Demonstration of Physiological Barrier Between Pulpal Odontoblasts and its Perturbation Following Routine Restorative Procedures: A Horseradish Peroxidase Tracing Study in the Rat

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Vascular injection of the macromolecular tracer, horseradish peroxidase (HRP), was used to study the permeability of the odontoblast cell layer in developing and mature rat molar teeth, and to investigate the effect of cavity preparations on the permeability of this epithelioid cell layer in adult animals. HRP injected into the vascular system of normal animals 28 days of age and older was localized histochemically (from 5 to 90 min after injection) throughout the extracellular spaces of the maxillary dental pulps; however, the tracer did not penetrate beyond the tight junctions at the apical region of the odontoblast cell layer, and was absent from the predentin and dentin. In contrast, HRP injected into very young neonatal animals (e.g., day 3) resulted in free passage of HRP between odontoblasts and into the overlying predentin and dentin. When Class V cavities had been prepared in adult maxillary molars after HRP was injected into the blood stream, HRP reaction product penetrated the predentin and dentin immediately beneath the cavity preparation; however, adjacent, untraumatized areas of predentin and dentin in the operated teeth were devoid of reaction product. These results provide evidence that: (1) a physiological barrier develops between the distal segments of odontoblast cell bodies in normal rat molar teeth between days 15 and 28 of postnatal life, and this barrier prevents the passage of macromolecules from the pulp into the predentin and dentin; and (2) this barrier is perturbed following routine restorative procedures in adult animals.

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Introduction.

The odontoblast is a unique cell that functionally appears to be a hybrid between a connective tissue cell—highly specialized for the synthesis and secretion of the organic components of dentin—and an epithelial cell, functioning as a covering or liner for the dental pulp. During dentinogenesis, the odontoblast cell layer acts as a semi-permeable barrier between the extracellular compartments of the pulp and predentin, and selectively regulates the passage of molecules, ions, and water into the predentin, in order to create the proper ionic environment for calcification. To perform this function, specialized junctional barriers exist between the odontoblasts. Three basic types of intercellular junctions (*i.e.*, gap junctions, desmosomes, and tight junctions) have been described in a variety of species, including humans (Arwill, 1967, 1968; Frank, 1968; Koling *et al.*, 1981; Koling and Rask-Andersen, 1984a,b; Calle, 1985), monkeys (Turner, 1982), cats (Holland, 1975, 1976; Sasaki *et al.*, 1982b; Bishop, 1987), rats (Jessen, 1967; Reith, 1968; Takuma and Nagai, 1971; Tanaka, 1980; Sasaki *et al.*, 1982a; Iguchi *et al.*, 1984), and mice (Garant *et al.*, 1968). Most recent studies of odontoblast junctional morphology and permeability have focused on the tight junction, and there is evidence to suggest that tight junctions contribute in an important way to (although they may not necessarily be the sole determinants of) this barrier function (*e.g.*, Bishop, 1985). Unfortunately, the precise nature of the odontoblast tight junction has been only partly elucidated, and efforts to examine them by use of electron microscopy (Jessen, 1967; Garant *et al.*, 1968; Reith, 1968; Frank, 1968; Takuma and Nagai, 1971; Holland, 1975; Bishop, 1987), freeze fracture (Koling *et al.*, 1981; Koling and Rask-Andersen, 1984a,b; Sasaki *et al.*, 1982b; Iguchi *et al.*, 1984; Calle, 1985), and intercellular tracer technics (Holland, 1976; Tanaka, 1980; Sasaki *et al.*, 1982a; Bishop, 1985) have so far yielded equivocal results. On the basis of these prior investigations, it has been variably concluded that tight junctions between odontoblasts are either absent (Koling *et al.*, 1981), "macular", or discontinuous (Frank, 1968; Reith, 1968; Sasaki *et al.*, 1982a,b; Iguchi *et al.*, 1984), or "zonular", forming complete rings about the apices of the cells (Calle, 1985; Bishop, 1985). The reasons for the contrasting nature of these observations are unclear, but may include differences in the methodologies used or species examined, as well as differences in the developmental stage of the particular tooth under investigation.

The normal structure and function of the odontoblast cell layer may be adversely affected by a variety of pathological processes, including dental caries, attrition, and abrasion, and by the restorative procedures and materials used to treat them. The acute histological changes that take place in these circumstances include a loss of integrity of the odontoblast layer and aspiration of the odontoblast cell bodies into the dentinal tubules (Brännström, 1963; Stewart, 1965; Eda and Saito, 1978). The displacement of the odontoblasts is thought to result both from the outward flow of dentinal fluid at the surface of the exposed dentin, and from the mobilization of capillary forces (Johnson *et al.*, 1973). Under these conditions, it seems likely that the tight junctions between adjacent odontoblasts would be stressed or broken, thereby compromising the epithelioid function of the odontoblast cell layer and increasing the potential for entry into the dental pulp of micro-organisms and their toxins. Unfortunately, our understanding of the short-term mechanisms by which odontoblasts respond to dentin trauma and restorations remains rudimentary.

