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The evaluation of fluoride dentifrices

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Synopsis

Four laboratory tests were investigated as indicators of the compatibility of FLUORIDE SOURCE and ABRASIVE SYSTEM in FLUORIDE DENTIFRICES. These were solubility of the fluoride in water, ability to reduce the solubility of dental enamel in acid *in vitro*, uptake of fluoride from the dentifrice *in vitro*, and a rat assay for anticaries efficacy. Dentifrices were formulated with 1000 ppm fluoride (as NaF, SnF_2, or Na_2PO_3F) and with abrasives known to either interact or not interact with those particular fluorides. Also, clinically proven commercial products were examined. The assays for water-solubility of the fluoride and the rat assay clearly distinguished between the dentifrices with respect to compatibility of fluoride source and dentifrice abrasive, in agreement with the results of clinical trials reported in the literature, involving different abrasives and sources of fluoride.

INTRODUCTION

Fluoride dentifrices, as opposed to purely cosmetic dentifrices, now account for approximately 80 to 85 per cent of the United States' market, and are becoming increasingly important in dentifrice markets throughout the world. The anticaries efficacy of dentifrices containing fluoride with compatible toothpaste excipients has been proven in numerous clinical trials. (Most of those which have been reported in the literature up to 1972 are summarized by Gershon *et al.* (1)). These trials demonstrated that a properly formulated fluoride dentifrice containing 1000 ppm fluoride incorporated as stannous fluoride, sodium fluoride, or sodium monofluorosphosphate can be expected to reduce the caries rate by some 15 to 30 per cent over the course of 2 to 3 years when used in normal dentifrice fashion by populations susceptible to caries.

Fluoride dentifrices in the United States, Canada, and elsewhere are categorized as drugs. As such, their formulation poses unique and difficult problems. Both safety and efficacy must be firmly established before marketing. There are only a few who at this time question either safety or efficacy of fluoride combined with a compatible cleaning system, but there is still no unanimity with respect to the means for proving compatibility and efficacy. Views have ranged from simply the presence of a prescribed amount of fluoride in a soluble form as adequate evidence of efficacy to the requirement of several clinical trials to prove such efficacy. One of the more important reasons for such a divergence of views is the lack in the literature of virtually any correlations between the results of chemical and animal assays for efficacy and those of clinical tests. The former types of tests have in some instances given results not subsequently satisfactorily confirmed by clinical test when applied to agents other than fluoride. On the other hand, caries clinical tests are far from precise, and they, too, have suffered from

lack of reliability, frequently not showing positive results for established fluoride products. But perhaps the greatest dilemma is that caries clinical trials on fluoride dentifrices are not undertaken unless the formulation has passed several laboratory tests; clinical testing of dentifrices is too consuming of time, financial resources, and personal effort for it to be undertaken on a purely exploratory basis. The consequence is that virtually all clinical trials conducted during the past decade or two employed fluoride systems which, on the basis of laboratory data, were expected to give positive results when tested clinically. More often than not, these expectations were realized, and in a sense, this provided evidence for the validity of laboratory tests as a predictor of clinical efficacy of fluoride dentifrice systems. But very few publications have appeared which present the other side of the coin, *viz.,* negative clinical findings, and it is only when studies are conducted to establish dose-response curves for fluoride in dentifrice, the effects of altering the excipients, and the role of other dentifrice properties (e.g. abrasion) can a scientifically cohesive and complete concept emerge for fluoride dentifrice formulation. It is doubtful that this ideal situation will ever be achieved; the costs and frailties of clinical trials are too great. Nonetheless, there is a substantial body of work, already available, from which one can draw upon to shed light on the problem.

Fluoride dentifrices have been formulated with abrasives and other excipients which result in minimal insolubilization of the fluoride ion because it is considered essential for efficacy that some minimal amount of "free" fluoride be present to react with the dental substrate, *viz.,* that availability of the fluoride depends on its being in soluble form.

