STANNOUS FLUORIDE DENTIFRICES

An Investigation into the Availability of the Fluoride and its Retention in Human Subjects

A thesis submitted for the

DEGREE OF DOCTOR OF MEDICINE

in the Faculty of Medicine of the University of Liverpool

by

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October 1964

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ABSTRACT

Soluble fluoride was extracted with water from samples of different brands of dentifrice, each containing stannous fluoride, which had been stored at 5, 20 and 37°C. There was a progressive decrease in the release of fluoride as the dentifrices aged and this age change was accelerated as the temperature of storage increased. Dentifrice samples of the same brand and age, stored at the same temperature, released less soluble fluoride to saliva than to water. Under comparable conditions of storage and extraction there were differences in the amounts of fluoride released by each brand of dentifrice; an attempt has been made to correlate these differences with the formulation of the dentifrices.

The uptake of dentifrice fluoride, as fluorine-18, by premolar teeth in situ was measured. No differences were found in the amounts of fluorine-18 taken up from the various toothpastes.

An investigation of the uptake of fluorine-18 by enamel in vitro from solutions of sodium fluoride and stannous fluoride and from dentifrices containing stannous fluoride showed that uptake is preferentially by iso-ionic exchange. Therefore, it was concluded that fluorine-18 was of little value in the determination of fluoride uptake by enamel.

In adult male subjects the mean retention of fluoride, as fluorine-18, during toothbrushing with labelled stannous fluoride dentifrice was approximately 18 per cent of the fluoride on the brush before brushing began. Excretion of fluoride in the urine was determined for 4 hours after brushing and found to be approximately 1 per cent.

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CHAPTER 1

STANNOUS FLUORIDE DENTIFRICES

A CRITICAL REVIEW OF PUBLISHED RESEARCH

WITH SUGGESTIONS FOR FURTHER INVESTIGATIONS

1.1. INTRODUCTION

The value of the fluoride ion in the prevention of dental caries is well recognised, but the mechanisms of its action are still not fully understood (Jenkins, 1961 and 1963; Hardwick, 1963). The two principal effects of fluoride are believed to be its ability to reduce the solubility of enamel in acid and to inhibit bacterial enzymes in the dental plaque. If these mechanisms operate when fluoride is applied topically, and if the fluoride in a fluoride dentifrice is free to enter the enamel and the plaque, the use of such a dentifrice should allow the individual to apply his own preventive agent. In recent years sodium fluoride, stannous fluoride, sodium monofluorophosphate and several organic fluoride compounds have been included in dentifrices formulated to test this hypothesis.

The addition of fluoride compounds to dentifrices is not a recent innovation, for fluorides were being added to a variety of toothpastes and mouthwashes at the close of the 19th century (Volker, 1960). Any useful effect that these preparations might have had in the control of dental caries was not demonstrated, because the testing of dentifrices in controlled clinical trials had not, at that time, been developed. In consequence, fluoride dentifrices were neglected until Bibby (1945) tested one containing sodium fluoride. During the last decade there has been renewed interest in fluoride dentifrices, particularly in those containing stannous fluoride. This interest, although

widespread, has not led to general acceptance of dentifrices containing stannous fluoride as caries-preventive agents.

If stannous fluoride dentifrices are to gain such acceptance it must be shown, beyond all reasonable doubt, that 1) they contribute to the control of dental caries and 2) they do so without causing undesirable side-effects. Some of the available evidence suggests that both criteria have been met, although recent work has failed to confirm that the dentifrices are able to reduce the incidence of dental caries.

These important data have been obtained in controlled trials, but such trials, concerned as they are with the assessment of clinical results provide little or no information upon mechanisms of action. However, these mechanisms must be fully understood if the present stannous fluoride dentifrices are to be improved and the maximum benefit, if any, offered to their users. Therefore, selected research reports relevant to the development and laboratory testing of these preparations, together with data upon the behaviour of dentifrice fluoride in the body will be evaluated in this review. The reports of the clinical trials will not be reviewed in detail.

1.2. REASONS FOR THE DEVELOPMENT OF STANNOUS FLUORIDE DENTIFRICES

In spite of the early laboratory and clinical data which suggested the usefulness of solutions of sodium fluoride in preventing dental caries (Volker, 1939; Bibby, 1944), this compound did not prove effective when included in dentifrices (Bibby, 1945; Winkler, Backer Dirks and Van Amerongen, 1953; Muhler, Radike, Nebergall and Day, 1955b; Kyes, Overton and MacKean, 1961).

Stannous fluoride dentifrices were developed after comparisons had been made between certain effects of stannous and sodium fluoride. Solutions of both were tested for their ability to reduce enamel solubility, to reduce experimental caries in animals and to reduce dental caries in human subjects after topical applications.

Stannous fluoride appears to be more effective than sodium fluoride in protecting enamel against acid decalcification (Muhler and Van Huysen, 1947; Segreto and Harris, 1959; Francis and Meckel, 1963) although enamel does not accept more fluoride from a solution of stannous fluoride than from an equivalent solution of sodium fluoride (Smith, Gardner, Leach and Hodge, 1957; Cooley, 1961).

Variable results have followed the addition of stannous fluoride to the drinking water of experimental animals. Muhler, Nebergall and Day (1953) and Radike and Muhler (1953) claimed that stannous fluoride was more effective than sodium fluoride in reducing the incidence of dental caries in rats and hamsters, but Pindborg (1958) failed to

confirm this.

In most of the clinical studies in which fluoride solutions have been applied topically to the teeth, stannous fluoride or other tin fluoride compounds have reduced the incidence of new DMF surfaces more than has sodium fluoride. Much of this work was done by Muhler and his associates and it has already been reviewed (Muhler, 1961a). Although Nevitt, Witter and Bowman (1958) were unable to show a significant difference in the caries-preventive effect of these two fluoride compounds, Muhler's work has received independent confirmation from McLaren and Brown (1955).

Thus, much, but not all, of the evidence obtained in chemical, animal and human experiments indicates that stannous fluoride confers upon enamel a degree of protection which is superior to that provided by sodium fluoride. However, it should be noted that most of these data have been supplied by one group of workers. To date, their findings, which have been applied to the formulation and testing of dentifrices containing stannous fluoride, have received scanty independent corroboration.

