Invasion of bacteria into dentinal tubules

Experiments in vivo and in vitro

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Penetration of bacteria into the tubules of intact dentin exposed by fracture was compared *in vitro* in pairs of teeth, one of which in each pair was mounted with an intrapulpal hydrostatic pressure equivalent to 30 mm Hg. The teeth were incubated at 37°C for 21 days. Intra-pair comparisons *in vivo* were made of bacterial invasion into dentinal tubules beneath ground dentin surfaces and beneath fractured or acid-treated surfaces, which were exposed for 1 week. It was found that an outward fluid flow in the dentinal tubules due to intrapulpal pressure may mechanically hinder bacterial growth into the tubules. Of greater importance as an obstruction to bacterial invasion was the blocking of the outer apertures of the dentinal tubules by grinding debris. This barrier, however, seemed to be removed after some days and this would allow bacterial ingrowth into intact vital dentin. It is concluded that dentinal surfaces that have been left unprotected and are covered with plaque for some days should be treated as carious dentin, and the surface layer should be ground off to remove widened apertures filled with bacteria.

Key-words: Bacteria; dentin; dental caries

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Opinions differ as to the extent to which bacteria from saliva and plaque can penetrate dentinal tubules. Some histological investigations of the caries process have suggested that living microorganisms do not occur in the non-demineralized dentin beneath a carious lesion. (*Dorfman et al.*, 1943, *Sarnat & Massler*, 1965, *Fusayama, Okuse & Hosada*, 1966). *Lundy & Stanley* (1969) left prepared cavities in human teeth exposed to saliva and plaque formation. The first evidence of bacterial invasion into the dentinal tubules was observed after 6 days' exposure. After 6—11 days bacteria had penetrated to a depth of 0.03—0.36 mm. They established that the average depth of penetration was 0.52 mm after an average period of 84.2 days; maximum penetration was noted after 210 days. They concluded that »the visualized bacteria moved through the patent dentinal tubules at no great speed.»

In an investigation of the effect of abrasion on the underlying dentin and pulp, (*Tronstad & Langeland*, 1971), bacteria were observed in individual dentinal tubules. In most cases bacterial invasion was observed in cracks in the dentin, and in these areas the penetration was confined to the incisal half of the dentin exposed by attrition.

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Wachtel & Brown (1963) observed in tooth sections with exposed dentin subjected to bacterial acid production that caries-like lesions and bacterial ingrowth occurred mainly in those areas where the bacteria had been supplied with niacin diffused through the dentin from the pulpal surface.

Accepting the assumption that pulpless teeth are more resistant to caries, the authors suggested that a prerequisite for bacterial invasion towards the pulp is »nourishment by means of diffusion of fluid from the pulp». Observations made in earlier investigations by these authors supported this assumption (*Brown, Wachtel & Wheatcroft*, 1962). A similar interpretation was presented some years ago by *Bodecker* (1934, 1960).

On the other hand, *Chirnside* (1961) conducted experiments in which teeth with exposed dentinal surfaces were placed in prostheses and he observed bacterial invasion of the dentinal tubules beneath the exposed dentine after 3 weeks. This would suggest that nutrition from the pulp by diffusion through the fluid phase of the dentin is not necessary for bacterial invasion towards the pulp.

Another factor which may be of significance in determining the extent of bacterial ingrowth is the outward fluid flow through the dentinal tubules, which may occur in exposed dentin of vital teeth. In an earlier investigation *in vitro* it was found that the applied intrapulpal pressure of 30 mm Hg, caused an outward fluid flow in the tubules beneath dentinal surfaces exposed by fracture. This flow was calculated as 0.6 μl/mm^2/d, which means that a fully open dentinal tubule is emptied of its contents about 10 times a day (*Johnson, Olgart & Brännström*, 1973). The existence of such a flow might mechanically inhibit invasion of bacteria through the wide open tubules. Furthermore such an outward flow may, as well as simple diffusion, not only distribute nutritional substrate for bacteria but also antibodies.

The conditions at the outer apertures of the dentinal tubules may also be significant in this context. In a dentin surface exposed by fracture the tubules are wide open. When dentin is ground the apertures of the tubules are blocked by debris. This may be removed completely by acid treatment during which the tubular apertures are not only opened but also widened (*Brännström & Johnson*, 1974). In another investigation (*Vojinovic, Nyborg & Brännström*, 1973) it was found that such »plugs» of grinding debris prevented bacterial invasion into dentinal tubules beneath cavities which had been filled with a composite material for 4 weeks. However, in teeth where the dentin had first been treated with acid, massive invasion of bacteria was observed in a large number of dentinal tubules after the same period.

