The effect of dentin depth on the permeability and ultrastructure of primary molars

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Abstract

The purpose of this study was to measure the permeability of primary molar and permanent premolar dentin at various thicknesses from the pulp and to correlate permeability with the tubule density and diameter of dentin using SEM. The data were examined for statistically significant differences using two-way analysis of variance, multiple comparison Scheffe, and regression analysis. The permeability of all teeth increased with decreasing dentin thickness. The removal of the smear layer resulted in a significant increase in the permeability. However, the permeability of the dentin in premolars was significantly higher than that in the primary molars. The density and diameter of the dentinal tubular in primary molars were lower than the values reported for permanent teeth and may account for the lower permeability of the primary molars. (Pediatr Dent 16:29–35, 1994)

Introduction

The intrinsic permeability of dentin is responsible for permitting bacterial or chemical substances to diffuse across dentin and irritate pulpal and periradicular tissues. If dentin was not permeable then the pulp would be spared a good deal of irritation. Normal dentin is more permeable than carious dentin[1] or sclerotic dentin.[2] The permeability of dentin tends to decrease with age due to physiologic sclerosis of dentin.[2, 3] Thus, the permeability of dentin is an important biologic variable that can be measured and used to compare the barrier properties of dentin within teeth, between teeth, or between species.[4]

Most of the previous measurements of dentin permeability have been carried out on the coronal[1-4] or radicular[5] dentin of human permanent teeth. However, no studies have been performed on primary teeth. Permeability is defined as the ability of a membrane to permit solutes or solvents to pass through it.[6] The presence of numerous fluid-filled cylindrical dentinal tubules makes dentin a very poor barrier. Rather, dentin can be regarded as a very porous membrane that can be studied by quantitating the ease with which fluid can pass through it under defined conditions. The most important variables that influence filtration across dentin are the pressure difference across the dentin (whether osmotic or hydrostatic), the length of the tubule (shorter tubules have less resistance than longer tubules), and the radius of the tubule raised to the fourth power.[6] (Although diffusion varies with the tubule radius raised to the second power, fluid filtration involves different mechanisms, one of which is the frictional resistance developed by concentric cylinders of fluid slipping past one another, which cause convective transport to vary with the fourth power of the

radius.) Since filtration varies with the fourth power of the tubular radius, small changes in tubule radius or diameter have a large effect on fluid flow. The number and diameter of dentinal tubules have an obvious effect on permeability. If the tubules of primary dentin are smaller and/or less numerous than in permanent dentin, then the permeability of primary dentin will be lower than that of permanent dentin. Dentinal tubules are less dense and narrower (1 μm) at the dentoenamel junction (DEJ), and become more dense and wider (3–4 μm) near the pulp.[7] This anatomical organization indicates that superficial dentin is less permeable than deep dentin, a notion that has been experimentally confirmed.[5] The rate at which fluid filters through dentin is also sensitive to the length of the tubules. As dentin thickness is decreased, filtration increases exponentially for both coronal[6] and radicular[5] permanent dentin.

A critical variable affecting permeability is the nature of the dentin surface—that is, whether or not the surface is coated with a smear layer. The smear layer is a microscopic layer of cutting debris that is produced whenever dentin is cut by hand or rotary instruments. Removal of the smear layer increases the hydraulic conductance of dentin by 30- to 40-fold.[4, 6, 8] Clinically, removing the smear layer results in increased sensitivity to osmotic, thermal, and tactile stimuli.[9] Smear layer loss can occur gradually through dissolution caused by microleakage around restorations or suddenly following the acid conditioning step common to most marketed dentin bonding systems. Since the permeability of primary dentin has never been reported, the purposes of this study were: to investigate the effect of dentin depth and the presence and absence of the smear layer on the permeability of primary molars;

to compare the permeability of primary molars with that of premolars; and to correlate permeability with density and diameter of primary molars.

Methods and materials

Tooth preparation

Fifteen extracted, human, posterior, noncarious primary teeth (from children aged 9 to 11 years) and 10 premolars (from children aged 12 to 15 years) were placed immediately after extraction into 4°C phosphate buffered saline (Dulbecco's Phosphate Buffered Saline, Gibco Laboratories; Grand Island, NY) containing 0.2% sodium azide to inhibit microbial growth. All teeth were removed for orthodontic reasons with informed consent from parents. The teeth were used within three months of extraction. The roots of the teeth were removed approximately 1 mm apical to the cementoenamel junction using a low-speed diamond saw (Isomet, Buehler Ltd., Lake Bluff, IL). The pulp tissue was removed with cotton forceps, avoiding contact with the walls of the coronal pulp chamber.

