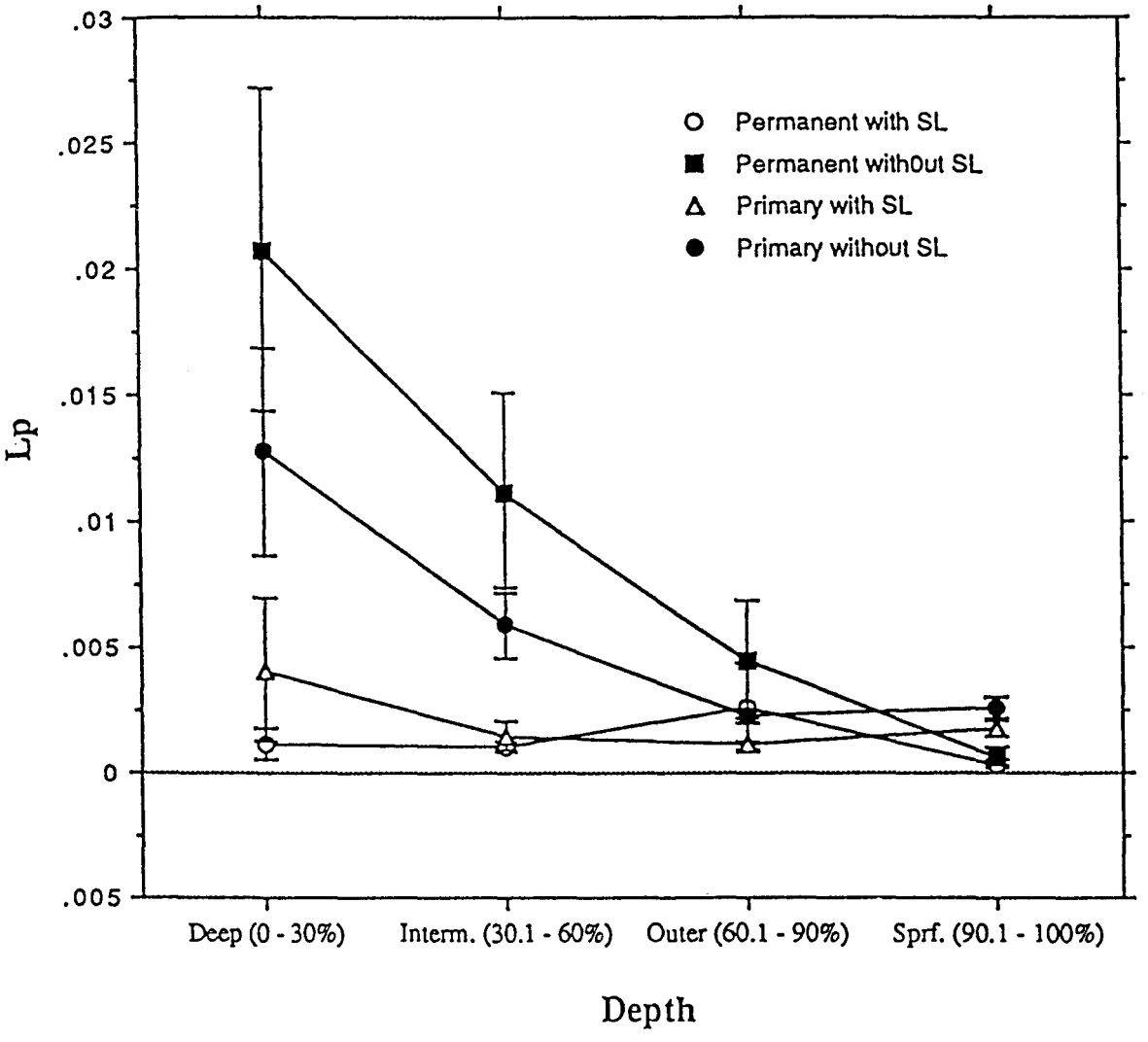


Figure 13. Correlation between Hydraulic Conductance (L_p) with smear layer and Dentin Depth for Primary Teeth.

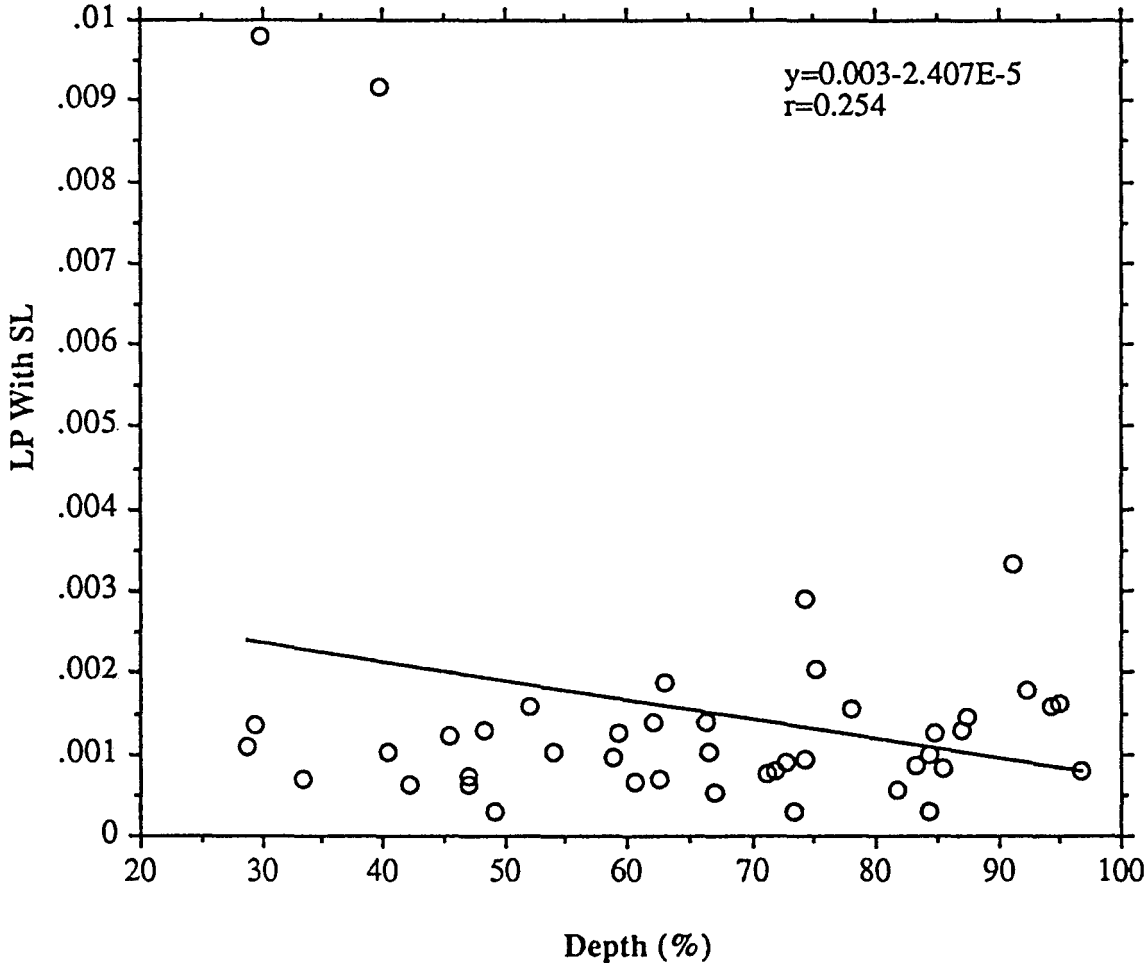


Figure 14. Correlation between Hydraulic Conductance (L_p) without Smear Layer and Dentin Depth for Primary Teeth

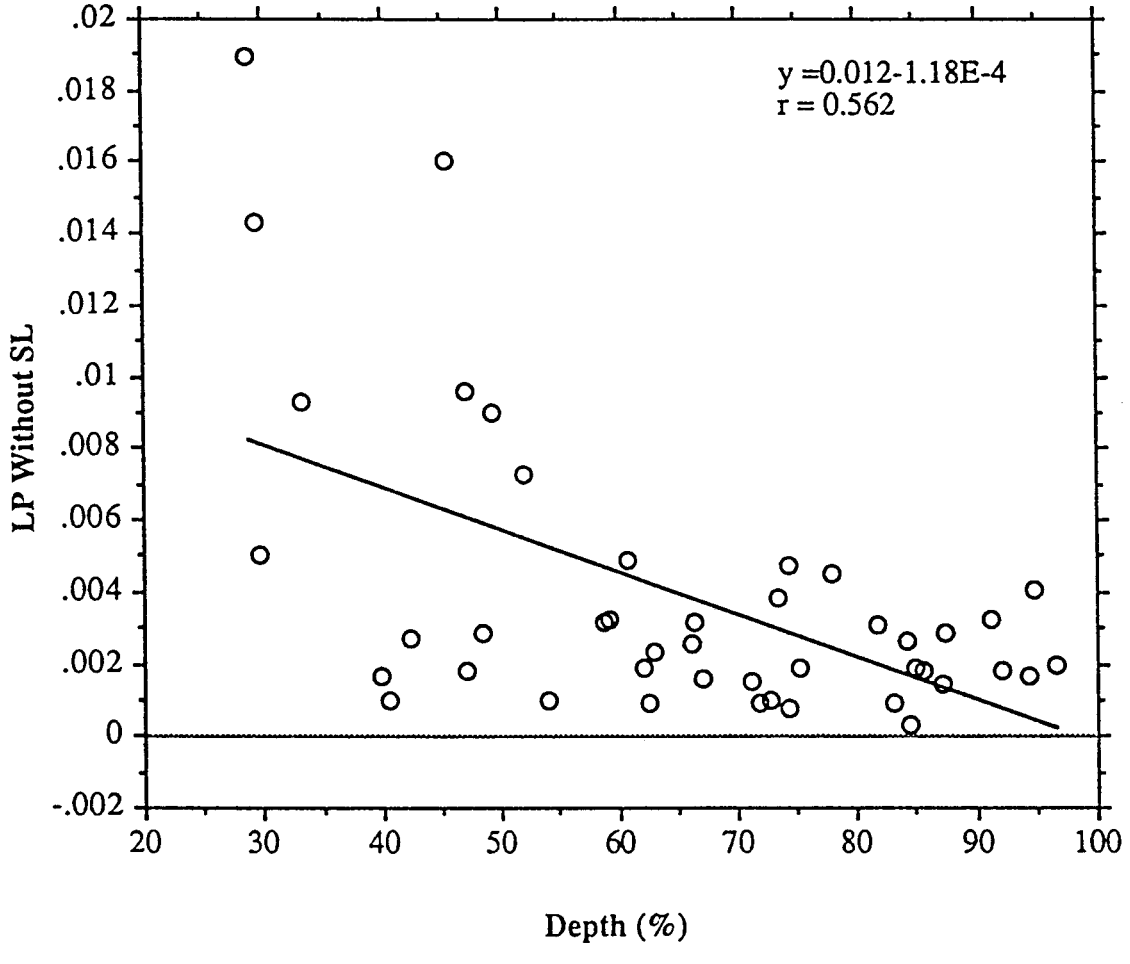


Figure 15. Correlation between Hydraulic Conductance (L_p) with Smear Layer and Dentin Depth for Permanent Teeth.