1.3. CHEMISTRY OF STANNOUS FLUORIDE

The chemical reactions of stannous fluoride become important when the formulation of a compatible dentifrice mixture is considered. Unfortunately knowledge of the chemistry of this fluoride compound is limited; this limitation being exemplified by the scant attention it receives in the latest review of fluorine chemistry (Simons, 1964). Much of the data which are available appear to have been provided by investigators with dental interests.

Commercial "stannous fluoride" has physical, chemical and biological properties which vary with the source of the product. Therefore, Nebergall, Muhler and Day (1952) prepared what they considered to be pure stannous fluoride from stannous oxide and hydrofluoric acid and they studied some of its properties. Their product was soluble in water in which it formed an acid solution, a 2 per cent solution having a pH of 2.9 (Howell, Gish, Smiley and Muhler, 1955).

The value of topically applied solutions of stannous fluoride in the prevention of dental caries would appear to depend upon the presence of ionic fluoride and perhaps upon the presence of stannous ions. Therefore, it is unfortunate that stannous fluoride in aqueous solution is unstable; its ions being readily removed from solution by hydrolysis, by oxidation and by the formation of complex ions.

A freshly prepared aqueous solution of stannous fluoride is clear at first, but hydrolysis soon renders it turbid. On standing the turbidity increases with the eventual formation of a white precipitate.

Although it has been reported that this precipitate contains less than 0.01 per cent fluoride (Nebergall et al., 1952) later work has shown that the loss of soluble fluoride is greater. Thus, 15 to 20 per cent of the fluoride was removed from solution in the precipitate which formed during a 30-day period (Hatton, Nebergall and Muhler, 1955). These authors report that this precipitate is probably a mixture of stannous hydroxide and stannous oxy-fluoride. Precipitation is influenced by pH, for, above pH 4 it is much increased (Muhler and Day, 1952; Muhler and Weddle, 1955 and Walsh, Nebergall, Muhler and Day, 1957). Hydrolysis is accompanied by the expected fall in pH which is proportional to the amount of stannous hydroxide formed (Muhler, Day and Nebergall, 1952).

The stannous ion is readily oxidised to the stannic state. Muhler and Day (1955) claim to have shown this, indirectly, by demonstrating that oxygen free solutions of stannous fluoride are more effective in the control of experimental rat caries than solutions prepared in the presence of oxygen. To minimize the effect of oxidation Muhler et al. (1953) and Muhler, Nebergall and Day (1954) have stressed the importance of using freshly prepared solutions.

That oxidation inactivates stannous fluoride solutions is not borne out by the results of some of the experiments where the solubility of treated enamel was investigated. Thus, Mörch, Torrell and Hals (1956) have reported that aged stannous fluoride solutions have an enhanced ability to reduce the dissolution of enamel in acid and ageing of aqueous solutions of stannous fluoride, over a period of 18 months,

appears to have been without influence upon their ability to reduce enamel solubility (Shannon and Gibson, 1963a). Even stannic fluoride appears to have been effective in caries prevention when applied topically (Hals, Torrell and Mörch, 1960). Clearly, it has not been established that the protective action of stannous fluoride in simple solution is entirely dependent upon its chemical state.

In addition to hydrolysis and oxidation, tin and fluoride ions may be removed from solution by the formation of complexes. In a polarographic study of solutions containing these ions Schaap, Davis and Nebergall (1954) showed that the stable and predominant ion was the trifluorostannate (II) ion. This ion was also demonstrated by Davis (1954), Torrell, Hals and Mörch (1958), Muetterties (1962) and Donaldson and O'Donaghue (1964). In addition the last authors provide preliminary data suggesting the presence of the penta-fluorostannate (II) ion in solutions containing stannous fluoride and alkali-metal fluoride. One other complex ion, SnF^+, has been demonstrated in aqueous tin fluoride solutions (Connick and Poulson, 1959).

Dentifrices, which are compounded in a watery base, may lose their fluoride and stannous ions by any of the mechanisms described above. In addition ionic fluoride in a dentifrice may be lost from solution by metathesis with calcium in the polishing agent to form the poorly soluble calcium fluoride, although the formation of tin fluoride complexes is said to minimize this change (Torrell et al., 1958). With some polishing agents the precipitation of tin phosphates may also contribute to the loss of stannous ions.

1.4. FORMULATION OF STANNOUS FLUORIDE DENTIFRICES

Only minimal information on the composition of the proprietary dentifrices has been released. Presumably, attempts must have been made by the manufacturers to reduce, or prevent, the loss of active ions by the reactions mentioned in the previous section. Thus, to minimize hydrolysis, some stannous fluoride dentifrices are acidified to a pH of 4.5 or 5 and new abrasives have been employed, or specially developed, to reduce the loss of fluoride by precipitation as calcium fluoride.

As an example of the formulation of a stannous fluoride dentifrice the composition of the original American preparation, as given by Muhler (1961a), may be considered.

Ingredients	Parts
Calcium pyrophosphate (polishing agent)	39.00
Stannous fluoride	0.40 (0.10 F)
Stannous pyrophosphate (Sn^{++} depot)	1.00
Glycerine (humectant)[1]	30.00[1]
Miscellaneous agents (detergent, binder, flavour)	4.63
Water	24.97
	100.00

1. The very recent report by the Council on Dental Therapeutics of the American Dental Association on the reclassification of this toothpaste (J. Amer. dent. Ass. 69, 195-196, 1964) shows that it now contains 20 parts of sorbitol and only 10 parts of glycerine.

Unlike dicalcium phosphate, which was used in a dentifrice in an unsuccessful clinical trial reported by Muhler (1957), calcium pyrophosphate is said to be compatible with stannous fluoride. Stannous pyrophosphate, not present in the original formula of this dentifrice, was added in an attempt to maintain its level of soluble stannous ion.

At least two other preparations contain sodium metaphosphate. The calcium sequestering action of this compound could mean that more fluoride would be available to the tooth. Although Muhler (1958) tested a modified stannous fluoride-calcium pyrophosphate dentifrice which contained 1 per cent sodium metaphosphate it has yet to be demonstrated that dentifrices containing larger amounts of sodium metaphosphate can confer enhanced resistance against dental caries. Indeed, Slack and Martin (1964) were unable to show that such a dentifrice had any preventive action.