The crowns were attached to 2x2x0.7-cm pieces of Plexiglas® (DuPont, Wilmington, DE) containing 15-mm lengths of 18-gauge stainless steel tubing through their centers, using fast-setting cyanoacrylate adhesive (Zapit, DVA, Yorba Linda, CA). This served to connect the tooth pulp chamber with the apparatus used to measure dentin permeability at 10 psi[5] and it ensured that the pulp chamber and dentin were always full of phosphate-buffered saline (Fig. 1).

Dentin reductions

A groove was prepared with a diamond bur in the lingual surface enamel 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). All reductions were done on the buccal surface, thereby avoiding any influence that occlusal abrasion might have had on the production of sclerotic or reparative occlusal dentin. All buccal surfaces were normal and intact. The enamel on the buccal surface of the teeth was removed using a diamond bur in a high-speed handpiece under copious air/water spray. When all enamel was removed and the first dentin could be seen, the tooth thickness was measured and recorded again, which defined the DEJ.

The buccal dentin was ground further until it was visually estimated that the dentin surface area was sufficient for permeability measurements (ca. 2–3 mm diameter). The buccolingual thickness was remeasured and the crowns were connected to the apparatus to measure the hydraulic conductance of the dentin covered with smear layer.[1] 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 min to remove the smear layer. The dentin surface was rinsed with distilled water for 5 sec and four additional measurements were taken. Then, the DEJ 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. 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
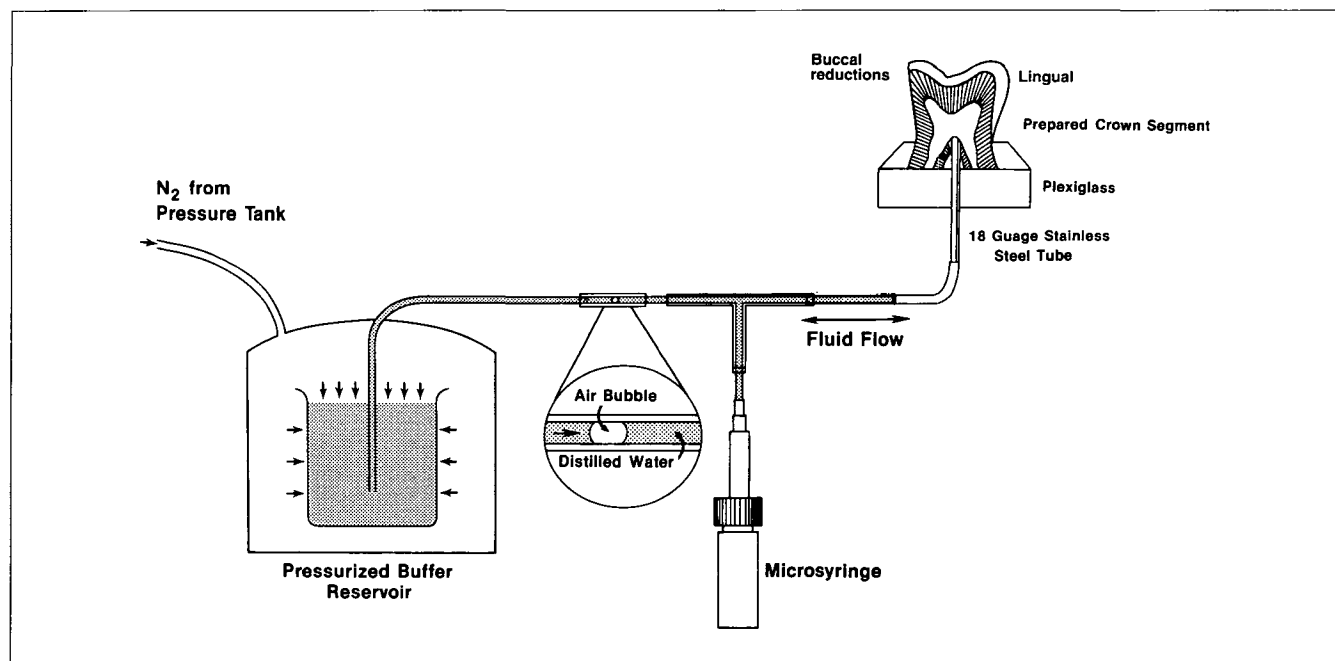

Fig 1. Schematic of the apparatus used to measure dentin permeability via fluid flow from a pressurized reservoir through a micropipette to the crown. The movement of an air bubble (insert) in the micropipette was proportional to the permeability.