The current investigation was undertaken in an effort to: (1) determine whether functional intercellular barriers exist between odontoblasts in mature, fully erupted teeth and, if so, to determine the approximate age at which these barriers first appear, and (2) determine whether the normal barrier function of the adult epithelioid odontoblast cell layer is altered following routine restorative procedures. To study these questions, we have investigated the ability of pre-eruptive—as well as mature—odontoblasts to prevent passage of the enzyme tracer, horseradish peroxidase (HRP, mw 43,000), from the dental pulp to the predentin and dentin in normal teeth. We have contrasted these findings with observations on the permeability

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of the odontoblast cell layer to HRP, following Class V cavity preparations. The results of these studies have been presented previously in abstract form (Sattelberg and Turner, 1984, 1985).

Materials and methods.

We used a total of 22 adult Sprague-Dawley rats (each weighing from 200 to 250 g) and 20 neonatal animals (ages from 3 to 28 days). All animals were carefully examined, and only animals with healthy dentition, free of obvious dental pathology, were selected for use. Each animal was anesthetized with an intraperitoneal dose of pentobarbital (30 mg/kg body weight).

We used the neonates and 16 of the adult animals to study the permeability of the odontoblast cell layer in developing and fully erupted molar teeth, and the remaining six adult animals to investigate whether there was altered permeability of the odontoblast layer following Class V cavity preparations. In each adult rat in the first group, we made a midline incision on the ventral surface of the neck from the hyoid bone to the jugular notch, and gently separated the underlying strap muscles to expose the external carotid artery. We then carefully inserted a fine-tipped glass micropipette through the wall of the artery, and slowly injected from 0.2 to 0.4 mL of 1 to 5% HRP (Sigma type VI) in saline into the lumen of the vessel. HRP injections in the neonatal animals were made into the inferior vena cava. Five, 15, 30, 45, 60, or 90 min after the cessation of the injection, the animals were perfused through the left ventricle with 200 mL of warm (37°C) heparinized saline, followed by 500 mL of 1% paraformaldehyde-4% glutaraldehyde in 0.1 mol/L phosphate buffer, pH 7.4, for 15-30 min. Finally, the fixation was terminated by perfusion of the animal for an additional 15-30 min with 500 mL of chilled (4°C) 0.1 mol/L phosphate buffer containing 10% sucrose.

Each of the six adult animals in the second group received Class V cavity preparations in the lingual surfaces of the first and second maxillary molar teeth. The preparations were made by use of a low-speed handpiece (Kerr Electrotorque) and a 33½ inverted cone dental bur under continuous saline spray. Each cavity was approximately one millimeter square and 0.75 mm deep, and extended, at its maximum depth, to within 0.15 mm (150 μm) of the predentin-dentin border. Immediately prior to cavity preparation, the abdominal cavity was opened *via* a ventral midline incision, and 0.6 to 1.0 mL of 1 to 2% HRP in saline was slowly infused with a glass micropipette into the inferior vena cava over a period of ten to 15 min. Fifteen, 30, or 45 min after cavity preparation, successive solutions of saline, fixative, and 10% sucrose were perfused through the left ventricle, as described in the experiments on normal animals (see above).

Following the perfusion, that portion of the maxilla containing the molar teeth was removed bilaterally and decalcified over a period of two to three weeks in daily changes of ice-cold, buffered 0.2 mol/L sodium ethylenediamine tetra-acetate (EDTA), at pH 7.4. The teeth were then sectioned individually at 30-40 μm in a cryostat, and the sections immediately reacted for HRP activity according to either the tetramethylbenzidine (TMB) technique of Mesulam (1978) or the cobalt chloride modification (Adams, 1977) of the diaminobenzidine (DAB, Graham and Karnovsky, 1966) procedure. After completion of the incubations, the sections were washed in three changes of either cold 5% acetate buffer (TMB procedure) or 0.1 mol/L phosphate buffer (DAB procedure). All of the neonatal material and approximately half of the sections from the normal and experimental adult animals were then mounted on chrome alum-gelatin-coated slides, dehydrated, cover-slipped, and examined with an Olympus BH-2 light microscope. The remaining sections were osmicated (Carson and Mesulam, 1982), dehydrated for seven min each (in a series of graded ethanols), and flat-embedded in Spurrs epoxy resin between two Teflon-coated cover slips. Following polymerization, the 80-100-μm-thick resin wafers were examined under a light microscope, and areas of interest were trimmed from the wafers and glued with quick-setting epoxy to the ends of epoxy resin blocks. Thin (80-100 nm) sections were cut with a diamond knife, collected on 150-mesh copper grids, and viewed, unstained, in a Phillips 300 electron microscope.

Two types of histochemical controls were performed to verify the specificity of the HRP-tracing procedure used in this study. So that we could test for the presence of endogenous peroxidase activity within the dental pulp, we subjected two adult control animals to vascular injections of 0.9% NaCl containing no exogenous HRP. The animals were then perfusion-fixed, and the teeth were sectioned and processed, as described above, for the demonstration of HRP activity. To investigate the possibility that some or all of the visualized reaction product may have resulted from the non-specific deposition of incubation media components, we reacted several sections from each experimental animal in an incubation medium lacking either the chromagen (TMB or DAB) or the substrate (H_2O_2). All the controls proved to be negative.