The success of manufacturers in preserving the activity of stannous fluoride in a dentifrice can only be determined by the results achieved in clinical trials, but measurements of the soluble fluoride and the stannous ion in the dentifrices may give a preliminary indication of their usefulness. Manly (1961) reported a study in which he attempted to assess the decrease in availability of these ions which occurred as the dentifrices aged during storage. Analyses of two commercial preparations showed surprising variations in the amounts of soluble fluoride. At the end of one year this fell to about 50 per cent of its original level in one dentifrice and in the other the soluble fluoride was at an even lower level throughout. A much more rapid decrease in

available stannous ions was found in both dentifrices, only 20 per cent or less being available at the end of one year. The ability of these dentifrices to reduce the rate of enamel solution also fell with ageing.

In two important papers Shannon and his colleagues (Shannon, Dirksen, Gibson and Hester, 1962 and Shannon and Gibson, 1963 b)have reported that certain dentifrice ingredients influence the action of stannous fluoride in reducing enamel solubility. Calcium pyrophosphate almost destroyed the ability of aqueous stannous fluoride solutions to protect enamel against decalcification by lactic acid. Although it has been claimed that the addition of glycerine to stannous fluoride solutions retards hydrolysis and loss of stannous ions (Gray, J.A., unpublished data cited by Cooley, 1961), glycerine reduced the protective effect of fresh solutions. The addition of glycerine to solutions of stannous fluoride containing calcium pyrophosphate did not prevent a deterioration in protection with time. Ericsson (1961) has stated that different commercial preparations of calcium pyrophosphate differ in the extent to which they interfere with the reaction between stannous fluoride and enamel but heat treatment of this polishing agent appears to reduce its interference. Although the calcium pyrophosphate used by Shannon and Gibson (1963b) was probably not identical with the abrasive included in the commercial stannous fluoride-calcium pyrophosphate toothpaste, their findings emphasize the difficulty of formulating a suitably abrasive dentifrice carrier for stannous fluoride.

Changes in formulation, notably in the type of polishing agent and the final pH of the mixture, could affect the value of stannous fluoride in a dentifrice but, from the data reviewed here, it is concluded that the ideal combination of dentifrice ingredients, to which stannous fluoride may be added without loss of activity, has yet to be found.

1.5. REACTIONS OF STANNOUS FLUORIDE DENTIFRICES WITH ENAMEL

If it becomes established that stannous fluoride dentifrices have a part to play in the prevention of dental caries a full understanding of the factors which influence their action will be required. A knowledge of these factors could contribute to improvements in formulation which might lead to the production of dentifrices with increased clinical effectiveness. However, very little information upon the reactions of stannous fluoride dentifrices with enamel is available although the reactions of enamel with stannous fluoride in solution have been quite fully explored.

When stannous fluoride in a dentifrice reacts with enamel it would seem possible that a mixture of a tin phosphate, tin oxide and calcium fluoride are formed at the surface and fluorapatite may be formed within its superficial layers. However, Brudevold, Steadman, Gardner, Rowley and Little (1956) were unable to show a significant uptake of tin and fluoride from an aqueous slurry of a stannous fluoride dentifrice.

Electron-photomicrographs of enamel surfaces which had been etched before and after treatment with a 25 per cent aqueous slurry of a stannous fluoride dentifrice (Cooper, 1958) suggest that its protective effect is similar to that provided by a solution of stannous fluoride (Gray, Schweizer, Rosevear and Broge, 1958). However, enamel solubility measurements reported by Wachtel (1961) showed that stannous fluoride dentifrices afforded enamel less protection against acid than

equivalent solutions of stannous fluoride unhindered by the presence of additional agents.

The hydrated tin oxide which is believed to be deposited on the surface of enamel may not give lasting protection. The lability of the protection afforded by topical applications of stannous fluoride has been noted in enamel solubility reduction tests reported, with few details, by Darrow, Stookey and Muhler (1961) and by Mercer (1961). The latter has shown that non-fluoride dentifrices rapidly remove the protective effect whereas in both reports it is claimed that the effect of the topical fluoride is prolonged by the use of a stannous fluoride dentifrice. However, Scott (1960) mentions that studies with the electron-microscope have shown that immersion of enamel in a stannous fluoride dentifrice provided better protection than when the dentifrice was applied by brushing.

A technique has been devised for measuring enamel solubility in situ (Holmes and Middleton, 1962) and it has been claimed that a measurable reduction in solubility followed the use of a stannous fluoride dentifrice for only 21 days. If a reduction in enamel solubility is related to caries resistance, and there are some observations which throw doubt upon the validity of this relationship (Hardwick, 1963), the dentifrice tested by Holmes and Middleton (1962) would be expected to be useful in the prevention of dental caries. However, when Slack and Martin (1964) tested the same preparation in a clinical trial they were unable to demonstrate that it had a preventive action.

An undesirable reaction of stannous fluoride in a dentifrice leads to staining of the margins of fillings and the cervical regions of sound teeth. Slack and Martin (1964) found significantly more staining of the teeth in children using a stannous fluoride dentifrice than in children using the control paste. It has been suggested that this staining is due to the precipitation of a brown tin oxide (Muhler, 1961a). Alternatively, sulphide, released by bacterial action in the mouth, may combine with stannous ions to form insoluble brown stannous sulphide, for Brudevold et al. (1956) have shown that when stannous fluoride is applied to enamel in the presence of hydrogen sulphide the treated enamel is stained.

The staining with stannous fluoride dentifrices, which has been attributed to the stannous ion, may be due to the inefficiency of the newer polishing agents in removing stain from the teeth. In support of this is the clinical observation that teeth can often be kept free of stain by brushing with a calcium carbonate containing dentifrice at weekly intervals. However, data on the relative polishing powers of stannous fluoride dentifrices and toothpastes with conventional abrasives do not appear to be available.

1.6. CLINICAL TRIALS OF DENTIFRICES CONTAINING STANNOUS FLUORIDE

The results of 12 double-blind controlled trials of dentifrices containing stannous fluoride are now available for evaluation. Muhler and his colleagues, or workers associated with the manufacturer of the first commercial stannous fluoride dentifrice, initiated 9 of these trials. Their findings appear in a series of reports published since 1954. A detailed assessment is not within the scope of this review but data from these trials are summarized here (Table 1.1)to show that promising results have been claimed. Hill (1959) gave an inadequate description of his methods, therefore the value of his trial is uncertain.