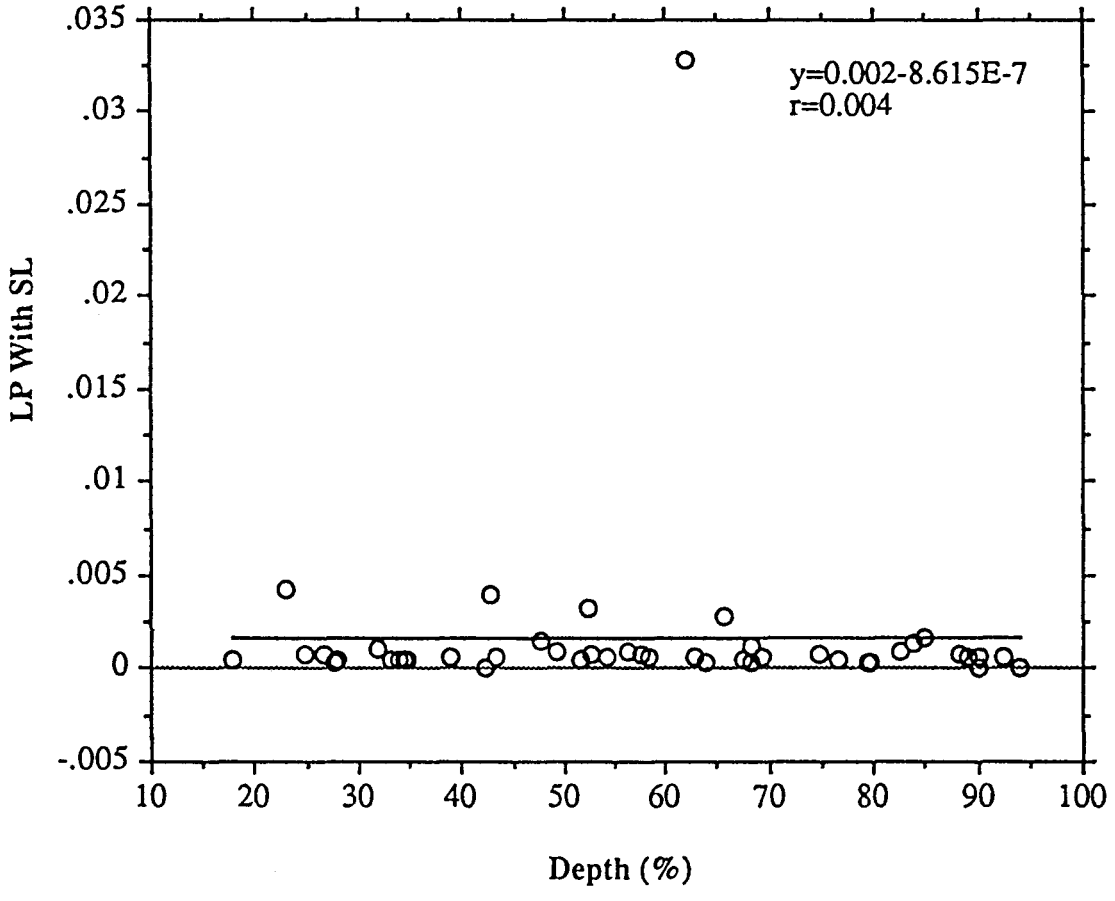


Figure 16. Correlation between Hydraulic Conductance (L_p) without Smear Layer and Dentin Depth for Permanent Teeth

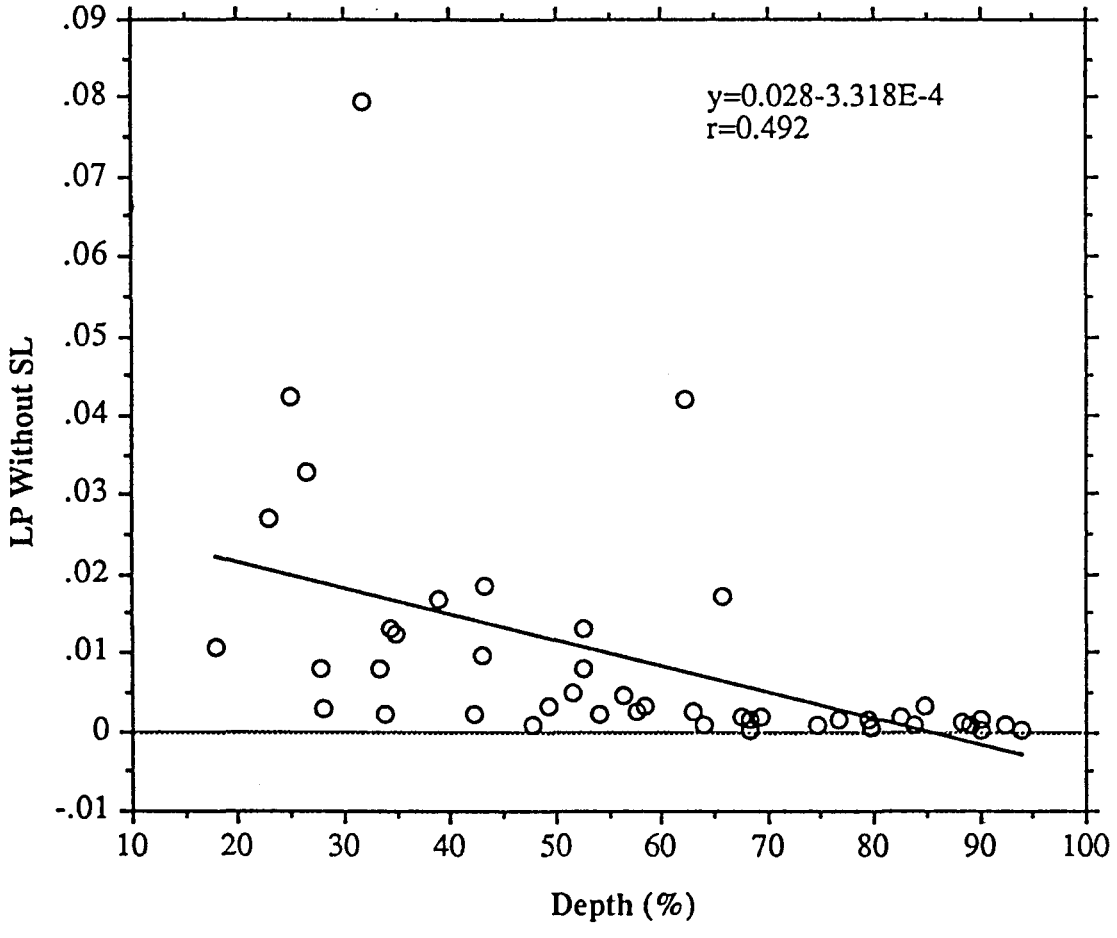


Figure 17. The Effect of Potassium Oxalate on the Hydraulic Conductance (L_p) of Deep Dentin (0-30%) of Primary and Permanent Teeth

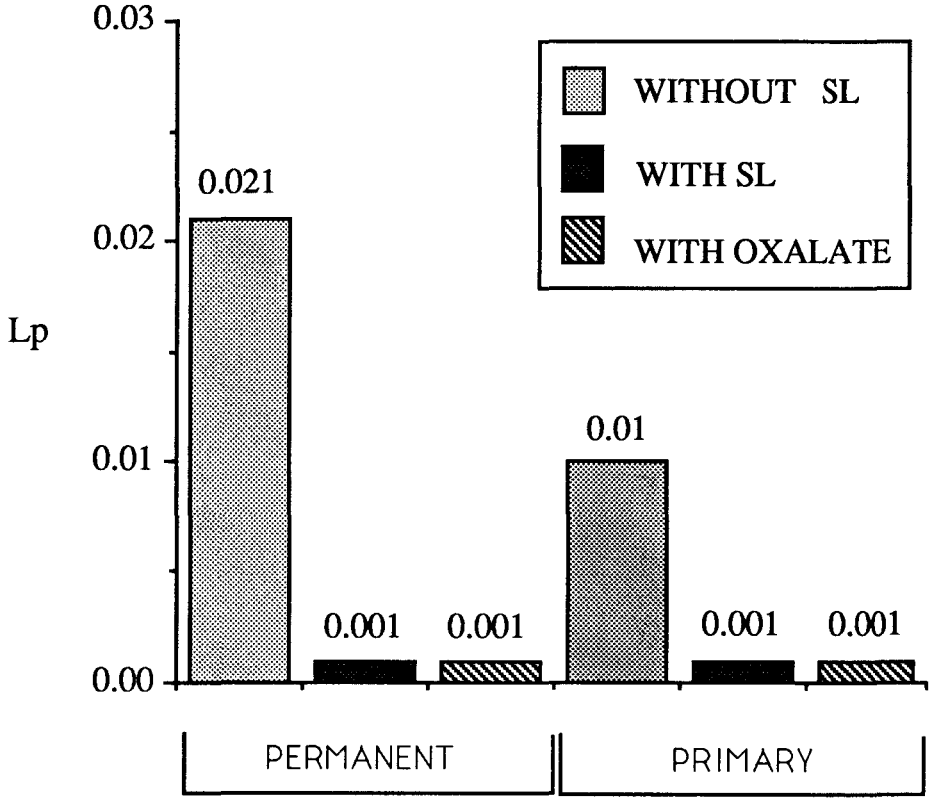


Figure 18. The Effect of Potassium Oxalate on the Hydraulic Conductance (L_p) of Intermediate Dentin (30.1-60%) of Primary and Permanent Teeth