Hefferren (2) pointed to the need for analyzing fluoride dentifrices for water-soluble anticaries species, and presented procedures for the assay of fluoride in solution. Hefferren (3), Cooley (4), and Gershon *et al.* (5) discussed and presented methodology for the assessment of the ability of fluorides to reduce the solubility in acid of dental enamel. König *et al.* (6) and others presented animal assays designed to evaluate the anticaries value of a fluoride preparation. Brudevold *et al.* (7) suggested that uptake of fluoride by dental enamel can be an important aspect of fluoride efficacy. In the current studies, several of these laboratory tests were conducted with dentifrices in which the fluoride was added at a level of 1000 ppm, but in which interaction of fluoride and abrasive reduced the amount of fluoride soluble in water to varying degrees. Thus, any differences in response in the assays could be attributed with reasonable assurance to differences in the amount of fluoride in soluble form. Appropriate responses, i.e., decrease in effect with decrease in water-soluble fluoride species, whether fluoride ion or monofluorophosphate ion, would lend support to the concept that availability of the fluoride species, i.e., its water solubility, is the prime requisite to dentifrice efficacy. The results are also discussed in relationship to the value of tests for bioavailability of the fluoride species in predicting the clinical efficacy of a dentifrice containing any of the three commonly used sources of fluoride—stannous fluoride, sodium fluoride, or sodium monofluorophosphate.

METHODS

EXPERIMENTAL DENTIFRICES

Dentifrices were prepared employing conventional abrasives, humectants, and foaming agents. They were packed into appropriate tubes and equilibrated at room temperature

before being subjected to assay. In some instances, where the assay required several weeks, the state of solubility of the fluoride was determined at the beginning and end of the assay period. The fluoride sources studies were SnF_2, NaF, and Na_2PO_3F. The abrasives examined were dicalcium phosphate dihydrate (DPD), chalk, calcium pyrophosphate, insoluble sodium metaphosphate (IMP), and silica gel. A commercial dentifrice was used as the calcium pyrophosphate product to be assured that the abrasive had the correct properties; this introduced an uncontrolled factor which did not appear to confuse the results of the study. Similarly, a commercial dentifrice was used as the IMP product. The compositions of these products have been reported (8).

ESTIMATION OF AVAILABLE FLUORIDE

Soluble or available fluoride was determined essentially as described by Hefferren (2). This involved dilution of the dentifrice 1:10 with water, centrifugation to obtain a clear supernatant solution, and analysis of the solution for fluoride by electrode or chemically, as appropriate. The major deviation from Hefferren's procedure was use of a 1:10 dilution; Hefferren recommended a 1:3 dilution. The higher dilution gave higher values for available fluoride, but in all instances the two methods ranked available fluoride content of the various products in the same way.

ESTIMATION OF ABILITY OF DENTIFRICE TO REDUCE SOLUBILITY OF DENTAL ENAMEL IN ACID (RES)

The RES method reported by Hefferren (3) was employed. Six enamel crowns were mounted in acrylic and placed in a vessel. The susceptibility of the enamel to dissolution was measured by exposing the teeth to a lactic acid-lactate buffer solution at pH 4.5, under standardized conditions of temperature, time, concentration, solution volume, pH, and stirring rate. The amount of phosphate dissolved by the buffer solution was determined. The same teeth were then exposed to a 25 per cent (W/W) slurry of fluoride dentifrice in water for a specific period of time. The teeth were then rinsed with water, and a second measurement of enamel dissolution was made. The amount of phosphate present in the initial and final buffer solutions provided the RES value:

$$\text{RES per cent} = \frac{\left[PO_4^{-3}\right] \text{ initial} - \left[PO_4^{-3}\right] \text{ final}}{\left[PO_4^{-3}\right] \text{ initial}} \times 100$$

FLUORIDE UPTAKE IN TOOTH ENAMEL

The degree of incorporation of fluoride into tooth enamel was evaluated using a modification of the procedure reported by Brudevold *et al.* (7). Noncarious enamel crowns were mounted in wax at the base of small glass vials. The crowns were then subjected to several successive etchings with 0.5 *M* $HClO_4$ until a constant amount of fluoride was removed at each etching. The fluoride concentration per million parts of surface enamel removed by etching was determined by analyzing the etching solutions for calcium, phosphorous, and fluoride. The teeth were then exposed to 25 per cent (W/W) slurry of fluoride dentifrice in water for 15 min. Following this exposure, the teeth were rinsed in dionized water and etched again with 0.5 *M* $HC1O_4$. The post-

treatment etch solution were analyzed. The difference between the fluoride content of the treated tooth surface and that of the untreated tooth surface gave the amount of fluoride incorporated into the enamel.