Only 3 trials have been reported by other workers (Kyes et al., 1961; Finn and Jamison, 1963; Slack and Martin, 1964). In none of these trials was it shown that a stannous fluoride dentifrice had a preventive action which was superior to that of any of the other preparations tested in the same trial.

To summarize the results of all the trials, it is clear that one group of workers has been able to report that a commercial stannous fluoride-calcium pyrophosphate dentifrice had a useful preventive effect in 7 out of 9 clinical trials. There is, as yet, no confirmation of these results in the work of others. Further trials of this dentifrice and other toothpastes containing stannous fluoride, by independent workers, are known to be in progress but their findings are not yet available.

TABLE 1.1c

Results of Clinical Trials of a Stannous Fluoride Dentifrice Published by Muhler and Associates.

Authors	Age in Years of Subjects at Beginning	Duration of Trial in Years	Supervision of Brushing	Per cent Reduction in New DMFS at the End of 1 year	2 years	3 years
Muhler, Radike, Nebergall and Day (1954 and 1955a)	5-15	1	None	49	-	-
Muhler, Radike, Nebergall and Day (1955b)	5-15	1	None	36	-	-
Muhler, Radike, Nebergall and Day (1956); Muhler and Radike (1957)	17-36	2	None	41	34	-
Jordan and Peterson (1957, 1959)	8-11	2	1/day	34	20	-
Jordan and Peterson (1959)	8-11	2	None	-	12 (not significant)	-
Muhler (1959, 1960)[1]	6-18	3	None	23	25	-[1]
Hill (1959)	9-16	2	None	14	15 (not significant)	-
Peffley and Muhler (1960) Peffley, G.E., cited by Muhler (1962)	10-19	2	3/day	47	46	-
Muhler (1962)	6-18	3	None	24	24	21

1. Detailed results for 3rd year not reported (Muhler, 1961b).

Considerable additional, but as yet, unpublished evidence of the preventive action of stannous fluoride-containing dentifrices has been cited in a very recent report by the Council on Dental Therapeutics of the American Dental Association (see J. Amer. dent. Ass. 69, 195-198, 1964). It appears that in 3 out of 4 two-year clinical trials substantially fewer DMF surfaces were found after the use of the proprietary dentifrice containing stannous fluoride and calcium pyrophosphate and in 3 trials of a dentifrice containing stannous fluoride, sodium metaphosphate and dicalcium phosphate a reduced caries incidence was also observed. The American Dental Association is now recommending both these dentifrices as effective agents in the prevention of dental caries although the statistical significance of some of the results cited appears to be in doubt. Clearly, judgement by others must be deferred until the detailed evidence is published.

In this review of factors which may influence the actions of fluoride dentifrices it is appropriate to consider whether our knowledge in this respect is enlarged by data from the clinical trials. These trials were not all carried out in the same way. There were variations in the composition of the dentifrices, including the addition of stannous pyrophosphate, and in some studies an initial cleaning of the tooth surfaces was performed. There is no evidence to show that these modifications improved the preventive action of the dentifrices tested and it would seem unlikely that the uptake of fluoride by enamel was significantly augmented by these changes in medicament and method.

In a clinical trial the composition of the control dentifrice

should, apart from the active agent(s), be similar to the composition of the test dentifrice. In the trials quoted, the low pH of the test preparations was not reproduced in the control dentifrices. However, this has been defended by Muhler (1961d) on the grounds that a dentifrice without stannous fluoride would be harmful to enamel if used at a pH around 4.5. The difference in pH is probably without significance when the actions of a stannous fluoride dentifrice and a bland control paste are compared. However, this pH factor may have been important in one trial in which the actions of stannous and sodium fluoride dentifrices were compared (Muhler et al., 1955b). The lower pH of the stannous fluoride paste may have raised the fluoride uptake by enamel and hence reduced the caries score.

A single topical application of a solution of stannous fluoride, at the time of each examination, combined with the use of a stannous fluoride dentifrice appeared to provide greater protection to the teeth than the use of either agent alone (Muhler, 1959, 1960, 1961b and c). This is in keeping with the laboratory findings of Darrow et al. (1961) and Mercer (1961) which suggest that stannous fluoride solutions applied topically have a synergistic action with stannous fluoride dentifrices. For children with rampant caries this could be a significant finding. However, as Bartlestone, Mandel and Chilton (1961) have pointed out, Muhler's clinical study lacked a proper control for the topical stannous fluoride. Therefore, the apparently superior protection could have been due to the topical applications alone.

1.7. TOXICITY OF STANNOUS FLUORIDE DENTIFRICES

1.7.1. Introduction.

In view of the relatively high fluoride content of stannous fluoride dentifrices (1000 ppm fluoride) it must be shown that their use is free from undesirable and even dangerous side-effects.

Four possible hazards should be considered.

1) The prolonged use of stannous fluoride in a dentifrice may be harmful to the soft tissues of the mouth.
2) Children using fluoride dentifrices may swallow sufficient fluoride to cause mottling of the developing enamel. This risk would appear to be greater when children are already drinking fluoridated water.
3) Adults or children may swallow enough of the dentifrice to produce symptoms of chronic fluorosis.
4) Children occasionally ingest large amounts of any dentifrice. When the dentifrice ingested contains fluoride the possibility of acute fluoride intoxication should be considered.

1.7.2. Effects of Stannous Fluoride Dentifrices upon the Soft Tissues.

The possible effects of stannous fluoride upon the soft tissues of the mouth have received very little consideration. However, it is known that the application of a 10 per cent solution of stannous fluoride to the teeth causes blanching of the adjacent gingival tissue but no lasting clinical or histological abnormality has been detected (Sweiterman,

Muhler and Swenson, 1961). It seems unlikely that the continued use of a dentifrice, containing a much lower concentration of stannous fluoride, could exert a harmful effect upon the soft tissues. However, Kraus (1962) has argued that fluoride in a dentifrice may interfere with the lactate-peroxide metabolism of the oral flora, making the mouth and throat more liable to infection. In a very brief clinical trial no evidence to support this contention was produced (Goose and Melville, 1963).