The purpose of the present investigation was (1) to compare bacterial invasion in contralateral pairs of teeth where there was an outward flow of dentinal fluid in only one tooth of the pair and (2), to see whether any difference could be demonstrated in the bacterial invasion from a ground dentin surface with its tubular apertures initially blocked by grinding debris and from a dentin surface with wide open tubules.

MATERIAL AND METHODS

1. *In vitro-experiment on the effect of an outward fluid flow in the dentinal tubules*

The material consisted of 10 contralateral, intact, single-rooted premolars, extracted for orthodontic reasons. Immediately after

extraction the teeth were cleaned mechanically and placed in hydrogen peroxide 30 % and in iodine tincture 10 % for 2 minutes, after which they were transferred to a 5 % sodium thiosulphate solution for 2 minutes to inactivate the iodine preparation. The specimens were stored in sterile physiological saline. Within one hour after extraction, a groove was ground with a diamond disk around the buccal cusp a short distance into the dentin; the cusp was then fractured with forceps.

In this way an area of dentin was exposed, which had a peripheral ground part and a central fractured surface of about 1 mm^2. These procedures as well as the following were performed under sterile conditions and all preparations were made under cooling with sterile physiological saline. Polyvinyl tubes were ligated over the crown and the root, the apical third of which had been cut off (Fig. 1). The coronal tube was filled with a suspension of caries-inducing streptococci (E49) in soy broth (Trypticase Soy broth, BBL) and sealed with a rubber stopper. The stopper was provided with a valve to permit equilibration of pressure. The tube connected with the root and the pulp was filled with sterile soy broth and sealed either with a rubber stopper (horizontal mounting) or connected with a glass capillary which was mounted vertically and produced a hydrostatic pressure in the pulp equivalent to 30 mm Hg, which according to *Beveridge* and *Brown* (1965), is the mean pulpal pressure in human teeth. The tooth of the pair to be selected for vertical mounting or horizontal mounting without pulpal pressure was decided by lot (Fig. 1). Evaporation from the glass capillary connected with the root was compensated for by addition of sterile soy broth.

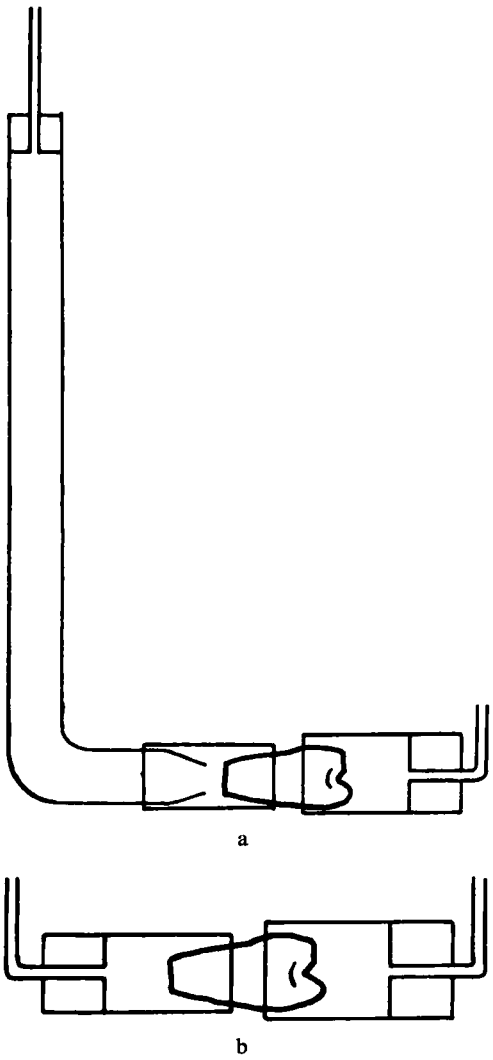

Fig. 1. Experimental arrangement in *in vitro* experiment.
a) vertical mounting of the tooth resulting in a hydrostatic pressure equivalent to 30 mm Hg in the pulp,
b) horizontal mounting without intrapulpal pressure.

The system was kept in a thermostatically controlled room at 37°C for 21 days, after which the experiment was discontinued and bacteriological samples were taken from the broth in the coronal tube of each tooth. 0.1 ml of the broth was transferred to soy-agar plates (Trypticase

soy agar BBL), which were incubated anaerobically for identification of micro-organisms and for counting of the number of colonies. Readings were made after 7 days.