ANIMAL MODEL SYSTEM FOR ASSESSING CARIES REDUCTION BY FLUORIDE DENTIFRICES

The animal assay system employed was essentially that of König *et al.* (6). It gave an excellent dose-response curve with aqueous fluoride solutions containing fluoride at levels occurring in dentifrices. (The method will only be outlined here; details will be reported elsewhere.) Osborne-Mendel albino rats were employed. They were maintained under conditions generally observed for specific pathogen-free animals. Females were fed a balanced vitamin-supplemented diet from mating to the end of the suckling period. Trials were started on the day of weaning. Weanlings were randomly distributed to the various treatments, the animals being distributed among the cages so as to equalize the stresses of weaning, treatment, and cariogenic diet. A cariogenic diet was fed *ad libitum.* It consisted of sucrose (56 per cent) plus milk powder and other essential nutrients. About 24 animals were subjected to each treatment. Twenty-second applications of the materials to be tested were applied to the lower jaws by means of a marten-hair brush, using about 15 to- and fro- motions of the brush. The rats were deprived of food and water for 1 h after treatment. The treatments were applied twice daily during the first 2 weeks and once daily during the third week (no treatment on Sundays). The rats were sacrificed, and the lower jaws were removed and prepared for sectioning and evaluation of carious lesions after staining. The severity of carious lesions in the first and second molar teeth was assessed by the method of König *et al.*, which grades carious lesions in terms of the stages of the carious process from start (in the enamel) through the next stage (in the dentin) to the conclusion (cavitation). In essence, 4 stages are recognized after staining: *A* lesions (limited to enamel, no staining of the adamantine border), *T* lesions (early dentinal lesion, involving color reaction at the adamantine border), *B* lesions (moderate dentin lesion, comprising progression of the lesion with decalcificiation of dentin bordering on the pulp), and *C* lesions (severe dentin lesion, involving destruction in the direction of both the pulp and occlusal surfaces, loss of enamel in the sulcus, and first signs of cavitation). In calculating the reduction of incidence of carious lesions produced by the test products compared to nonfluoride products in the current study, only the more severe lesions (B and C) were considered. These values were combined, and reduction in carious lesions calculated as:

$$\text{Per cent reduction lesions} = 100 \times \frac{(\text{B + C lesions on nonfluoride dentifrice}) - (\text{B + C lesions on fluoride dentifrice})}{(\text{B + C lesions on nonfluoride dentifrice})}$$

Of course, the values obtained by this procedure are valid for only within-trial comparisons. Also, it should be recognized that the magnitude of the reduction in numerical terms can depend on the value attributed to the various lesions; in this study it was found advantageous to base the calculatons of fluoride effect in the rat on only the B and C lesions.

Table I

	A	B	C	D	E	F	G	H_1	H_2	I[a]	J[b]
Abrasive	DCPD 47 per cent	DCPD 47 per cent	DCPD 47 per cent	DCPD 47 per cent	$CaCO_3$ 37.5 per cent	$CaCO_3$ 37.5 per cent	Silica gel 21 per cent	Silica gel 21 per cent	Silica gel 21 per cent	IMP+DCP 42 per cent 5 per cent	$Ca_2P_2O_7$ 39 per cent
Fluoride	None	NaF 0.22 per cent	SnF_2 0.40 per cent	MFP 0.80 per cent	NaF 0.22 per cent	MFP 0.80 per cent	—	SnF_2 0.41 per cent	MFP 0.80 per cent	MFP 0.76 per cent	SnF_2 0.4 per cent
Other ingredients											
Humectants	27 per cent	27 per cent	27 per cent	27 per cent	21 per cent	21 per cent	72 per cent	72 per cent	72 per cent	22 per cent	30 per cent
Miscellaneous formulating ingredients	3.6	3.6	3.6	3.6	4.0	4.0	4.0	4.8	4.8	[a]	[b]
Water to 100 per cent	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

[a]Colgate toothpaste; composition taken from *Accepted Dental Therapeutics,* 36th Edition, American Dental Association.
[b]Crest toothpaste; composition taken from *Accepted Dental Therapeutics,* 36th Edition, American Dental Association.