1.7.3. Chronic Toxicity.

To evaluate the risk of producing chronic fluorosis by the continued use of a stannous fluoride dentifrice data are required upon the amount of fluoride retained during toothbrushing, the amount of fluoride absorbed and the amount subsequently excreted.

Each time the teeth are cleaned, up to approximately 1 gram of dentifrice will be used. Therefore, if the teeth are brushed three times per day with a stannous fluoride dentifrice containing 1 mg of fluoride per gram, up to 3 mg of fluoride could be taken into the mouth each day. If sufficient of the dentifrice is swallowed the daily ingestion of fluoride during toothbrushing could be greater than the intake from drinking water containing 1 ppm fluoride.

In what appears to be the only reported study of the retention of fluoride during brushing with a dentifrice containing stannous fluoride Ericsson (1961) found a maximum retention in the body of 35 per cent of the fluoride present on the brush at the beginning. The mean

retention for 10 subjects was 12 per cent. The preparation used in this experiment was a laboratory prepared stannous fluoride-calcium pyrophosphate dentifrice. It was not a commercial product. In a similar experiment, this time with a sodium fluoride dentifrice, Ericsson (1961) found the mean retention of fluoride by 10 subjects to be 8 per cent. This was less than the 15 to 23 per cent retention reported by Eichler, Appel and Burschel (1955); in their experiment the amount of fluoride retained by children aged 4 to 7 years was only 3 per cent greater than the amount retained by the adult subjects. The actual amount of retained fluoride is more important than the percentage retained and this has been put as high as 0.3 mg per day when the teeth were brushed twice daily (Hill Top Research Institute, 1954, cited by Büttner, Schülke and Soyka, 1961).

The fate of the retained fluoride is uncertain. Presumably some is taken up by the teeth, some by the dental plaque or by other organic material in the mouth and the rest is swallowed.

The amount of fluoride absorbed, during and after toothbrushing, has not been determined by measuring the amount of dentifrice fluoride ingested and the amount recovered in the faeces. However, indirect evidence of fluoride absorption has been obtained by determining the amount of fluoride excreted in the urine of subjects using a stannous fluoride dentifrice. In studies of this type, changes in the amount of fluoride absorbed are reflected by changes in urinary fluoride excretion.

When children aged 2 to 5 years used a stannous fluoride dentifrice no increase in their urinary fluoride output was detected (Schweinsberger and Muhler, 1957). Some of the children were already exposed to fluoridated water but it was claimed that their relatively high output of fluoride was not further increased by the use of the dentifrice. However, it is possible that evidence of high fluoride excretion by a few children was hidden in these results, for the study was reported in terms of the mean fluoride excretion by groups of children, without any indication of the ranges over which the individual values were spread. Therefore, the risk of enamel fluorosis, produced by the ingestion of dentifrice fluoride, was not excluded for all the children. Although sufficient time has elapsed since the introduction of the first stannous fluoride dentifrice, no long-term clinical investigation of enamel mottling has been reported. A comparison is required of the incidence of mottling in the permanent teeth of children who used the dentifrice up to the age of 6 with the incidence in children of similar age who had never used a fluoride dentifrice.

The output of fluoride in the urine of individual adults, before and during the period when a stannous fluoride dentifrice was being used, has been measured by Büttner et al. (1961). The normal variations in fluoride excretion were greater than any added variation produced by the ingestion of dentifrice fluoride. Therefore, it was concluded that the subjects absorbed little or no fluoride whilst toothbrushing and that there was no risk of chronic fluorosis due to ingestion of dentifrice fluoride.

1.7.4. Acute Fluoride Intoxication.

In considering this possibility allowance must be made for the most perverted appetites. Children can, and occasionally do, eat substantial quantities of dentifrice. Therefore, the risk involved when the dentifrice contains fluoride must be evaluated.

Acute toxicity studies in animals are mentioned by Cooper (1958). The LD_{50} value for a slurry of fluoride dentifrice (presumably stannous fluoride) was found to be 100 mg/kg of body weight for rats and 70 mg/kg for mice. The equivalent intake of dentifrice for a 15 kg child would be contained in between 7 and 10 large tubes. However, a child, in contradistinction to these experimental animals which are unable to regurgitate, would be partly protected by vomiting caused by the fluoride and essential oils in the dentifrice. Hence, the human lethal dose would be even greater than the dose suggested from the results of the animal experiments and the possibility of acute lethal poisoning has therefore been dismissed as a virtually non-existent danger (Cooper, 1958).

Confirmatory calculations by Büttner et al. (1961), based upon data reported by Cox and Hodge (1950), show that even the smallest child would not die after ingesting the contents of one large tube of stannous fluoride dentifrice. This calculation errs on the side of safety because it assumes that all the fluoride in a dentifrice is soluble and therefore readily absorbed, whereas there is evidence to show that fluoride absorption is reduced by the presence of calcium (Weddle and Muhler, 1957; Wagner and Muhler, 1960). With some dentifrices this may minimize fluoride absorption (Büttner et al., 1961).

1.8. SUGGESTIONS FOR FURTHER INVESTIGATIONS

Earlier work, in which the ageing of stannous fluoride in solution was connected with a progressive reduction in its power to protect enamel against decalcification, has been challenged in the light of recent studies. These later results require confirmation but if they are substantiated it would appear that the principal limitation upon the action of stannous fluoride in a dentifrice lies in the presence of the other constituents. The extent to which these constituents interfere is only beginning to become apparent and the solitary report of decreasing availability of stannous and fluoride ions with ageing of a dentifrice requires confirmation.

The effect of storage conditions, particularly the temperature of storage, upon the availability of the useful ions does not appear to have been investigated. Nevertheless, this may be of considerable practical importance when stannous fluoride dentifrices are stored in hot climates.

There is a very limited amount of data on the behaviour of dentifrice stannous fluoride in the saliva-enamel environment. The influence of saliva upon both the availability of the active ions and the uptake of tin and fluoride by enamel and plaque in vivo has not been determined. A satisfactory method for measuring such uptake is of potential importance as a screening test for the assessment of new dentifrices. Other screening tests may be developed from measurements of the availability of stannous

and fluoride ions in saliva and the ability of dentifrice-saliva slurries to reduce enamel solubility both in vitro and in vivo.