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. The teeth were removed from the Plexiglas and sectioned buccolingually into two pieces with a diamond disc. The minimum remaining dentin thickness between the pulp chamber and the cut surface 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 as a per cent of the total dentin thickness. Photographic enlargements (5x7-in. prints) were made, and the DEJ that had been marked with the pencil was traced with a movable cursor on a digitizing tablet. The dentin surface area then was calculated in cm^2, utilizing a computer program (Sigma Scan, Jandel Scientific, San Rafael, CA). The fluid flow (μL) per min was then expressed per cm^2. The hydraulic conductance (Lp) of each specimen at each dentin thickness was calculated in $\mu L\ cm^{-2}\ min^{-1}\ cmH_2O^{-1}$. Due to variations in permeability between different teeth, results were expressed as per cent changes of the maximum hydraulic conductances of the EDTA treated surfaces for each tooth. Thus, each tooth served as its own control.

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. Four reductions of buccal dentin were made for each tooth. After each reduction, the diamond bur-created smear layer was removed using 320-grit aluminum oxide sandpaper to create a new smear layer. The exposed dentin surface was placed on the sandpaper and it was moved 10 cm along the wet sandpaper 10 times with finger pressure. This procedure was utilized to create a smear layer that was more easily removed by sonication.[10]

Each crown segment was placed in the cuphorn attachment of a powerful sonicator (Sonifer, Model 450 watts, Branson Ultrasonics Corporation; Danbury, CT). During sonication, the cup was kept filled with ice water with the tooth submerged. Each crown was sonicated for approximately 15 min at 70% power to remove the smear layer and smear plugs to permit SEM measurement of tubule density and diameter.

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 480 to 6000x. This procedure was repeated at four depths for each of the five teeth. Three 480x and six 6000x micrographs were taken for each of the 20 surfaces examined. The cervical third of the tooth was the area most frequently examined. Visu-

ally, this region was found to be the most permeable, confirming the results of Maroli et al.[11] SEM examination of the most permeable area permitted 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. 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 6000x. A computer program was utilized to measure the diameter in μm. 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 ellipse that best fit the traced tubule orifice. This measurement of the smallest diameter across the tubule orifice minimized the error caused by tubules fractured obliquely. The diameters 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.

Scanning electron microscopy was not done on the premolars because this information was already available in the literature.[7, 12-14]

Statistical methodology

Dentin depths were stratified into four groups: 1) 0–30% of the distance from the pulp, designated as deep dentin; 2) 30.1–60% from the pulp, designated as intermediate dentin; 3) 60.1–90% from the pulp, designated as outer dentin; and 4) 90–100% from the pulp, designated as 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 (primary or permanent) and dentin surface (with smear layer, without smear layer) and dentin depth on hydraulic conductance (Lp). A one-way ANOVA (repeated measurement) also was used to test the difference of the Lp values between primary and permanent teeth with smear layer and without smear layer. Whenever there was a significant difference, the pairwise multiple comparison Scheffe test was used for each of the factors, with level of significance at $P = 0.05$.

Regression analysis was used to evaluate graphically the effect of dentin depth on Lp for primary and permanent teeth with and without smear layer.

Results

Effects of dentin depth and smear layer

The effects of dentin depth are shown in Fig 2. Because the thickness of the dentin in primary teeth was

less than that of permanent teeth, the thickness data were expressed as a percentage of the total dentin thickness to permit meaningful comparisons. In both types of teeth, the hydraulic conductance (Lp) of the dentin was very low (i.e., 0.001–0.003 µL cm^{-2}min^{-1}cmH$_2$0^{-1}) when measured within 10% of the DEJ (90.1–100% of the full dentin thickness). The permeability of primary dentin with or without a smear layer was slightly higher ($P < 0.05$) than that of the premolars. Although the permeability of the teeth was higher in the absence of the smear layer than in its presence, the difference was not statistically significant in superficial dentin (Fig 2). At the next dentin thickness (60–90%), there were no statistically significant differences between the permeabilities of the primary and permanent dentin in the presence of the smear layer, although the permeability of permanent dentin was higher after removal of the smear layer. In deeper dentin (30–60% of the total thickness), the differences between the two types of dentin became greater. In the presence of the smear layer, there was no difference between the two types of dentin (Fig 2). After removing the smear layer, the permeability of both types of dentin increased significantly ($P < 0.01$), but the permanent dentin revealed a higher permeability ($P < 0.05$) than did the primary dentin. In the deepest dentin (0–30% of the total thickness), when the smear layer was present, primary dentin exhibited a higher permeability than permanent dentin, although the difference was

