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The laboratory evaluation of prophylactic dentifrices

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Synopsis—The need for laboratory methods of assessing dental prophylactic products is discussed and a review of some of the methods used to evaluate fluorine containing toothpastes given. The techniques mentioned include *in vitro* and *in vivo* solubility studies using chemical, physical and electron microscopical techniques to evaluate the action of the products. The advantages of establishing *in vitro* methods which do not depend directly on acid solubility, e.g. some form of artificial mouth, are explained.

Introduction

From writings which have survived from the Ancient World it is apparent that dental products designed to care for teeth have been made by man from earliest times. In Chinese and Indian records of ca. 3000 B.C. and in Egyptian manuscripts of ca. 1500 B.C. there are references to dental topics including anatomy, treatments with drugs and by acupuncture, dentifrices, etc. The Romans were also versed in such matters and Pliny records the formula for a dentifrice which includes the ashes of oxen hooves, myrrh, burned eggshells, pumice, etc. The formulator of today may not recognise all of these as ingredients which he might want to use in toothpaste but such a mixture is likely to have an abrasive, and therefore cleaning, action on teeth. That cleaning the teeth is desirable for other than social reasons has been shown by Fosdick (1) and Mansbridge (2) who found that children about 12–14 years of age, practising good oral hygiene, i.e.

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regular tooth brushing, experienced a lower caries incidence than those with poor habits in this respect.

The greatest contribution so far made by manufacturers of dental products has been to provide the public with toothbrushes and dentifrices which are reasonably efficient and pleasant to use. However, with increase in knowledge and in sophistication additional attributes, such as sweet breath, healthy gums and freedom from caries, have been claimed. These have been based, usually, upon some scientific fact or hypothesis but their efficiency has not necessarily, in the past, been proved rigorously. A genuine desire on the part of the manufacturer to make his product as effective as possible and the explicit demand of bodies such as the Independent Television Authority that his claims be related to user experience has resulted in industrial organisations undertaking *in vitro* and *in vivo* studies on problems of oral health and hygiene.

The only satisfactory method for testing products designed to improve dental health is a clinical trial. Because the changes achieved by prophylactic agents may be difficult to assess and indeed may be small, large numbers of subjects are required if a statistically significant result is to be obtained. Thus a full scale clinical trial to demonstrate, say, the effectiveness of a caries inhibitory agent will be expensive, not only in terms of time and money, but also in the numbers of highly trained research workers required to conduct it. The thorough screening of potential agents by whatever laboratory means are available or can be devised, is therefore essential if we are to be sure that these expensive, cumbersome and sometimes scarce facilities are used to the best advantage.

The crucial factor, of course, is the selection of appropriate tests, the results of which will correlate with user experience. In this field the results which will permit correlation are acquired only slowly and so at the start of a project the experimenter is left with the problem of selecting tests which, on his knowledge at that time, are likely to be most relevant to the mode of action of the active ingredient. As this knowledge is often scanty, a battery of tests to cover all possible modes of action, whether likely or not, may be the safest way of dealing with this problem.

A further requirement for laboratory methods is the need to test hypotheses which might involve the use of materials which could never be employed in the mouth for reasons of toxicity but which might establish principles and therefore, lead to the development of safe derivatives.

Prophylactic toothpaste means any product which has a specific action

against caries, gingivitis, calvulus, etc., but only products intended to inhibit caries are considered here.

To test the caries inhibiting properties of a product efficiently two requirements are necessary:

(a) Knowledge of the aetiology of the carious process.
(b) Knowledge of the mechanism by which the product is intended to interfere with this process.

If these were known, specific tests could be designed to check that the product does, in fact, perform its allotted function. Although Miller's acidogenic theory of caries is largely accepted as being true, the detail is by no means established.

However, the wide acceptance of the acidogenic theory and the undoubted effect of fluoride in reducing dental decay has led to the popularity of the study of enamel solubility in acid in the investigation of potential cariostatic agents.

Enamel solubility

In essence the method is simple in that the solubility of enamel in a suitable acid buffer – usually lactate or acetate – before and after treatment with the test material is determined. Practical difficulties arise, however, in that no two teeth seem to behave in the same way even when efforts are made to standardise conditions. The use of ground enamel, prepared by the method of Manly and Hodge (3) was introduced to overcome this problem.

By this technique a large batch of ground enamel, free from fines and graded by sieving to give a standard material, can be prepared and its solubility characteristics in acid buffers determined accurately. A large number of tests can then be run on the rest of the batch of enamel. Further, if a suitable mesh size is selected (e.g. 25–72 mesh) the enamel can easily be separated from toothpaste solids by sedimentation and this avoids tedious filtration procedures. In determining solubility changes, some of the early work was performed on a weight-loss basis, e.g. Manly and Bibby (4), but most studies now depend on a determination of calcium, usually by a microtitration with ethylenediamine tetraacetic acid (EDTA), or of phosphorus, by a colorimetric procedure. Radiotracer techniques have also been used (5).

Numerous criticisms and drawbacks to powdered enamel are apparent, e.g. a large surface area is exposed compared with intact enamel and

agitation during treatment may cause attrition of the enamel pieces with the result that minute particles appear in suspension: these are difficult to remove and affect results. The use of intact enamel would certainly be more realistic.

Of the many studies made to investigate the solubility characteristics of tooth enamel, those of Gray (6, 7) in which he showed that the rate of solution is predominantly diffusion controlled, are probably the most detailed. Using pieces of enamel of constant area and by careful control of the experimental conditions, particularly agitation, he proved that sound enamel from different teeth dissolved in acid buffer at a similar rate, provided the surface enamel was first removed. This is necessary because of adsorption or incorporation into surface enamel of salivary calcium phosphate, salivary organic material and numerous trace elements which tend to reduce its solubility.

