The Effects of Desensitizing Agents on the Hydraulic Conductance of Human Dentin in vitro

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The hydrodynamic theory of dentin sensitivity states that a stimulus applied at the orifice of exposed dentinal tubules causes movement of tubular fluid which stimulates nerve receptors. The fluid should obey principles of fluid movement through capillary tubes. Any decrease in the functional radius of the dentinal tubules should greatly reduce the rate of fluid flow, thus reducing dentinal sensitivity.

The purpose of this study was to evaluate the ability of agents that have been used previously for clinical dentin desensitization to reduce the rate of fluid flow through dentin in vitro.

Dentin discs prepared from extracted human third molars were treated with 50% citric acid to remove debris from tubular orifices. After placing the discs in a split chamber device, the rate at which buffer solution could filter across the dentin under 240 cm of water pressure was measured. The occlusal side of the disc was then treated with an agent thought to desensitize dentin to determine if it reduced fluid flow rate. Discs that had more than a 50% reduction in flow rate were examined by scanning electron microscopy to determine if those agents that decreased fluid flow also partially occluded tubular orifices. This in vitro model provided a useful quantitative method for screening a host of preparations that have been used in the past to decrease dentin sensitivity.


Introduction.

Many different empirical treatments have been used in the past to decrease or eliminate dentin sensitivity.¹⁻⁶ None of these remedies, however, works predictably. The greatest problem regarding the development of dentin desensitizing agents is that the mechanism of dentin sensitivity is not clearly understood.

Basically, there are two theories of the mechanism of dentin sensitivity.⁷ The first proposes that the dentin tubules contain neural receptors which, upon direct stimulation by a noxious agent, conduct pain impulses.⁸ These neural receptors are said to be either nerve terminals from the dental pulp,⁸⁻⁹ odontoblastic processes joined to nerves from the dental pulp,¹⁰ or odontoblastic processes alone (presumably of neural origin).¹¹ The second theory is the hydrodynamic theory¹² which states that stimuli applied to dentin tubules result in movement of dentinal fluid, which then stimulates nervous processes in the more pulpal areas of the dentin and/or nerves in the dental pulp itself, resulting in pain impulse transmission.

In an attempt to determine the distribution of nerves in dentin, Byers and Kish¹³ injected radioactive proline into the trigeminal ganglion of rats. The proline was then traced peripherally through the distribution of nerve endings in molar teeth. The nerves were seen to extend fairly evenly into all dentinal tubules in the coronal dentin. In general, their findings indicated that although dentin is in fact innervated, the peripheral two-thirds of dentin is devoid of nervous structures. This does not mean that odontoblastic processes attached or unattached to nerves do not extend into the peripheral dentin and act as pain receptors. However, Holland¹⁴,¹⁵ showed that the odontoblastic processes in cats also remain limited to the pulpal half of the dentin.

It is evident then that pain receptors, whether free nerve endings (which are extensions of pulpal nerves) or processes of the specialized odontoblast, do not extend into the peripheral one-half of the dentin. Since stimuli cannot directly stimulate pain receptors, there must be an indirect stimulation of these receptors in the pulpal one-half of the dentin and/or the pulp itself.

Brannstrom's analysis of his own work and that of Anderson's group⁷ led him to

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postulate the hydrodynamic theory of dentinal sensitivity which states that any stimulus causing an inward or outward movement of dentinal tubule fluid stimulates nerves either in the pulpal one-half of the dentin and/or the nerves in the pulp to elicit pain. Thus, the coupling of stimulus application and nerve stimulation is via an intermediary mechanism (i.e., fluid movement through the tubules). If one assumes that fluid movement is an important transducing mechanism in the production of dentinal pain, then reductions in fluid flow should reduce dentin sensitivity.

The aim of this project was to screen a host of preparations and methods which have been used in clinical dentistry to desensitize dentin. The evaluation of their efficiency was based strictly on the ability of these agents or procedures to decrease the rate of fluid filtration through discs of dentin held in a split-chamber device.

Materials and methods.

Dentin discs. — One hundred twenty-three coronal dentin discs, 1 mm in thickness, were prepared from maxillary and mandibular unerupted third molars. The teeth were obtained from both sexes ranging in age from 17 to 28 years of age. All teeth had been stored for varying periods of time in Krebs-Ringer's phosphate buffer† (pH 7.4) with 1000 units/ml penicillin and 1 mg/ml streptomycin at 8°C.

The dentin discs were prepared by sectioning off the roots with a high speed #557 carbide burr under water spray. The remaining coronal segment (Fig. 1) was sectioned with a diamond saw* parallel to the occlusal surface at a point just coronal to the pulp horns and another point just cervical to the dentino-enamel junction. The resulting disc was then reduced in thickness on both the pulpal and enamel side with a high speed 557 carbide burr under water spray until it was 1.0 ± 0.1 mm in thickness, as measured by a pincer-type micrometer to the nearest 0.1 mm.