At present far too few data are available to attempt to correlate the findings in possible screening tests for stannous fluoride dentifrices with their clinical effectiveness. Therefore, these tests should be applied to all the available preparations and the results should be compared with the results eventually obtained in clinical trials. If screening tests can be shown to indicate the probable preventive action of a dentifrice only the more promising preparations would need to be submitted to a full clinical trial.

Information upon the retention, absorption and excretion of dentifrice fluoride is incomplete because there are very few reports of such work upon the commercial stannous fluoride dentifrices. It would appear that the technique of cleaning the teeth and the thoroughness of subsequent rinsing are the factors which determine the amount of fluoride retained. Although the risks of swallowing these dentifrices have been minimized the one report of quite high fluoride retention at the end of toothbrushing needs checking.

Therefore, in some of the experimental investigations recorded in this thesis attempts have been made to:-

1) Compare the loss of fluoride during the ageing of three commercially available stannous fluoride dentifrices (Chapter 3). These dentifrices are currently being tested clinically.

2) Compare the amount of soluble fluoride in water and saliva slurries prepared from the same three dentifrices (Chapter 3).

3) Show the influence of storage temperature upon the amount of soluble fluoride in the dentifrices (Chapter 3).

4) Compare the uptake of fluoride by enamel in vivo when the three dentifrices are used in toothbrushing (Chapter 4).

5) Investigate the retention and excretion of fluoride from a commercial stannous fluoride dentifrice (Chapter 6).

CHAPTER 2

THE DETERMINATION OF MICROGRAM AND SUBMICROGRAM AMOUNTS OF FLUORIDE

2.1. INTRODUCTION

Research into the problems outlined at the end of the previous chapter is dependent upon the availability of suitable methods of fluoride analysis. Information will be required upon the fluoride content of dentifrice extracts, enamel, saliva and urine samples. Extracts from dentifrices do not present any serious analytical problems but difficulties arise with biological samples. In these, small changes in fluoride content, produced by treatment with fluoride, are difficult to detect against the large natural variations in the amounts of fluoride often present in the control samples.

At the commencement of this investigation it appeared that the analytical requirements would probably be met by a chemical method capable of measuring microgram quantities of fluoride in small volumes of solution and a radioisotope method employing fluorine-18.

2.2. MICRO-DIFFUSION METHODS IN THE ANALYSIS OF FLUORIDE

Horton (1960) has reviewed recent trends in the determination of fluoride but his review was written before the development of all but one of the new micro-diffusion methods. The use of diffusion methods will be evaluated here because two of these have been employed in the present work, and one has been used by Manly (1961), to determine soluble fluoride in dentifrices.

The essential steps for the successful analysis of fluoride in samples containing organic material are:

1) The removal of the organic material,
2) The separation, recovery and concentration of the fluoride free of anions which may interfere with
3) The estimation of fluoride by methods based upon the formation of coloured fluorocomplexes.

In the micro-diffusion methods of analysis the sample reacts with a strong acid and hydrogen fluoride is released. This diffuses within a closed vessel and is trapped by alkali. Fluoride separated and recovered in this way is then estimated by titrimetric or colorimetric techniques.

2.2.1. Ashing.

Fluorine-18 has been used to show that high losses of fluoride can occur when fluoride containing samples are ashed (Singer and Armstrong, 1959; Kudahl, 1961, personal communication). Such losses

constitute one of the main hazards of fluoride analysis. Although these losses can be reduced by ashing with calcium or magnesium oxides as fixatives, such fixatives free of fluoride, are often difficult to prepare.

In the classical methods of fluoride analysis one of the reasons for ashing, prior to distillation with hot perchloric acid, was the removal of organic material which might otherwise contribute to the formation of an explosive mixture. This risk no longer exists when small samples are heated to only 60°C or less in a micro-diffusion cell and satisfactory separation of fluoride is possible without ashing (Wharton, 1962; Rowley and Farrah, 1962). Singer and Armstrong (1962) appear to have analysed dentine and urine samples without preliminary ashing and Manly (1961), using the method described by Frere (1961 and 1962), did not ash extracts from dentifrices before diffusion. Therefore, in the experiments to be described later similar extracts will not be ashed.

2.2.2. Diffusion Separation of Fluoride.

Gautier and Claussmann (1912) appear to have been the first to describe a method for the separation of fluoride by diffusion as hydrogen fluoride but, possibly because they used a large and expensive platinum cell, interest in this method waned until polymer laboratory-ware became available.

Singer and Armstrong (1954) were the first to use polyethylene bottles as cells for the separation of fluoride. In their method

70 per cent perchloric acid and the ashed sample are heated to 50°C for 20 hours in a capped bottle. The diffusing hydrogen fluoride is recovered on a polyethylene strip treated with 2 per cent sodium hydroxide. Similar methods, using polyethylene bottles, have been described by Stegemann and Jung (1959) and Hall (1960). These authors recommend the addition to the cell of silver as perchlorate (Stegemann and Jung, 1959) or sulphate (Hall, 1961, personal communication) to prevent the diffusion of other halogens which, if in sufficient quantity, may interfere with the recovery of fluoride. Diffusion in polyethylene bottles permits the recovery of 1 µg or more of fluoride in volumes of about 1 ml. Hall (1963) has extended the range of his method to the submicrogram level by using magnesium succinate to trap the diffused fluoride. This is then determined by a new direct colour reaction.

Frere (1961 and 1962) reported the use of a diffusion cell made of polypropylene, fashioned according to a modification of the Conway dish (Öbrink, 1955). The fluoride-containing sample and 80 per cent sulphuric acid are placed in the inner, annular chamber and sodium hydroxide is the trapping agent in the central well. The sealed cell is kept at room-temperature for 24 hours. Polypropylene diffusion vessels of the Öbrink pattern have also been used for the separation of fluoride by Wharton (1962 and 1963), Singer and Armstrong (1962) and Leach and Griffiths (1963). Unlike Frere (1961 and 1962), these authors encourage diffusion of hydrogen fluoride by heating the sealed dish to 55 or 60°C for 16 - 24 hours. Indeed, Wharton (1963) showed that with his method heating is essential for complete diffusion.