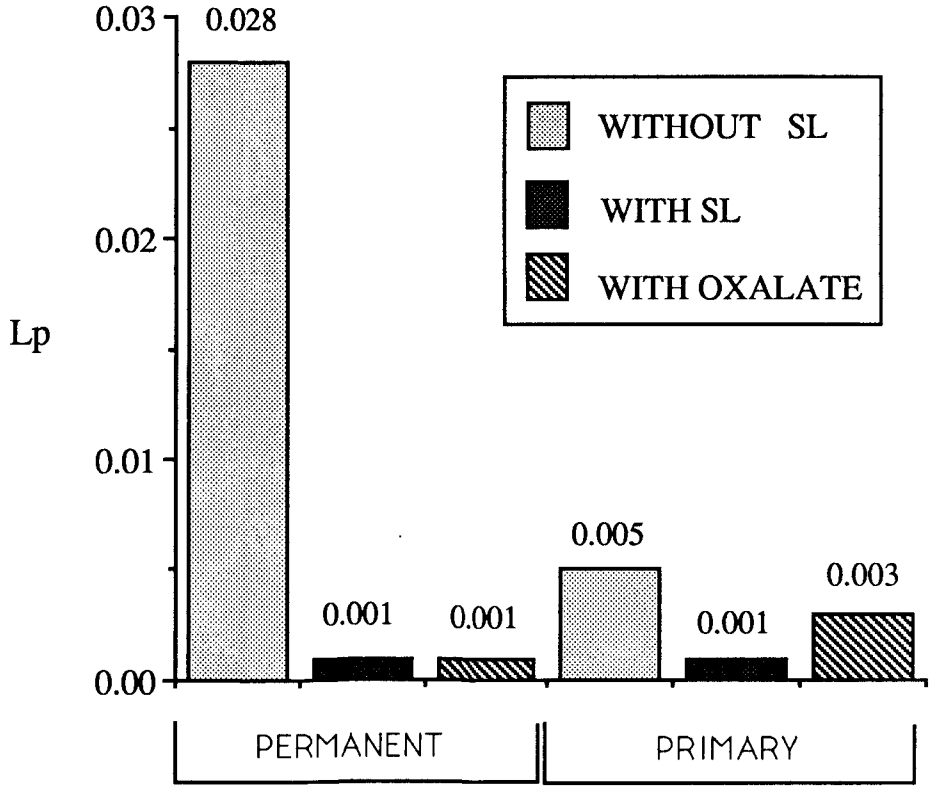


Figure 19. The Effect of Depth on the Dentinal Tubule Density in Primary Teeth

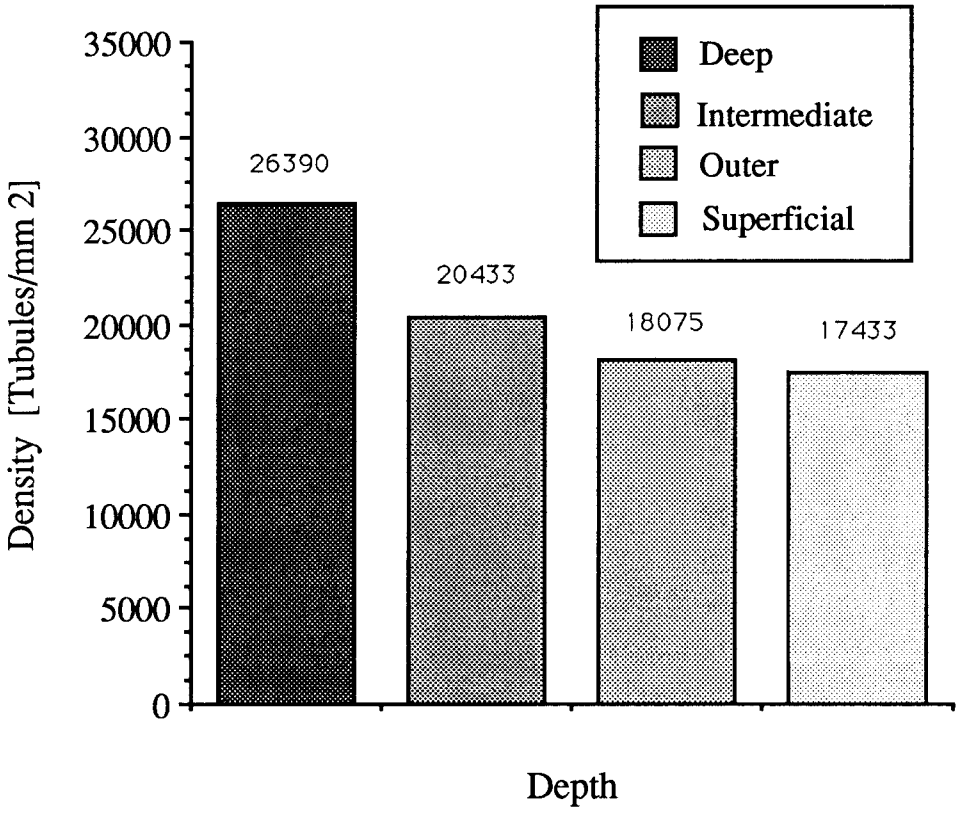
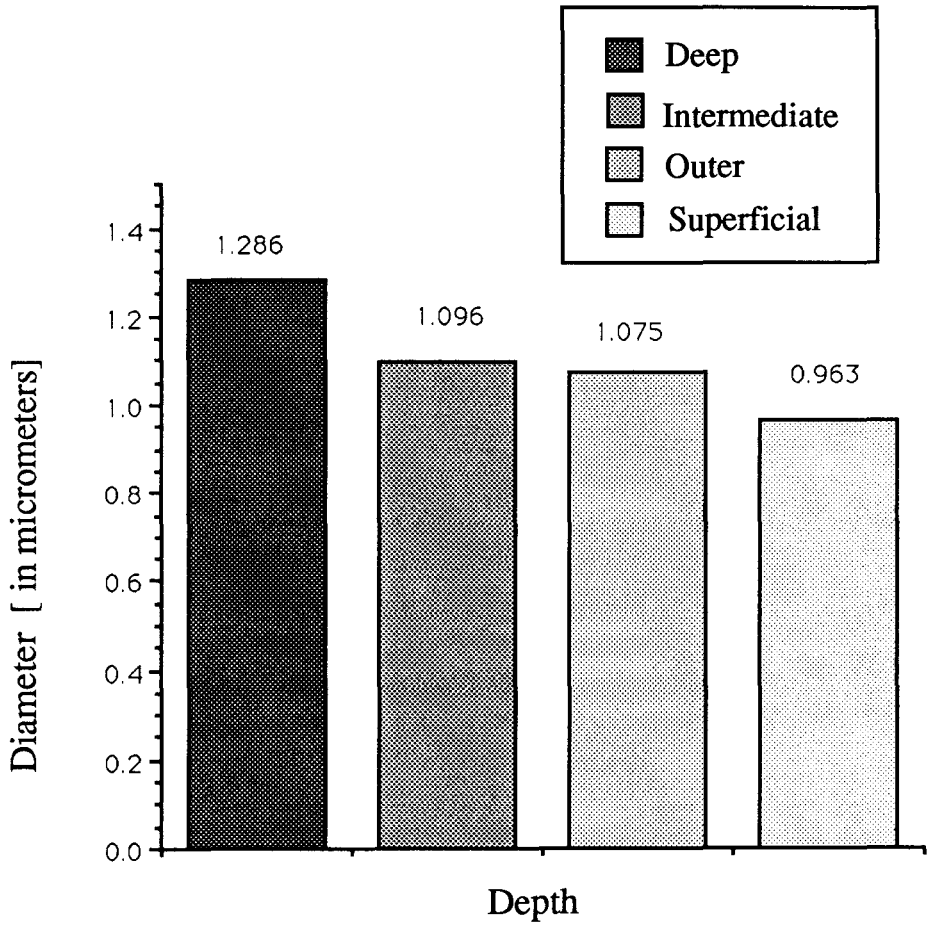


Figure 20. The Effect of Depth on the Dentinal Tubule Diameter in Primary Teeth



CHAPTER IV

DISCUSSION

Our results concerning the relationship between dentin permeability and dentin depth for both primary and permanent teeth confirm the results of previous work on human coronal dentin of permanent teeth (Outhwaite, Livingston, and Pashley, 1978) showing that permeability increases as dentin becomes thinner. This is due both to an increase in tubule diameter and an increase in tubule density as dentin is thinned toward the pulp chamber. It is also due to a reduction in the frictional resistance of the tubule walls to flow as thickness is reduced. The tubule diameter is more important than the density since the fluid movement through dentin varies with the fourth power of the tubule radius. Small changes in tubule radius have more profound effects on fluid shifts across dentin than large changes in thickness (Pashley, 1984).

Garberoglio and Brännström (1976) studied fractured surfaces of human coronal dentin under the SEM. Close to the pulp they found approximately 45,000 tubules/mm^2 with a diameter of 2.5 μm, in contrast to our observations in primary teeth of 26,390 tubules/mm^2 with a diameter of only 1.3 μm. The greater density and diameter of the permanent teeth could explain the greater permeability of

permanent dentin in comparison to that of the dentin of the primary teeth.

Although the density and diameter of the dentinal tubules of the primary teeth are smaller than those reported for permanent teeth, our observations are in agreement with the findings of Garberoglio and Brännström (1976) who reported that tubule density and diameter decrease progressively from the pulp to the enamel in coronal dentin. Carrigan et al. (1984) reported that the number of dentinal tubules decreases corono-apically. This may explain the difference between the study by Garberoglio and Brännström (1976) on occlusal dentin and our study on cervical coronal dentin.

The data obtained from measurements of this type are subject to certain errors, which may influence the mean values of the number of tubules, as well as the tubule diameter. Some of the errors such as the magnification value and the angle of the area studied, are associated with the SEM procedure. Other problems relate to the difficulties in precisely defining the diameter of the tubules in the photographs. In this study, since all the specimens were examined in a standardized manner, the errors inherent to the methodology might tend to cancel one another. However, a direct comparison of the absolute numbers reported in this study for primary teeth to those reported in other studies for permanent teeth is not possible due to differencies in methodology. Our method for determining the density of the dentinal tubules is very similar to the one used by most researchers. However, the computer program, utilized to measure the tubule diameter on the

scanning electron micrographs, has never been utilized before for that purpose, to our knowledge.

Although the measured tubule diameter and density of primary teeth correlate well with the observed low values in hydraulic conductance for primary teeth, compared to permanent teeth, at various depths from the pulp, some variability might be explained by the differencies in functional *versus* anatomical radii (Michelich, Pashley, and Whitford, 1978). Functional radius refers to the effective cross section radius of the tubule actually available for fluid movement after partial tubule blockages, such as collagenous bundles and irregularities of the tubule wall, are taken into account.

Anatomic radii, as measured microscopically in this study, provide a maximum boundary for the functional radii. However, if one assumes that the amount of intratubular blockages were similar at different depths from the pulp, the observed differencies in hydraulic conductance can be largely explained by differencies in the tubule diameter and density.

Intratubular deposits account for 6% of the overall resistance to fluid movement across dentin (Michelich, Pashley, and Whitford, 1978), and they can restrict significantly the functional diameter of the dentinal tubules. The odontoblast and its process may not represent a significant resistance to fluid movement through dentinal tubules relative to other intratubular resistances (Pashley, Livingston, and Greenhill, 1978). In the present study, saline storage of

the teeth allowed the odontoblasts and their processes to undergo autolysis. Since the presence of the intact odontoblast processes do not restrict significantly the functional radius of the tubules, it is unlike that the products of their autolysis will cause a significant restriction. Thus, the hydraulic conductances measured *in vitro* should be regarded as maximum limits of the *in vivo* values.

The functional diameter of tubules determined using hydrodynamic techniques is only 5-10% that of the anatomical diameter (Michelich, Pashley, and Whitford, 1978). An important consequence of this constriction is that dentin under a dental restoration with significant microleakage is very effective in trapping bacteria from saliva. If the tubules appear, by SEM observation, to be approximately 1μm, their functional diameter is less than 0.2μm. Therefore, dentin functions much like a 0.2 μm Millipore filter which is commonly used to sterilize fluids. When bacterial-laden oral fluid passes through dentin it exits the pulpal side sterile (Michelitch, Schuster, and Pashley, 1980), thus demonstrating the efficacy of dentin as a barrier for bacterial penetration.

The presence of a smear layer on the dentin surface and smear plugs in the dentinal tubules also has a protective role for the pulp because it prevents massive invasion of bacteria through the dentinal tubules (Vojinovic, Nyborg, and Brännström, 1973). However, it does not prevent bacterial toxins from diffusing into the pulp. When intact teeth are cut experimentally the smear layer produced is free of bacteria. On the other hand, under clinical conditions, especially when operating

on carious teeth, there is a great risk of bacteria surviving in the smear layer (Brännström, 1984). Several investigations have been carried out to find a suitable cleanser that would retain the smear plugs and remove only the superficial smear layer, so that an antiseptic component in the cleanser could reach and kill any bacteria present in the smear plugs (Brännström, 1984). Mechanical retention or bonding of cavity liners, luting cements, and restorations would be stronger because the bond strengths would not suffer from the intrinsic weakness of the smear layer.

