J. Soc. Cosmet. Chem., 24, 815–828 (December 9, 1973)

Interfaces of Laboratory and Clinical Assessment of Therapeutic Dentifrices

JOHN J. HEFFERREN, Ph.D.*

Presented December 11, 1972, New York City

Synopsis—The character and frequency of use has led many researchers to consider the dentifrice as a vehicle to deliver specific agents to the oral cavity. FLUORIDE-containing DENTIFRICES are an accomplished fact illustrating the success of this approach. The dentifrice oral vehicle approach requires a consumer-acceptable formulation, one that does its job of cleaning the teeth and refreshing the mouth while delivering agents to the oral cavity. Supervised brushing studies have always provided a more positive agent response than unsupervised studies, because good supervision ensures consistent adherence to study protocol. Laboratory assessment of a dentifrice formulation involves the usual physical criteria of a cleansing dentifrice, i.e., dispersion, cohesion, extrusion, flavor, etc., as well as other procedures designed to reflect the character and quantity of special agents added to the formulation. Total ion or entity content will assess manufacturing control; soluble ion content will indicate availability. Chemical availability, however, may differ from clinical availability and thus additional laboratory procedures must be designed to assess clinical availability. Whether these procedures are called *in vitro, in vivo,* or bioavailability, clinical reference should be established. The interfaces of LABORATORY and CLINICAL EVALUATIONS constitute the milieu of today's and tomorrow's products.

Introduction

The human oral cavity is a dynamic place. Air, food, and drink necessary for life pass through this orifice. It is not surprising that the soft and hard tissues of the mouth require periodic refreshment. The most general oral cleansing aid has been the dentifrice. The abrasive-detergent system of the dentifrice has been used to remove the oral debris collecting on the tooth surfaces, while the dentifrice-flavoring system provides general refreshment to the oral cavity. Dental floss, oral irrigators, and mouthwashes have also been used to clean and refresh the oral cavity.

The dynamic interaction of food substrates, salivary fluids, and the oral microbiota results in periodic dissolution and remineralization of tooth surfaces. When the balance of the interaction overshifts to dissolution, dental

*Council on Dental Therapeutics, American Dental Assn., 211 East Chicago Ave., Chicago, Ill. 60611.

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caries occurs. This carious process is not a generalized process but one restricted to specific tooth sites. At other sites, often on the same tooth, mineralization may be favored.

Tooth mortality studies in Sweden (1) have indicated that dental caries is the cause for 75% of the tooth extractions occurring in the first 25 years of life. After 50 years of age, tooth loss due to soft tissue (periodontal) disease becomes more significant, but even then dental caries remains a major cause of tooth loss.

Dental caries is probably not a single disease entity. Socransky (2) has listed four types of dental caries differentiating them on the basis of the specific site of activity and the microorganisms present at the site, i.e., smooth surface, pit and fissure, root surface (cementum), and deep dentin caries. Smooth surface dental caries requires dental plaque with good adhesive properties. A few strains of streptococci produce this adhesive dextran plaque from sucrose. Pit and fissure dental caries occurs when acid-producing microorganisms are packed into the tooth crevices. These crevices are located primarily on the occlusal or chewing surfaces of the teeth and thus the term occlusal caries is frequently used. These microorganisms have a less specific biochemical character, and because of the protective shelter of the tooth crevice they do not require plaque for adhesion to the tooth surfaces. Dental caries occurring on the root surfaces of teeth afflicted with periodontal disease may be a by-product of a somewhat different, mildly acidiogenic group of microorganisms common to the gingival crevice area. The fourth type of dental caries, deep dentin caries, may be caused by microorganisms different from those initiating the lesion in the enamel surface.

In the design of an anticaries agent, there would appear to be a number of routes and points of attack. Some of these general approaches might include: antibacterial, antibacterial metabolite, substrate (tooth structure) alteration, fluid phase (saliva) alteration, and diet control. Many of these approaches and their ramifications have been reviewed previously (3). At present, substrate alteration is most important and successful in retardation of the dental caries process. Although physical protection of the tooth surface with the use of a sealant on the occlusal surface has enjoyed some success (4), improvement of the chemical resistance to dissolution is most important.