All dentin segments were stored in Krebs-Ringer's phosphate (0.01 M phosphate, pH 7.4) with 1000 units/ml penicillin and 1 mg/ml streptomycin at 8°C.

Chamber device. — Dentin discs were placed in split-chamber devices as shown in Fig. 1. The chambers were closed except for inlet and outlet tubules made from 20 gauge hypodermic needle stainless steel tubing. The discs were clamped between two paired rubber “O” rings to give a constant exposed dentin surface area of 0.178 cm². Both sides of the chamber were filled with sterile Krebs-Ringer’s phosphate buffer (KRB, pH 7.4).

Pressure application. — Filtration of buffer through the dentin disc was accomplished by raising a buffer reservoir bottle, connected by polyethylene tubing, 240 cm above the midpoint of the chamber. Attached to the side of the reservoir bottle was a 25 ml disposable plastic syringe barrel connected to polyethylene tubing and filled with mercury to provide higher hydrostatic pressures than could be accomplished with KRP buffer for filtering fluid across root dentin.

The reservoir bottle was filled with KRP buffer (pH 7.4) with 1000 units/ml penicillin and 1 mg/ml streptomycin. The bottle had a sterilizing Nucleopore filter‡ (0.2 μm) attached to it approximately 5 cm in front of the chamber device. This removed any bacterial or fungal growth which could contaminate the disc surface and occlude dentinal tubules. There was no measurable pressure drop across the filter at the filtration rates used in this study.

Acid etching. — Just prior to assembling the split chamber device, the dentin discs were etched by submerging them in 50% (w/v) citric acid for two min to remove microcrystalline debris occluding tubular orifices. They were then removed from the acid and rinsed with KRP buffer.

Medicaments. — The following medica-

†Dulbecco's solution, Gibco, Grand Island, NY
*Isomet Saw, Buehler, Ltd., Evanston, IL
‡Nucleopore Corp., Pleasanton, CA
tions were applied to the occlusal surface of the coronal discs:
  1) Protein precipitants previously used clinically:
     a) Ammoniacal Silver nitrate (AgNO₃);
     b) Formaldehyde solution, 10% w/v in water;
     c) Zinc chloride (ZnCl₂), 10% w/v in water followed by Potassium ferro-
        cyanide (K₃Fe(CN)₆), 40% w/v in water;
     d) Phenol, 90% w/v, liquid;
     e) Strontium chloride (SrCl₂), 10% w/v in water; and
     f) Zinc chloride (ZnCl₂), 10% w/v in water.
  2) Tubule occluding agents previously used clinically:
     a) Sodium silicofluoride (Na₂SiF₆), saturated in water;
     b) Sodium fluoride (NaF), 2% w/v, neutral pH;
     c) Sodium fluoride (NaF), 2% w/v, made up in 0.1 N H₃PO₄ (pH 3.2);
     d) Calcium hydroxide paste, 90% w/v in water;
     e) Calcium phosphate paste (monobasic) CaH₄(PO₄)₂ · H₂O, 190% w/v in water;
     f) Potassium nitrate, 30% in water;
     g) Sensodyne toothpaste, active ingredient, SrCl₂;
     h) Thermodent toothpaste, active ingredient, Formalin;
     i) Luride gel, active ingredient 1.23% NaF in H₃PO₄;
     j) Protect toothpaste;
     k) Silver nitrate (AgNO₃) 5.9 M followed by Formalin 10% to reduce it to
elementsal silver; and
     l) Sodium fluoride, 2% neutral, ionto-
     3) Experimental tubule occluding agents:
        a) Potassium oxalate, 30% w/v in water;
        b) Barium chloride (BaCl₂), 1 M in water;
        c) Sodium sulfate (Na₂SO₄ • 10 H₂O), 1 M in water;
        d) Barium chloride followed by Sodium sulfate;
        e) Sodium sulfate followed by Barium chloride;
        f) Sodium carbonate (Na₂CO₃), 2 M in water;
        g) Calcium chloride (CaCl₂), 2% w/v in water;
        h) Sodium carbonate followed by Calc-
           i) Potassium carbonate (K₂CO₃ • 1½
           j) Potassium carbonate followed by Calcium chloride.

Unless otherwise indicated, all the above chemicals were certified ASC grade. All
medicament solutions were applied to the dentin surface with a medicine dropper to
fill the “O” ring area. This was done after partially disassembling the split-chamber so
as to expose the occlusal side of the disc. The solution was left in contact with the
disc for two min, and it was then rinsed off with 25 cc of distilled water from a syringe
with a 25 gauge needle, followed by 25 cc of KRP buffer from a similar syringe and
needle. The chamber was then re-assembled and refilled with KRP buffer from the
reservoir bottle-polyethylene tubing appara-

tus.