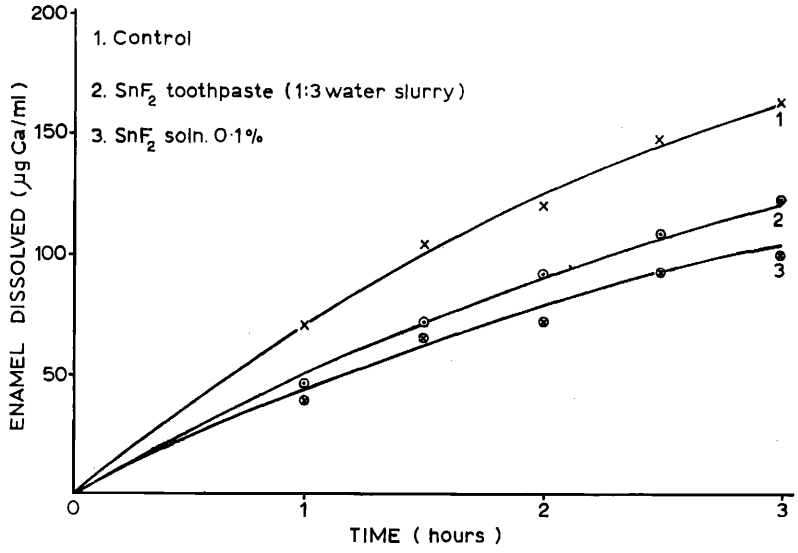

Figure 2. Solubility of untreated and treated tooth enamel (continuous immersion in acid).

The equipment shown in *Fig. 1* was constructed to carry out this type of work and it consists of a constant speed motor (1300 rpm) geared to drive six *Perspex* rods. The tubes which hold the acid have a baffle at the bottom of each to limit vortex formation. The apparatus spans a constant temperature water bath so that the lower two thirds of the tubes are

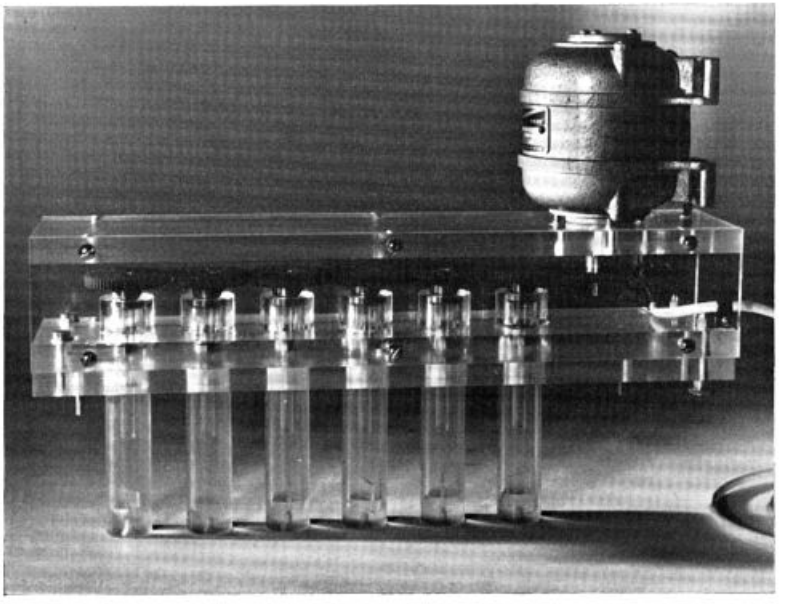

Figure 1. Apparatus used to determine the solubility of intact tooth enamel.

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immersed. The pieces of enamel are attached to the ends of the stirrers with blue inlay wax, only the required area of sound enamel being exposed.

The results obtained with the equipment are shown in *Fig. 2.* The upper curve is that for untreated enamel while the lower one, indicating a lower solubility rate, is for enamel treated with a 0.1% solution of stannous fluoride. The third line represents the effect of a stannous fluoride toothpaste.

A slightly different technique, also mentioned by Gray, is that of immersion of a tooth or piece of tooth in successive aliquots of acid buffer.

Fig. 3 is reproduced from Gray's paper and it is apparent that the protection provided by SnF_2 survived only the first two exposures to acid.

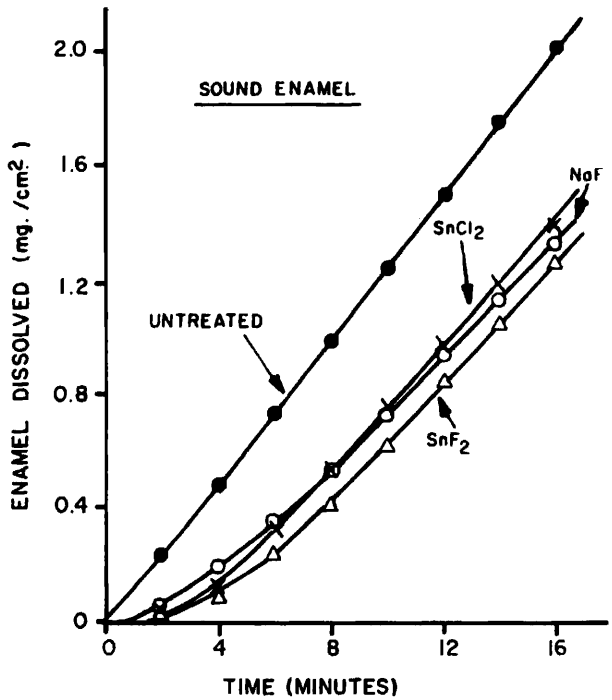

Figure 3. Solubility of untreated and treated tooth enamel (successive immersion in acid).