Dietary fluoride through communal fluoridation is the most effective and efficient route to the reduction of dental caries (5). The dietary fluoride is incorporated into the tooth structure at the time of mineralization and effectively produces a more perfect structure. Subsequent topical administration of fluoride at substantially higher concentrations (dentifrice—0.1% fluoride, and professionally applied preparations—1.2 to 10% fluoride) supplement the dietary fluoride.

It should be recognized that the prime site for fluoride activity is the tooth mineral. However, the effect of fluoride in some vehicles may be due in part to

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inhibition of metabolic processes of the oral microorganisms. The development of a compatible stannous fluoride dentifrice abrasive system, which was demonstrated to reduce the dental caries rate, was one of the most significant anticaries dentifrice advances (5). Because of the vast amount of laboratory and clinical data on stannous fluoride, the various facets of stannous fluoride and its incorporation into dentifrice formulations will be used to illustrate some of the sequences and interactions operative in the assessment of a therapeutic dentifrice. Other fluoride salts will be presented as contrasts when appropriate.

Background

A thorough knowledge of the physical and clinical characteristics of the agent as a chemical entity is the basis for all investigations (6). The more complete this knowledge is, the fewer will be the surprises and unfortunate losses of time and effort later. Of particular importance are the reactions of the active ingredient at the conditions of dentifrice manufacture, storage, and use. Essential data are derived from the interaction of the active ingredient with the other ingredients in the dentifrice. With fluoride-containing dentifrices, the primary interaction occurs with the abrasive system, the major ($\approx$50%) dentifrice ingredient.

Stannous fluoride dissolves easily in water to give acidic solutions (6). Thus, from a solubility viewpoint, it could be used at high concentrations, but the acidic nature of its solutions could cause some immediate formulation and packaging problems. Why not simply neutralize the stannous fluoride solution and eliminate these problems? Stability studies examining such variables as pH, concentration, and solution content indicate that stannous fluoride is more stable in a glycerin-containing solution at an acidic pH (6). With hydrolysis of the stannous ion to insoluble hydroxides and reactions of fluoride with cations such as calcium to form insoluble compounds (7), the definition of the formulation restraints are relatively well defined, or at least well enough to make some initial formulation approaches. Needless to say, simple incorporation of stannous fluoride into a chalk dentifrice formulation would be contraindicated. The chalk (calcium carbonate) system would have a basic pH with easily solubilized calcium to effectively rid the preparation of stannous and fluoride ions (7).

Once the best formulations are developed, i.e., formulations with low or no soluble interfering cations and other ingredients compatible to the required somewhat acidic pH ($\approx$ 4.5), then routine product stability studies are indicated. In the case of stannous fluoride, the available stannous and fluoride ion concentrations would be examined as a function of time and temperature. Chemical availability is here defined as the ionic concentration obtained by preparing a slurry of 1 part paste to 3 parts water (an approximate dentifrice-use concentration ratio). The stannous ion is determined by iodometric titra-

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tion (an oxidation-reduction procedure), rather than by atomic absorption or other methods not distinguishing between tin II and IV, because the stannous ion is more active than the stannic ion (6). The fluoride ion is usually separated by diffusion and determined electrometrically or colorimetrically (6).

These analyses will give precise data on the soluble ions chemically available as a function of dentifrice formulation, pH, temperature, and time. It is important to recognize that both stannous and fluoride ions contribute to the anticaries activity (8,9). If a formulation is selected to sacrifice the stannous ions, then sodium fluoride should be chosen as the active ingredient. In fact, the stannous ion does inhibit some of the fluoride from reacting with enamel (8).

If the stannous ion stability is of prime concern, why not tie it up in a complex which will provide indefinite stability? This situation is not unlike the difference between investing money in a growth situation or placing it in a freeze. Complexes will increase stability, but this may be accomplished at the expense of clinical availability. Thus, a decision must be made in an effort to balance chemical stability and clinical availability. Admittedly the two factors are not mutually exclusive, and chemical instability would result in a lack of clinical availability. Polarography (6, 10) would be the natural laboratory tool to study the strength of complexes. However, such studies would not provide direct information on the availability of the stannous ion to dental enamel.