Results.

Permeability of the odontoblast cell layer in normal, mature dentition.—Injection of horseradish peroxidase into either the external carotid artery or the inferior vena cava of the adult rats resulted in the rapid and extensive deposition of HRP reaction product throughout all areas of the dental pulp (roots, chamber, and horns). Increasing the survival time following tracer infusion (from 5 to 90 min) had no noticeable effect on the distribution of reaction product. Tissue sections processed according to the TMB or DAB procedures yielded comparable results, although the quality of ultrastructural preservation was slightly better in sections reacted with DAB.

Light microscopic examination of the tissue revealed that the reaction product crystals were distributed relatively uniformly throughout the extracellular compartment of the dental pulp (Figs. 1a,b). Conspicuous deposits of reaction product were present in the periphery of the pulp, immediately beneath the odontoblast cell layer, and within the narrow intercellular spaces between adjacent odontoblasts; however, the predentin and dentin were in every animal devoid of reaction product (Figs. 1c,d).

Electron microscopic (EM) observations confirmed and extended the findings made at the light microscopic level. At the ultrastructural level, the crystals of HRP reaction product were easily distinguishable because of their superior electron density and characteristic crystalline (TMB) or flocculent (DAB) morphology. Reaction product crystals were observed throughout the extracellular spaces of the pulp (Fig. 2a), and in the odontoblast cell layer, they were often observed in the narrow intercellular gaps between adjacent odontoblast cell bodies (Fig. 2b). However, penetration of reaction product between the apical regions of adjacent odontoblasts was prohibited by areas of membrane fusion that resembled tight junctions (Fig. 2b). No reaction product was observed in the predentin or dentin.

Permeability of the odontoblast cell layer in neonates.—The experiments which used neonatal animals provided evidence that functional barriers between developing odontoblasts first appeared between days 15 and 28 of neonatal life. Intraventricular injections of HRP in 3–15-day-old animals resulted

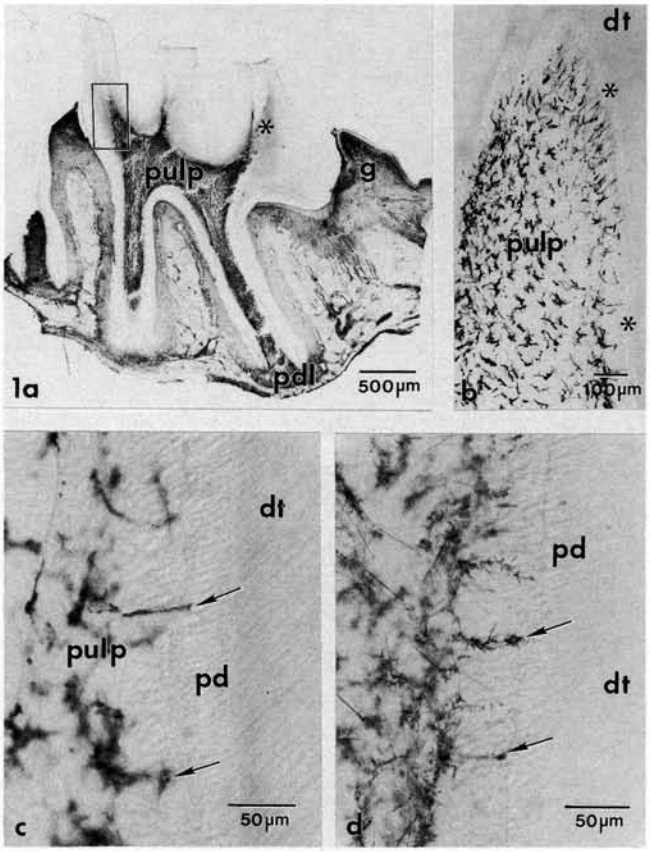

Fig. 1 — Light micrographs showing HRP reaction product in the maxillary molar teeth of normal, adult animals, 15 min after HRP was injected into the external carotid artery. (a) HRP-TMB reaction product (dark staining material) is distributed heavily and uniformly throughout the dental pulp, periodontal ligament (pdl), and gingiva (g). The slightly darkish area indicated by the asterisk is caused by optical refraction of the dentin and should not be confused with reaction product. An area of pulp horn similar to that enclosed by the box is shown at higher magnification in (b). (b) HRP-DAB reaction product is distributed widely throughout the extracellular spaces of the pulp horn (pulp), but has not entered the predentin (asterisks) or dentin (dt). (c) High-magnification micrograph of the peripheral dental pulp, predentin (pd), and dentin (dt) in a mesial pulp horn. HRP-DAB reaction product is visible in the pulp and between adjacent cells of the odontoblast layer (*e.g.,* arrow), but is not visible distal to the odontoblast/predentin border zone. (d) Comparable section as in (c) but with TMB as the chromogen.

in widespread deposition of HRP throughout the dental pulp, predentin, and dentin (Fig. 3), whereas injections of animals 28 days and older produced results identical to those seen in the adult, *i.e.,* there was a total absence of reaction product in the predentin and dentin.