Table 1. Tubule density and diameter as a function of dentin depth in primary molars

Dentin Depth	Tubule Density*		Tubule Diameter†	
Superficial	17,433 ± 1370	(17,335-18,530)	0.96 ± 0.03	(0.94–0.99)
Outer	18,075 2,415	(15,266-21,132)	1.08 0.12	(0.89–1.17)
Intermediate	20,433 2,568	(16,794-22,782)	1.10 0.09	(1.00–1.20)
Deep	26,391 ± 6,605	(18,816-36,650)	1.29 ± 0.10	(1.13–1.39)

* Values are tubules/mm², $\overline{x}$± S.D. (range) derived from 5 teeth. † Values are in µm, $\overline{x}$± S.D. (range) derived from 5 teeth. Groups connected by vertical lines are not significantly different. Groups not connected by vertical lines are different at $P < 0.05$.

not statistically significant. After removing the smear layer, the permeability of both types of dentin increased significantly ($P < 0.01$). Although the permeability of the permanent dentin was higher than that of primary deep dentin, the difference was not statistically significant.

Regression analysis of the two types of dentin indicated that there were no significant correlations between dentin permeability and per cent relative dentin thickness in the presence of a smear layer (not shown). In the absence of the smear layer, the two variables were significantly but not strongly correlated (Fig 2) in primary dentin ($r = -0.56$, $P < 0.001$) and in the dentin of permanent teeth ($r = -0.49$, $P < 0.001$).

Discussion

Our results concerning the relationship between dentin permeability and dentin depth for both primary and succedaneous permanent teeth confirm the results of previous work on dentin of permanent teeth[2, 5, 6] 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 (Table 1). It is also due to a reduction in the frictional resistance of the tubule walls to flow as dentin 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.[6] Small changes in tubule radius have more profound effects on fluid shifts across dentin than large changes in thickness.[6] This was demonstrated by the poor correlation between reductions in dentin thickness and increases in dentin permeability in the presence (but not the absence) of the smear layer (Fig 2).

Garberoglio and Brännström[7] studied fractured surfaces of human coronal dentin via SEM. At a distance of 0.4–0.5 mm from the pulpal surface they found approximately 40,000–41,000 tubules/mm² with diameters of 1.6–1.7 µm, in contrast to our observations in primary teeth of 26,390 tubules/mm² with a diameter of only 1.3 µm. Fosse et al.[13] measured the tubule density of premolars from patients aged 10–14 years, 0.3 mm from the pulpal wall. Their average value was
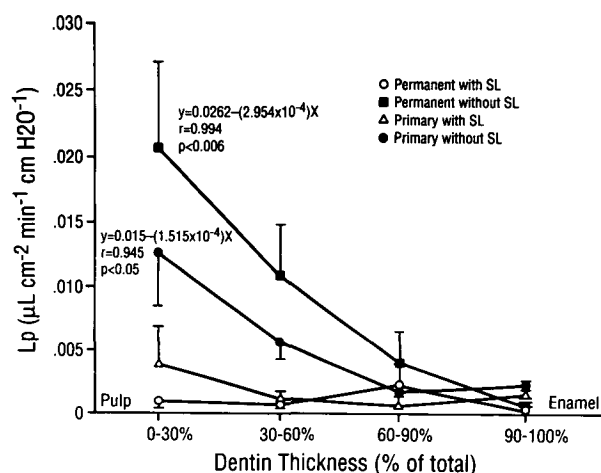

Fig 2. The effect of dentin depth and presence or absence of smear layer on the hydraulic conductance (Lp) of primary and permanent dentin.