Further simplifications of the technique of separating fluoride by diffusion as hydrogen fluoride have been described by Rowley and Farrah (1962) and by Weatherell and Hargreaves (1964). The diffusion cell recommended by the former workers is a disposable methacrylate Petri dish. The sample, perchloric acid and silver perchlorate are placed in one half, whilst sodium hydroxide, previously evaporated on to the lid of the dish, is the trapping agent. This method appears to offer advantages over those employing the Öbrink-Conway polypropylene dishes for disposable dishes eliminate contamination errors. In addition, splashing the trapping agent with acid, which may affect the subsequent colour estimation, is unlikely. The large surface area and shallow depth of the perchloric acid-sample solution should facilitate rapid diffusion in this system.

The use of diffusion methods in fluoride analysis have greatly simplified the separation procedure. Older distillation methods, based upon the technique of Willard and Winter (1933), are difficult with microgram quantities of fluoride in small volumes of fluid. The diffusion technique has made possible the quantitative recovery of 1 µg of fluoride or less and the rapid analysis of many samples concurrently. Hence its use in the experiments to be described in Chapter 3.

2.2.3. Fluoride Estimation.

A variety of techniques have been employed for the quantitative determination of fluoride after its separation by diffusion. Most depend upon the bleaching of coloured metal complexes by fluoride. Thorium-alizarin or zirconium-eriochrome cyanine-R complexes have been

used. The colour changes can be measured by titration (Hall, 1960) or, more simply by using colorimetric apparatus.

Two new metal complexes have recently been employed in the determination of diffused fluoride. A method using zirconium-SPADNS [4, 5-dihydroxy-3-(p-sulphophenylazo)-2, 7-naphthalene disulphonic acid, trisodium salt] complex was introduced by Bellack and Schouboe (1958). This reagent has been used by Wharton (1962 and 1963). It appears to represent a considerable advance because it consists of a single phase which is easily prepared, highly stable, well buffered at a pH less than 1 and no separate correction of the pH of the diffusate is required.

Belcher, Leonard and West (1958) introduced alizarin complexone (3-amino-methyl alizarin N:N-diacetic acid) for complexiometric estimations of fluoride. This lanthanum-alizarin complexone has been used in fluoride determinations by Frere (1961 and 1962), Hall (1963) and Quentin (1964). It appears to be the first colorimetric agent to give a direct colour reaction with the fluoride ion but its use involves a greater number of steps than the use of the Zr-SPADNS reagent.

Thus micro-diffusion methods of separating fluoride, followed by its colorimetric estimation, appeared to be suitable for the analysis of soluble fluoride in dentifrices containing stannous fluoride. Indeed, at the time this study began Frere's methods had already been used in such experiments by Manly (1961). The best of the methods available during the early stages of the work to be reported in the next chapter appeared to be the one described by Hall (1960). In later analyses the Zr-SPADNS method of Wharton (1962 and 1963)was used because it proved to be simpler and more accurate than Hall's method.

2.3. FLUORINE-18 MEASUREMENTS

Fluorine-18 has been used by previous workers to measure submicrogram changes in the fluoride content of biological samples after treatment with agents containing labelled fluoride.

According to Myers, Hamilton and Becks (1952) one of the advantages of this technique over chemical analysis is that labelled fluoride, added to a sample by an experimental procedure, can be distinguished from the fluoride already present in the sample. This would appear to overcome the difficulty of measuring small changes in fluoride content by chemical methods against a background of widely variable fluoride levels in the untreated control samples.

The outstanding disadvantage in the use of fluorine-18 is its short half-life of 112 minutes (Allen, Smith and Hiscott, 1961). This gives an approximate working time of only 10 hours. Therefore, the number of experimental observations which can be made with one consignment is severely curtailed. The half-life also limits the use of fluorine-18 to workers with easy access to equipment for the production of short-lived radioisotopes.

In spite of these limitations fluorine-18 has been used to measure the uptake of fluoride by enamel in vitro and in vivo.

The uptake of fluoride by enamel, from very dilute solutions, is difficult to measure directly by chemical methods, although it can be measured indirectly by determining the loss of fluoride from the solution. To provide direct evidence of such uptake by whole enamel

Brudevold, Hein, Bonner, Nevin, Bibby and Hodge (1957) used a fluorine-18 labelled solution of sodium fluoride which contained 1 ppm fluoride. Other studies of fluoride uptake by enamel in vitro have been made with the aid of radiofluorine (Ericsson, 1958, 1961 and 1962a and b; Hardwick, Fremlin and Mathieson, 1958; Hardwick and Fremlin, 1959) and Fremlin, Hardwick and Suthers (1957) have used fluorine-18 in an ingenious method for the analysis of fluoride in μl volumes of solution.

Fluorine-18 has been employed in clinical studies to measure the uptake of fluoride by enamel from topical applications (Myers et al., 1952), from mouthwashes (Hellstrom, 1960) and from chewing gum (Emslie, Veall and Duckworth, 1961). Additional investigations in which fluorine-18 has been used in human subjects include a study of the absorption and excretion of sodium fluoride by Carlson, Armstrong and Singer (1960) and measurements of the retention of fluoride during toothbrushing with fluoride dentifrices (Ericsson, 1961).

From a consideration of the data presented in these reports it was concluded that fluorine-18 could be used to measure

1) The uptake of fluoride by enamel in vivo from stannous fluoride dentifrices (Chapter 4).
2) The retention of dentifrice fluoride, its absorption and excretion after toothbrushing (Chapter 6).

However, Fremlin, Hardwick and Mathieson (1959) have warned intending users of fluorine-18 of the inherent difficulties in the interpretation of results obtained with this radioisotope. An appraisal of these difficulties is included in a later discussion of the experimental findings recorded in Chapter 5.

2.4. RADIATION HAZARDS WITH FLUORINE-18

A possibly serious disadvantage of the use of any radioactive-isotope in experimental work involving human subjects is the risk of exposing the subjects to ionizing radiation. Fortunately, this risk with fluorine-18 is low because of its short half-life.