Thereafter the slopes of this and the control sample become parallel, indicating that the rates of solution are the same and that the protective effect of SnF_2 is confined to the surface of the enamel.

Electron microscopy

The application of electron microscopy to the assessment of dental products provides a fascinating insight into what is happening on the tooth surface although interpretation of the results requires considerable care.

Fig. 4 shows a polished tooth surface which, in this case, is rather featureless except for surface scratches. *Fig. 5* is of a similar tooth after exposure to 0.2M lactate buffer (pH=4) for 15 min and shows a roughened, etched appearance. The enamel prisms are very obvious. *Fig. 6* shows a tooth surface, half of which was masked during immersion in sodium fluoride solution (0.2%: pH=4). The masked area can be seen to be similar to the polished surface while the treated area is covered with a deposit of calcium fluoride crystals. This is undesirable as it means that erosion of the enamel is taking place. This is apparent in *Fig. 7* where the sodium fluoride treated area has been brushed to remove the calcium fluoride and reveal the etched region below. Treatment with stannous fluoride on the other hand (*Fig. 8*) results in no apparent change in the enamel structure and exposure to lactate buffer now has apparently little effect (*Fig. 9*). However, if this surface is now brushed with a toothbrush and re-examined it is seen that etching has occurred (*Fig. 10*). Thus stannous fluoride has deposited a film, probably of hydrated tin oxides, which is quite adherent to the tooth because it can still be found after several replicas have been taken from the one area but which is permeable to acid. *Fig. 11* shows a tooth in which part of the enamel was masked while a stannous fluoride treatment was given to the exposed area. The mask was then removed and the whole surface exposed to lactate buffer. The micrograph was taken by a reflectance technique, i.e. the elctron beam was directed at an angle onto the enamel surface and the reflected electrons produced the micrograph. There is obviously a marked difference between the etched surface and the stannous fluoride treated surface. The critical brushing experiment has not been done so that it cannot be said with certainty that the 'cliff' effect is not due entirely to the film of hydrated tin oxides; the other chemical evidence strongly suggests that this film reduces the rate of penetration of the acid to the tooth surface.

Fluoride in enamel

The pattern of distribution of fluoride in tooth enamel has been studied by a number of workers. Early results were based on analyses performed

Figure 4. A polished enamel surface.

Figure 5. A polished enamel surface after etching with 0.2M lactate buffer (pH 4) for 15 min.

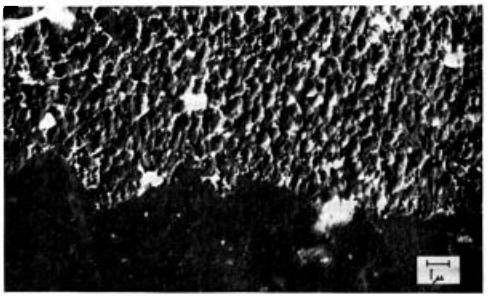

Figure 6. Polished enamel surface; the lower half was masked while the upper was exposed to 0.2% NaF.

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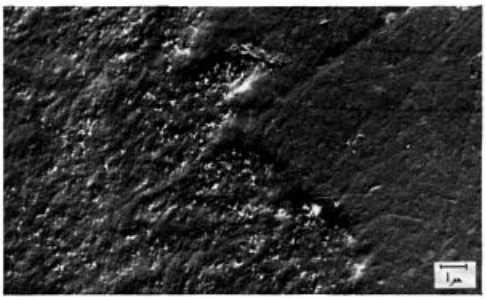

Figure 7. Similar preparation to that in *Fig. 6* after brushing with a toothbrush. The sodium fluoride treated area is on the left of the micrograph.

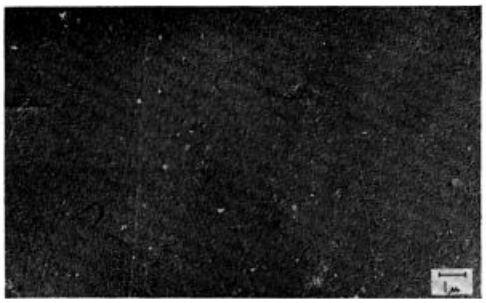

Figure 8. Polished enamel surface after exposure to 0.1 % aqueous SnF_2 solution.

Figure 9. Polished enamel surface after treatment with 0.1 % SnF_2 solution and immersion in lactate buffer (0.2M, pH=4).

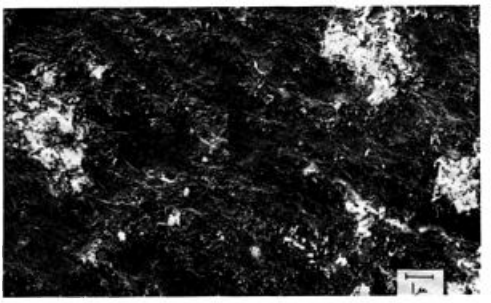

Figure 10. Preparation shown in *Fig. 9* after brushing.

Figure 11. A reflectance electron micrograph of an enamel surface. The area to the right was exposed to a 0.1 % SnF_2 solution, that to the left was masked. The complete tooth was then exposed to lactate buffer.