Clinical Evaluation

As physical and chemical data are accumulated on the active ingredient alone and in its projected formulations, it is necessary to select methodology to define the clinical availability of the active ingredient to the clinical substrate, the tooth. The selection of *in vitro* and *in vivo* test methodology to define bioavailability is dependent upon the multiplicity of variables present in the human use situation. No one test should be expected to satisfy the evaluation requirements of one or several active ingredients in suitable vehicles.

The most direct approach to the measurement of stannous and fluoride ion availability from dentifrice formulations to enamel would appear to be ion uptake studies (11). There is very little of this type of data in the published literature. The two main reasons for this situation would appear to be the difficulty of measuring ion uptake from dentifrice slurries under use conditions, (12, 13) and the availability of other methods such as enamel solubility tests, which are sensitive to the ion levels in dentifrices.

Since the prime mode of dental caries attack is acid dissolution of tooth structure, one of the most frequently used criteria for evaluation of anticaries activity has been the use of enamel solubility reduction (ESR) procedures. The types and modifications of the ESR are extensive. In a review of ESR tests and their significance to dental caries, Brudevold and McCann (14) con-

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cluded that enamel dissolution testing was useless in predicting clinical effectiveness of anticaries agents. One of the major concerns of these authors was the number of false positive results. Nevertheless, the judicious selection of operational variables integrated into a group of test procedures (14) can provide the desired information on bioavailability.

A wide variety of powdered enamel solubility tests have been described in the literature (15). One procedure useful with dentifrice slurries is that described by Gershon (16). Since the dissolution rate is dependent upon particle size (Fig. 1), powder passing a 125-mesh sieve and held up on a 225-mesh sieve was used. Enamel powder treated with dentifrice slurries was then freed of the dentifrice ingredients by simply rinsing the enamel powder on a 225-mesh sieve. Some of the problems arising with powdered enamel include: reduction of surface area due to reaction with active ingredient, decrease in particle size due to acid dissolution during the test, contamination of the powder with treatment reaction products, and difficulties in observing changes in the physical characteristics of the substrate (15). With powdered enamel as well as other substrates, it is important to determine the full dissolution profile of all treated substrates (Fig. 2). These curves are necessary for selection of specific test conditions which may be used for routine screening (Fig. 3).

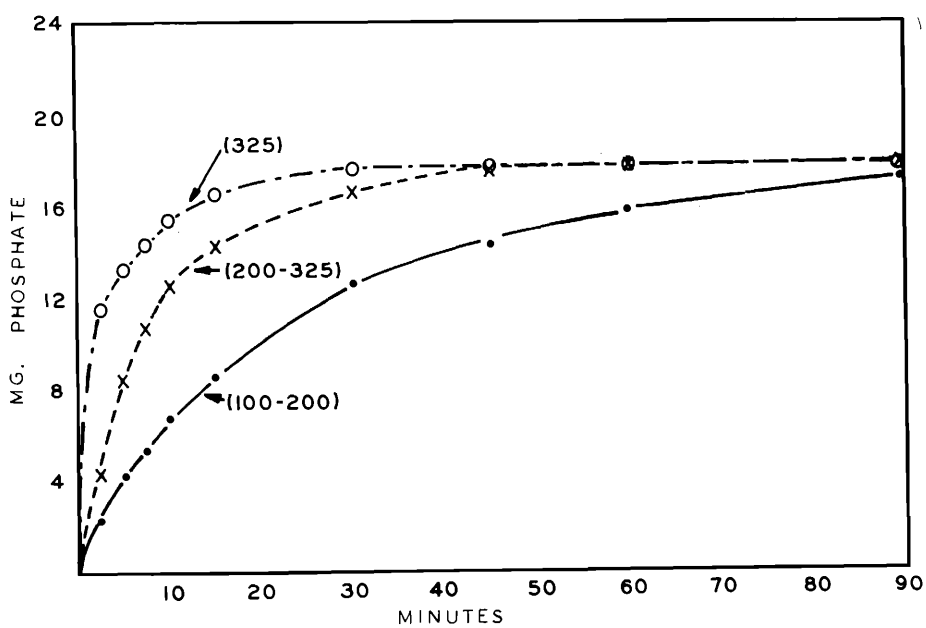

Figure 1. Dissolution curves of powdered human dental enamel with three different particle sizes, (100–200) passing 100-mesh and retained by 200-mesh sieve, (200–325) passing 200-mesh and retained by 325-mesh sieve, and (325) passing 325-mesh sieve