Alteration of odontoblast permeability following Class V cavity preparations. — The dental pulps of animals that received Class V cavity preparations after HRP was injected into the blood stream contained deposits of reaction product in the extracellular compartment that were similar in appearance and distribution to those described in the normal adult animals (see above). However, additional quantities of reaction product were also observed in the predentin and dentin immediately beneath the cavity preparation (Fig. 4). Electron microscopic examination of these areas revealed that the HRP reaction product was located in the periodontoblastic spaces surrounding the odontoblast processes (Fig. 5). In contrast, the dentin and predentin in all other areas of the tooth, and in control (nonperturbed) teeth from the same animal, were devoid of reaction product.

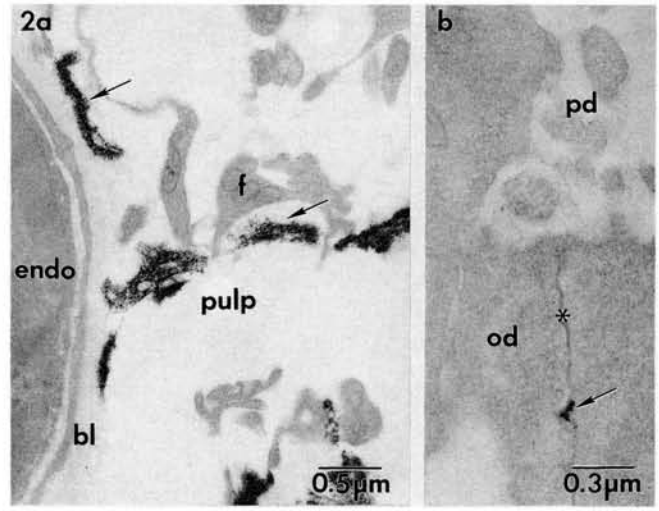

Fig. 2 — Electron micrographs showing HRP reaction product in the dental pulp of normal adult rat molars, 15 min after tracer was injected into the vascular system. (a) Electron-dense crystals of HRP-DAB reaction product (*e.g.,* arrows) are scattered within the extracellular spaces of the pulp. Fibroblast processes (f) and the basal lamina (bl) of a capillary endothelial cell (endo) are also visible. Unstained section. (b) Electron micrograph of the odontoblast-predentin border zone. A crystal of HRP-DAB reaction product (arrow) is visible in the intercellular space between two odontoblasts (od). The odontoblast cell membranes distal to the reaction product are fused in a five-layered complex (asterisk) that suggests a tight junction. Reaction product is absent from the predentin (pd).

Discussion.

Intravascular injection of the macromolecular tracer, HRP, was used in this investigation to study the permeability of the odontoblast cell layer in developing and mature rat molar teeth, and to examine the effects of cavity preparations on the permeability of this epithelioid cell layer in adult animals. The results have shown that in 3–15-day-old molar teeth, HRP enters the developing dental pulp through the vascular system and penetrates freely between adjacent odontoblasts to enter the predentin and dentin. In contrast, in animals aged 28 days and older, HRP enters the pulp and passes between adjacent odontoblasts as far as the pulp-predentin border, but no further. When Class V cavity preparations were performed on adult teeth immediately after HRP infusion, the tracer penetrated the predentin and dentin subjacent to the base of the cavity; however, remaining (nontraumatized) areas of dentin and predentin remained devoid of reaction product. We conclude from these investigations that: (1) a functional barrier develops between the distal segments of pulpal odontoblastic cell bodies in normal rat molar teeth between days 15 and 28 of postnatal life, and these barriers prevent the passage of macromolecules from the pulp into predentin and dentin, and (2) this barrier is perturbed following routine restorative procedures.

Technical considerations. — In this study, the light and electron microscopic visualization of HRP was accomplished with use of both DAB and TMB histochemical procedures. The chromagen DAB possesses the advantage of forming a discrete reaction product which, at the ultrastructural level, is confined in its distribution to the subcellular compartment(s) involved in the uptake or transport of the HRP (Graham and Karnovsky, 1966). The chromagen TMB, in contrast, gives