Although the presence of the smear layer in the interface of most restorative materials and the dentin matrix is not desirable by dental material standards, it does reduce dentin permeability more than most commercially available cavity liners (Pashley, 1989). Pashley, Livingston, and Greenhill (1978) reported that the smear layer accounts for 86% of the total resistance to flow of fluid through dentin. Thus, after etching with acid, the rate of flow of fluid increased 15-fold, 32-fold in another study (Reeder et al., 1978), and 42-fold in a more recent *in vitro* study (Pashley, Thomson, and Stewart, 1983). In these studies, the magnitude of change in the rate of fluid flow across dentin before and after etching indicated the thickness or density of the smear layer (Pashley, 1984).

In the present study, when the hydraulic conductance of primary teeth were compared to that of permanent teeth in the presence of smear layer, no significant difference was found. The smear layer was very effective in reducing the

permeability at all depths, for both primary and permanent teeth. After removal of the smear layer, the permeability of the primary teeth increased with the depth, in a manner similar to that of the permanent teeth. However, the hydraulic conductance of the permanent teeth was significantly higher than that of primary teeth. This result can be attributed to the smaller density and diameter of the dentinal tubules of primary teeth. These are factors which determine the theoretical area of diffusional surface.

Our findings concerning the permeability characteristics of the dentin of primary teeth are of more than academic interest. As the amount of bacterial toxins that can diffuse across dentin each minute is proportional to the available diffusional area, the deeper the carious destruction of a primary tooth, the greater will be the amount of toxins that can diffuse into the pulp per unit time. According to Pashley (1979), the degree of the inflammatory response of the pulp will be determined by two factors. The first is the rate of permeation of the bacterial toxins through the tubules which can be defined as the dentin permeability. The second factor is the rate at which the toxins are removed from the pulpal interstitial fluid by the pulpal circulation. If the rate of delivery of the toxin exceeds the rate of removal by the pulpal circulation, then the pulpal concentration will increase. This will cause a more severe inflammatory response with further reductions of blood flow resulting in further increases in the pulpal concentration of the toxins. Therefore, inflammed pulps with compromised blood flow may be

more susceptible to damage by toxic agents.

This situation may be encountered clinically during procedures used to restore a carious tooth. Even though the carious part is removed, the dentinal tubules beneath the affected area still contain soluble bacterial products. The reduction in blood flow in the pulp, that follows infiltration of local anesthetics with vasoconstrictor, may permit the accummulation of bacterial products in the pulp. The concentration of these toxins might induce even more significant tissue damage, before microcirculation is restored. The inflammatory response of the pulp could lower the pain threshold of the tooth, thus, making it more sensitive than before the placement of the restoration.

Etching the dentin clinically before placement of restorations, as well as by the action of microorganisms of plaque on exposed cervical dentin or under leaking restorations, can lead to an even more significant sensitivity to osmotic, thermal, and tactile stimuli (Johnson and Brännström, 1974). If dentin is sensitive, then according to the hydrodynamic theory of dentin sensitivity, the dentinal tubules must be patent and therefore allow movement of fluid across dentin. Sensitivity is probably a result of the fluid shifts, as well as of the easier permeation of bacterial toxins through the tubules, towards the pulp. Therefore, etching of dentin before placement of restorations should be avoided.

The goal of treatment of dentin sensitivity should be the restoration of the original impermeability of the tubules. This can be accomplished, therapeutically,

using topical application of many agents, such as soluble salts of oxalates. Application of 30% dipotassium oxalate (pH 7) or 3% monopotassium-monohydrogen oxalate (pH 2) onto etched dentin can result in reduction of the hydrualic conductance up to 98.4% (Greenhill and Pashley, 1981; Pashley and Depew, 1986). Our results indicate that 3% monopotassium-monohydrogen oxalate reduced the permeability of dentin by 93.2% in the permanent teeth. The reduction of permeability in primary teeth was less, 79.4%, but nevertheless significant. This difference may be attributed to the small sample size examined and the variability between teeth. The oxalate was as effective as the smear layer in reducing the permeability.

While no precipitate is observed visually on the dentin surface treated with oxalate, SEM examination reveals calcium oxalate salts partially occluding the tubule orifices (Greenhill and Pashley, 1981). According to Pashley and Galloway (1985), the source of calcium may be ions in the fluids within the smear layer. The reaction of the soluble oxalate salts with the dentin seems to occur within seconds and is probably a surface phenomenon. The large crystals which form following application of 30% dipotassium oxalate are likely to be calcium-oxalate. The smaller crystals that form after topical application of 3% monopotassium-monohydrogen oxalate are probably a complex mixture of calcium-phosphates and calcium oxalates. Thus, the latter solution may be acid-etching the smear layer and replacing it with a layer of crystaline precipitates. The crystalline covered dentin

surface is resistant to acid attack (Pashley and Galloway, 1985). Although the attention of most investigators has been directed toward evaluating the mechanism of tubule occlusion with oxalates, there is another possible explanation for the desensitization of dentin currently under investigation. There is a theoretical possibility that nerve depolarization caused by elevation in intratubular potassium might lead to release of substance P or other neuroactive/vasoactive peptides from local intratubular nerves. These peptides may subsequently modify local blood flow or nerve excitability long after potassium concentration is restored to normal (Pashley, 1986).

Oxalates have been tested for use as cavity liners due to their ability to greatly reduce dentin permeability. They were found to be very effective in reducing microleakage of amalgam restorations (Sandoval, Cooley, and Barnwell, 1989). In addition to the reduction in microleakage, oxalates, when applied under cements, commonly used in operative and prosthetic dentistry, do not affect the bond strength of glass-ionomer or polycarboxylate cements but produce a large decrease in the bond strength of zinc phosphate cement.

Although the previous research on oxalates, used as desensitizing agents or cavity liners, has been limited to studies *in vitro*, two recent papers demonstrate their effectiveness *in vivo*. Hasson (1987) and Muzzin and Johnson (1989), reported that oxalates significantly reduced the hypersensitivity of cervical root dentin to thermal stimuli at 4 weeks after periodontal treatment. There have been

no *in vivo* studies to evaluate the short and long-term effects of oxalates on reducing hypersensitivity under restorations although unpublished clinical reports suggest them to be effective.

Because of their desirable properties, oxalates may soon be used routinely under restorative materials to provide a second line of defense against the ability of their components to chemically irritate the pulp and the possibility that these materials may not bind to or adapt to enamel and dentin sufficiently to prevent microleakage and percolation.

SUMMARY AND CONCLUSIONS

The permeability of buccal coronal dentin at various depths from the pulp was tested experimentally in primary molars and premolars. The crowns of the teeth were connected to a pressurized device, described by Pashley and Galloway (1985), and the permeability was measured in the presence of smear layer, after its removal, and after applying 3% monopotassium-monohydrogen oxalate on the dentin surface. The density and diameter of the dentinal tubules of primary molars was also measured at various depths from the pulp, utilizing the scanning electron microscope. The results indicate that:

1. The permeability of both primary and permanent teeth increased with increasing dentin depth.

2. The smear layer reduced significantly the permeability of primary and permanent teeth, regardless of the dentin depth.

3. The removal of the smear layer resulted in a significant increase in the permeability of both primary and permanent teeth. However, the permeability of the premolars was significantly higher of that of the primary molars.

4. The application of 3% monopotassium-monohydrogen oxalate for two minutes on EDTA-treated dentin resulted in a 79.4% and 93.2% reduction of

permeability, for primary and permanent teeth respectively.

5. The density and diameter of the dentinal tubules in primary molars were found to be lower than the values reported in the literature for permanent teeth.

6. The smaller density and diameter may account for the lower permeability of the primary molars, when compared to the premolars.

BIBLIOGRAPHY

Ampiro R, and Camanni F. 1956. Historadiographic autoradiographic researches on hard dental tissues. *Acta Anat* 28:217-258.

Anderson DJ, and Matthews B. 1967. Osmotic stimulation of human dentine and the distribution of dental pain thresholds. *Arch Oral Biol* 12:417-426.

Anderson DJ, and Ronning GA. 1962. Osmotic excitants of pain in human dentine. *Arch Oral Biol* 7:513-523.

Anderson DJ, Matthews B, and Gorretta C. 1967. Fluid flow through human dentin. *Arch Oral Biol* 12:209-216.

Andringa HJ. 1987. *Regional variability of Dentin Permeability.* Master's Thesis Medical College of Georgia, Augusta, GA.

Avery JK. 1986. Dentin. In *Orban's Oral Histology and Embryology.* 10th Ed. Bhaskar SN Ed, Mosby. St. Louis, MO.

Avery JK. 1987. *Oral Development and Histology.* Williams and Wilkins. Baltimore, MA.

Bang G, and Ramm E. 1970. Determination of age in humans from root dentin transparency. *Acta Odont Scand* 28:3-35.

Bergenholtz G, and Lindhe J. 1975. Effect of soluble plaque factors on inflammatory reactions in the dental pulp. *Scand J Dent Res* 83:153-158.

Bergenholtz G, Mjör IA, Cotton WR, Hanks CT, Kim S, Torneck CD, and Trowbridge HO. 1985. Consensus report: Dentin-predentin complex and its permeability. *J Dent Res.* 64; Special Issue:631-635.

Bergenholtz G. 1977. Effect of bacterial products on inflammatory reactions in the dental pulp. *Scand J Dent Res* 85:122-129.

Bergenholtz G. 1981. Inflammatory response of the dental pulp to bacterial

irritation. *J Endod* 7:100-104.

Berggren G, and Brännström M. 1965. The rate of flow in dentinal tubules due to capillary attraction. *J Dent Res* 44:408-415.

Bergman G. 1959. Studies on mineralized dental tissues. *J Dent Berge* 50:75-85.

Bird MJ, French EL, Woodside MR, Morrison ML, and Hodge HC. 1940. Chemical analysis of deciduous enamel and dentin. *J Dent Res* 19:413-423.

Black GV. 1895. An investigation of the physical characteristics of the human teeth in relation to their diseases and to practical dental operations, together with the physical characters of filling materials. *Dent Cosmos* 37:353-421.

Blake GC. 1958. The peritubular translucent zones in human dentine. *Br Dent J* 109:57-64.

Bornstein P, and Sage H. 1980. Structural distinct collagen types. *Annual Rev Biochem* 49:957-1003.

Boskey AL, and Posner AS. 1976. In vitro nucleation of hydroxyapatite by a bone calcium-phospholipid-phosphate complex. *Calcif Tissue Res* 225:197-201.

Boskey AL, and Posner AS. 1977. The role of synthetic and bone-extracted Ca-phospholipid-PO_4 complexes in hydroxyapatite formation. *Calcif Tissue Res* 23:251-258.

Bowen RL. 1978. Adhesive bonding of various materials to hard tooth tissues-solubility of dental smear layer of dilute acid buffers. *Int Dent J* 28:91-107.

Boyde A, and Lester KS. 1967. An Electron Microscope Study of fractured dentinal surfaces. *Calc Tiss Res* 1:122-136.

Bradford EW. 1955. The interpretation of decalcified sections of human dentine. *Br Dent J* 98:154-159.

Bradford EW. 1958. The maturation of dentine. *Br Dent J* 105:212-216.

Brännström M, and Åstrom A. 1964. A study on the mechanism of pain elicited fron the dentin. *J Dent Res* 43:619-625.

Brännström M, and Åström A. 1972. The hydrodynamics of dentine: Its possible relationship to dentinal pain. *Int Dent J* 22:219-227.