The teeth were decalcified in EDTA and serial sectioned bucco-lingually through the centre of the tooth. The sections were stained with Htx-eosin or with a modified gram stain according to *Brown* and *Brenn* (1931). The dentinal tubules were examined for micro-organisms by two examiners who did not know which of the teeth in the pairs had been mounted vertically.

II. *In vivo-experiment on bacterial growth from ground, fractured and acid-treated dentin surfaces*

The material consisted of pairs of intact permanent teeth in young individuals.

In 5 pairs one tooth of each pair was selected at random and the dentin was exposed on the buccal cusp by grinding with a diamond wheel under water cooling. In the contralateral tooth the dentin was exposed by fracturing with forceps after the buccal cusp had been undermined by a shallow groove. In 5 pairs the dentin of both teeth was exposed by grinding, after which the exposed surfaces were rubbed with citric acid 50 % for 2 minutes. The exposed dentin on one of the teeth in the pair — selected by lot — was ground again to remove the surface of the dentin affected by the acid. In 7 pairs of teeth buccal cavities were prepared to a depth of about 2 mm with a round diamond bur and water cooling. One cavity in each pair of teeth — selected by lot — was treated with citric acid 50 % for 2 minutes. All the exposed areas of dentin were left unprotected for 1 week. The teeth were then extracted and placed in formalin after two thirds of the root had been cut off. The teeth were decalcified in EDTA, serially sectioned bucco-lingually through the exposed areas of dentin and alternate sections were stained with Htx-eosin and with a bacterial stain according to Brown and Brenn for gram positive bacteria.

Two pairs of teeth with cavities were frozen in propane and afterwards fractured longitudinally through the centre of the cavity. One half was decalcified and treated in the same way as the other teeth, while the other half was examined in a scanning electron microscope using the same technique as described by *Johnson et al.* (1973).

RESULTS

Effect of an outward fluid flow in the dentinal tubules

In 7 of the 10 teeth mounted with an intrapulpal pressure no bacterial growth was found in the dentinal tubules. In 2 teeth a few bacteria were seen on the dentin surface adjacent to superficial cracks in the dentin and in 1 tooth the bacteria had penetrated up to 1.5 mm into the dentin. In the 10 contralateral teeth, which were mounted without pressure in the pulp, bacteria were demonstrated in the dentinal tubules in 7 teeth. Penetration was 0.1—2.0 mm (M = 1.05 mm). In 7 of the 8 teeth with micro-organisms in the tubules, this was only demonstrated underneath the fractured central part of the dentin surface.

In all cases viable micro-organisms were demonstrated in the coronal broth when the experiment was discontinued. No significant difference in the number of colonies on solid substrate was found between the two groups of teeth.

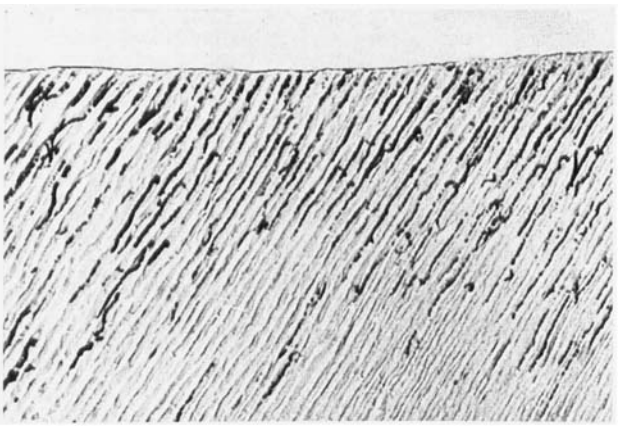

Fig. 2. Acid-treated dentinal surface exposed by grinding of buccal cusp. exposed for 1 week. Bacteria in most dentinal tubules. Brown & Brenn stain. ×375.

Bacterial growth from ground, fractured and acid-treated dentin surfaces

Of the 10 pairs of teeth with dentin exposed on the cusps, 7 pairs showed more extensive bacterial growth in a larger number of dentinal tubules beneath the fractured or acid-treated dentin surface than underneath the ground surface. In 1 pair no difference was found, and in 2 pairs bacterial growth was seen in a larger number of tubules beneath the ground dentin.