51,368 tubules/mm². Whittaker and Kneale[12] obtained a tubule density of 43,000 tubules/mm² at the pulpal surface. Thus, the tubule densities that we report for primary molars were much lower than those reported for premolars. The greater density and diameter of the permanent teeth could explain the greater permeability of permanent dentin compared with that of the dentin of the primary teeth. Carrigan et al.[14] reported that the number of dentinal tubules decreased coronoapically. This also may have contributed to the difference between the study by Garberoglio and Brännström[7] on occlusal dentin and our study on cervical coronal dentin. Hirayama et al.[15] reported that the tubules of primary dentin had smaller diameters because the peritubular dentin matrix was wider than that of permanent dentin.

The presence of a smear layer on the dentin surface and smear plugs in the dentinal tubules prevents invasion of bacteria through the dentinal tubules.[16, 17] However, it does not prevent bacterial toxins from diffusing into the pulp. When intact healthy teeth are cut, the smear layer produced is bacteria free. On the other hand, under many clinical conditions, especially when operating on carious teeth, there is a great risk of bacteria becoming incorporated into the smear layer.[17] If restorations leak, the bacteria may survive and multiply. They also may contribute to the solubilization of the smear layer, which might leave a gap under restorative materials. Several investigations have been carried out to find a suitable cleanser that would retain the smear plugs and remove only the contaminated superficial smear layer.[17] Mechanical retention or bonding of cavity liners, luting cements, and restorations would be stronger after removing the smear layer because the bond strengths would not suffer from the smear layer's intrinsic weakness.[18]

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.[19] However, many recently marketed dentin bonding systems include acidic solutions designed to remove smear layers. Thus, primary dentin should be studied under both conditions.

In the present study, when the hydraulic conductance of primary teeth was compared with that of per-manent 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 removing 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 same anatomical factors determine the theoretical area for diffusional permeation of substances across dentin.

The theoretical hydraulic conductance of dentin can be calculated from measured tubule densities and radii (Table 2). First, the fluid flow that would be obtained at the applied pressure (10 psi or 6.89×10^5 dynes cm⁻²) per tubule was calculated as:

$$(1) \quad J_v = \frac{\pi \Delta P r^4}{8 h l}$$

where J_v = fluid flow across dentin in cm³ sec⁻¹ tubule⁻¹

π = 3.1416

ΔP = 10 psi or 6.89×10^5 dynes cm⁻²

r = tubule radius in cm at a known distance from pulp

h = dynamic viscosity of PBS, 1×10^{-2} dynes sec cm⁻²

l = length of tubule from pulp in cm

This value was then multiplied by the number of tubules cm⁻², converted to μL min⁻¹ and then divided by the applied pressure (10 psi or 701 cm H_2O) to obtain the fluid flow per unit area per cm H_2O per min, which is the hydraulic conductance of dentin. This was done for

Table 2. Comparison of calculated* and measured hydraulic conductance (Lp) of dentin

	Tubule			Lp		
	Length	Density	Radius†	Calc.*	Measured	%
A. Primary dentin						
Superficial	0.170 cm	1.743	0.48	0.126	0.003	2.4
Outer	0.113	1.807	0.54	0.315	0.002	0.6
Intermediate	0.041	2.043	0.55	1.055	0.006	0.6
Deep	0.025 cm	2.639	0.64	4.102	0.013	0.3
B. Permanent dentin						
Superficial	0.170	3.100‡	0.67	0.851	—	—
Outer	0.113	3.550	0.70	1.746	—	—
Intermediate	0.041	4.100	0.05	12.088	—	—
Deep	0.025	4.200	1.05	47.291	—	—

* Calculations based on equation (1). Lp units are μL cm⁻² min⁻¹ cm H_2O^{-1}. See text for details.
† Tubule radius x 10⁻⁴ = tubule radius in cm. ‡ Tubule density x 10⁶ = tubules/cm² from Garberoglio and Brännström 1976.

all of the data in Table 1 for primary dentin and for the same dentin thicknesses in premolars, using the data of Garberoglio and Brännström.[7] These calculated data are listed in Table 2 under "calculated Lp." The right column of Table 2 lists the measured Lp as a per cent of the calculated Lp.