RESULTS AND DISCUSSION

In these experiments, model toothpaste systems were formulated in which the abrasive and the fluoride were either compatible or incompatible. Compatibility was shown by a high proportion of the added fluoride remaining in a water-extractable form, and incompatibility was shown by the fluoride being complexed with the abrasive and being insolubilized thereby. Separate experiments (not reported here) demonstrated very clearly that the humectants (glycerol and sorbitol), binding agent, and foaming agent, and flavor did not influence the interaction between the fluoride and the dentifrice abrasive in formulations typical of those investigated in this study. Other trials, with the silica-SnF_2 system, gave results which demonstrated that the nature of the nonfluoride ingredients other than the abrasive did not detectably affect the response to the fluoride in the assays for fluoride solubility, reduction of enamel solubility, or rat caries inhibition.

DENTIFRICES STUDIED

Table I shows the compositions of the dentifrices investigated in terms of abrasive, fluoride source, and other ingredients such as humectant and miscellaneous formulating ingredients, the latter including binding agent, flavor, and foaming agent. The compositions of the dentifrices were selected (1) to provide a basis for a placebo, i.e. nonfluoride paste, to be used for the animal caries trials, and (2) to provide compositions which would reveal differences in response between dentifrices containing different dentifrice abrasives and different sources of fluoride. The dentifrices were assayed in groups, the number of products per group being limited by the logistics of the rat caries test. Thus, for example, dentifrices A through F were tested in the rat caries test alongside each other in order to achieve maximum comparability of results and statistical validity.

Dentifrices A through F represent products with either (1) no fluoride, (2) abrasives which are known to interact with free fluoride ion, or (3) sources of fluoride ion which have been shown to be reactive with calcium-containing dentifrice abrasives on one hand and nonreactive on the other hand.

Dentifrice A is the placebo in the A-F series. It contains as abrasive dicalcium phosphate dihydrate, and contains no fluoride. Dentifrice B comprises dicalcium phosphate dihydrate and, as fluoride source, sodium fluoride. Sodium fluoride is known to interact with dicalcium phosphate dihydrate. Dentifrices C and D both contain dicalcium phosphate dihydrate as the abrasive; C contains stannous fluoride while D contains sodium monofluorophosphate. Dentifrices E and F contain calcium carbonate as the abrasive, E containing sodium fluoride as source of fluoride ion and F containing sodium monofluorophosphate.

Dentifrices G through I represent a group of dentifrice compositions in which the fluoride source and the abrasive are known to be compatible. In this series, dentifrice G represents the placebo. The last test series of dentifrices is represented by dentifrices G, H_1, H_2, and J. Both H_1 and J contain stannous fluoride and a compatible abrasive. Dentifrice G, again, represents the placebo.

Table II
Evaluation of DCPD and $CaCO_3$ Dentifrices

Product	Abrasive/ Fluoride[a]	Available Fluoride Initially ppm	Available Fluoride After 7 Weeks ppm	Reduction in Enamel Solubility Per Cent	Reduction in Carious (B + C) Lesions in Rat Trial Per Cent
A	DCPD/–	—	—	−4.2	—
B	DCPD/NaF	155	143	15.6	6
C	DCPD/SnF_2	130	111	23.0	4
D	DCPD/MFP	864	888	16.9	44
E	$CaCO_3$/NaF	378	354	8.5	27
F	$CaCO_3$/MFP	856	844	6.4	51

[a]All dentifrices formulated with 1000 ppm added fluoride, as NaF, SnF_2, or MFP.

"COMPATIBLE" VERSUS "INCOMPATIBLE" SYSTEMS

The results with dentifrices A through F are given in Table II. Data are given for water-soluble fluoride at the beginning and at the end of the rat caries trial (which covered a period of 6 weeks), reduction in enamel solubility, and reduction in carious lesions in the rat trial.

Water-solubility of the fluoride:

The incompatibilities of certain of the fluoride sources and the dentifrice abrasive are clearly demonstrated. In each instance (except for the nonfluoride control, dentifrice A), fluoride was added at a level of 0.10 per cent (fluoride basis). Substantial interaction and insolubilization of the fluoride occurred very rapidly as demonstrated by lowered values for water-soluble fluoride. Both sodium fluoride and stannous fluoride reacted with the dicalcium phosphate dihydrate, and over 85 per cent of the fluoride was insolubilized within 1 week after manufacture of the dentifrice. Sodium fluoride showed less reactivity with calcium carbonate than it did with dicalcium phosphate dihydrate, but even in this instance, almost 60 per cent of the fluoride was insolubilized by the abrasive. Sodium monofluorophosphate, in contrast to sodium fluoride and stannous fluoride, showed excellent compatibility with the dicalcium phosphate dihydrate and calcium carbonate, and not more than about 15 per cent of the fluoride ion contained therein was rendered insoluble over the course of the trial.