Bacterial penetration was deeper beneath the fractured and acid-treated surfaces. In most cases bacteria were found in nearly all the tubules beneath these surfaces (Fig. 2). The depth of penetration, however, varied from one tubule to another and the maximum depth was 1.8 mm (Fig. 2). Beneath the ground surfaces bacteria were generally seen in only a few tubules and the depth of penetration was as a rule very small. However, in occasional tubules bacteria could be observed at a greater depth in the dentin, maximum depth being 1 mm.

In all pairs of teeth with cavities exposed for one week, there were a clearly larger number of tubules with a deeper penetration of bacteria beneath the acid-treated surfaces than beneath the untreated cavity surfaces (Figs. 3 and 4). Beneath all the

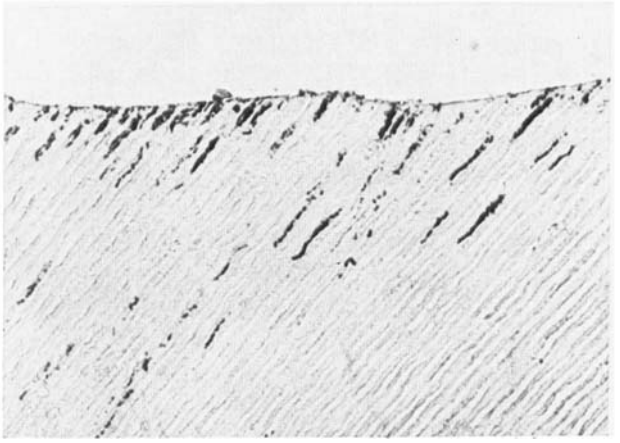

Fig. 3. Bacteria in dentinal tubules beneath the base of an acid-treated cavity, exposed for 1 week. ×375.

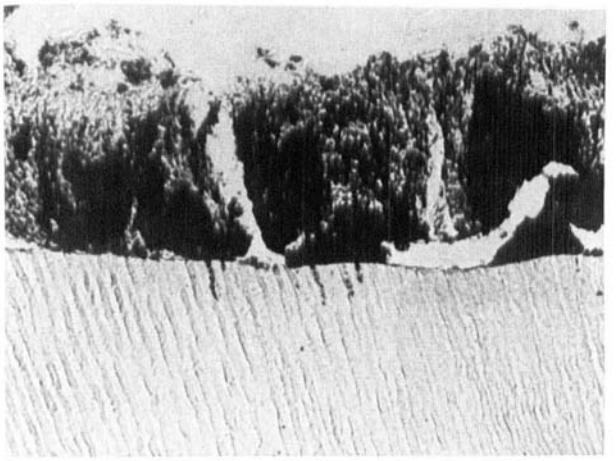

Fig. 4. Base of a cavity not treated with acid in the tooth contralateral of Fig. 3. Heavy bacterial coating on the surface with bacteria only in a few tubules. Brown & Brenn stain. ×375.

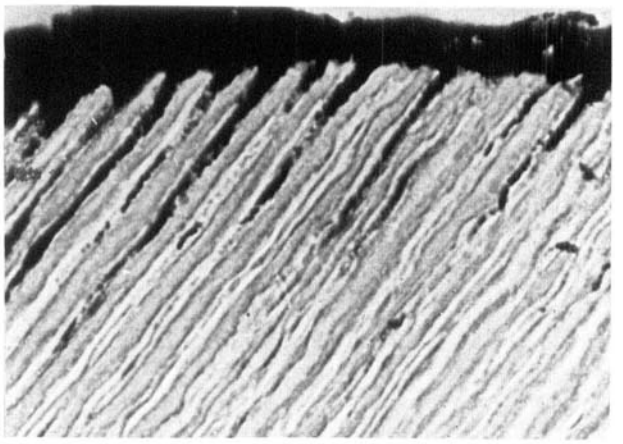

Fig. 5. Acid-treated dentinal surface exposed by grinding of buccal cusp, exposed for 1 week. Dense bacterial growth on the surface and bacteria in several funnel-shaped, widened dentinal tubules. Brown & Brenn stain. ×950.