After partial disassembly of the chamber to permit access to the disc, all pastes and
gels were applied with a cotton swab to fill the O ring area and then gently rubbed on
the dentin surface for two min. Unless indicated otherwise, the pastes and gels were
rinsed off the discs in the same man-
ner as the solutions. The chambers were
also re-assembled and refilled with phos-
phate buffer in the same manner as pre-
viously described.

For the two-step medications, the first
medicament was applied for two min fol-
lowed by application of the second medica-
ment to fill the O ring area. Using the cotton swab and the one-half turn back and forth
rotational mixing movement for two min, the two medicaments were allowed to react
with each other to form a precipitate.

Hydraulic conductance. — In order to
determine whether any of the medicaments have any effect on the ease with which
fluid can be filtered across dentin, the fil-
tration data were expressed as hydraulic conductances (Lp) where:

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Howes Formula, Shein, Inc., Port Washington, NY
Block Drug Co., Inc., Jersey City, NJ
Chas. Pfizer & Co., Inc., New York, NY
Hoyt Laboratories, Needham, MA
International Pharmaceutical Corp., Warrington, PA
\[ Lp = \frac{Q}{A(P_1-P_2)} \]

Q = fluid flow rate in \( \mu l \) min\(^{-1}\), \( A \) = surface area, in \( cm^2 \), \( P_1 \) = hydrostatic pressure above chamber in \( cm \) H\(_2\)O or Hg, and \( P_2 \) = atmospheric pressure. The flow rate of the phosphate buffer through the dentinal tubules under known pressures of either 240 cm water or mercury was determined by connecting a capillary tube of known length and radius (10 \( \mu l \) or 25 \( \mu l \) micropipette)** to the outlet side of the split-chamber. By measuring the distance that the fluid moved in the tube over set increments of time and multiplying by a proportionality constant, the linear displacement was converted into a volume flow rate. Four filtration rates were made successively on each disc. The hydraulic conductance\(^{16} \) was expressed in \( \mu l \) \( cm^{-2} \) min\(^{-1} \) \( cm \) H\(_2\)O\(^{-1} \).

**Scanning electron microscopy.** — Following the gathering of the physiological data, discs showing a decrease in \( Lp \) of 50% or more were removed from the split-chamber with tweezers (touching the periphery only), and, while being held in the tweezers, they were rinsed under flowing distilled water for 20 sec. The discs were air-dried and fractured with a sharp blade placed perpendicular to the disc surface. One sharp blow by a hammer on top of the blade resulted in fracturing the discs into two equal halves. One half of each of these discs was then glued, coronal or cementum side up, on a scanning electron microscope stage with silver paint and coated with gold/ palladium and examined under a Cambridge Stereoscan Mark II.

The discs were first scanned at low powers (1000X – 500X) to observe the control areas (outside the O ring), treated areas (inside the O ring), and the fractured surface. Appropriate representative photographs were taken at approximately 1000X and 3000X, since this magnification provided a good compromise between viewing a relatively large surface area of disc and detail of dentinal tubules. If there was no evident difference between control and treated areas, no photographs were taken.

Statistics. — Due to the biological variability of the depth of the enamel and the height of pulp horns, each disc (although 1.0 mm thick) is nevertheless different from every other disc. This introduces an inherent biological variability in the data when analyzed as a group. To decrease the variability, the data were calculated as percent change in hydraulic conductance (\( Lp \)) after treatment, using each disc as its own control. The mean and standard error (\( x \pm SEM \)) of the percent changes in \( Lp \) were calculated. Unpaired data were tested using Student's \( t \) test for means of two samples, while the data obtained on individual discs before and after treatment were evaluated using the paired \( t \) test. Statistical significance was given to any \( t \) test having a \( p \) value \( \leq 0.05 \).

Results.

**Hydraulic conductance.** — The mean percent change of hydraulic conductances from pre-treatment to post-treatment values (Table) ranged from 0.00% for potassium nitrate and calcium chloride to a -98.40% for potassium oxalate. Those treatments that did not result in a statistically significant reduction in hydraulic conductance included treatments with distilled water, potassium nitrate, calcium chloride, saturated sodium silicofluoride, iontophoresis of NaNO\(_2\), calcium phosphate paste, phenol, formalin, sodium sulfate, and strontium chloride. None of these treatments reduced hydraulic conductances more than 5.2%. Those treatments that reduced \( Lp \) more than 6% were statistically significantly different from treatment with distilled water or buffer and included: Sensodyne toothpaste, Thermodent toothpaste, Luride gel, 2 M potassium carbonate, 2 M sodium carbonate, 2% neutral sodium fluoride, Protect toothpaste, calcium hydroxide paste, zinc chloride, and acidulated sodium fluoride.