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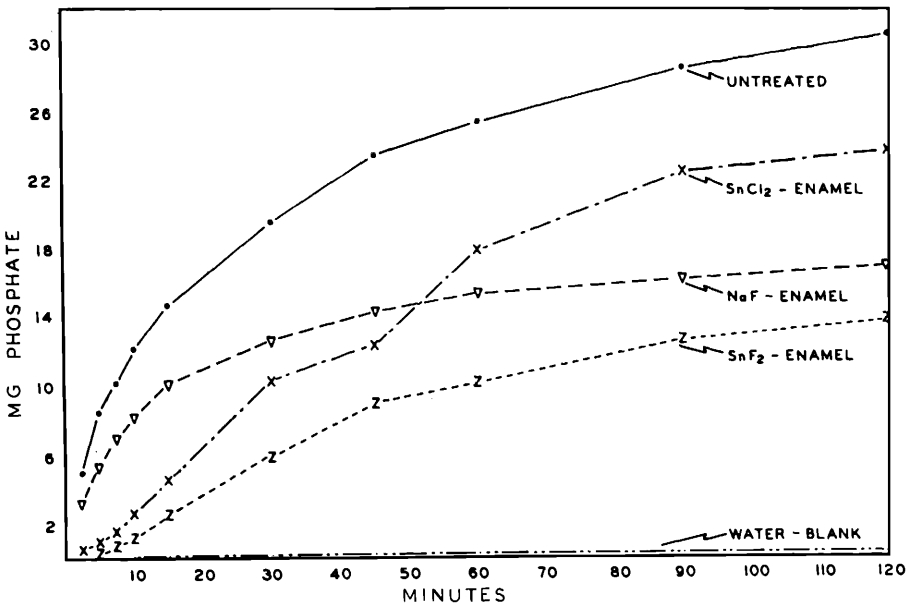

Figure 2. Dissolution curves of powdered human dental enamel treated with 0.1% fluoride and 0.3% stannous equivalent aqueous concentrations of sodium fluoride, stannous fluoride, and stannous chloride in pH 4, 0.2*M* acetate

Although the general shape of dissolution curves of powdered enamel treated with tin and fluoride compounds (Fig. 4) are similar to comparable data with intact enamel surfaces (9, 17), the reaction rate and extent of reaction are substantially different. Partially demineralized enamel (white spot enamel) has been used as the prime substrate for enamel solubility reduction tests of dentifrice slurries. The test (18) described in the Methods section responds to the stannous and fluoride ions in the concentrations available in dentifrices (9). By utilizing the etched enamel surface solubility as a base line, multiple tests can be run with the same set of teeth, providing the teeth are conditioned (etched) between tests to remove all traces of previous treatments.

In vivo enamel solubility tests have been suggested as providing evidence of protection against acid attack in the mouth (19, 20). These tests are useful in confirmatory studies but the physical nature of the test makes it difficult for detailed studies.

Considerable effort has been spent in developing procedures for the study of anticaries agents in experimental animals (21). The albino rat is the most frequently used animal with the cotton rat and Syrian hamster distinct second and third choices. The experimental animal has been particularly useful in determining the effect of microorganisms and diet on the caries process. By utilizing particular strains of caries-susceptible rats and innoculating them