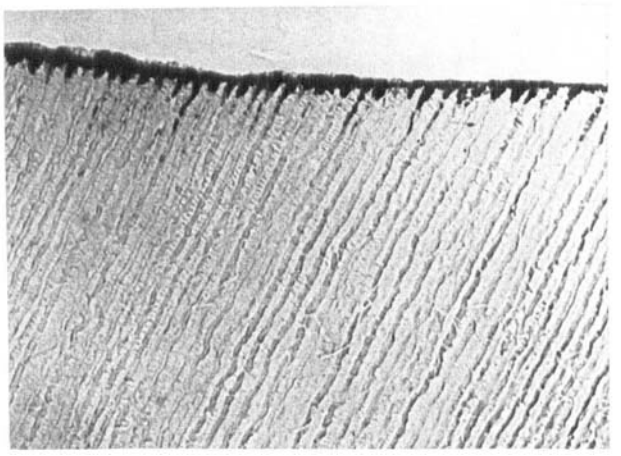

Fig. 6. Base of an untreated cavity, exposed for 1 week. Thin layer of bacteria on surface and bacteria in funnel-shaped widened tubular apertures. Brown & Brenn stain. ×375.

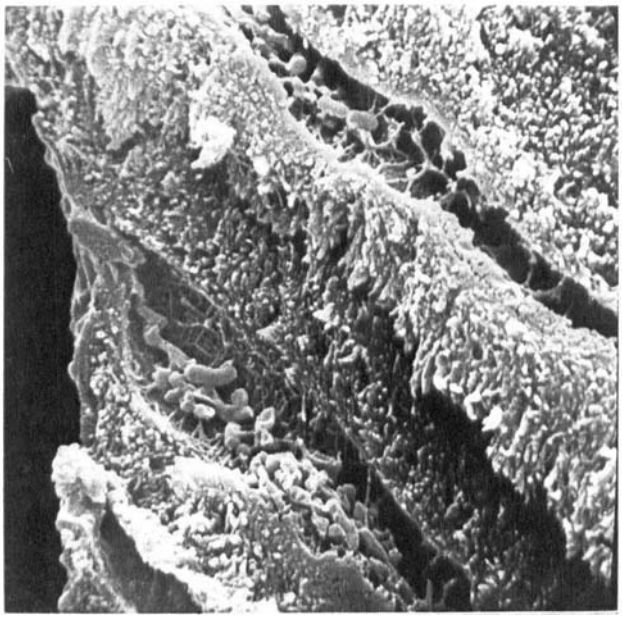

Fig. 7. Profile view of base (left) of acid-treated cavity exposed for 1 week, photographed in scanning electron microscope. Same cavity as in Fig. 3. Micro-organisms of varying form in longitudinally fractured, widened dentinal tubules. Peritubular zone removed. × 5400.

acid-treated surfaces the apertures of the tubules were funnel-shaped and widened about 20 μm into the dentin (Fig. 5). Such widening could also be observed in some cases in which the dentin had only been ground; the widening was obvious from the mass of bacteria it contained (Figs. 5 and 6).

Mild to moderate local inflammation had developed in the pulp beneath all of the exposed dentin areas. In the pairs of teeth with cavities the reaction was more

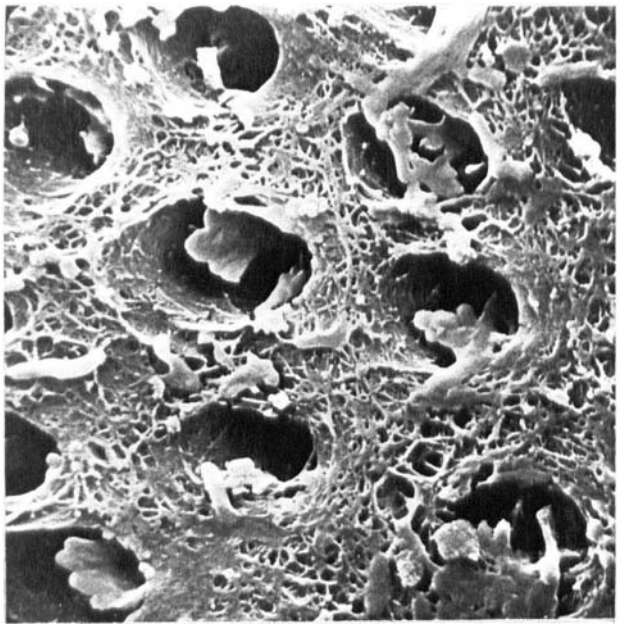

Fig. 8. Base of same cavity as ni Fig. 3 and Fig. 7.
The superficial layer of the dentin is dissolved partly by the effect of citric acid, partly by other means, probably bacterial products. The tubules are widened and the fibrillar structure of the intertubular dentin is visible. Clusters of bacteria on the surface and in the apertures of the tubules. × 5500.

pronounced beneath the acid-treated cavities than beneath the untreated ones.