Reduction in enamel solubility (RES):

The RES test, the ability to so affect dental enamel as to make it more resistant to attack by acid, has been a valuable criterion for assessing the utility of potential anticaries compounds. It is not the intent here to explore in depth relations between the RES test and other anticaries agent assays. They do not necessarily measure the same parameters. For example, the RES test involves a single parameter, *viz.,* attack by acid on dental enamel, while the rat caries assay involves multiple parameters related to the carious process. Factors which quantitatively affect one test markedly may be less important in another. A case in point is the nature of the cation associated with the fluoride. The RES value is markedly affected by the presence of the stannous ion in an ac-

tive form. Hefferren (3) showed that the rate and extent of dissolution of dental enamel in acid varied with the treatment (SnF_2, NaF, $SnCl_2$), and that the $SnCl_2$ was a very effective inhibitor of dissolution when the time of acid attack was limited to a few minutes, but not when it was extended to 2 h. SnF_2 produces greater reductions in RES than an equivalent amount of MFP. The mechanism whereby MFP influences resistance of enamel to acid differs from that of the simpler fluorides.

Table III
Reduction of Enamel Solubility by SnF_2 Dentifrices

Dentifrice Type	Abrasive	Available Fluoride ppm	Reduction of Enamel Solubility Per Cent
C	DCPD	130	23.0
H_1[a]	Silica gel	Average 790 Range 750–880 n = 8	64 54–72 n = 20
J[a]	$Ca_2P_2O_7$	Average 710 Range 640–750 n = 8	38 14–60 n = 4

[a]Commercial products, selected randomly for analysis. J reportedly contains stannous pyrophosphate in addition to stannous fluoride.

In Table III, the SnF_2 dentifrices C (DCPD/SnF_2), H_1 (silica/SnF_2), and J ($Ca_2P_2O_7$/SnF_2) show the importance in the RES test of having the active ions in water-soluble form. The response to the test was directionally in proportion to the amount of fluoride ion available. However, strong conclusions cannot be drawn, since the data are limited and the role of tin, whether soluble or insoluble in the dentifrice, was not assessed.

In Table II it can be seen that MFP-containing dentifrices (D and F) yielded only relatively small reductions in enamel solubility compared to SnF_2 containing dentifrices despite the high levels of available active ion(s) in the former. In Table II it is also apparent that on a strictly numerical basis the results of the RES test did not correlate with the rat caries assay results. It can be concluded that results of the RES test should be examined independently of those of the rat caries assay, as evaluating different parameters of activity of anticaries agents.

Rat caries trials:

Of greatest significance are the results of the rat caries trials. Here, a definite relationship was established between the ability of a dentifrice to reduce the incidence of carious lesions and the level of water-soluble fluoride or fluorophosphate ion. In Table II are the results of a series of trials in which NaF, SnF_2, and MFP were combined with abrasives which are incompatible with NaF and SnF_2 but compatible with MFP. A key finding was that fluoride ion bound to the abrasive was inactive in protecting the rat against caries. This effect has been hypothesized on many occasions, but it is believed that the series of tests reported here provides the first published clear cut evidence for

Table IV
Rat Caries Trial Number 1

Dentifrice	Type	Reduction in Carious Lesions (Per Cent)	Available Fluoride (as F) (ppm)
G	Silica, no F	—	Nil
H	Silica/SnF_2	50	880
I	IMP + DCP/MFP	70	950[a]
J	$Ca_2P_2O_7$/SnF_2	73	730

[a]Not determined specifically for this trial. The water-soluble content of this commercial dentifrice has been found on several occasions to be about 950 ppm.

Table V
Rat Caries Trial Number 2

Dentifrice	Type	Reduction in Carious Lesions (Per Cent)	Available Fluoride (as F) (ppm)
G	Silica, no F	—	Nil
H_1	Silica/SnF_2	37	790[a]
H_2	Silica/MFP	37	960
I	IMP + DCP/MFP	31	950[a]
J	$Ca_2P_2O_7$/SnF_2	29	710[a]

[a]Available fluoride contents of the commercial dentifrices (H_1, I, J) were not determined specifically for this trial; the available fluoride values are average values for the commercial products (cf. Table III).