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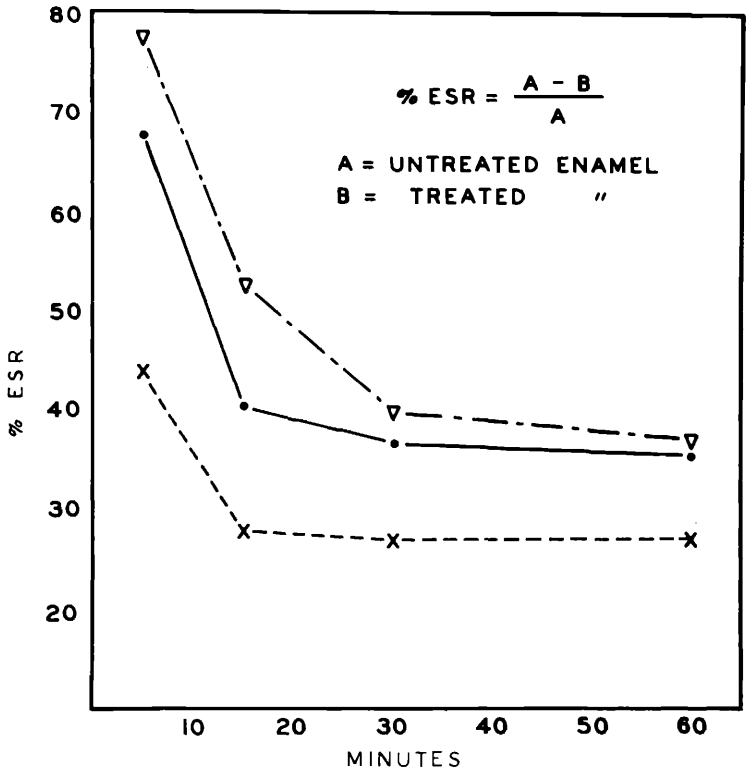

Figure 3. Dissolution curves of powdered human dental enamel treated with dentifrice-water (1 : 3) slurries in pH 4, 0.2*M* acetate buffer

X — — — X Control
• ——— • Stannous fluoride paste A
Δ — — — Δ Stannous fluoride paste B

with known caries-producing microorganisms, the reproducibility of animal studies in defining anticaries activity is increased (21).

After complete physical and chemical characterization of the active ingredient alone and in its dentifrice formulation, demonstration of anticaries activity for the formulation in experimental animals, and determination of bioavailability by a combination of carefully chosen *in vitro* and *in vivo* tests, the question arises of what else must be done to prove anticaries effectiveness. The immediate response has been two, or better three, clinical studies lasting 3 years with several hundred children. Even this quantity of clinical evidence may not be adequate for a new type of active ingredient. The initial review of the stannous fluoride dentifrice data was based upon seven clinical studies (22). How much data is required for minor modifications of an existing dentifrice system which has already been demonstrated to be clinically effective?

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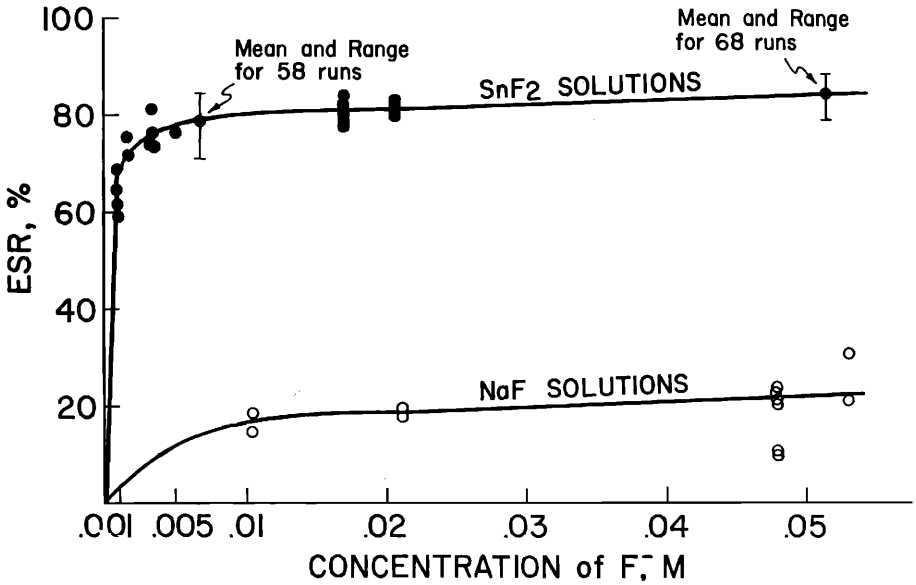

Figure 4. Dissolution curves of intact human dental enamel treated with sodium and stannous fluoride solutions [from (9)]

Certainly if clinical studies are required to verify laboratory documented improvements of the basic dentifrice system, few if any of these improvements will be made or utilized.