Scanning electron microscopy of the cavities in 2 pairs of teeth revealed bacteria as well as plaque and debris on the surface in all 4 cavities. Microorganisms of varying form were seen in most of the dentinal tubules beneath the acid-treated cavities (Fig. 7). Beneath the cavities which had been ground but not acid-treated, bacteria occurred in only an occasional tubule. On the acid treated surfaces the fibrillar structure of the intertubular dentin could readily be distinguished (Fig. 8). The tubular apertures were wide open and the peritubular zone was dissolved. The diameter of the tubules was 3—4 μm (Fig. 8) compared with 1—2 μm normally (*Fromme & Riedel*, 1970, *Brännström & Garberoglio*, 1972).

DISCUSSION

An earlier *in vitro* investigation revealed an outward fluid flow in the dentinal tubules at an intra-pulpal pressure equivalent to 30 mm Hg (*Johnson et al.*, 1973). The experimental conditions were the same in the present investigation which showed that bacterial invasion in the dentinal tubules was in most cases more frequent in the teeth without intrapulpal pressure than in those under pressure. This suggests that, all other factors being equal, penetration of micro-organisms into the dentinal tubules is hindered by an outward fluid flow. On the other hand, the possibility should be borne in mind that a larger supply of fresh substrate from the pulpal chamber occurred in teeth with an intrapulpal pressure than in those without. This may so have enhanced the growth conditions in the coronal substrate that the bacteria did not need to grow for fresh substrate in the dentinal tubules. However, no difference in the amount of bacteria could be demonstrated in the coronal broth in teeth with and without intrapulpal pressure.

In an earlier *in vitro* experiment it was found that the flow caused by the intrapulpal pressure was greater in tubules beneath fractured surfaces than beneath ground surfaces (*Johnson et al.*, 1973). One may therefore assume that the flow *in vivo* through tubules beneath fractured and acid-treated surfaces is greater than in tubules under ground surfaces. Nevertheless, the present *in vivo* investigation revealed that bacterial invasion was heavier beneath the fractured and acid-treated surfaces. This would suggest that the ability of the outward flow to hinder mechanically the ingrowth of bacteria is a less significant factor.

It has been found earlier that acid treatment of dentin facilitates penetration of the bacteria into dentinal tubules beneath cavities filled with a composite material for 4 weeks (*Vojinović et al.*, 1973). The observation made in the present investigation that the conditions at the outer apertures of the tubules seem to affect the ingrowth of micro-organisms into dentinal tubules, is in accordance with the above findings. This is further confirmed in the *in vitro* experiment where in 7 of the 8 teeth with bacteria in the tubules, micro-organisms could be demonstrated only beneath that part of the dentinal surface that had been fractured, *i.e.* in open tubules, and not beneath the ground part where the tubule apertures were blocked. Comparisons made within pairs *in vivo* also showed that in most cases there was a more extensive penetration of bacteria beneath those dentin surfaces where the apertures of the tubules

were wide open from the beginning, *i.e.* beneath fractured and acid-treated surfaces. That acid treatment facilitates ingrowth of bacteria into dentin is presumably not due to changes in the contents of the tubules further in the dentin, since both exposed dentin areas had been washed with acid, after which one of the areas was ground again in the 5 pairs of teeth in which ground and acid-treated, exposed cusps were compared for bacterial ingrowth.

In 2 of the 10 pairs in which the dentin of the cusps had been exposed, the bacterial ingrowth was greater beneath the ground surfaces. This might be due to plugs of grinding debris having been washed off after a few days' exposure. It might, however, be of interest to note that such plugs appear to persist for 4—6 weeks when the dentin is covered by a filling (*Vojinović et al.*, 1973). In the present study the plugs of grinding debris may also have been removed by the effect of acid from the bacteria on the unprotected dentin surface, indicating that a carious process had started after one week including widening of the apertures of the tubules (Fig. 6) and dissolution of intertubular dentin (Fig. 8). In other words, the results obtained in the present investigation suggest that bacteria can grow into tubules in intact vital dentin, regardless of whether the tubules are wide open from the beginning or whether they are initially blocked by grinding debris. These results are in accordance with the aforementioned findings of *Lundy & Stanley* (1969) and with the observation of bacteria in occasional tubules beneath dentin exposed by abrasion (*Tronstad & Langeland*, 1971). Dentin surfaces left unprotected for some days and covered with plaque should therefore be treated clinically as carious dentin and the superficial layer should be ground away to remove widened tubule apertures filled with bacteria.

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