The use of two medicaments applied sequentially required that each medicament be tested separately and that the order of application be evaluated. Generally, combinations of two medicaments reduced \( Lp \) more than either agent used separately with the exception of barium chloride/sodium sulfate combinations. Sodium sulfate alone (Table) reduced \( Lp \) only by 3.1% in contrast

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**Microcaps, Fisher Scientific Co., Pittsburgh, PA.
<table>
<thead>
<tr>
<th>MEDICAMENT</th>
<th>$\bar{x}$</th>
<th>SEM</th>
<th>n</th>
<th>p Values (Compared to H₂O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>-0.20</td>
<td>0.92</td>
<td>(6)</td>
<td>N.S.</td>
</tr>
<tr>
<td>30% Potassium nitrate</td>
<td>0.00</td>
<td>0.00</td>
<td>(3)</td>
<td>N.S.</td>
</tr>
<tr>
<td>2% Calcium chloride</td>
<td>0.00</td>
<td>0.00</td>
<td>(3)</td>
<td>N.S.</td>
</tr>
<tr>
<td>Saturated Sodium silicofluoride</td>
<td>0.67</td>
<td>0.67</td>
<td>(3)</td>
<td>N.S.</td>
</tr>
<tr>
<td>Iontophoresis of 1% Sodium nitrate³</td>
<td>1.00</td>
<td>1.00</td>
<td>(3)</td>
<td>N.S.</td>
</tr>
<tr>
<td>Calcium phosphate</td>
<td>+1.67</td>
<td>1.67</td>
<td>(3)</td>
<td>N.S.</td>
</tr>
<tr>
<td>90% Phenol</td>
<td>-2.00</td>
<td>2.00</td>
<td>(3)</td>
<td>N.S.</td>
</tr>
<tr>
<td>10% Formalin</td>
<td>-2.57</td>
<td>1.29</td>
<td>(3)</td>
<td>N.S.</td>
</tr>
<tr>
<td>1 M Sodium sulfate</td>
<td>-3.10</td>
<td>1.65</td>
<td>(3)</td>
<td>N.S.</td>
</tr>
<tr>
<td>10% Strontium chloride</td>
<td>-5.20</td>
<td>2.27</td>
<td>(5)</td>
<td>N.S.</td>
</tr>
<tr>
<td>Sensodyne</td>
<td>-6.81</td>
<td>2.49</td>
<td>(4)</td>
<td>&lt;.05 &gt;.025</td>
</tr>
<tr>
<td>Thermodent</td>
<td>-7.07</td>
<td>1.93</td>
<td>(3)</td>
<td>&lt;.025&gt;.01</td>
</tr>
<tr>
<td>Luride (gel)</td>
<td>-7.67</td>
<td>1.67</td>
<td>(3)</td>
<td>&lt;.005&gt;.01</td>
</tr>
<tr>
<td>2 M Potassium carbonate</td>
<td>-8.00</td>
<td>1.53</td>
<td>(3)</td>
<td>&lt;.005&gt;.001</td>
</tr>
<tr>
<td>2 M Sodium carbonate</td>
<td>-11.67</td>
<td>2.67</td>
<td>(3)</td>
<td>&lt;.005&gt;.001</td>
</tr>
<tr>
<td>2% Neutral Sodium fluoride</td>
<td>-17.77</td>
<td>2.60</td>
<td>(3)</td>
<td>&lt;.001</td>
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<tr>
<td>Protect</td>
<td>-19.25</td>
<td>3.64</td>
<td>(4)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Calcium hydroxide</td>
<td>-21.00</td>
<td>1.78</td>
<td>(4)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>40% Zinc chloride</td>
<td>-23.50</td>
<td>1.19</td>
<td>(4)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>2% Sodium fluoride in 0.1 M Phosphoric acid</td>
<td>-24.50</td>
<td>1.70</td>
<td>(4)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>1 M Sodium sulfate + 1 M Barium chloride</td>
<td>-24.33</td>
<td>2.19</td>
<td>(3)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>2 M Potassium carbonate + 2% Calcium chloride</td>
<td>-26.00</td>
<td>2.89</td>
<td>(3)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>1 M Barium chloride</td>
<td>-28.50</td>
<td>2.72</td>
<td>(4)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>2 M Sodium carbonate + 2% Calcium chloride</td>
<td>-30.75</td>
<td>2.17</td>
<td>(4)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Iontophoresis of 2% neutral Sodium fluoride³</td>
<td>-33.00</td>
<td>1.53</td>
<td>(3)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>40% Zinc chloride + 20% Potassium ferrocyanide</td>
<td>-38.00</td>
<td>3.21</td>
<td>(3)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>2% Sodium fluoride in 0.1 M H₃PO₄ + 2% CaCl₂</td>
<td>-39.00</td>
<td>2.00</td>
<td>(3)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>5.9 M Silver nitrate</td>
<td>-47.38</td>
<td>1.75</td>
<td>(8)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>1 M Barium chloride + 1 M Sodium sulfate</td>
<td>-56.33</td>
<td>7.06</td>
<td>(3)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