Brännström M, and Garberoglio R. 1972. The dentinal tubules and odontoblastic processes: A scanning electron microscopic study. *Acta Odont Scan* 30:291-311.

Brännström M, and Johnson G. 1970. Movements of the dentine and pulp liquids on application of thermal stimuli. *Acta Odont Scand* 28:59-70.

Brännström M, and Johnson G. 1974. Effects of various conditioners and cleaning agents on prepared dentin surfaces: A scanning electron microscopic investigation. *J Prosth Dent* 31:422-430.

Brännström M, Johnson G, and Linden LA. 1969. Fluid flow and pain response in the dentine produced by hydrostatic pressure. *Odont Rev* 20:15-30.

Brännström M, Linden L, and Johnson G. 1968. Movement of dentinal and pulpal fluid caused by clinical procedures. *J Dent Res* 47:679-682.

Brännström M, Linden LA, and Åstrom A. 1967. The hydrodynamics of the dentinal tubule and of pulp fluid. *Caries Res* 1:310-317.

Brännström M, Nordenvall KJ, and Glantz P. 1980. The effect of EDTA-containing surface-active solutions on the morphology of prepared dentin: An *in vivo* study. *J Dent Res* 59:1127-1131.

Brännström M. 1961. Dentinal and pulpal response V: Application of pressure to exposed dentin. *J Dent Res* 40:960-970.

Brännström M. 1962. The elicitiation of pain in human dentine and pulp by chemical stimuli. *Arch Oral Biol* 7:59-62.

Brännström M. 1966. Sensitivity of dentine. *Oral Surg* 21:517-526.

Brännström M. 1984. Smear layer: Pathologic and treatment considerations. *Oper Dent; Supplement* 3:35-42.

Burnett GW, and Zenewitz J. 1958. Studies on the composition of teeth: VII. The moisture content of calcified tooth tissues. *J Dent Res* 37:581-589.

Butler NT. 1973. Concerning the high level of hydroxylysine in dentin collagen.

Ala J Med Sci 10:103-106.

Butler WT. 1987. Dentin specific proteins. *Methods Enzymol* 145:290-303.

Carrigan PJ, Morse DR, Furst ML, and Sinai IH. 1984. A scanning electron microscopic evaluation of human dentinal tubules according to age and location. *J Endod* 10:359-363.

Chan DCN, and Jensen ME. 1986. Dentin permeability to phosphoric acid. *Dent Mater* 2:251-256.

Charbeneau GT, Peyton FA, and Antony DH. 1966. Profile characteristics of cut tooth surfaces developed by rotating instruments. *J Dent Res* 36:951-966.

Chirnside IM. 1961. Bacterial invasion of non-vital dentin. *J Dent Res* 40:134-140.

Coffey CT, Ingram MJ, and Björndal AM. 1970. Analysis of human dentinal fluid. *Oral Surg* 30:835-837.

Daculsi G, Kerebel B, LaCabella MT, Kerebel LM. 1979. Qualitative and quantitative data on arrested caries in dentin. *Caries Res* 13:190-202.

Dippel HW, Borggreven JMPM, and Hoppenbrouwers PMM. 1984. Morphology and permeability of the dentinal smear layer. *J Prosthet Dent* 52:657-662.

Dirksen TR. 1963. Lipid contents of sound and carious dentin. *J Dent Res* 42:128-132.

Eastoe JE. 1963. The amino acid composition of proteins from the oral tissues: II. The matrix proteins in dentine and enamel from developing human deciduous teeth. *Arch Oral Biol* 8:633-652.

Eastoe JE. 1969. The amino acid composition of bone and tooth. *Adv Fluor Res* 3:5-17.

Eick JD, and Welch FH. 1986. Dentin adhesives-Do they protect the dentin from acid etching? *Quint Inter* 17:533-544.

Eick JD, Bowen RL, Erickson R, and Codd CM. 1987. TEM of the smear layer and the dentin - adhesive interface. *J Dent Res* 66:Special Issue; 268, Abstr# 1295.

Eick JD, Wilko RA, Anderson CH, and Sorensen SE. 1970. Scanning electron microscopy of cut tooth surfaces and identification of debris by use of the electron microprobe. *J Dent Res* 49:1359-1368.

Elliot JC. 1973. The problems of the composition and structure of the mineral components of the hard tissues. *Clin Orthop* 93:313-345.

Ericson SG. 1965. Quantitative microradiography of cementum and abraded dentine a methodological and biological study. *Acta Radiol* Suppl 246.

Fogel HM, Marshall FJ, and Rashley DH. 1988. Effects of distance from the pulp and thickness on the hydroculic conductance of human radicular dentin. *J Dent Res* 67:1381-1385.

Forssell - Alberg K, Brännström M, and Edwall L. 1975. The diameter and number of dentinal tubules in rat, cat, dog and monkey. *Acta Odont Scand* 33:243-250.

Fosdick LS, Blackwell RQ, and Wachtl C. 1959. The effect of age and exposure to oral environment on the permeability of teeth. *J Dent Res* 38:676, Abstr# 60.

Frank RM, and Nalbandian J. 1963. Comparative aspects of development of dental hard tissues. *J Dent Res* 42:422-437.

Frank RM, and Sognnaes RF. 1960. Electron microscopy of matrix formation and calcification in rat enamel. *Arch Oral Biol* 1:339-348.

Frank RM. 1966. Etùde au microscope électronique de l'odontoblaste et du canalicule dentinaire humain. *Arch Oral Biol* 11:179-199.

Frank RM. 1980. Contribution of electron microscopy to oral biology, a review. *J R Soc Med* 73:265-272.

Fromme HG, and Riedel H. 1970. Messungen über die weite der dentinkanälchen an michtenmineralisierten bleibenden zähnen und milchzähnen. *Dt zahnärztl Z* 25:401-405.

Garberoglio R, and Brännström M. 1976. Scanning electron microscopic investigation of human dentinal tubules. *Arch Oral Biol* 21:355-362.

Gilboe DB, Svare CW, Thayer KE, and Drennon DG. 1980. Dentinal smearing:

an investigation of the phenomenon. *J Prosth Dent* 44:310-316.

Greenhill JD, and Pashley DH. 1981. The effects of desensitizing agents on the hydraulic conductance of human dentin *in vitro*. *J Dent Res* 60:686-698.

Gwinnett AJ. 1973. Structural changes in enamel and dentin of fractured anterior teeth after acid conditioning *in vitro*. *JADA* 86:117-122.

Gwinnett AJ. 1984. Smear layer: Morphologic considerations. *Oper Dent; Supplement* 3:3-12.

Haljamae H, and Rockert H. 1970. Potassium and sodium content in dentinal fluid. *Odont Revy* 21:369-377.

Harcourt JK. 1964. Further observations on the peritubular translucent zone in human dentin. *Austr Dent J* 9:387-392.

Hasson RE. 1987. *The assessment of the subjective nature of pain associated with cervical root dentin hypersensitivity and the evaluation of the effectiveness of dipotassium oxalate in the reduction of cervical root dentin hypersensitivity.* Master's Thesis. University of Michigan, Ann Arbor, MI.

Hirvonen TJ, and Narhi MVO. 1986. The effect of dentinal stimulation on pulp nerve function and pulp morphology in the dog. *J Dent Res* 65:1290-1293.

Höhling HJ. 1966. Die Baulemente von Zahnschmelz und dentin aus morphologischer, chemischer, und struktureller sicht. *C. Hanser*, München.

Isokawa S., Toda Y, and Kubota K. 1970. A scanning electron microscopic observation of etched human peritubular dentin. *Arch Oral Biol* 15:1303-1306.

Jenkins GN. 1978. Chemical composition of teeth. In *The Physiology and Biochemistry of the Mouth.* Blackwell/Lippincott, Philadelphia.

Jessen H. 1967. The ultrastructure of odontoblasts in perfusion fixed, demineralized incisors of adult rats. *Acta Odont Scand* 25:491-523.

Johannessen JV, and Bang G. 1972. Transmission electron microscopy of sound demineralized guinea pig dentin. *Scand J Dent Res* 80:222-229.

Johnson G, and Brännström M. 1974. The sensitivity of dentin: Changes in

relation to conditions at exposed tubule apertures. *Acta Odont Scand* 32:29-38.

Johnson G, Olgart L, and Brännström M. 1973. Outward fluid flow in dentin under a physiologic pressure gradient: Experiments *in vitro*. *Oral Surg* 35:238-248.

Kaye H, and Herold RC. 1966. Structure of human dentine: I. Phase contrast, polarization, interface and bright field microscopic observations on the lateral branch system. *Arch Oral Biol* 11:355-368.

Kennedy JJ, Teuscher GW, and Fosdick LS. 1953. The ultramicroscopic structure of enamel and dentin. *JADA* 46:423-431.

Ketterl W. 1961. Studie über das dentin der permanenten zähne des menschen. *Stoma* 14:79-112.

Komrska J. 1972. Derivativographic analysis of dentin. *J Dent Res* 51:148-150.

Kuboki K, and Mechanic G. 1982. Comparative molecular distribution of cross-links in bone and dentin collagen structure-junction relationships. *Calcif Tissue Int* 34:306-308.

Kuttler Y. 1959. Classification of dentine into primary, secondary and tertiary. *Oral Surg* 12:996-1001.

La Fleche RG, Frank RM, and Steuer P. 1985. The extent of the human odontoblast process as determined by transmission electron microscopy: The hypothesis of a retractable suspensor system. *J Biol Buccale* 13:293-305.

Le Ferre ML, Ball WF, and Hodge HC. 1937. The chemical nature of the inorganic portion of fetal tooth substance. *J Dent Res* 16:85-110.

Leaver AG. 1969. Studies on certain peptide fractions isolated from human dentine. *Arch Oral Biol* 14:501-511.

Lee HL, Orlawski JA, Scheidt GC, and Lee JR. 1973. Effect of acid etchants on dentin. *J Dent Res* 52:1228-1232.

Levine RS. 1971. The distribution of hydroxyproline in sound human coronal dentine. *Arch Oral Biol* 16:473-478.

Linde A, Ed. 1984. Dentin and Dentinogenesis, Vol. I & II. CRC Press, Boca Raton.

Linden LA, and Brännström M. 1973. Fluid movements in dentine and pulp. *Odont Revy* 28:227-236.

Loevy HT, and Kovitz A. 1988. Dentistry on Stamps: Purkinye. *JADA* 117:179-182.

Maniatopoulos C, and Smith DC. 1983. A scanning electron microscopic study of the odontoblast process in human coronal dentine. *Arch Oral Biol* 28:701-710.

Manley RS, and Hodge HC. 1939. Density and refractive studies on dental hard tissues. I. Methods for separation and determination of purity. *J Dent Res* 18:133-141.

Martens PJ, Bradford EW, and Frank RM. 1959. Tissue changes in dentine. *Int Dent J* 9:330-348.

Mechanic G, Gallop PM, and Tomzer ML. 1971. The nature of crosslinking in collagens from mineralized tissues. *Biochem Biophys Res Commun* 45:644-653.

Merchant VA, Livingston MJ, and Pashley DH. 1977. Dentin permeation: Comparison of diffusion with filtration. *J Dent Res* 56:1161-1164.

Meryon SD. 1984. The influence of dentine on the *in vitro* cytotoxicity testing of dental restorative materials. *J Biomed Mater Res* 18:771-779.

Michelich V, Pashley DH, and Whitford GM. 1978. Dentin permeability: A comparison of functional versus anatomical tubular radii. *J Dent Res* 57:1019-1024.