Table VI
Rat Caries Trial Number 3

Dentifrice	Type	Reduction in Carious Lesions (Per Cent)	Available Fluoride (ppm)
G	Silica, no F	—	Nil
H_1	Silica/SnF_2	42	820
H_2	Silica/MFP	34,30	950,990
I	IMP + DCP/MFP	36	950

differentiation of soluble and bound fluoride in a dentifrice using an animal system. Dentifrices D (DCPD/MFP) and F($CaCO_3$/MFP), both containing MFP at a level of 0.8 per cent (1000 ppm fluoride) produced significant reductions in caries. Dentifrices B (DCPD/NaF) and C (DCPD/SnF_2), representing pastes with sodium fluoride and stannous fluoride in an incompatible base gave negligible reductions in carious lesions despite the fact that they, too, contained 1000 ppm fluoride. It must be concluded that their low order of efficacy was attributable only to the low amount of available fluoride. Dentifrice E, which contained sodium fluoride in a calcium carbonate base, did yield a modest reduction in rat caries. However, the level of available fluoride at the time of the test was reasonably high, i.e. intermediate between that of the DCPD/NaF dentifrice and DCPD/MFP dentifrices. Presumably, it would decrease on aging, as reaction between the fluoride and abrasive progressed.

Table VII
Fluoride Uptake After 5 Min Exposure

Group Treated with Product J				Group Treated with Product H_1			
Sample Numbers	Pretreatment ppm F	Post-Treatment ppm F	Uptake ppm F	Sample Numbers	Pretreatment ppm F	Post-Treatment ppm F	Uptake ppm F
III-1	147.4	253.8	106.4	IV-1	69.4	136.7	67.3
III-2	81.9	126.6	44.7	IV-2	51.5	181.0	129.5
III-3	98.4	142.8	44.4	IV-3	75.9	148.7	72.8
III-4	34.0	97.6	63.6	IV-4	75.5	167.5	92.0
III-5	62.0	162.0	100.0	IV-5	46.5	147.9	101.4
III-6	34.0	120.4	66.4	IV-6	87.2	241.5	154.3
III-7	56.2	152.4	96.2	IV-7	233.6	336.0	102.4
III-8	113.5	348.8	235.3	IV-8	195.6	460.3	244.7
III-9	37.7	158.1	120.4	IV-9	42.7	151.2	108.5
III-10	40.8	106.5	65.7	IV-10	79.7	171.4	91.7
III-11	79.5	176.6	97.1	IV-11	45.8	159.0	113.2
III-12	52.8	105.5	52.7	IV-12	118.3	225.7	107.4
III-13	53.4	75.0	21.6	IV-13	70.7	307.5	236.8
III-14	151.8	240.3	88.5	IV-14	103.0	261.4	158.4
III-15	69.7	121.3	51.6	IV-15	125.9	351.2	226.3
III-16	72.7	144.1	71.4	IV-16	67.7	230.9	163.2
III-17	80.0	123.0	43.0	IV-17	135.1	556.6	421.5
III-18	186.1	184.7	Nil	IV-18	42.1	168.1	126.0
III-19	180.5	180.6	Nil	IV-19	59.3	276.6	217.3
III-20	55.0	116.8	61.8	IV-20	45.2	767.3	722.1
III-22	83.5	165.6	82.1	IV-21	77.1	180.7	113.6
III-23	45.6	169.1	123.5	IV-22	328.1	339.8	11.7
III-24	132.9	221.6	88.7	IV-23	57.2	287.7	230.5
				IV-24	63.3	154.3	91.0
Average			75.0	Average			171.0
N			(23)	N			(24)
S.D.			48.4	S.D.			144.2

"COMPATIBLE" SYSTEMS

Tables IV, V, and VI give data on additional trials with compatible abrasive/fluoride systems. The data are organized according to products assayed simultaneously in a single rat caries trial. All of the reductions in carious lesions over the nonfluoride product were signiflcant at $p < 0.05$. Comparison of data from trial-to-trial was not statistically valid. The results given in Tables IV to VI show: (1) compatible abrasive/fluoride systems respond positively in the rat caries test; (2) the response is positive regardless of whether the fluoride source is SnF_2 or MFP; and (3) the response is positive regardless of the abrasive when the abrasive is compatible with the fluoride. The results confirm those of Table II, and in conjunction with the latter results support current concepts which require that clinical fluoride efficacy in a dentifrice critically depends on the fluoride and abrasive being compatible.