(continued on next page)
to barium chloride alone which reduced Lp 28.5%. Applying sodium sulfate first, followed by barium chloride, did not reduce Lp any more (24.3%) than barium chloride alone. However, applying barium chloride first, followed by sodium sulfate, reduced Lp by 56.3% — a value significantly different from either barium chloride treatment alone (P < 0.001) or treatment with sodium sulfate first followed by barium chloride (P < 0.001). Treating the dentin surface with zinc chloride alone resulted in a reduction in Lp of 23.5%, while the combination of zinc chloride first, followed by potassium ferrocyanide treatment, reduced Lp by 38.0%.

The combinations of sodium sulfate and barium chloride (in either order), potassium carbonate plus calcium chloride or sodium carbonate plus calcium chloride produced white precipitates which appeared to completely wash off the discs with rinsing. The combination of silver nitrate plus formalin produced a black precipitate with a round bead of metallic silver metal in the center of the O ring. When viewing the fractured edge of discs treated with this combination, the black precipitate was seen to extend into the dentin a minimum of two-thirds of the disc thickness. Discs treated with silver nitrate followed by buffer immediately developed a white precipitate. This precipitate was rinsed off and hydraulic conductances reetermined. Upon observing the same discs 12 h later, the treated area was completely black. Observation of the edge of the disc after fracturing revealed that the black precipitate penetrated at least two-thirds of the thickness of the disc. None of the medicaments or combinations of medicaments other than those mentioned above showed any evidence of a precipitate.

**Scanning electron microscopy.** — Both the treated and fractured surfaces of representative discs that decreased the Lp more than 50% were examined with a scanning electron microscope (SEM). Areas outside the O ring served as control areas, while those inside the O ring served as experimental areas. Representative photomicrographs of the more than 200 taken during the course of these experiments are shown in the following figures.

Fig. 2A shows the area outside the O ring (control area) of a treated disc. All untreated areas of all discs examined had a similar appearance, showing a relatively clean dentin surface with open tubule orifices, all being approximately the same diameter for each disc.

Fig. 2B shows the treated surface of a disc that had a two-minute topical application of 1 M barium chloride followed by a two-minute topical application of 1 M sodium sulfate. Examination of the micrograph reveals the heterogenous appearance of the disc with some areas having heavy precipitate and others having practically none. Fig. 2C shows another area of the same disc in which there was very heavy precipitate covering the dentin surface. Fig. 2D is a photomicrograph of the fractured edge of a disc treated with barium chloride for two min which was followed by rubbing in sodium sulfate for two min. This figure shows the deep penetration of precipitate into many tubules which was estimated.
Fig. 2A – Appearance of acid-etched dentin outside rubber O ring. Discs of dentin treated with distilled water had a similar appearance within the confines of the O ring. (1000X)
Fig. 2B – Surface of dentin treated with barium chloride followed by sodium sulfate. This represents an area of light precipitation. (1000X)
Fig. 2C – Another area of the same specimen shown in Fig. 2B, exhibiting areas of very heavy precipitate occluding tubule orifices. (3000X)
Fig. 2D – This shows the appearance of a disc of dentin treated with barium chloride/sodium sulfate. The disc was fractured in an area of heavy precipitate similar to that shown in Fig. 2C. The top of the figure is the surface of the disc. The crystals are assumed to be barium sulfate. (3500X)

Fig. 2E is representative of the surface of a disc treated with silver nitrate followed by formalin. A heavy precipitate appeared to cover practically all the tubule orifices. The fractured edge of the same disc is shown in Fig. 2F and demonstrates some of the precipitate partially occluding tubule orifices.

Fig. 2A shows the surface of a disc treated with zinc chloride followed by potassium ferrocyanide. The photomicrograph demonstrates the amount of precipitate, the approximate percentage of tubules covered, and the varying sizes and shapes of the crystalline precipitate. In almost all cases the crystals appear significantly larger than the tubule orifices, sometimes giving a plate-like appearance (Fig. 3B).

Fig. 3C shows the surface of a disc treated with silver nitrate followed by phosphate buffered saline. This resulted in a very homogenous, but thin, precipitate on the dentin surface. Some of the precipitate appeared to adhere to the periphery of the tubule orifices.
DESENSITIZING AGENTS & FLUID FLOW

Fig. 2E - A dentin surface treated with silver nitrate followed by formalin. The surface is covered with such a heavy precipitate of silver grains that only a few open tubules can be seen (open arrows).