Because of the widespread availability of anticaries agents, it is becoming increasingly difficult to conduct a clinical study. It will become more difficult to establish clinical anticaries activity. For this reason, continued emphasis must be placed on the design of the clinical study to maximize the information received and clinical studies should be undertaken only when the need is indicated. There must be greater reliance upon definitive laboratory studies. The basis for this greater reliance upon laboratory studies is the correlation of experimental data of the new product with the clinically established product. Comparison with activity of the stannous fluoride dentifrices seems indicated.

Laboratory Evaluation

Laboratory data on sodium fluoride and monofluorophosphate are less extensive than those for stannous fluoride and probably less impressive. Since these compounds have been demonstrated to be clinically effective (5) with an activity somewhat comparable to stannous fluoride dentifrices, then new laboratory procedures must be developed to assess this activity.

The following methods have been useful in characterizing dentifrice formulations containing stannous fluoride. The enamel solubility test is a good meas-

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ure of the bioavailabilty of stannous fluorde because of the correlations with clinical and other studies using different types and ages of stannous fluoride formulations (23).

Methods

Chemical Analysis (24)

A slurry is prepared of 10 g of paste and 30 ml of water in a 50-ml centrifuge tube using a stirring rod fitted with polyethylene tubing cut in strips to facilitate mixing. Stirring follows for 2 min or until a uniform slurry is obtained. The tube is capped and centrifuged at 15,000 rpm for 5 min (total time about 15 min.) to obtain a clear supernatant.

For fluoride ion analysis (6, 13), an accurately measured aliquot of the supernatant or dilution thereof equivalent to about 1-2 μg of fluoride is transferred to a suitable diffusion vessel (25, 26) and the fluoride ion is separated from the interfering ions. The diffused fluoride is collected and determined colorimetrically using the SPADNS reagent or fluoride electrode. Total fluoride concentration can be determined by fixing the paste with alkali, ashing, and determining the fluoride by diffusion–colorimetric or electrode procedure (24). For stannous ion analysis (7), an aliquot of the supernatant equivalent to about 3 mg of stannous ion is transferred to a titration assembly and is titrated potentiometrically using a platinum combination electrode and 0.005*N* iodate reagent. Total stannous and tin concentrations in paste can be determined by dissolving the paste in 6*N* hydrochloric acid and titrating a diluted aliquot with iodate reagent (24).

Enamel Solubility Reduction

In general, it is convenient to run enamel solubility tests in groups of four. Twenty-four caries-free teeth which have a minimum number of defects are selected. Teeth with larger enamel surface areas are preferred. These are prepared for mounting by scaling the enamel surface with a suitable dental instrument and pumicing lightly using the rubber cup procedure.

Preparation of Tooth Mounts (Fig. 5)

Dental stone is mixed and transferred with gentle tapping to remove air bubbles to an aluminum pan (55 mm in diameter x 17 mm deep*) to a depth of about 12 mm. The bottom and sides of a 50-ml beaker (o.d. 42 mm)† are coated with petrolatum and forced into the dental stone to a depth of about 10 mm. After the dental stone has reached a final set, the beaker is removed.

*S-25725, Sargent-Welch Scientific Co., Skokie, Ill. 60076.
†No. 1000, Corning Glass Works, Corning, N.Y. 14830.

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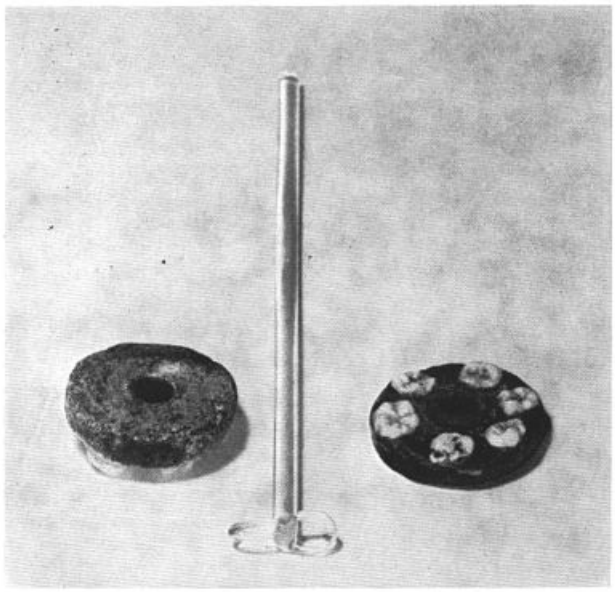