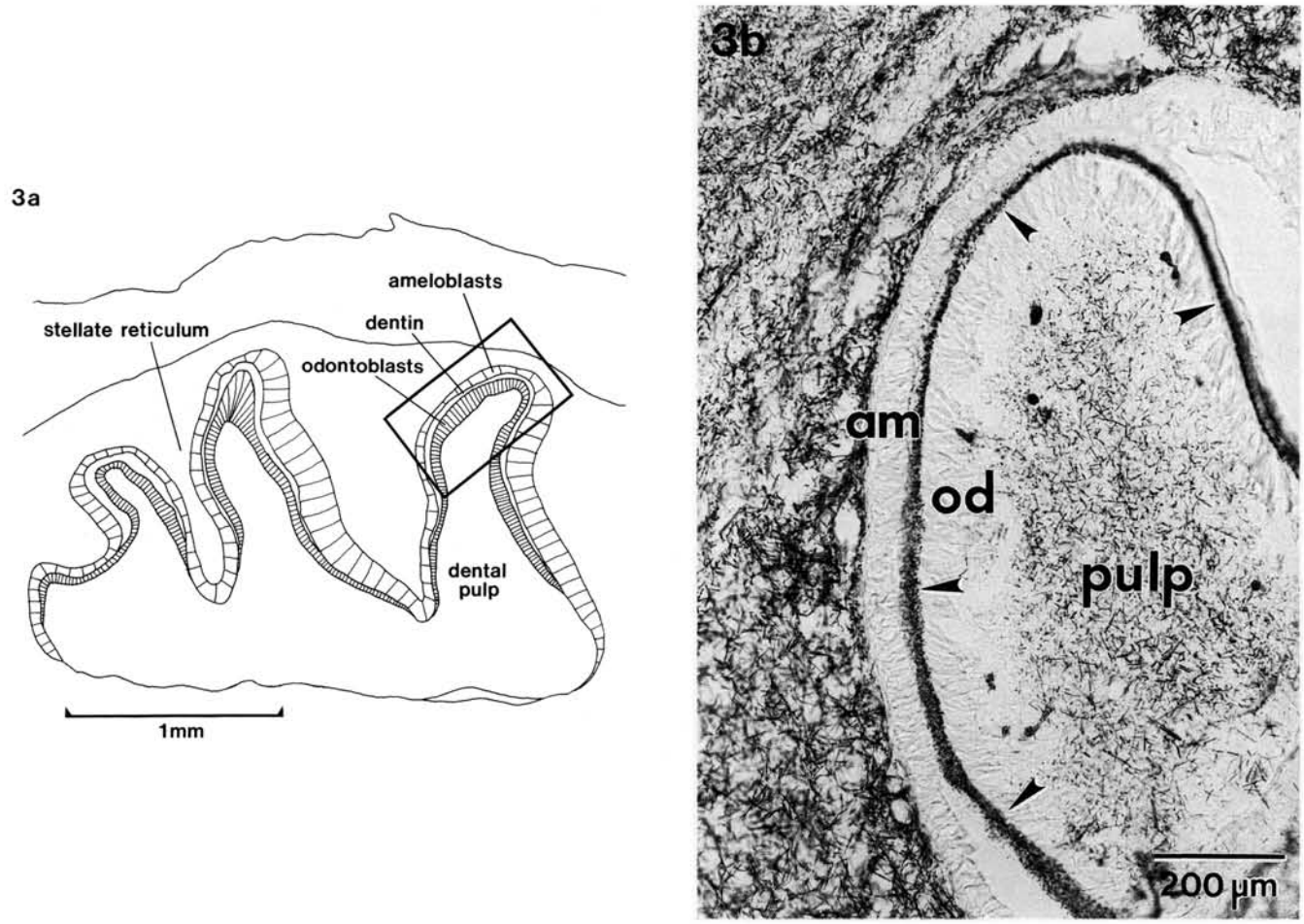

Fig. 3 — Distribution of HRP-TMB reaction product in the first maxillary molar tooth bud of a 3-day-old rat. The tooth bud is in the bell (dentinogenesis) stage of tooth development and is characterized by the presence of an ameloblast (am) layer, an odontoblast (od) layer, and a thin, intervening layer of predentin and dentin. The ameloblasts have not yet begun to form enamel. The area of tooth bud illustrated in the right figure (3b) is outlined by the box in the orientation diagram (3a). A dense band of HRP-TMB reaction product is located in the predentin/dentin (arrowheads). The tracer most likely reached the dentin by passing through the odontoblast cell layer (as opposed to entering *via* the ameloblast layer), since at higher magnification numerous granules of reaction product were observed in the intercellular spaces between odontoblasts, but only rarely were they seen between ameloblasts.

rise to a reaction product that usually exceeds in size the distribution of the exogenous HRP (Carson and Mesulam, 1982). However, the use of TMB offers two distinct advantages that made it particularly attractive for use in this study: (1) It is generally felt that, at both the light and electron microscopic levels, TMB affords a level of sensitivity considerably higher than that of DAB (Mesulam, 1978; Carson and Mesulam, 1982); and (2) the crystalline substructure of the TMB reaction product at the ultrastructural level is unique, which greatly facilitates its differentiation from endogenous tissue components (Sakumoto *et al.*, 1980; Marfurt *et al.*, 1988). The results of this study and of earlier work in our laboratories (Marfurt and Turner, 1983; Marfurt *et al.*, 1988) have shown that the HRP enzyme will survive the decalcification procedure used in this study, in quantities sufficient to catalyze the oxidation of a suitable chromagen, *i.e.*, TMB or DAB. Nevertheless, we acknowledge that some loss of enzyme activity may have occurred following decalcification. Therefore, small amounts of HRP could have diffused into the predentin space and gone undetected by the less-sensitive DAB protocol. However, we believe that, by using TMB and taking advantage of the "phenomenon of histochemical magnification" (Carson and Mesulam, 1982), even small amounts of the enzyme can be localized and identified.