Fig. 2F - A similar disc treated with silver nitrate and fractured to show silver grains in some, but not all, of the tubules. (3000X)

Fig. 3A - The surface appearance of dentin treated with zinc chloride followed by potassium ferrocyanide. Most of the crystals appear to be larger than the tubular orifices (arrows). (2500X)

Fig. 3B - Another specimen treated with zinc chloride/potassium ferrocyanide exhibited plate-like crystals. (1000X)

Fig. 3D is a photomicrograph of a disc treated with dipotassium oxalate, which decreased the Lp by 98.4%. This demonstrates the approximate number of tubules covered by crystals and also the size of the crystals. The crystal size appears fairly regular and has approximately the same dimensions as the actual tubule orifices (Fig. 3D). This can be seen more readily in Fig. 3E which was taken at a higher magnification than Fig. 3D. Still higher magnification (Fig. 3F) reveals thread-like structures connecting a calcium oxalate crystal to the walls of the tubule.

Discussion.

The hydrodynamic theory of dentin sensitivity12 states that stimuli applied to exposed dentin tubule orifices cause a movement of fluid in the tubules which, in turn, stimulates nerves in the pulp. Fluid flow through these tubules should obey Poiseuille's law and therefore should vary with the fourth power of the radius of the tubules.17 Reducing the functional radius of the tubule by partially occluding the tubule orifice should greatly reduce fluid flow and, therefore, dentin sensitivity. The in vitro
Fig. 3C — Dentin treated with silver nitrate followed by phosphate buffered saline exhibited a very fine precipitate. (2000X)

Fig. 3D — Dentin treated with 30% potassium oxalate revealed crystals of calcium oxalate that closely match the size of tubule orifices. (1000X)

Fig. 3E — Higher magnification of the specimen in Fig. 3D more closely reveals penetration of crystals of calcium oxalate into the dentinal tubules. (3000X)

Fig. 3F — Still higher magnification of an oxalate-treated dentin surface shows strands of material connecting the calcium oxalate crystal to the walls of the tubule. (6000X)

model used in this study held all of the variables in the Poiseuille relationship constant. Thus, surface area, dentin thickness, filtration pressure, and the viscosity of the fluid were all constant. Only two parameters varied; fluid flow, the dependent variable, varied directly with changes in tubular radius, the independent variable. In addition to screening a host of medicaments which have been used clinically to desensitize dentin due to their ability to occlude dentinal tubules in vivo, this study was also designed to include the evaluation of positive controls (i.e., agents that precipitate on dentin).

To understand how exogenous salts might react with endogenous ions present in dentin tubular fluid, one needs to know the composition of tubular fluid. Coffey et al.18 reported that dentin fluid contains 150 mEq/L sodium, 3 mEq/L potassium, and 100 mEq/L chloride and is similar to extracellular fluid. These authors did not, however, report the concentrations of either calcium or phosphorus even though nearly two-thirds of dentin is composed of hydroxyapatite calcified around a collagen matrix. Since hydroxyapatite is simply one form of calcium phosphate, one would expect that the tubule fluid would be in dynamic equilibrium with
the solid (hydroxyapatite) phase of the tubule walls and that the tubule fluid would be saturated with respect to calcium and phosphate. Raising either the ionized calcium or phosphate concentrations would upset the equilibrium (Fig. 4), exceed the solubility product constant for calcium phosphate, and lead to the development of a precipitate. At the tubule orifice, addition of a solution high in calcium should theoretically cause the development of a calcium phosphate precipitate. The quantity of precipitate formed, however, may be too small to reduce the functional radius of the tubule \(^{17}\) significantly. Thus, the concentration of endogenous phosphate might be rate limiting as may the rate of diffusion of the exogenous medicament in a two to four min exposure.

Strontium chloride was tested because it is the main ingredient of a widely used dentifrice \(^{6}\) for decreasing dentin sensitivity.

Although there was a small difference in the change of hydraulic conductance caused by two strontium chloride-containing medications (Strontium chloride 5.20% and Sensodyne\(^{5}\) 6.81%), only the latter was significantly different from the control. This difference possibly resulted because all of the abrasive filler in the toothpaste was not rinsed off. The same criticism can be made of the observation that Thermodent\(^{8}\) toothpaste decreased hydraulic conductance 7.07% as opposed to the effect of its active ingredient, formalin (2.57%).

Luride gel\(^{9}\) is a commercial product containing 1.23% fluoride in 0.1 M phosphoric acid. The gel is widely used to give topical fluoride treatments and to desensitize dentin. In the present work, the gel did cause a decrease in hydraulic conductance. The active ingredient of the gel is sodium fluoride, which presumably reacts with ionized calcium in the tubular fluid to form insoluble calcium fluoride (Fig. 4). The observation that Luride gel produced a smaller decrease in Lp (7.67%) than 2% neutral sodium fluoride (17.77%, Table) may be due to the fact that Luride gel is quite viscous and may have decreased the rate at which fluoride could diffuse into the tubules.