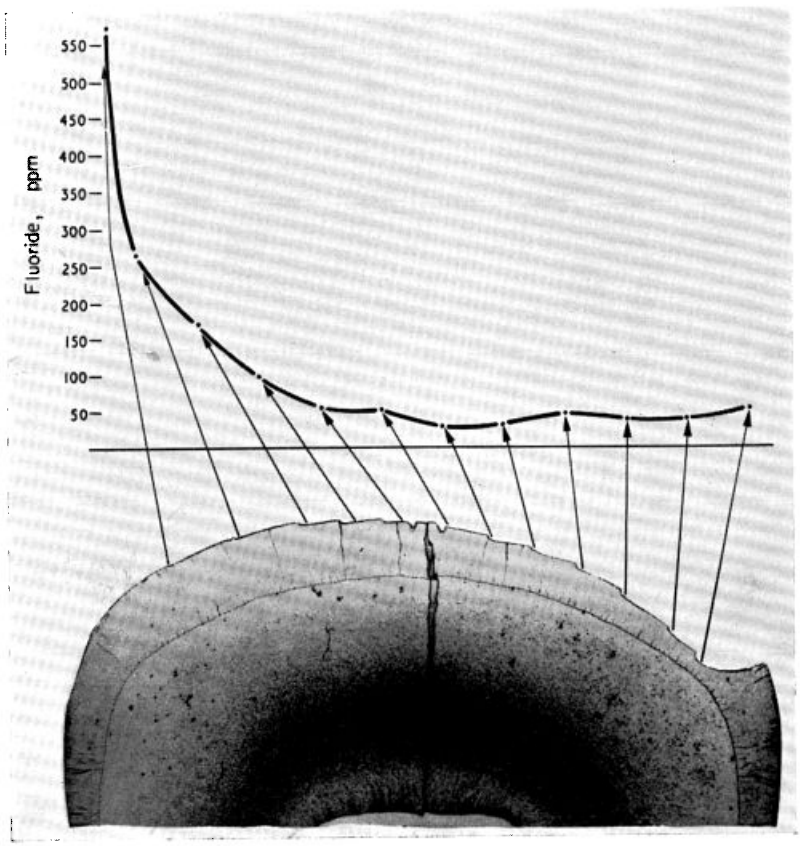

Figure 12. Distribution of fluoride in the labial enamel of a permanent incisor (14).

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on bulked enamel which suggested that the average fluoride content of enamel is low but more refined techniques used by Jenkins and Spiers (9), Brudevold *et al* (10, 11), Spiers (12), Weatherell and Hargreaves (13, 14) and Mühlemann (15) have proved that fluoride acquired by enamel from natural sources is concentrated in the outer layers.

The technique devised by Weatherell and Hargreaves is elegant and worthy of description. A tooth is covered with nail varnish except for the particular area on which fluoride is to be determined. This can conveniently take the form of a rectangular area on, say, the labial surface of an incisor. A small spot of nail lacquer is also placed within the test area.

The area is then dipped in strong (6N) perchloric acid for a predetermined time (e.g. 5 sec), rinsed with a drop or two of water and dried. A second spot of nail varnish is applied beside the first one and the etching process repeated in fresh acid. This sequence of masking and etching is repeated as often as desired. A section cut through the row of spots gives a histological picture from which can be derived the depth of each layer from the surface. The amount of fluoride in the acid solutions is determined colorimetrically using the SPADNS reagent of Wharton (16), provided the fluoride is first separated from the components of the tooth mineral also present. This can be accomplished very neatly using the Conway diffusion principle.

Fluoride in strong perchloric acid will volatilise as hydrogen fluoride and can be absorbed quantitatively in an alkaline medium. Although numerous variations have been proposed, the original Conway design of diffusion cell, provided it is made from an unreactive material such as polyethylene or better, polypropylene is probably the most convenient apparatus in which to carry out the diffusion. Sodium hydroxide solution (about 1.5N) is placed in one well and the acid fluoride in the other. The lid, suitably greased to prevent leakage, is fitted on top and the unit is then incubated at 60°C for 24 hr during which time the fluoride is transferred quantitatively to the alkaline reagent. The colorimetric procedure can then be carried out on this solution. The amount of fluoride found can be related to the amount of enamel dissolved at each stage either by determining phosphorus in the acid residue from the diffusion stage or by weight loss.

The results quoted by Weatherell show that 500 to 2500 ppm of fluoride may be present in surface enamel and that this dwindles to about 50 ppm in the deeper layers. There is a nice correlation in Weatherell's results between depth of the etch as seen on the photomicrograph and fluoride content (*Fig. 12*).

This technique is obviously applicable to the study of the possible uptake of fluoride by enamel from a fluoride-containing product and some preliminary results which were obtained to prove the feasibility of the method can be described.

Upper central incisors free from caries were selected and cut in half, one half to act as control and the other as test. The test halves were attached to the circumference of a disc fitted to the end of a stirrer spindle. When the stirrer motor was switched on, the test surfaces were arranged to brush lightly against the tips of toothbrush bristles set in the bottom of the beaker which contained the test solution. In the results which follow, the treatment was continued for 24 hr in an effort to ensure that some fluoride would be incorporated in the enamel.