The nature of the barrier function: a role for junctional complexes? — Specialized areas of membrane contact between odontoblasts have been known since the initial electron microscopic studies of Frank (1968) and his contemporaries (Jessen, 1967; Arwill, 1967, 1968; Garant *et al.*, 1968; Reith, 1968). These early observations have subsequently been confirmed and extended by a number of different workers who used both conventional transmission electron microscopy (Takuma and Nagai, 1971; Holland, 1975; Sasaki *et al.*, 1982a; Bishop, 1987) and freeze-fracture techniques (Sasaki *et al.*, 1982a,b; Iguchi *et al.*, 1984; Calle, 1985), and as a result, three basic types of odontoblastic junctional complexes are now widely recognized: desmosomes, gap junctions, and tight junctions. Desmosomes and gap junctions are numerous along the lateral and basal aspects of the cells. The former junctional contacts are important in cell-to-cell adhesion and in maintaining the polarity and epithelioid nature of the odontoblast cell layer. Gap junctions represent between-cell areas of low electrical resistance through which ions and metabolites move freely, and although their precise function in odontoblast physiology

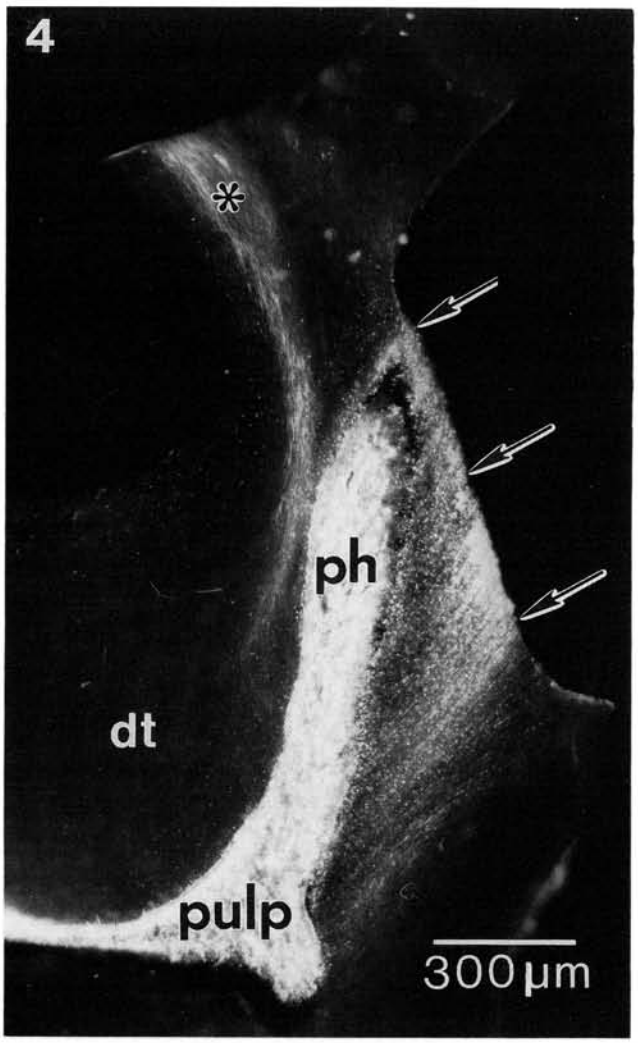

Fig. 4 — Darkfield light micrograph of a pulp horn from a molar tooth that received a Class V cavity preparation after HRP injection into the blood stream. HRP-TMB reaction product fills the pulp chamber (pulp) and pulp horn (ph), and extends into the dentin subjacent to the base of the cavity preparation (arrows). The surrounding, unperturbed areas of dentin (dt) are devoid of reaction product. The whitish area indicated by the asterisk is a region of refractile dentin and is not to be confused with reaction product.

remains speculative, it has been postulated (Sasaki *et al.,* 1982b) that they may play important roles in the cytodifferentiation of odontoblasts and in the synchronization of odontoblast function, especially with regard to the elaboration and mineralization of dentin. However, because desmosomes and gap junctions are distributed in spotlike fashion, and because narrow intercellular spaces exist between the outer lamellae of the adjacent plasma membranes, it is unlikely that they contribute in any appreciable fashion to the odontoblastic barrier function. A more likely candidate for the barrier function seen in the current investigation is the tight junction, where the adjacent outer lamellae of apical odontoblast cell membranes appear to fuse and, therefore, obliterate the intercellular space for a variable distance. Tracer studies in other epithelia have shown that HRP or lanthanum placed in the tissue space cannot cross the tight junctions to reach the free surface of the tissue (*e.g.,* Goodenough and Revel, 1970). In the odontoblast cell layer, some authors claim that odontoblast tight junctions form continuous rings (or belts) of membrane fusion about the circumference of the cell apex (*i.e.,* "true" tight junctions, on zonula occludens—Frank, 1966; Reith, 1968; Calle, 1985); however, others feel that they are arranged in a series of focal cellular fusions ("macular" tight junctions) analogous to "spot rivets" (Garant *et al.,* 1968; Sasaki *et al.,* 1982; Iguchi *et al.,* 1984). The resolution of this apparent controversy is perhaps beyond the capabilities of conventional morphological techniques, because these methods reveal only very small areas of cell membrane in a given section; thus, it is difficult to ascertain by means of this procedure, to what extent the intercellular junctions encircle the cells in three dimensions.