Uptake of fluoride by dental enamel:

Only limited data were obtained on the uptake of fluoride by human tooth enamel. This technique is difficult to carry out for many reasons, the major one being the large number of tooth samples required to assure statistical confidence. There is, as would be expected, great variability from tooth to tooth, even within teeth from the same

person. Soundness of the tooth surface, history of exposure to fluoride, and other factors come into play.

The results of a comparison of dentifrice H_1 and J are given in Table VII. They reveal that fluoride can be taken up *in vitro* from dentifrice slurries containing a substantial amount of available fluoride. Further studies will be necessary to determine the extent to which this uptake is dependent on the availability of the fluoride and/or other factors; the data in Table VII suggest that such may be so, but are far from adequate to establish a case with any degree of confidence. The data in Table VII clearly demonstrate the extreme variability in fluoride uptake from tooth sample to tooth sample, and thus the importance of conducting studies of new formulations with sufficient numbers of teeth and appropriate controls.

DISCUSSION

The foregoing results help evaluate the utility of 3 tests used to assess the anticaries activity of a fluoride dentifrice, *viz.,* reduction in enamel solubility (RES), uptake of fluoride by enamel, and animal caries assay.

The RES test gave results which cannot be interpreted readily. The values failed to correlate well with either the water-soluble fluoride content or the rat assay values. The RES values in Table II seem to reflect more an abrasive effect than a fluoride effect. At this time, it is probably safest to conclude that the RES test as applied to a fluoride dentifrice can show whether that dentifrice exerts an effect on the substrate (tooth enamel), but that this may not be translated to a positive anticaries effect.

The ability of the enamel to take up fluoride has been investigated extensively as a tool to evaluate fluoride treatments. Insufficient data are presented here to draw firm conclusions. What results are shown certainly do not point to a quantitative correlation between the amount of fluoride taken up from a dentifrice and the degree to which that dentifrice inhibits the development of carious lesions in the rat on a cariogenic diet. Additional investigations are required before the results of fluoride uptake *in vitro* can be interpreted with confidence.

The rat caries assay as conducted in our laboratories (details to be published elsewhere) gave results which are consistent and readily interpretable. They show a correlation with available fluoride, and do not seem to show a response to fluoride which is insolubilized by the abrasive. Of greater importance is the observation that the rat assay exhibits a positive response to all fluoride dentifrice systems which have been reported in the literature to be clinically effective, such as $Ca_2P_2O_7/SnF_2$ and IMP + DCP/MFP, and $CaCO_3$/MFP. Furthermore, the magnitude of effect of such clinically tested dentifrices such as $Ca_2P_2O_7/SnF_2$ and IMP + DCP/MFP is about the same when determined by the rat assay and about the same when determined by human clinical trial; thus, the rat assay has shown the equivalence of certain formulations which has been shown clinically.

More extensive and exact experiments than those reported here are needed to establish quantitative correlation between the RES and fluoride uptake tests on one hand and the rat caries assay on the other. The RES test and the fluoride uptake test measure the effect of fluoride dentifrices on specific parameters involved in the fluoride effect on caries. The rat caries assay, on the other hand, measures efficacy of a fluoride denti-

frice in terms of the totality of the carious process. The RES and fluoride uptake tests are extremely important in showing ability of an agent to interact beneficially with the tooth substrate; they are limited as an evaluative tool in providing less than a full picture. The rat assay can be criticized on the same basis that all animal systems can be criticized, i.e., differences between human and animal systems. But compared to the other, *in vitro,* tests which involve only an enamel substrate, it alone gives a complete assessment of efficacy which is compatible with current concepts of fluoride dentifrice formulation and the results of human clinical trials.

The carious process is extremely complex. Hefferren (3) has summarized more recent thinking on the carious process and on the design of anticaries agents. He has also pointed to the incompleteness of our knowledge on the mechanism of fluoride action. And he has indicated the need for greater reliance on definitive laboratory studies, the basis for which is correlation of experimental data on the new product with the clinically established product. The studies reported above clearly define such a correlation.