Figure 5. Tooth mount

The cavity area of the dental stone is coated with petrolatum and a 5-mm length of 10-mm i.d. polyethylene tubing is placed in the center of the cavity. Acrylic monomer and polymer are added around the tubing to a depth of about 3–4 mm. When the acrylic hardens to the dough stage, 6 carious-free crowns obtained by cutting mechanically extracted teeth at the cemento-enamel junction are spaced equally around the polyethylene tubing. The crowns should be immersed to a depth of 1–2 mm to achieve adequate adhesion and overall uniformity of crown height. After the acrylic has hardened (20 min), the "acrylic donut" with the embedded crowns is removed and the acrylic is coated with a thin layer of hard inlay wax. The excess wax is carved from around the necks of the crowns. A suitable instrument such as a dental excavation spoon is heated with a small flame and wax is flowed into the occlusal pits and fissures of the mounted crowns. Throughout the procedure, care should be taken to minimize the drying of the crowns.

Dissolution Apparatus (Fig. 6)

A 180-ml tall form beaker (Corning #1140) is prepared to support the wax-covered donut-like mounts by making 4 equidistant 5-mm indentations about 30 mm from the bottom. This can be done by heating the area with an oxygen-air torch and pushing a metal probe such as the tip of a rat tail file into the beaker wall to the desired depth. This beaker is fitted with a glass stirrer (Sargent #5-76667-A) cut to a shaft length of about 130 mm and a

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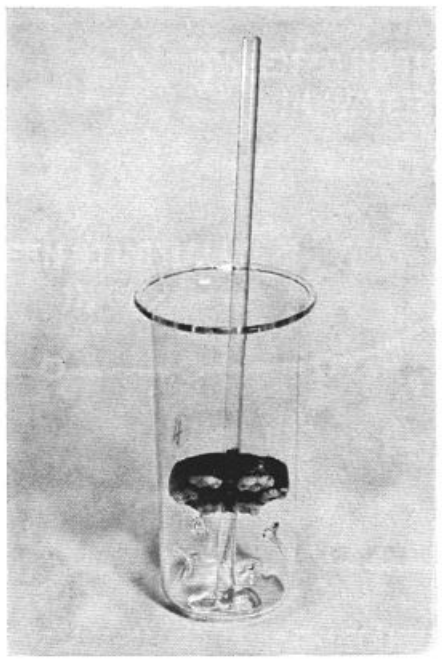

Figure 6. Assembled dissolution unit

constant-speed 1800-rpm motor.* The glass stirring rod is attached to the motor with about 45 mm length of 6-mm i.d., 16-mm o.d. pressure rubber tubing. It is convenient to make up dissolution units in sets of four which are mounted in a 37°C constant temperature bath (Fig. 7).

Lactate Buffer

To a 2-l. volumetric flask is transferred 211 g (2 moles) of 85.2% lactic acid, analytical reagent grade, and 400 ml of water is added. Next, 84 g (2.07 moles) of sodium hydroxide, analytical reagent grade, is dissolved in 600 ml of water and transferred completely to the lactic acid–containing volumetric flask. If the pH of the solution is not above 10.5, 1 g of sodium hydroxide is added and the pH is measured after standing 30 min. The solution is then diluted to 1 l. with water. Next, 211 g of 85.2% lactic acid reagent is transferred to a 2-l. volumetric flask and diluted to the mark with water to give 1*M* lactic acid. To a 3-l. beaker is transferred the 2 l. of 1*M* sodium lactate solution and about 425 ml of the 1*M* lactic acid solution is added to bring the pH of the mixture to 4.50. It is preferable to age this solution about a month or more to reduce the lactic acid polymer content. The 1*M* lactate buffer should be diluted

*Dayton 1/100 H.P. shaded pole #4K903, Dayton Motor Co., Dayton, Ohio.