Both potassium carbonate and sodium carbonate were tested separately because they were to be combined later with calcium chloride. The rationale for their use was that the carbonate ions in these concentrated (2 M) solutions might react with the endogenous calcium in the tubule fluid to form a precipitate of insoluble calcium carbonate. Both carbonate solutions did reduce Lp significantly, although the degree of reduction was modest (Table).

Protect toothpaste\(^{11}\) contains a polyglycol as its active ingredient. The chemical is a long chain polymer which has been used in the past to precipitate proteins out of solution without denaturing them. One might speculate that its theoretical action may be to decrease dentin sensitivity by precipitating protein in the nerve receptors or to possibly precipitate mucins out of saliva which

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\(^{6}\)Block Drug Co., Inc., Jersey City, NJ

\(^{8}\)Chas. Pfizer & Co., Inc., New York, NY

\(^{9}\)Hoyt Laboratories, Needham, MA

\(^{11}\)International Pharmaceutical Corp., Warrington, PA
could adhere to the dentin surface and decrease functional tubule radii. Neither of these actions would be detected in our model system. Since we were not measuring dentin sensitivity, and there were no salivary mucins present, the decrease in Lp was presumably due to abrasive fillers in the paste partially occluding tubules.

Calcium hydroxide treatment produced a moderate reduction in fluid flow. The ionized calcium concentration produced in tubule fluid by this paste may have been somewhat higher than that produced by treatment with 2% calcium chloride. Additionally, the high pH (12-14) of the paste would tend to convert phosphate present in tubule fluid from the more soluble HPO$_4^{2-}$ and H$_2$PO$_4^{-1}$ to the much less soluble tribasic phosphate. Both of these factors may have contributed to the precipitation of calcium phosphate in the tubules. This result is of some interest because the generally accepted clinical explanation for the desensitizing effects of Ca(OH)$_2$ has been that it irritates the odontoblasts, causing them to produce irritation dentin$^{20,21}$ which has fewer dentinal tubules than normal dentin and would presumably result in decreased fluid flow.

Zinc chloride has been used to desensitize dentin,$^4$ presumably by precipitating the protein of the odontoblastic process. This explanation cannot be tested in the present model system since the dentin discs are devoid of odontoblastic processes, yet it reduced hydraulic conductances by 23.5% (Table). Even though a precipitate was not observed with the unaided eye, the zinc chloride could have reacted with endogenous phosphate in the tubule fluid to form any of several zinc phosphates, all of which are relatively insoluble.

Acidulated sodium fluoride solution reduced hydraulic conductance 24.50%, compared to neutral NaF (17.77%). The 0.1 M phosphoric acid in the medication probably decalcified the dentin, raising the ionized calcium concentration to a point where it exceeded the solubility product constant for calcium fluoride, which then precipitated on and in the tubules. While it is theoretically possible that calcium phosphate could also precipitate, the quantitative significance of this would be minor in the face of the very high (2% NaF = 0.48 mol/l F$^{-}$) fluoride concentration employed.

The combination of sodium sulfate followed by barium chloride decreased hydraulic conductance (24.33%) to a greater degree than sodium sulfate alone (3.1%), but no more than barium chloride alone (28.50%). Although no precipitate was seen with the unaided eye following barium chloride treatment alone, it is possible that barium phosphate crystals could have been formed within dentin tubules.

The combination of potassium carbonate plus calcium chloride formed a gel-like precipitate. Similarly, the combination of sodium carbonate plus calcium chloride reduced hydraulic conductance (30.75%) more than either of its constituents alone. The mean reductions in the percent change in Lp of these two combinations (sodium carbonate/calcium chloride, 31% vs. potassium carbonate/calcium chloride, 26%) were not significantly different from each other. Both of the resulting precipitates were presumably calcium carbonate.

Iontophoresis of 2% neutral sodium fluoride reduced the percent change in Lp to a greater degree (33%) than non-iontophoresed NaF (17.77%). The negative fluoride ion presumably moved down the electrical gradient created by the apparatus so that the fluoride penetrated further into the tubule than occurred by diffusion alone.

The combination of zinc chloride and potassium ferrocyanide formed a flocculent orange precipitate which was presumably zinc ferrocyanide. Scanning electron micrographs of these discs revealed a dense highly crystalline precipitate covering the acid-etched surface (Fig. 3A). Most of the crystals were too large to enter the tubule orifice (Fig. 3B), and some of the crystals formed large plate-like structures (Fig. 3B) which covered many tubule orifices without extending down into the tubules.

The use of 2% sodium fluoride (acidified) alone reduced Lp by 24.5%. The combination treatment of 2% sodium fluoride (acidified with 0.1 M phosphoric acid), followed by 2% calcium chloride, decreased the hydraulic conductance (39%) more than either of its constituents alone. Since the addition of calcium chloride reduced Lp even more, one might speculate that, even when acid is available to raise the ionized calcium level locally by acid dissolution, the calcium concentration is limiting the overall reaction(s). Presumably calcium fluoride
crystals are responsible for the decrease in hydraulic conductance observed with fluoride solutions.