Michelich VJ, Schuster GS, and Pashley DH. 1980. Bacterial penetration of human dentin *in vitro*. *J Dent Res* 59:1398-1403.

Miles AEW. 1967. Structural and chemical organization of teeth, Vol. I & II. Academic Press, New York.

Miller NA, and Massler M. 1962. Permeability and staining of active and arrested lessions in dentin. *Br Dent J* 112:187-197.

Miller WA, Eick JD, and Neiders MA. 1971. Inorganic components of the peritubular dentin in young human permanent teeth. *Caries Res* 5:264-278.

Mjör IA. 1966. Microradiography of human coronal dentin. *Arch Oral Biol*; 11:225-234.

Mjör IA. 1967a. Histologic studies of human coronal dentine following cavity preparations and exposure of ground facets *in vivo*. *Arch Oral Biol* 12:247-263.

Mjör IA. 1967b. Histologic studies of human coronal dentine following the insertion of various materials in experimentally prepared cavities. *Arch Oral Biol* 12:441-452.

Mjör IA. 1972. Human coronal dentine: Structure and reactions. *Oral Surg* 33:810-823.

Mjör IA. 1985. Dentin - predentin complex and its permeability: Pathology and treatment overview. *J Dent Res* 64;Special Issue:621-627.

Munksgaard EC, Rhodes M, Mayne R, and Butler WT. 1978. Collagen synthesis and secretion by rat odontoblasts in organ culture. *Eur J Biochem* 82:609-617.

Muzzin KB, and Johnson R. 1989. Effects of potassium oxalate on dentin hypersensitivity *in vivo*. *J Periodontol* 60:151-158.

Nagi N, and Frank RM. 1974. Electron microscopic autoradiography of Ca^{45} during dentinogenesis. *Cell Tiss Res* 155:513-523.

Nalbadian J, Gonzales F, and Sognanes RF. 1960. Sclerotic age changes in root dentin of human teeth as observed by optical, electron, and X-ray microscopy. *J Dent Res* 39:598-607.

Neides ME, Eick JD, Miller WA. 1972. Electron probe microanalysis of cementation and underlying dentin in young permanent teeth. *J Dent Res* 51:122-130.

Odutuga AA, and Prout RE. 1974. Lipid analysis of human enamel and dentine. *Arch Oral Biol* 19:729-731.

Olgart L, Brännström M, and Johnson G. 1974. Invasion of bacteria into dentinal tubules: Experiments *in vivo* and *in vitro*. *Acta Odont Scand* 32:61-70.

Outhwaite WC, Livingstone MJ, and Pashley DH. 1976, Effects of changes in surface area, thickness, temperature, and post-extraction time on human dentine permeability. *Arch Oral Biol* 21:599-603.

Pashley DH, and DePew DD. 1986. Effects of the smear layer, copalite and oxalate on microleakage. *Oper Dent* 11:95-102.

Pashley DH, and Galloway S. 1985. The effects of oxalate treatment on the smear layer of ground surfaces of human dentine. *Arch Oral Biol* 30:731-737.

Pashley DH, and Livingston MJ. 1978. Effect of molecular size on permeability coefficients in human dentine. *Arch Oral Biol* 23:391-395.

Pashley DH, Andringa H, and Derkson GB. 1987. Regional variability in permeability of human dentin. *Arc Oral Biol* 32:519-523.

Pashley DH, Kehl T, Pashley E, and Palmer P. 1981. Comparison of *in vitro* and *in vivo* dog dentin permeability. *J Dent res* 60:763-768.

Pashley DH, Leibach JG, and Horner JA. 1987. The effects of burnishing NaF/Kaolin/Glycerin Paste on dentin permeability. *J Periodontol* 58:19-23.

Pashley DH, Livingston MJ, and Greenhill JD. 1978. Regional Resistances to fluid flow in human dentine *iv vitro*. *Arch Oral Biol* 23:807-810.

Pashley DH, Livingston MJ, and Outhwaite WC. 1977. Rate of permeation of isotopes through human dentin *in vitro*. *J Dent Res* 56:83-88.

Pashley DH, Livingston MJ, and Whitford GM. 1979. The effect of molecular size on reflection coefficients in human dentine. *Arch Oral Biol* 24:455-460.

Pashley DH, Livingston MJ, Reeder WO, and Horner J. 1978. Effects of the degree of tubule occlusion on the permeability of human dentine *in vitro*. *Arch Oral Biol* 23:1127-1133.

Pashley DH, Michelich V, and Kehl MS. 1981. Dentin permeability: Effects of smear layer removal. *J Prosthet Dent* 46:531-537.

Pashley DH, Nelson R, and Kepler EE. 1982. The effects of plasma and salivary constituents on dentin permeability. *J Dent Res* 61:978-981.

Pashley DH, Nelson R, and Pashley EL. 1981. *In vivo* fluid movement across dentine in the dog. *Arch Oral Biol* 26:701-710.

Pashley DH, Nelson R, and Williams EC. 1981. Dentin hydraulic conductance: Changes produced by red blood cells. *J Dent Res* 60:1797-1802.

Pashley DH, O'Meara JA, Kepler EE, Galloway SE, Thompson SM, and Stewart FP. 1984. DEntin permeability: Effects of desensitizing dentrifrices *in vitro*. *J Periodontol* 55:522-525.

Pashley DH, Tao L, Boyd L, King GE, Horner JA. 1988. Scanning electron microscopy of the substructure of smear layers in human dentin. *Arch Oral Biol* 33:265-270.

Pashley DH, Thompson SM, and Stewart FP. 1983. Dentin permeability: Effects of temperature on hydraulic conductance. *J Dent Res* 62:956-959.

Pashley DH. 1979. The influence of dentin permeability and pulpal blood flow on pulpal solute concentrations. *J Endod* 5:355-361.

Pashley DH. 1984. Smear layer: Physiological considerations. *Operative Dentistry; Supplement* 3:13-29.

Pashley DH. 1984. The effect on dentine permeability of time following cavity preparation in dogs. Arch Oral Biol 29:65-68.

Pashley DH. 1985. Dentin-predentin complex and its permeability: Physiologic overview. *J Dent Res* 64; (Special Issue):613-620.

Pashley DH. 1986. Dentin permeability, dentin sensitivity, and treatment through tubule occlusion. *J Endod* 12:465-474.

Pashley DH. 1989. Dentin: A dynamic substrate - a review. *Scanning Microsc* 3:161-176.

Piez KA, and Likins RC. 1960. The nature of collagen. II. Vertebrate collagens, in calcification of biological systems. Sognnnaes RF Ed. American Association for Advancement of Science. Washington D.C., 411.

Polhagen L, and Brännström M. 1971. The liquid movement in desiccated and rehydrated dentin *in vitro*. *Acta Odont Scand* 29:95-102.

Prout RES, and Shutt ER. 1970. Separation of enamel and dentin using cadmium tungstoborate solution. *Arch Oral Biol* 15:559-561.

Reeder OW, Walton RE, Livingston MJ, and Pashley DH. 1978. Dentin permeability: Determinants of hydraulic conductance. *J Dent Res* 57:187-193.

Richardson DW, Tao L, and Pashley DH. 1990. Bond strengths of luting cements to potassium oxalate-treated dentin. *J Prosth Dent* 63:418-422.

Sandoval VA, Cooley RL, and Barnwell SE. 1989. Evaluation of potassium oxalate as cavity liner. *J Prosthet Dent* 62:283-287.

Sarnat H, and Massler M. 1965. Microstructure of active and arrested dentinal caries. *J Dent Res* 44:1389-1401.

Scherft JP. 1972. The lamina limitants of the organic matrix of calcified cartilage and bone. *J Ultra Struct Res* 28:318-331.

Scott DB. 1955. The electron microscopy of enamel and dentine. *Ann NY Acad Sci* 60:575-584.

Seiberg BH, and Schilder H. 1974. An evaluation of EDTA in endodontics. *Oral Surg* 37:609-620.

Seltzer S, and Bender IB. 1975. The dentinal pulp. 2nd Ed. Lippincott. Philadelphia.

Selvig KA. 1968. Effect of fenoride on the acid solubility of human dentin. *Arch Oral Biol* 14:1297-1310.

Shortall AC. 1981. Cavity cleansers in restorative dentistry. *Br Dent J* 150:243-247.

Sigal MJ, and Chernecky R. 1988. Terminal end of the odontoblast process. *J Endod* 14:543-545.

Sigal MJ, Aubin JA, and Ten Cate, AR. 1985. An immunocytochemical study of the human odontoblast process using antobodies against tubulin, actin, and vimentin. *J Dent Res* 64:1348-1355.

Söremark R, Söremark C, Martin-Löj S, and Myrberg N. 1973. Thermal

expansion of dental tissues. *Anat NY Acd Sci* 204:169-190.

Speter von Krendenstein T, and Stüben J. 1955. Dentinstoffwechselstudien. *Dtsch Zahnärtzi Ztschr* 12:500.

Stanley HR, Pereira JC, Spiegel E, Broom C, and Schultz M. 1983. The detection and prevalence of reactive and physiologic sclerotic dentin, reparative dentin and dead tracts beneath various types of dental lesions according to tooth surface and age. *J Path* 12:257-289.

Stanley HR. 1985. Toxicity testing of dental materials. *CRC Press*, Boca Raton, FL.

Stevenson TS. 1965. Fluid movement in human dentin. *Arch Oral Biol* 10:935-944.

Sturdevant JR, and Pashley DH. 1989. Regional dentin permeability of class I and class II preparations. *J Dent Res* 68:203, Abstr #173.

Symons NBB. 1961. A histochemical study of the intertubular and peritubular matrices in normal human dentine. *Arch Oral Biol* 5:241-250.

Takuma S, and Eda S. 1961. Structure and development of the peritubular matrix in dentin. *J Dent Res* 45:683-692.

Takuma S. 1960a. Preliminary report on the mineralization of dentin. *J Dent Res* 39:964-972.

Takuma S. 1960b. Electron microscopy of the structure around the dentinal tubule. *J Dent Res* 39:973-981.

Tanaka T. 1980. The origin and localization of dentinal fluid in developing rat molar teeth studied with lanthanum as a tracer. *Arch Oral Biol* 25:153-162.

Tao L, Pashley DH, and Boyd L. 1988. Effect of different types of smear layers on dentin and enamel shear bond strengths. *Dent Mater* 4:208-216.

Ten Cate AR. 1989. *Oral Histology: development, structure, and function.* AR Ten Cate Ed., 3rd Ed. Mosby, St Louis. MO.

Thomas HF, and Carella P. 1983. A scanning electron microscope study of

dentinal tubules from erupted human premolar teeth. *Arch Oral Biol* 28:1125-1130.

Thomas HF, and Carella P. 1984. Correlation of scanning and transmission electron microscopy of human dentinal tubules. *Arch Oral Biol* 29:641-646.

Thomas HF, and Payne RC. 1983. The ultrastructure of dentinal tubules from erupted human premolar teeth. *J Dent Res* 62:532-536.

Thomas HF. 1984. The lamina limitans of human dentinal tubules. *J Dent Res* 63:1064-1066.

Thomas HF. 1985. Dentin-predentin complex and its permeability: Anatomic overview. *J Dent Res* 64; Special Issue:607-612.

Tomes J. 1856. On the presence of fibrils of soft tissue in the dentinal tubes. *Philos Trans R Soc London* 146:515-522.