In this study, therefore, we have investigated the nature of the odontoblast permeability barrier in molar teeth of normal adult and developing animals, by using a more three-dimensional approach, *i.e.,* by introducing the exogenous tracer, HRP, into the dental pulp, and monitoring its passage through the odontoblast cell layer and into the predentin and dentin. The results of earlier studies of this type, which used the same or different tracer substances, resulted in the publication of what appears to be yet another collection of contradictory reports (Tanaka, 1980; Sasaki *et al.,* 1982b; Bishop, 1985). However, the results of our study strongly suggest that the differences in odontoblast permeability reported by other investigators may be largely explained by variation in the developmental stage of the tooth examined. Thus, we propose that the odontoblast cell layer is permeable to horseradish peroxidase and lanthanum nitrate in developing, pre-eruptive-stage teeth (Sasaki *et al.,* 1982; Tanaka, 1980; current investigation), but that it is impermeable to the passage of these same compounds in adult, fully developed teeth (Bishop, 1985; current investigation). Furthermore, we hypothesize that the appearance of this barrier may be related to the development of zonular tight junctions between the apical regions of the odontoblasts. This theory receives support from electron microscopic and freeze-fracture studies: Macular (discontinuous) tight junctions have been observed between odontoblasts of developing teeth (Sasaki *et al.,* 1982b; Iguchi *et al.,* 1984), whereas zonular tight junctions are seen between the apical regions of the cells in mature teeth (Calle, 1985). It is not known whether the macular tight junctions observed between young odontoblasts enlarge and fuse to form zonular tight junctions between mature odontoblasts. However, assemblage (or "maturation") of zonulae occludens from multiple focal tight junctions has been reported in other epithelial tissues (Tice *et al.,* 1977; Dermietzel *et al.,* 1977).

Functional considerations. — The appearance of zonulae occludens in mature dental pulp likely indicates a shift in function, or the addition of new functions, for the odontoblasts. Macular tight junctions between odontoblasts of immature teeth are probably important for cell-to-cell attachment (Sasaki *et al.,* 1982b), a role consistent with the proposed function of tight junctions in other types of epithelia (Grynszpan-Wynograd and Nicolas, 1980). Indeed, tight junctions show considerable strength and are the last elements of the junctional complex to break under tension (Farquhar and Palade, 1963). However, it is unlikely that macular tight junctions, because of their discontinuous nature, are able to regulate fluid and ion movements between the pulp and dentin. Thus, during dentinogenesis, minerals, metabolites, and other components of dentinal fluid can pass freely through the odontoblast layer by both extracellular (Sasaki *et al.,* 1982a; Tanaka, 1980) and intracellular (Reith, 1968; Nagai and Frank, 1974) routes to reach the mineralization front. In contrast, zonulae occludens between mature, secretory odontoblasts form continuous, beltlike barriers between the pulp and predentin. Functionally, zonular tight junctions may partially regulate fluid movement