A light brushing with distilled water was given before the fluoride determination was carried out as described above. Three consecutive 10 sec etches were given and the fluoride in each determined. In some experiments

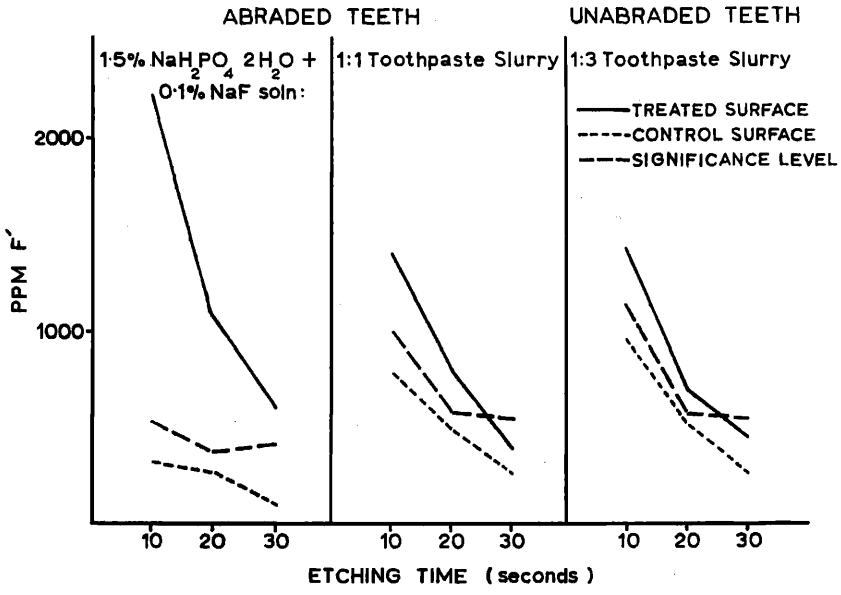

Figure 13. The uptake of F^- on enamel surfaces.

the surface of the teeth was abraded to remove the layer of high fluoride content and so help to achieve more consistent results. Typical results, the average of six teeth per treatment, are shown in *Fig. 13*. The treatment in the first experiment was with a sodium fluoride/sodium phosphate

solution as suggested by Brudevold (17). This is obviously more effective than when incorporated in a dentifrice though even then there is a significant uptake compared with the controls. The dotted line indicates the fluoride level above which the test teeth have acquired a significant increase in fluoride.

Tin uptake by enamel

A similar type of experiment can be performed to demonstrate the presence of tin derived from stannous fluoride preparations though the analytical procedure is not so simple. The technique of electron probe microanalysis is applicable and the results can be obtained either as a photograph in which the atoms of tin appear as white spots on a dark ground or as a recorder trace obtained as the electron beam traverses the selected area. An example of the latter is shown in *Fig. 14*. A polished

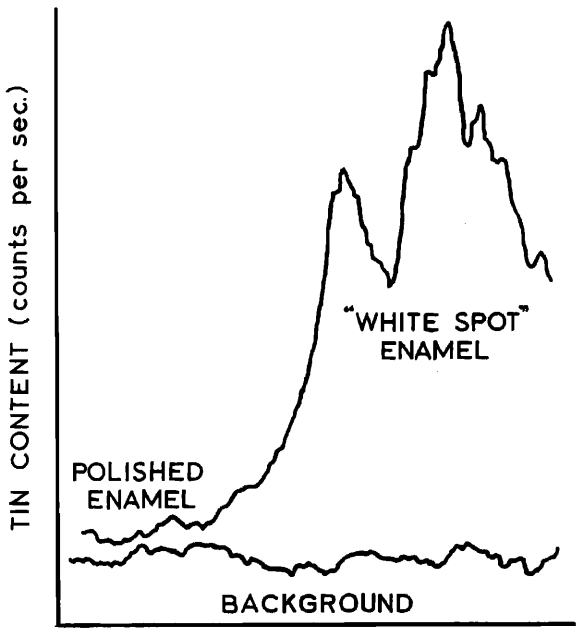

Figure 14. Tin uptake on polished and on etched enamel.

tooth surface was etched with dilute acid to produce a white spot. A stannous fluoride treatment was given and, after rinsing, the area was

scanned. The peaks on the trace indicate the presence of tin on the tooth surface and it can be seen that greatest uptake occurs in the white spot area. This may well be due to the increased surface available on this etched region rather than to any specific affinity of white spot enamel for tin.

In vivo methods

All the tests mentioned are test-tube experiments and however much the conditions are varied in an effort to test the permanence of the treatment, they give no unequivocal indication of what will happen in the mouth.

Walter (18) was the first to describe a feasible technique which went some way to answering this criticism, in that during the period of the trial, the treated teeth were exposed to all the dynamic conditions normally present in the mouth. His test was again dependent upon solubility changes of tooth enamel but on humans it was conducted as follows:

A 2mm diameter filter paper disc impregnated with an acid base indicator and with 2μl of 0.3N HCl, was placed on the selected tooth surface after it had been dried with a tissue. The time taken by the indicator to change colour (Colour Reaction Time) is a function of the tooth's 'resistance' to the acid attack. In a test using a panel of school children Mühlemann (19), using this method, reported the effectiveness of certain amine hydrofluorides.

In our hands this test gave a positive result for a topical stannous fluoride treatment but even with half the amount of acid used by Mühlemann it had a (literally) marked effect on the subjects' teeth and had to be abandoned.

An alternative method was then developed in which a weak acid was used in place of hydrochloric acid and the change in pH was recorded after a predetermined time. The technique is described by Middleton and Holmes (20) and Morley and Holmes (21), and depends upon clamping a well to an upper central incisor so that leakage cannot occur (*Fig. 15*). With the well clamped in position, 0.1 ml acetic acid (pH=3.4) is added from a syringe and is stirred by a stream of bubbles from the air line during the 3 min of the test. At the end of this time the air stream is stopped and a sample of the acid is sucked into the microcapillary glass electrode for pH measurement. This is a rather elaborate micro-electrode supplied by Messrs. Pye of Cambridge.