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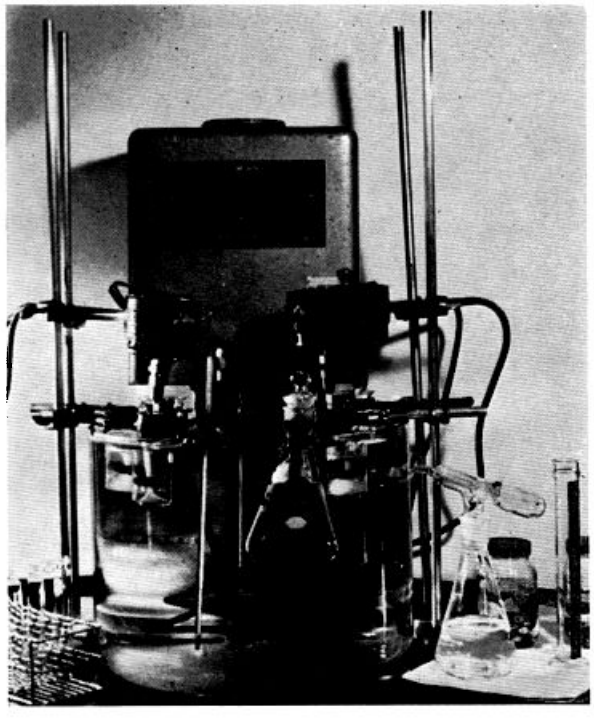

Figure 7. Constant-temperature bath with four dissolution units

tenfold just prior to use. Prolonged storage of the 0.1*M* lactate buffer may result in mold growth.

Conditioning of Mounts

Each new tooth mount is conditioned in the dissolution apparatus with 40-ml portions of 0.1*M* pH 4.5 lactate buffer for 2 one-hour periods, with fresh buffer solution added after one hour of conditioning. Immediately after each enamel solubility test, the individual mount should be conditioned for 2 one-hour periods. At the time when no further tests are contemplated on the same or successive days, the mounts are conditioned for 2 one-hour periods. Since there is the danger of effects from previous treatments, it is very important to condition the teeth properly before each test. Most mounts can be used for 10 individual tests before discarding.

Enamel Solubility Test

After proper pretest conditioning, the conditioned tooth mount is etched in the dissolution apparatus with 40 ml, accurately measured, of 0.1*M* pH 4.5 lactate buffer for 15 min at 37°C to determine the pretreatment solubility. A

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reservoir of lactate buffer is maintained at 37°C for pre- and post-treatment use. The apparatus is disconnected; the lactate etch solution is collected and saved for calcium and phosphate analyses. The mount, stirrer, and beaker are thoroughly rinsed with purified water, the rinse is discarded, and the apparatus is reassembled. A 20-ml mixed slurry of 10 g of paste and 30 ml of purified water is added. The mount is treated for 5 min at 37°C with 20 ml of the uniform 1 : 3 aqueous slurry of the paste (10 g of paste and 30 ml of purified water thoroughly mixed with flared plastic tipped stirring rod).

The apparatus is then disconnected, the treatment mixture is discarded, and the mount is thoroughly rinsed with purified water to remove all traces of the treatment mixture. It is important that this rinsing step be thorough for traces of treatment paste will inhibit post-treatment etching. The apparatus is reassembled and the pretreatment exposure of the mount is repeated, in exact detail, to 40 ml of 0.1*M* pH 4.5 lactate buffer for 15 min at 37°C. The apparatus is disconnected; the lactate etch solution is collected and saved for calcium and phosphate analyses. The mount, stirrer, and beaker are then thoroughly rinsed with purified water. The rinse is discarded, the apparatus is reassembled, and the mount is conditioned with lactate buffer in order to prepare it for another enamel solubility test. The concentration of phosphate and calcium in the pre- and post-treatment lactate etch solutions are determined using the Lucena-Conde molybdate method and atomic absorption spectrometry. The per cent enamel solubility reduction (ESR) is calculated as follows:

$$\% \text{ ESR} = \frac{A - B}{A}$$

A = mg of phosphate/calcium in pretreatment etch

B = mg of phosphate/calcium in post-treatment etch

Summary

The dentifrice formulation which helps clean teeth and refresh the mouth has been used to bring anticaries agents to the mouth. Laboratory tests can be used to assess the compatibility of the formulation with the agent and the availability of the agent to the teeth. Correlation of laboratory tests with the results of clinical studies of the same and comparable agents permits a more meaningful interpretation and extrapolation of laboratory data and may reduce the future need for clinical studies.

(Received February 1, 1973)

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