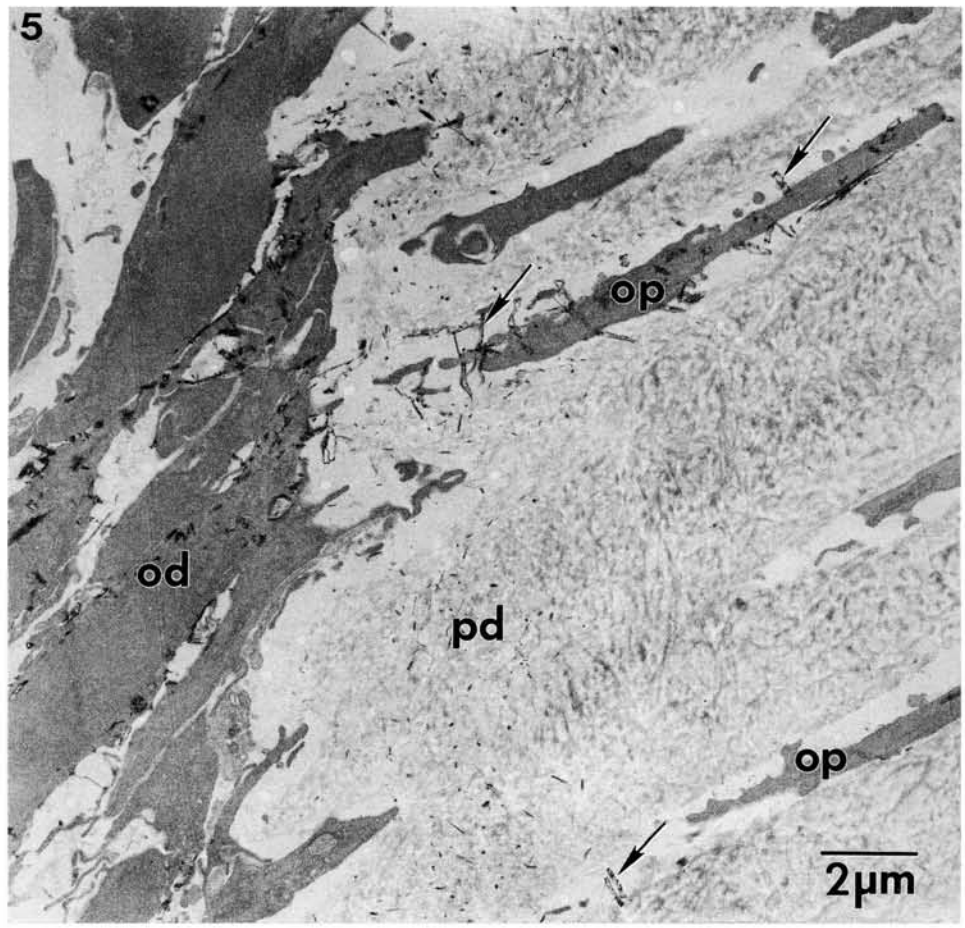

Fig. 5 — Electron micrograph illustrating crystals of HRP-TMB reaction product (arrows) surrounding odontoblast processes (op) in the predentin (pd) beneath a cavity preparation. Od, odontoblast.

through dentinal tubules. Fluid movement through dentin is thought to be regulated or "resisted" by a combination of three factors: surface resistance, intratubular resistance, and pulpal resistance (Pashley *et al.*, 1978, 1981). The tight junctions demonstrated experimentally in this study may well be the source of the "pulpal" resistance. Zonulae occludens also function to maintain chemical and electrochemical gradients across the odontoblast cell layer, by impeding the back-diffusion (leakage) of substances along the intercellular spaces between odontoblasts. Thus, in fully formed teeth, the passage of fluid, ions, and other molecules along the extracellular pathway is hindered, and transfer of these substances from pulp to dentin must occur largely through intra-odontoblastic, transcellular mechanisms. The composition of dentinal fluid in the adult, therefore, is apparently highly regulated and is not simply a transudate of pulpal capillaries (Bishop, 1987).

Odontoblasts may also play a role in dentin sensitivity by regulating the ionic composition of the dentinal fluid that surrounds intradentinal nerve fibers. However, the importance of the odontoblast cell layer in this regard is uncertain, since pain can be evoked from dentin with no odontoblasts beneath it (Brännström and Åström, 1964), and nerves can continue to be activated by dentinal stimulation despite damage to the peripheral pulp and odontoblasts (Hirvonen and Närhi, 1986). Interestingly enough, the impermeability of the adult odontoblast cell layer to HRP and lanthanum (Bishop, 1985; current investigation) is maintained even in the pulp horns, where sensory fibers pass between adjacent odontoblasts to enter the dentinal tubules (see Byers, 1984, for review). The mechanism responsible for maintaining the barrier to exogenous substances between these disparate cell types is presently unknown. Although gap junctions between odontoblasts and "nerve-like" processes have been reported by some workers (Holland, 1975, 1976; Matthews and Holland, 1975; Koling and Rask-Andersen, 1984a), more recent anatomical (Byers, 1977, 1979; Holland, 1980; Turner, 1982) and experimental denervation (Holland, 1987) studies have been unable to confirm these observations.

Breakdown of zonulae occludens in the odontoblast layer following Class V cavity preparations. — The results of the present experimental tracer study have demonstrated that the functional barrier to HRP passage that exists between pulp and dentin in normal adult teeth is perturbed following routine restorative procedures. We hypothesize that the loss of the odontoblast barrier function is probably caused, at least in part, by the disruption of the zonulae occludens that normally link these cells together. The short-term physiological and metabolic responses of the odontoblasts to cavity preparation and severance of the distal aspect of the odontoblast process are unknown. However, the present results suggest that, when perturbed by a Class V cavity preparation, zonulae occludens between odontoblasts rapidly dissolve (or re-arrange to become "macular"), as evidenced by the immediate, prominent, and highly localized passage of HRP from the pulp into the peritubular spaces of the overlying dentin. Functionally, this phenomenon of enhanced permeability may indicate an attempt by the odontoblast cell layer to increase the rate of transfer of reparative compounds and associated ions into the overlying predentin, as a first step toward repairing the damage to the overlying tooth matrix.

The elegant *in vivo* studies of Pashley *et al.* (1983a,b, 1984) are consistent with the observations of this study. The latter workers have shown that dentin permeability is high immediately following routine cavity preparations, then falls progressively to a level of about 20% of zero-time values in five to six h. Considering the data presented here, these observations could be explained by the perturbation of zonulae occludens following routine cavity preparation, and the subsequent restoration of this barrier with time. This hypothesis raises broader questions concerning the dynamic properties of odontoblast tight junctions under normal and pathological conditions, and additional studies are needed to learn to what extent odontoblast tight junctions can assemble, disassemble, and re-assemble in response to functional demand.

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