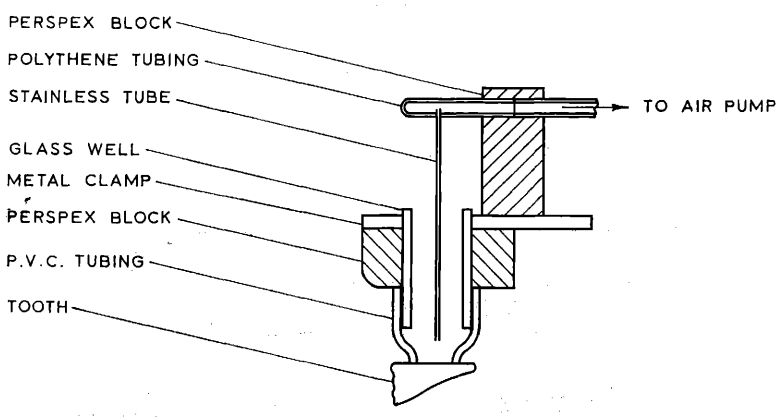

Figure 15. Apparatus used to maintain a weak acid solution in contact with a tooth surface

The results obtained from a panel of about 50 people, who used a proprietary stannous fluoride toothpaste and a control, are illustrated in *Figs. 16–19*. *Fig. 16* shows the design of the panel test. All used the control paste first and, after 5 weeks, base line measurements were made. Half the subjects were then given the test paste while the remainder continued with the control paste and measurements were taken after 4 to 8 weeks. The pastes given to the two groups were then interchanged and after a further 4-6 weeks, final measurements were made.

The results for the subjects on the sequence–control, fluoride, control are given in *Fig. 17* and it is evident that the rise in pH is much less after the use of the test paste and that after a further spell on the control paste, there is a trend towards the original values. For those on the sequence-control, control, fluoride (*Fig. 18*) the trend continues towards small decreases in rise of pH at each stage. The overall effects can be summarised in graphs of the population distribution of pH values (*Fig. 19*). The shift in the peak populations towards lower pH values denotes a reduction in apparent enamel solubility. Statistical analysis proved these differences to be significant.

Herd and Overell (22) report results obtained for a monofluorphosphate paste using a somewhat similar well to maintain acid in contact with a tooth but their estimate of the amount of enamel dissolved was based on phosphorus determinations. They claimed a significant reduction in enamel solubility after one brushing with the test paste.

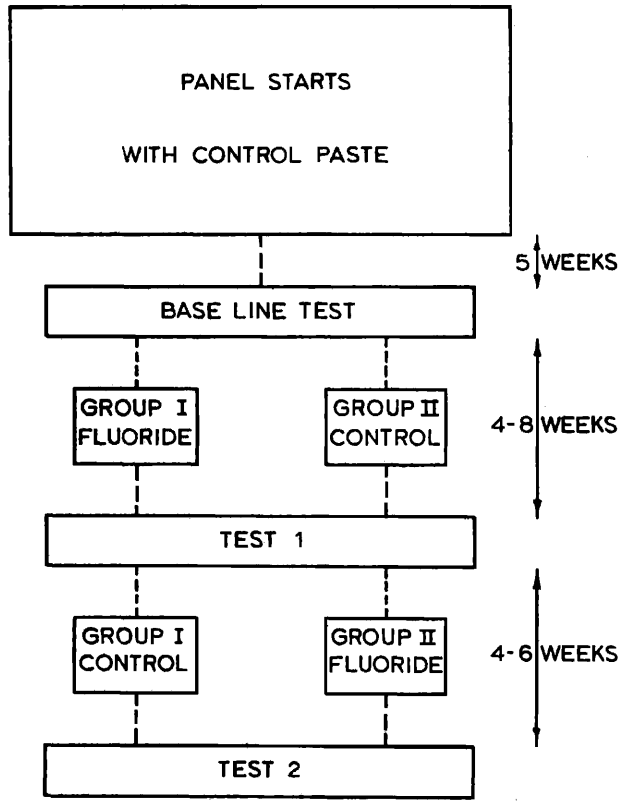

Figure 16. Design of panel trial.

Most of the tests mentioned depend upon assessing changes in enamel solubility. There are probably numerous reasons why this principle is so popular, e.g. Miller's acidogenic theory of caries emphasises the destructive role of acid; naturally occurring fluoride affects the structure of teeth – excessive amounts cause mottling – and these teeth are more resistant to caries. However, some agents have been found which produce a marked reduction in solubility, e.g. lead, indium and zinc, but which have no effect on caries (23). The ability of fluoride to reduce enamel solubility is well documented, but the really critical caries inhibiting effect may not be this at all. Indeed, Jenkins (24) quotes a number of ways in which fluoride could inhibit the carious process, viz:

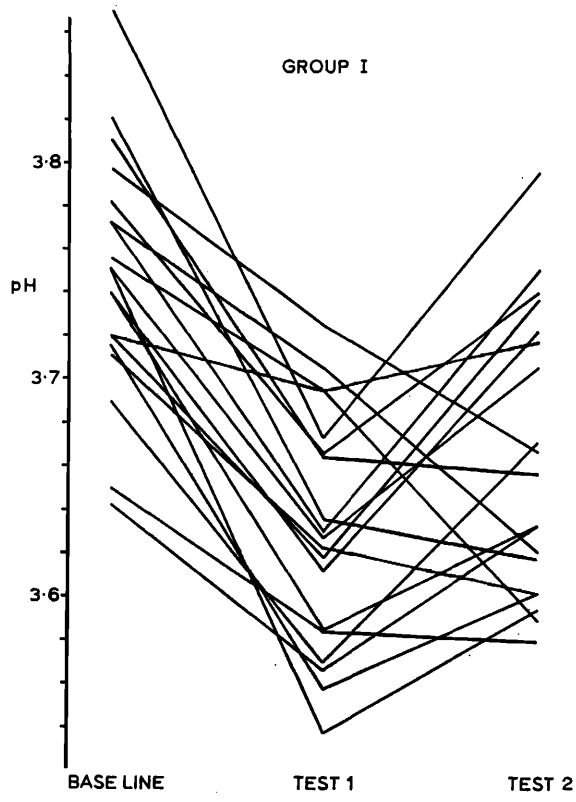

Figure 17. 19 persons on sequence—control, fluoride, control.

1. Cause morphological changes in teeth resulting in shallower fissures.
2. Slow decalcification and accelerate recalcification.
3. Promote apatite formation and improve crystallinity.
4. Inhibit the production of polysaccharide and acid by plaque bacteria.

The second and fourth processes could possibly be achieved by a topical application. That bacteria are essential to the carious process is known from studies on germ-free animals and so this is obviously an important area to consider when techniques are being selected to evaluate cariostatic agents.

The need for a method to assess the effect of agents on the carious process as a whole is shown by the various attempts which have been made

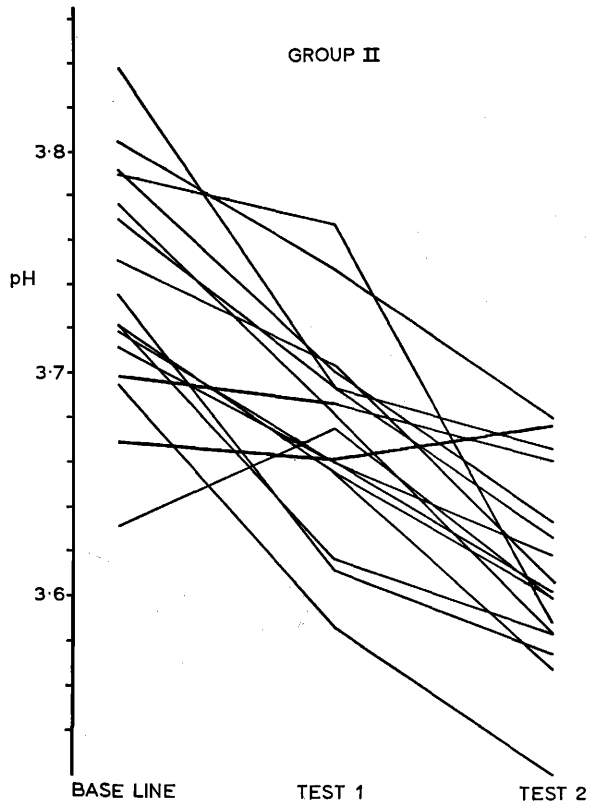

Figure 18. 17 persons on sequence – control, control, fluoride.

to produce caries under controlled conditions. Von der Fehr (25) described a technique in which he induced lesions in premolars which were to be extracted for orthodontic reasons. The buccal surface of the tooth was pumiced, rinsed with water and dried. One half of the surface selected as the control was covered with wax while the other was treated with the test solution. The wax was removed and a gold onlay cemented in place over the test and control areas in such a way as to leave a space about 0.5 to 1 mm wide between the enamel surface and the gold. The teeth were extracted after periods of three to five weeks and the test areas examined micro-

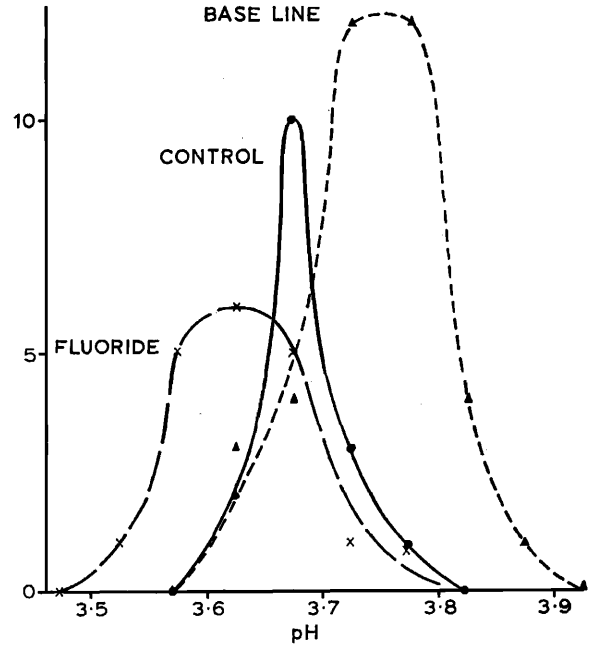

Figure 19. pH distribution of fluoride and control users.

scopically and macroscopically to assess the degree of carious attack. This seems a valid method but is impracticable for studying large numbers of test materials.

The artificial mouth

Numerous attempts have been made to produce carious lesions in an artificial mouth system for the purpose of studying the carious process. Pigman *et al* (26) quote some twenty references, the first dating from 1878, to work involving the formation of carious lesions in 'a laboratory apparatus'. Some report merely general decalcification while others claim to produce lesions identical to natural ones.