Tronstad L. 1973. Ultrastructural observations on human coronal dentin. *Scand J Dent Res* 81:101-111.

Vogel JJ, and Boyan-Salyers BD. 1976. Acidic lipids associated with the local mechanism of calcification. *Clin Orthopaed* 118:230-241.

Vojinovic O, Nyborg H, and Brännström M. 1973. Acid treatment of cavities under resin fillings: Bacterial growth in dentinal tubules and pulpal reactions. *J Dent Res* 52:1189-1193.

Weber DF, and Zaki AL. 1986. Scanning and transmission electron microscopy of tubular structures presumed to be human odontoblast processes. *J Dent Res* 65:982-986.

Weber K, and Osborn M. 1981. Microtubule and intermediate filament network in cells by immunofluorescence microscopy. In: *Cytoskeletal elements and plasma membrane organization.* G Poste and GL Nicolson Eds. North Holland Publ Co., Amsterdam, NY, pp 1-54.

Wetherell JA, and Robinson C. 1973. The inorganic composition of teeth, in *Biological Mineralization*, Zipkin. I Ed. John Wiley & Sons. New York, 43.

White, RK, Senia ES, Zislis T, Fox Lee T, and Zeagler JW. 1986. A study of the

odontoblast process with transmission electron microscopy. *Oral Surg* 62:569-579.

Whittaker DK, and Kneale MJ. 1979. The dentine-predentine interface in human teeth. *Brit Dent J* 146:43-46.

Young RA. 1975. Biological apatite vs hydroxyapatite at the atomic level. *Clin Orthopaed* 113:249-262.

APPENDIX

Table 1. Mean L_p Values (M) for Primary Teeth

Tooth/Surface	Depth† (%)	N	M	SD
Primary with SL	Deep (0-30%)	3	.004	.005
Primary with SL	Interm (30.1-60%)	15	.001	.002
Primary with SL	Outer (60.1-90%)	23	.001	.001
Primary with SL	Sprf (90.1-100%)	5	.002	.001
Primary without SL	Deep (0-30%)	3	.013	.007
Primary without SL	Interm (30.1-60%)	15	.006	.005
Primary without SL	Outer (60.1-90%)	23	.001	.001
Primary without SL	Sprf (90.1-100%)	5	.003	.001
Primary with oxalate	Deep (0-30%)	3	.001	2.33E-4
Primary with oxalate	Interm (30.1-60%)	3	.001	2.198E-4

† from the pulp

Interm = intermediate

Sprf = superficial

N = number of surfaces studied
M = mean L_p
SD = standard deviation
L_p measured in [$\mu L*cm^{-2}*min^{-1}*cm\ H_2O^{-1}$]

Table 2. Mean L_p Values (M) for Permanent Teeth

Tooth/Surface	Depth† (%) from the Pulp	N	M	SD
Permanent with SL	Deep (0-30%)	6	.001	.002
Permanent with SL	Interm (30.1-60%)	19	.001	.001
Permanent with SL	Outer (60.1-90%)	18	.003	.008
Permanent with SL	Sprf (90.1-100%)	4	3.3E-4	3.357E-4
Permanent without SL	Deep (0-30%)	6	.021	.016
Permanent without SL	Interm (30.1-60%)	19	.011	.017
Permanent without SL	Outer (60.1-90%)	18	.005	.010
Permanent without SL	Superficial (90.1-100%)	4	.001	.001
Permanent with oxalate	Deep (0-30%)	6	.001	.002
Permanent with oxalate	Interm (30.1-60%)	6	.001	.002

† from the pulp

Interm = intermediate

Sprf = superficial

N = number of surfaces studied
M = mean L_p
SD = standard deviation
L_p measured in [$\mu L*cm^{-2}*min^{-1}*cm\ H_2O^{-1}$]

Table 3. Hydraulic Conductance (L_p) of Primary Teeth with and without Smear Layer at 0-30% from the Pulp (Deep Dentin)

Specimen	Depth† (%)	L_p with SL	L_p without SL
D	29.8	0.0098	0.00504
F	29.4	0.00136	0.01433
G	28.9	0.00108	0.01895
	N	3	3
	M	0.004	0.013
	SD	0.005	0.007

† from the pulp

N = number of surfaces studied
M = mean L_p
SD = standard deviation
L_p measured in [$\mu L*cm^{-2}*min^{-1}*cm\ H_2O^{-1}$]

Table 4. Hydraulic Conductance (L_p) of Primary Teeth with and without Smear Layer at 30.1-60.0% from the Pulp (Intermediate Dentin)

Specimen	Depth† (%)	L_p with SL	L_p without SL
A	52.1	.0016	.00726
B	59.3	.00126	.00322
C	40.4	.00103	.00103
C	55.7	.00054	.00255
D	42.3	.00062	.0027
E	48.4	.0013	.00288
E	47	.00074	.0018
F	33.3	.00068	.00933
F	58.8	.00095	.0032
G	47.1	.00063	.00958
G	56.6	.0122	.01816
H	45.5	.0122	.01604
H	54.1	.00102	.00102
I	49.2	.00030	.00902
J	39.8	.00916	.00168

Specimen	Depth† (%)	L_p with SL	L_p without SL
	N	15	15
	M	.001	.006
	SD	.002	.005

† from the pulp

N = number of surfaces studied
M = mean L_p
SD = standard deviation
L_p measured in [$\mu L*cm^{-2}*min^{-1}*cm\ H_2O^{-1}$]

Table 5. Hydraulic Conductance (L_p) of Primary Teeth with and without Smear Layer at 60.1-90% from the Pulp (Outer Dentin)

Specimen	Depth† (%)	L_p with SL	L_p without SL
A	84.9	.00126	.00188
A	75.1	.00204	.00189
A	63	.00188	.00232
B	87.4	.00144	.00286
B	74.3	.00292	.00475
C	84.5	.0003	.0003
C	71.1	.00077	.00154
D	85.6	.00082	.00178
D	66.5	.00102	.00317
E	87.1	.0013	.00146
E	74.3	.00091	.00077
E	62.7	.0007	.0009
F	67.1	.00052	.00162

Table 5. (contin.)

Specimen	Depth† (%)	L_p with SL	L_p without SL
G	84.3	.00098	.00265
G	71.9	.0008	.00093
H	78	.00154	.00454
H	66.3	.00139	.0026
I	81.7	.00056	.0031
I	73.3	.0003	.00387
I	60.7	.00067	.00488
J	83.2	.00086	.00091
J	72.8	.00085	.001
J	62.2	.0014	.00192
	N	23	23
	M	.001	.002
	SD	.001	.001

† from the pulp

N = number of surfaces studied
M = mean L_p
SD = standard deviation
L_p measured in [$\mu L*cm^{-2}*min^{-1}*cm\ H_2O^{-1}$]

Table 6. Hydraulic Conductance (L_p) of Primary Teeth with and without Smear Layer at 90.1-100% from the Pulp (Superficial Dentin)

Specimen	Depth† (%)	L_p with SL	L_p without SL
A	94.3	.0016	.0017
B	94.9	.00161	.00403
H	91.1	.00333	.0326
I	96.6	.00078	.00194
J	92.2	.00179	.00185
	N	5	5
	M	.002	.003
	SD	.001	.001

† from the pulp

N = number of surfaces studied
M = mean L_p
SD = standard deviation
L_p measured in [$\mu L*cm^{-2}*min^{-1}*cm\ H_2O^{-1}$]

Table 7. Hydraulic Conductance (L_p) of Permanent Teeth with and without Smear Layer at 0-30% from the Pulp (Deep Dentin)

Specimen	Depth† (%)	L_p with SL	L_p without SL
1	26.6	0.0008	0.03297
2	23	0.00424	0.02721
4	17.9	0.00049	0.01091
7	27.8	0.00029	0.00812
8	24.9	0.00084	0.04228
9	27.9	0.00045	0.00293
	N	6	6
	M	0.001	0.021
	SD	0.002	0.016

† from the pulp

N = number of surfaces studied
M = mean L_p
SD = standard deviation
L_p measured in [$\mu L*cm^{-2}*min^{-1}*cm\ H_2O^{-1}$]

Table 8. Hydraulic Conductance (L_p) of Permanent Teeth with and without Smear Layer at 30.1-60.0% from the Pulp (Intermediate Dentin)

Specimen	Depth† (%)	L_p with SL	L_p without SL
1	56.3	.00088	.00465
1	43.2	.00071	.01857
2	34.4	.00044	.01297
2	52.7	.00086	.00816
4	47.6	.00158	.00079
4	33.2	.00056	.00789
3	42.9	.00399	.00966
3	33.9	.00049	.0024
5	52.5	.00326	.01303
5	31.8	.00102	.07935

Specimen	Depth† (%)	L_p with SL	L_p without SL
6	42.3	.00014	.00222
6	34.8	.00044	.01234
6	54.2	.00058	.00213
7	58.5	.00059	.0033
7	49.4	.00099	.00327
8	47.8	.00086	.00712
9	51.5	.00049	.0048
10	57.6	.00079	.00266
10	38.8	.00059	.01699
	N	19	19
	M	.001	.011
	SD	.001	.017

† from the pulp

N = number of surfaces studied
M = mean L_p
SD = standard deviation
L_p measured in [$\mu L*cm^{-2}*min^{-1}*cm\ H_2O^{-1}$]

Table 9. Hydraulic Conductance (L_p) of Permanent Teeth with and without Smear Layer at 60.1-90% from the Pulp (Outer Dentin)

Specimen	Depth† (%)	L_p with SL	L_p without SL
1	88.4	.00077	.00119
2	67.6	.00045	.00177
3	83.8	.00137	.00084
3	62.9	.00059	.00247
3	88.5	.00081	.00067
4	76.7	.00052	.00169
4	62.1	.03288	.04197
5	84.9	.00163	.00338
5	65.7	.00285	.01705
6	68.2	.00034	.00034
7	89.2	.0006	.0008
7	74.7	.00077	.00098

Table 9. (contin.)

Specimen	Depth† (%)	L_p with SL	L_p without SL
8	82.6	.00101	.0197
8	68.2	.00127	.00158
9	79.8	.00029	.00043
9	63.9	.00036	.00073
10	79.5	.00035	.00141
10	69.2	.00064	.000206
	N	18	18
	M	.003	.005
	SD	.008	.010

† from the pulp

N = number of surfaces studied
M = mean L_p
SD = standard deviation
L_p measured in [$\mu L*cm^{-2}*min^{-1}*cm\ H_2O^{-1}$]

Table 10. Hydraulic Conductance (L_p) of Permanent Teeth with and without Smear Layer at 90.1-100% from the Pulp (Superficial Dentin)

Specimen	Depth (%) †	L_p with SL	L_p without SL
2	91.1	.0006	.00172
6	91.1	.00006	.00008
9	94	.00002	.00006
10	92.4	.00064	.00082
	N	4	4
	M	3.300E-4	0.001
	SD	3.357E-4	0.001

† from the pulp

N = number of surfaces studied
M = mean L_p
SD = standard deviation
L_p measured in [$\mu L*cm^{-2}*min^{-1}*cm\ H_2O^{-1}$]

Table 11. Two-way Analysis of Variance Test for Independent Factors Tooth/Surface and Depth on the Hydraulic Conductance (L_p) at $\alpha=0.05$

Source	df	Sum of ...	Mean Square	F-Value	P-Value
Tooth\Surface	3	0.001	3.974E-4	6.719	0.0003
Depth	3	0.001	2.840E-4	4.801	0.0031
Tooth\Surface * Depth	9	0.001	1.185E-4	2.003	0.0417
Residual	170	0.010	5.915E-5		

Dependent: L_p

Table 12. The Effect of Depth on the Hydraulic Conductance (L_p) of Dentin for Primary and Permanent Teeth

Depth† (%)	N	M	SD
Deep (0-30%)	18	0.010	0.013
Intermediate (30.1-60%)	68	0.005	0.010
Outer (60.1-90%)	82	0.003	0.006
Superficial (90.1-100%)	18	0.001	0.001

† from the pulp

N = number of surfaces studied
M = mean density
SD = standard deviation

Table 13. Scheffé Multiple Comparison Test: The Effect of Depth on Hydraulic Conductance (L_p)

	Vs.	Diff.	Crit. Diff.	P-Value	
Sprf. (90.1-100%)	Outer	0.001	0.006	0.9626	
	Interm.	0.004	0.006	0.3740	
	Deep	0.09	0.07	0.0111	S
Outer (60.1-90%)	Interm.	0.003	0.04	0.2595	
	Deep	0.08	0.06	0.0030	S
Interm. (30.1-60%)	Deep	0.005	0.006	0.1075	

S = Significantly different at this level.