It is becoming increasingly apparent that caries is a specific disease state, caused by specific disease organisms. The route to cure of this disease is not unlike that of any other disease–eliminate the causative microorganisms(s), combat the microorganism(s) by making the environment unfavorable, and/or increase the resistance of the substrate to the action of the microorganism(s). The mechanism of anticaries activity for a fluoride, while not totally understood, is generally considered to involve, at a minimum, the incorporation of fluoride into the dental enamel and strengthening of the enamel thereby against acid attack, which acid is generated by specific microbial populations under favorable environmental conditions. A direct effect of fluoride on the microbial metabolism has not been ruled out as another route of fluoride action. Other mechanisms may be involved, such as interactions of effects of fluorides and effects of variations in substrate (tooth) characteristics.

The tests described above follow the pattern commonly used in the assessment of drug efficacy when formulating products containing drugs of established efficacy. Fluoride "availability" assures that the drug is in an active (noncomplexed) state. Reduction in enamel solubility (*in vitro*) and fluoride uptake (*in vitro*) give further assurance that the drug is in a state wherein it can act on the substrate, and the animal assay gives final assurance that the drug is active against the disease process *in vivo.*

The ideal situation in correlating the results of *in vitro* and animal assays with the human assay is a series of studies in which dose-response curves are available for all the test situations. Such is not feasible in the fluoride arena, and only one real attempt to establish a dose-response to fluoride in a (compatible) abrasive system appears to have been reported. Reed (9) conducted two-year clinical trials with calcium pyrophosphate dentifrices containing 0, 250, 500, or 1000 ppm added fluoride (as NaF). All 3 fluoride products resulted in a significant reduction of the parameter "decayed, missing, and filled teeth." Only the product with 1000 ppm fluoride, however, gave a significant reduction in the more relevant parameter, "decayed, missing and filled tooth surfaces" (DMFS). Trends, however, pointed to a dose-response relationship with both parameters. DMFS reductions, for example, were: 7.5 per cent for 250 ppm F, 8.5 per cent for 500 ppm F, and 20.0 per cent for 1000 ppm F. Extension of the study to a third

year might have established a good dose-response curve. With the exception of Reed's work, no clinical trials have been found in the literature to establish a dose-response in fluoride dentifrices.

Lacking totally appropriate experimental clinical data on which to base judgments of the effect of fluoride in a dentifrice on dental caries, i.e., lacking dose-response clinical data of the type available for most drugs, one can assess the value of *in vitro* and animal data for fluoride dentifrices in relationship to clinical efficacy only by reference to the treatment of comparable data for other drug products. This has already been done in a sense by fluoride dentifrice investigators; it is universally accepted that a prime prerequisite for formulation of any fluoride dentifrice is that the fluoride source must not interact excessively with the abrasive and thereby become insolubilized. The experimental data presented above, particularly the data in Table II, support this view in its entirety. No thinking dentifrice formulator today would, for example, incorporate sodium fluoride into a DCPD dentifrice, or SnF_2 into a DCPD dentifrice.

The results in Table II are especially important in that they point out definitively that *in vitro* and animal data disclose compatibility relationships between fluoride source and abrasive which do tend to qualitatively correlate with clinical trial data reported in the literature. Thus, for example, MPF is shown to be compatible with every abrasive tested (DCPD, $CaCO_3$, IMP + DCP), and clinical results have been reported establishing the clinical efficacy of MFP not only in dentifrices containing these abrasives (e.g. 10, 11), but also with another compatible abrasive, *viz.,* alumina trihydrate (12). On the other hand, there are reports of negative findings with NaF in dentifrices containing DCPD as the abrasive (13). This type of correlation should not be overlooked in assessing the significance of the *in vitro* and animal tests reported here.

SUMMARY

Four laboratory assays were applied to dentifrices formulated with a variety of abrasives and fluoride sources. These were: (1) fluoride availability; (2) reduction in enamel solubility (RES), (3) fluoride uptake by human dental enamel; and (4) rat caries assay. The assays for fluoride availability and the rat assay very clearly distinguished between the dentifrices based on compatibility of the fluoride source with the abrasive. The results of the RES and fluoride uptake assays gave valuable information on the ability of a fluoride in a dentifrice to interact with the dental enamel. The results clearly support current views of fluoride dentifrice formulation, i.e., that the fluoride must not be insolubilized by the abrasive if it is to have anticaries activity. They also correlate qualitatively with results of clinical trials reported in the literature, involving different abrasives and sources of fluoride.

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