It is clear that the measured Lp values generally are less than 1% of the theoretical values (Table 2). Presumably this is because dentinal tubules contain a great deal of debris and collagen fibers that contribute much more to the resistance of fluid movement than the microscopic dimensions of the tubules would predict. What is important is the functional radius rather than the histologic or SEM radius.[20] Also shown in Table 2 are the calculated Lp of premolar dentin using the data of Garberoglio and Brännström.[7] They are the only authors to have carefully measured tubule density and diameter as a function of distance from the pulp. Using their linear regressions, we were able to calculate the theoretical hydraulic conductance of permanent dentin at the same distance from the pulp as our primary dentin was prepared. This is the reason we did not measure the tubule density and diameters of our premolars. Although doing so would have permitted us to directly calculate theoretical Lps, it is unlikely that we would have been able to prepare the dentin surfaces of the premolars at the same distance from the pulp as was the primary dentin. Clearly, the calculated Lp of premolars is 5- to 11-fold higher than those of primary dentin due to higher tubule densities and radii. When Lps were measured in superficial to deep primary dentin (Table 2), there was a 4.3-fold increase. Examining the increase in tubule density between superficial and deep dentin revealed a 1.5-fold increase. Presumably, the remaining (4.3 - 1.5 = 2.8) nearly threefold increase in Lp must have been due to the increase in tubule radii raised to the fourth power [$(0.64)^4 \div (0.048)^4$ = 3.16]. The above calculations confirm this presumption and indicate the importance of tubule radius in determining Lp.

Because primary enamel and dentin are thinner than that of permanent dentition, may clinicians believe that primary dentin seems to be penetrated more rapidly during the carious process. The results of this study suggest that the rapid advance of caries is not due to a higher intrinsic permeability of primary dentin. However, the primary dentin studied in this report was that of teeth nearing exfoliation. That is, the teeth had been in occlusion for about 8–9 years, which may have lowered the permeability of the primary dentin due to apposition of additional peritubular dentin matrix. The permeability of newly erupted primary dentin may be higher than was reported here. More research is required on the permeability of primary dentin as a function of posteruption age.

Conclusion

1. The permeability of both primary and permanent teeth increased with increasing dentin depth (or reductions in remaining dentin thickness).
2. The smear layer significantly reduced the permeability of primary and permanent teeth, regardless of the dentin depth.
3. Removing 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 than that of the primary molars in intermediate to deep dentin.
4. 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.
5. The smaller density and diameter may account for the lower permeability of the primary molars, when compared with the premolars.

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New techniques help hospitals fight infections
Traditional epidemic investigations may lead to false conclusions

Molecular epidemiology is more effective than traditional methods in identifying the source of *Staphylococcus aureus* infections in nurseries and hospitals, according to a pair of studies published in a recent *Journal of the American Medical Association*.

An editorial that accompanies the articles says "in the next few years, we are likely to witness an explosion in the clinical application of molecular typing." The editorial by James R. Lupski, MD, PhD, Baylor College of Medicine and the Texas Children's Hospital, Houston, says: "In essence, molecular typing provides a DNA fingerprint of the particular bacterial strain. Like the bar code used to identify consumer products, the DNA fingerprint should act as a signature to enable strain identification."

In one of the studies, by Nancy A. Back, RN, BSN, College of Medicine, University of Cincinnati, Ohio, and colleagues, clinical epidemiologic findings were compared with molecular epidemiologic analysis in a well-baby nursery in a 700-bed university teaching hospital.

Researchers studied the cases of newborn infants that developed Erythromycin-resistant *Staphylococcus aureus* (ERSA) infections during 1990 and 1991.

In the first epidemic, 15 infants were infected with ERSA. A nursing assistant who cared for most of the infants was found to be a carrier of ERSA. She was removed from the nursery and the epidemic was resolved. Fifteen months after the first epidemic ended, another involving 11 infants began. The attending physician was found to be a carrier of ERSA. Molecular tests (plasmid and genotyping) showed that the ERSA organisms from both epidemics were the same. The employee implicated in the first epidemic did not have the epidemic strain, but the physician who attended during both epidemics did.

The researchers concluded: "The present study demonstrates that results of traditional (or 'shoe leather') epidemiology may be misleading and that molecular epidemiology may correct these misinterpretations."

A second study, by Ferric C. Fang, MD, University of California—San Diego Medical Center, and colleagues, evaluated two molecular epidemiologic methods used in the analysis of a nosocomial (hospital-related infection) methicillin-resistant *Staphylococcus aureus* (MRSA) outbreak that involved 28 patients at the UC—San Diego Medical Center.

The study says: "Since the first reported nosocomial outbreak in 1963, MRSA has become a major cause of hospital-acquired infections worldwide."

The researchers concluded: "Although traditional epidemiologic methods retain their central role in modern hospital infection control, molecular epidemiologic analysis can significantly enhance the ability of infection control officers to analyze and terminate hospital epidemics."