Table 14. Scheffé Multiple Comparison Test: The Effect of type Tooth and Dentin Surface on Hydraulic Conductance (L_p)

	Vs.	Diff.	Crit. Diff.	P-Value	
Primary with SL	Permanent with SL	1.030E-4	0.005	0.9999	
	Primary without SL	0.003	0.005	0.4266	
	Permanent without SL	0.007	0.005	0.0001	S
Permanent with SL	Primary without SL	0.003	0.005	0.4574	
	Permanent without SL	0.007	0.004	0.0001	S
Primary without SL	Permanent without SL	0.005	0.005	0.0328	S

S = Significantly different at this level.

Table 15. The Effect of Potassium Oxalate on the Hydraulic Conductance (L_p) of the Deep Dentin (0-30%) for Primary Teeth

Specimen	Depth† (%)	L_p with SL	L_p without SL	L_p with oxalate	Per cent L_p reduction ‡
G	28.9	.00108	.00812	.00127	84.3
F	29.4	.00136	.01234	.00082	93.4
D	29.8	.00098	.01091	.00115	89.5
	N	3	3	3	3
	M	.001	.010	.001	89.1
	SD	1.970E-4	.002	2.33E-4	4.565

† from the pulp

‡ compared to L_p without SL

N = number of surfaces studied
M = mean L_p
SD = standard deviation
L_p measured in [$\mu L*cm^{-2}*min^{-1}*cm\ H_2O^{-1}$]

Table 16. The Effect of Potassium Oxalate on the Hydraulic Conductance (L_p) of the Intermediate Dentin (30.1-60%) for Primary Teeth

Specimen	Depth† (%)	L_p with SL	L_p without SL	L_p with oxalate	Per cent L_p reduction ‡
E	33.3	.00068	.00933	.00086	90.8
H	39.8	.00102	.00102	.00150	‡
B	40.4	.00103	.00103	.00098	‡
C	42.3	.00062	.00270	.00166	38.5
I	49.2	.00030	.00902	.00154	82.9
J	51.6	.00092	.00169	.00076	55.0
A	52.1	.00160	.00726	.00134	81.5
	N	7	7	7	5
	M	.001	.005	.003	69.7
	SD	4.095E-4	.004	.005	22.069

† from the pulp

‡ compared to L_p without SL

‡ Data excluded due to obvious experimental error

N = number of surfaces studied
M = mean L_p
SD = standard deviation
L_p measured in [$\mu L*cm^{-2}*min^{-1}*cm\ H_2O^{-1}$]

Table 17. The Effect of Potassium Oxalate on the Hydraulic Conductance (L_p) of the Deep Dentin (0-30%) for Permanent Teeth

Specimen	Depth† (%)	L_p with SL	L_p without SL	L_p with oxalate	Per cent L_p reduction ‡
4	17.9	.00049	.01091	.00490	55.1
2	23	.00424	.02721	.00237	91.3
8	24.9	.00084	.04228	.00036	99.1
1	26.6	.00080	.03297	.00027	99.1
7	27.8	.00029	.00812	.00037	95.4
9	27.9	.00045	.00293	.00031	89.4
	N	6	6	6	6
	M	.001	.021	.001	88.2
	SD	.002	.016	.002	16.710

† from the pulp

‡ compared to L_p without SL

N = number of surfaces studied
M = mean L_p
SD = standard deviation
L_p measured in [$\mu L*cm^{-2}*min^{-1}*cm\ H_2O^{-1}$]

Table 18. The Effect of Potassium Oxalate on the Hydraulic Conductance (L_p) of Intermediate Dentin (30.1-60%) for Permanent Teeth

Specimen	Depth† (%)	L_p with SL	L_p without SL	L_p with oxalate	Per cent L_p reduction ‡
5	31.8	.00102	.07935	.00091	98.9
3	33.9	.00049	.00240	.00244	‡
6	34.8	.00044	.01234	.00041	96.7
10	38.8	.00059	.01699	.00014	99.2
	N	4	4	4	3
	M	.001	.028	.001	98.2
	SD	2.641E-4	.035	.001	1.359

† from the pulp

‡ compared to L_p without SL

‡ Data excluded due to obvious experimental error

N = number of surfaces studied
M = mean L_p
SD = standard deviation
L_p measured in [$\mu L*cm^{-2}*min^{-1}*cm\ H_2O^{-1}$]

Table 19. One-way Analysis of Variance Test for the Hydraulic Conductance (L_p) with, without Smear Layer, and with Oxalate, of Deep Dentin (0-30%) in Primary Teeth

Source	df	Sum of Squares	Mean Square	F-test	P value
Between subjects	2	2.800E-6	1.400E-6	0.046	0.9551
Within subjects	6	1.813E-4	3.022E-5		
Treatments	2	1.747E-4	8.736E-5	52.956	0.0013
Residual	4	6.599E-6	1.650E-6		
Total	8	1.814E-4			

Reliability Estimates for All treatments: -20.59 Single Treatment: -0.466

Table 20. Scheffé Multiple Comparison Test: The Effect of Potassium Oxalate, related to the presence or absence of Smear Layer, on the Hydraulic Conductance (L_p) of Primary Teeth

Comparison	Mean Diff.	Fisher PLSD	Scheffé F-test	Dunnett test
With SL vs Without	-0.009	0.003*	39.461*	8.884
With SL vs with Oxalate	6.000E-5	0.003	0.002	0.057
Without SL vs With Oxalate	0.009	0.003*	39.971*	8.941

* Significant at 95%

Table 21. One-way Analysis of Variance Test for the Hydraulic Conductance (L_p) with, without smear layer, and with Oxalate, of Deep Dentin (0-30%) in Permanent Teeth

Source	df	Sum of Squares	Mean Square	F-test	P value
Between subjects	5	4.259E-4	8.517E-5	0.436	0.8149
Within subjects	12	0.002	1.952E-4		
Treatments	2	0.002	0.001	9.077	0.0057
Residual	10	0.001	8.318E-5		
Total	17	0.003			

Reliability Estimates for All treatments: -1.291 Single Treatment: -0.231

Table 22. Scheffé Multiple Comparison Test: The Effect of Potassium Oxalate, related to the presence or absence of Smear Layer, on the Hydraulic Conductance (L_p) of Permanent Teeth

Comparison	Mean Diff.	Fisher PLSD	Scheffé F-test	Dunnett test
With SL vs Without	-0.02	0.012*	6.893*	3.713
With SL vs with Oxalate	-2.450E-4	0.012	0.001	0.047
Without SL vs With Oxalate	0.019	0.012*	6.721*	3.666

* Significant at 95%

Table 23. One-way Analysis of Variance Test for the Hydraulic Conductance (L_p) with, without Smear Layer, and with Oxalate, of Intermediate Dentin (30.1-60%) in Primary teeth

Source	df	Sum of Squares	Mean Square	F-test	P value
Between subjects	6	5.186E-5	8.644E-6	0.486	0.808
Within subjects	14	2.489E-4	1.778E-5		
Treatments	2	4.872E-5	2.436E-5	1.46	0.2706
Residual	12	2.002E-4	1.668E-5		
Total	20	3.008E-4			

Reliability Estimates for All treatments: -1.057 Single Treatment: -0.207

Table 24. One-way Analysis of Variance Test for the Hydraulic Conductance (L_p) with, without Smear Layer, and without Oxalate, of Intermediate Dentin (30.1-60%) in Permanent Teeth

Source	df	Sum of Squares	Mean Square	F-test	P value
Between subjects	3	0.001	4.078E-4	0.745	0.5547
Within subjects	8	0.004	0.001		
Treatments	2	0.002	0.001	2.386	0.1728
Residual	6	0.002	4.064E-4		
Total	11	0.006			

Reliability Estimates for All treatments: -0.342 Single Treatment: -0.093

Note: 2 cases deleted with missing values

Table 25. Density and Diameter of Dentinal Tubules at Various Depths from the Pulp of Primary Teeth

Specimen	Depth† (%)	Depth† (mm)	Density‡	Diameter*
1	12.2	0.25	18815	1.298
5	14.8	0.35	36680	1.37
4	18.4	0.45	27348	1.13
2	23.2	0.40	23096	1.387
3	23.8	0.45	26042	1.243
1	42.4	0.87	16794	1.198
5	43.9	1.04	22782	1.14
4	56.5	1.38	20764	1.004
2	59.0	1.03	21391	1.044
1	65.8	1.35	15266	1.138
3	70.9	1.34	21132	1.173
4	72.1	1.76	17601	0.894
5	83.1	1.97	18303	1.097
2	91.3	1.57	17335	0.986
3	91.5	1.83	17530	0.94

† from the pulp

‡ tubules/mm^2

* [μm]

Table 26. Tubule density of Primary Teeth at various Depths from the pulp

Depth† (%)	Density (tubules/mm^2)				
	N	M	SD	R	
Deep (0-30%)	5	26390	6605	18815	36650
Intermediate (30.1-60%)	4	20433	2568	16794	22782
Outer (60.1-90%)	4	18075	2415	15266	21132
Superficial (90.1-100%)	2	17433	137	17335	17530

† from the pulp

N = number of surfaces studied
M = mean density
SD = standard deviation
R = range

Table 27. Tubule Diameter of Primary Teeth at various Depths from the Pulp

Depth† (%)	Diameter (μm)				
	N	M	SD	R	
Deep (0-30%)	155	1.286	0.104	1.13	1.387
Intermediate (30.1-60%)	134	1.096	0.089	1.004	1.198
Outer (60.1-90%)	88	1.075	0.125	0.894	1.173
Superficial (90.1-100%)	36	0.963	0.033	0.94	0.986

† from the pulp

N = number of tubules studied
M = mean density
SD = standard deviation
R = range

VITA

The author, Vasiliki Koutsi, daughter of Nikolaos and Vasiliki Koutsi, was born in Korinth, Greece, on April 11, 1964. Her elementary education was obtained in the public schools of Korinth. After graduation from the Second Lyceum, in September 1981 she entered the School of Dentistry of the National Kapodestrian University of Athens, Athens, Greece, and received her degree of Doctor of Dental Surgery in March 1987.

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APPROVAL SHEET

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

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

April 17, 1991
Date

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