Such a system is attractive as an assessment technique in that the overall effect of an agent, whether it be on enamel structure, enzymes or bacteria, could be assessed without understanding in detail the mechanisms involved. Pigman (27) has developed an artificial mouth apparatus in which a nutrient medium is fed to a culture of oral organisms in contact

with teeth. The medium was dripped into a two part acrylic box which held the teeth in the lower compartment. A piece of muslin impregnated with plaque derived from saliva was in contact with the teeth while the upper part of the box maintained a pool of nutrient in contact with the plaque and teeth. In this paper Pigman used enamel hardness measurements to assess the effect of topical treatments but other workers, for example Francis and Meckel (28), have reported the production of lesions in this type of equipment.

Conclusion

The techniques mentioned have been used to assess products containing fluorides which became popular as a topical treatment because of the observed reduced caries experience of people living in areas with fluoride in the water supply. This effect was observed and exploited before the mechanism of its action was fully understood and it is possible that, based on the suggestions of Jenkins referred to previously, biochemical or bacteriological tests would have been at least as appropriate as the chemical ones described.

Any new caries inhibiting agents which may be proposed are likely to be based on knowledge derived from a study of the carious process. The point at which they are intended to attack this process will be known and so specific tests will be defined to study their efficiency. The laboratory assessment procedures will consequently be realistic so that, in the clinical trial which will still be necessary, these expensive and scarce facilities will be used to test only those materials likely to have a marked beneficial effect on dental health.

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Figure 3 – The editor, *Journal of Dental Research;*

Figure 12 – The editor, *Advances in fluorine research and dental caries prevention;*

Figure 15 – The editor, *British Dental Journal.*

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DISCUSSION

MR. N. J. VAN ABBÉ: You mentioned that you use pH measurements rather than phosphate determinations to assess *in vivo* tooth solubility. I wonder how far the spread of results that you have, which seemed to be unaccountable, was due to trying to measure pH values accurately on small quantities of weak acids.

THE LECTURER: We are fairly satisfied that, with attention to detail, we can achieve a good degree of reproducibility in determining pH values. We do, of course, rely on our statistician to show that the differences reported are statistically significant.

MR. N. J. VAN ABBÉ: One has seen an occasional statement that the case hardening effect due to fluoridation makes it more difficult to recognise a cavity with the probe. Is there any suggestion that this can be true in the case of a fluoride toothpaste?

THE LECTURER: The recognition of what is a carious lesion is a vexed one in the context of clinical trials and has been the subject of much learned discussion. The phenomenon of 'reversals' in clinical trials shows that the development and recording of lesions is not a simple matter. If the suggestion were true then the apparent benefit derived from fluoride toothpastes would be inflated.

MRS. H. BUTLER: You were suggesting that fluoride in water prevented dental caries. I wondered if you were referring to work that has been done since the war in areas in this country where fluoride has been added to water or whether you were basing it upon areas known to have natural calcium fluoride in the water.

THE LECTURER: My understanding of the position is that if fluoride is present at the right concentration in the water supply irrespective of whether it occurs natrually or is added, then a reduction caries incidence will be obtained in that area.

MRS. H. BUTLER: I should like to counter by saying that before the war in areas in this country where fluoride occurred at more than 5 ppm there was evidence of very bad dental caries in the children, and that it might be a bad thing to perpetuate this fiction. I should like to know what positive results have been obtained in areas where 1 ppm has been put into the water.

THE LECTURER: A fairly high incidence of mottling of tooth enamel would be expected with 5 ppm of fluoride in the water supply. I have not read reports which suggest that this would lead to the high level of caries you mentioned. A British mission studied several areas in the U.S.A. where fluoride occurred naturally or was added to the water supply. They found no health hazard associated with the presence of about 1 ppm of fluoride in water while the caries rate in teeth which had developed in this environment was much less than in non-fluoride areas. The Ministry of Health report on the five year studies conducted in this country records a marked improvement in the condition of teeth which had developed during these experiments and confirms that 1 ppm of fluoride, whether natural or added, is nothing but beneficial.

MR. R. CLARK: Extensive data has been collected in the U.S.A. in epidemiological studies where fluoride occurs naturally. The results are very significant and show that where 1 ppm or thereabouts of fluoride occurs in water, the incidence of caries is some 60% less than that where fluoride falls to about 0.2 ppm. This has been confirmed by extensive studies, again in America, where fluoride has been added artificially to water previously low in fluoride. When you have a high level of fluoride in the water supply you get tooth mottling and things like that but this is not the intention of those who want to fluoridate water. The evidence is incontrovertible.

DR. K. H. R. WRIGHT: Do you have any evidence of improved mechanical properties of an enamel surface after treatment with a fluoride toothpaste?

THE LECTURER: I have no results of my own on this topic but there are references in the literature to the remineralisation of enamel which can occur, or rather which is accelerated, in the presence of fluoride. This has been shown by hardness measurements on partly demineralised enamel. The hardness increased after exposure to fluoride solutions.

DR. K. H. R. WRIGHT: How much more effective is a toothpaste than a simple mouthwash?

THE LECTURER: I have no knowledge of *in vivo* tests which would answer this. In *in vitro* tests, fluoride is more effective in a simple solution in reducing enamel solubility than when mixed with other ingredients, e.g. toothpaste constituents.

MR. J. M. BLAKEWAY: Have electron microscopic studies been made on the effect of monofluorophosphate on enamel on the same basis as for stannous fluoride and sodium fluoride?

THE LECTURER: Not by us. The electron micrographs (*Figs. 4–11*) were taken at an early stage of the development of a fluoride toothpaste. We have done chemical studies on the action of monofluorophosphate on hydroxyapatite which showed that the fluorophosphate ion seems to be incorporated into surface enamel.