Application of silver nitrate alone resulted in a 47.38% reduction in Lp compared to pre-treatment controls. It was also associated with a slight, but easily visible, white precipitate formation on the dentin surface. Presumably, the precipitate was silver chloride — the chloride coming from the dentin (i.e., the tubule fluid). When both sides of the chamber were filled with phosphate buffer (the main constituent was sodium chloride) to insure the presence of high concentrations of chloride in the tubules, application of 5.9 M silver nitrate led to the production of a heavy white precipitate of silver chloride. This resulted in a 94.67% reduction in the Lp change from pre-treatment values (Table). The SEM appearance of discs treated in this manner (Fig. 3C) does not indicate that the tubules were more than slightly occluded, yet this treatment reduced Lp more than most medicaments. Thus, in this example, there was a poor correlation between the degree of tubule occlusion revealed by SEM and the reduction in Lp.

The reduction of 5.9 M silver nitrate by formalin produced a large decrease in Lp (59.2%). This was associated with the formation of a dense black precipitate which was presumably elemental silver. Examination of these discs by scanning electron microscopy did show a heavy surface precipitate (Fig. 2E) and tubular penetration (Fig. 2F) by the precipitate.

Discs treated with potassium oxalate showed the largest reduction in Lp (98.4%) recorded in this study. While no precipitate was observed visually, SEM examination revealed a homogeneous, moderately dense precipitate covering the dentin surface (Fig. 3D). The crystal size was approximately the size of the etched tubules (Fig. 3F), and crystals were frequently observed partially occluding the tubules. Although the penetration was normally no further than the funneled portion of the tubules, the crystals occluded a large portion of the diameter of the tubules, thereby reducing their functional diameter.

The functional data represented by the changes in Lp tend to support the hypothesis that agents that can form precipitates in or on dentin should decrease the ease with which fluid can filter through dentin discs in vitro. There was not, however, a very good correlation between the amount of precipitate formed and the degree of reduction in Lp. Furthermore, the agent that reduced Lp the most (potassium oxalate) did not produce any precipitate that could be seen with the naked eye.

The SEM examination provided clues as to why different precipitates occluded tubules to varying degrees. The large crystals of zinc ferrocyanide seen in Fig. 3A and 3B are too large to fit into tubules, whereas the smaller crystals of barium sulfate (Fig. 2B) easily fall into the tubules (Fig. 2D), frequently partially occluding the tubules (Fig. 2D). It must be emphasized that reduction in Lp can be achieved by the precipitation of a single large crystal at any point along the approximately 1000 µm long tubule.

Those crystals which penetrate further into dentinal tubules might be expected to reduce Lp more than surface precipitates. The results do not support this concept, however, since crystals of barium sulfate decreased Lp more than reduced silver nitrate, even though the latter penetrated into dentin much further than barium sulfate. Calcium oxalate crystals seemed to form only on the dentin surface, yet they reduced Lp more than any other agent tested. Thus, even though the depth of tubule penetration by crystals might theoretically have a great influence on reducing fluid movement, other variables, such as crystal size, are apparently more important. Also, because SEM did not show deep tubule penetration by reduced silver nitrate (but it was seen macroscopically), SEM evaluation of depth of penetration may be deceiving due to the possibility that during the process of fracturing the disc, the precipitate was dislodged from the tubules.

Another variable affecting the ability of medicaments to alter Lp may be the manner in which they are applied. When barium chloride was applied topically for two min and then sodium sulfate topically for two min a heterogeneous surface covering of precipitate was seen on SEM (Fig. 2B). However, when these agents were applied by rubbing in for five min each, a very homogeneous covering was observed (Fig. 2C). Whether rubbing would be effective for other precipitates may depend on their crystal size. Crystal size may depend upon the rate at
which salts react with each other, the concentration of the reactants, and whether the resulting precipitate tends to restrict further diffusion of ions into the tubules. Modification in the method of application may alter the morphologic and physiologic results. The fact that some medicaments did not significantly alter the $L_p$ does not necessarily mean that they would not clinically decrease dentin sensitivity. Some of these agents may modify pulpal nerve excitation by modifying the interstitial fluid ionic environment and/or blood supply, or they may actually injure or modify nerve receptors in the predentin or pulp. The model system used in the present study was designed only to evaluate the effects of agents used for dentin desensitization on fluid movement.

Conclusions.

The use of this in vitro model system provided a rapid method of screening agents which have been used to desensitize sensitive dentin in the past, as well as to evaluate newer agents for their ability to reduce fluid flow through dentin. The data demonstrate the effectiveness of fluoride, barium sulfate, silver nitrate, and oxalate. In this regard, oxalate was the most effective agent tested.

REFERENCES