According to Allen, Millett and Smith (1959) fluorine-18 is a member of the group of radioisotopes which has the lowest toxicity. The maximum permissible body burden is given by Morgan (1954) as 24 μC. However, as fluorine-18 is a bone-seeking radioisotope this should be reduced by a factor of 5 to 4.8 μC. Therefore, when not more than 1-2 μC of fluorine-18 is given to a human experimental subject, at any one time, there is no radiation risk. For the purpose of this study permission to use such a dose in clinical experiments was granted by the Advisory Board on the Allocation of Radioactive Isotopes for Clinical Use of the Medical Research Council.

The dose of external radiation received by all laboratory workers who were exposed to ionizing radiation from fluorine-18 was monitored by the use of photographic film badges and found to be negligible.

CHAPTER 3

THE RELEASE OF SOLUBLE FLUORIDE IN VITRO BY DENTIFRICES CONTAINING STANNOUS FLUORIDE

3.1. INTRODUCTION

If a chemical in a dentifrice is to contribute to the prevention of dental caries it must be conveyed in solution in saliva to the dental plaque or the enamel surface. Therefore, the amount of fluoride in a dentifrice which is soluble at the time of use would appear to be more important than the total amount of fluoride added to the mixture during its manufacture.

The present investigation was designed to fulfil two aims. The first was to measure and compare the amounts of soluble fluoride which could be eluted by water and by saliva from samples of stannous fluoride dentifrices. The second aim was to determine the influence of the duration and temperature of storage of the dentifrices upon the quantities of soluble fluoride released.

A preliminary investigation is described below in Section A. When more suitable dentifrice samples and an improved method of fluoride analysis became available the investigation was repeated and its scope extended. These more recent experiments and the data obtained are recorded in Section B.

SECTION A

A PRELIMINARY INVESTIGATION INTO THE RELEASE OF SOLUBLE FLUORIDE BY DENTIFRICES CONTAINING STANNOUS FLUORIDE

3.2. MATERIALS AND METHODS

3.2.1. The Stannous Fluoride Dentifrices.

Three commercial brands of dentifrice (designated A, B and C throughout this thesis) were used. According to the manufacturers each contained approximately 0.1 per cent fluoride as stannous fluoride. The experiments were started in 1961 about one year before such dentifrices were on sale in this country. Therefore, samples of known production date of two dentifrices (A and C) had to be obtained from the United States. Production of Dentifrice B, in commercial quantities, commenced in the United Kingdom late in 1961. Samples of this paste, together with their production dates, were supplied by the manufacturer concerned.

The conditions under which the various dentifrice samples had been stored, prior to reaching this laboratory, were not known; once in the laboratory they were stored at room temperature. Relatively few tubes of dentifrice were available. Therefore, in order to obtain a sufficient number of samples of different ages, many of the tubes were kept after analysis and their contents were analysed a second time after a period of further storage.

3.2.2. Extraction of Soluble Fluoride from the Dentifrices.

Fluoride was eluted from four samples of dentifrice taken from the same tube, by suspending 100 ± 5 mg of paste in 2 ml of distilled-deionized water or 2 ml of freshly expectorated human saliva. All the saliva was taken from one subject except when large volumes were required for the investigation of the method. For these experiments saliva from three subjects was pooled. After allowing the suspension to stand for at least 30 minutes most of the insoluble material was removed by centrifuging for 5 minutes at 3000 r.p.m. The supernatants were decanted and centrifuged again for a further 30 minutes at 5,500 r.p.m. At this stage the supernatants obtained from the water extracts were clear, but the saliva supernatants remained slightly turbid. However, on visual examination this turbidity appeared to be equal to the turbidity of the same saliva, without dentifrice, centrifuged as above. Therefore, it was not thought to be due to suspended insoluble matter from the dentifrices. Where necessary 1 ml of each supernatant was diluted with water to bring the fluoride concentration within the range of the analytical method (1 - 10 µg F).

Before this elution procedure was used routinely the following investigations were made.

1) After initial separation of the gross insolubles, the effect of varying the centrifuging time was explored by centrifuging a series of similarly prepared water and saliva slurries for 15, 30, 45 and 60 minutes at 5,500 r.p.m.

2) In the method of elution described above the concentration of dentifrice in the slurry was 5 per cent w/v. To determine the effect of altering the concentration of the paste upon the release of soluble fluoride, some slurries were also prepared with a dentifrice concentration of 2.5, 10, 15 and 25 per cent w/v.

3) The effect of conditions favouring oxidation of stannous fluoride upon the amount of soluble fluoride recovered from the dentifrices was determined. Weighed samples, from the same tube of dentifrice, were exposed to the air for different periods up to 4 hours before preparation of the slurry and subsequent analysis.

3.2.3. Analysis of Fluoride.

The fluoride in the supernatant solutions obtained by centrifuging the slurries was estimated by the micro-diffusion method of Hall (1960) as modified by the same author (Hall, 1961, personal communication).

Reagents. Analytical grade reagents and distilled-deionized water were used.

Thorium nitrate, 0.001N, was prepared by dissolving 147 mg of $Th(NO_3)_4.6H_2O$ in 1 litre of water.

Monochloracetate buffer solution at pH 3.0 was prepared by adding 120 ml N sodium hydroxide to 22.7 g of monochloroacetic acid dissolved in 800 ml of water. This was then diluted to 1 litre.

Buffered alizarin sulphonate solution. 5 ml of the thorium nitrate solution and 10 ml of the buffer were added to 20 ml of a 0.01 per cent w/v aqueous solution of alizarin sulphonate. This was diluted with water to 100 ml.

Potassium hydroxide, 3N, to which was added 1 ml of 0.5 per cent w/v ethanolic solution of phenolphthalein per 100 ml.

Perchloric acid -silver sulphate. To 4 ml of water, 2 g of silver sulphate and 80 ml of perchloric acid (72 per cent w/v) were added. After heating to 80°C and cooling, more perchloric acid was added to 100 ml.

Standard fluoride solution. 22.1 mg of sodium fluoride dissolved in 1 litre of water to give a fluoride concentration of 10 µg per ml.

Analytical Procedure.

Diffusion of Fluoride. The solutions for analysis (1 ml) were pipetted into diffusion cells. These were 20 ml polyethylene bottles with specially adapted screw-caps (Fig. 3.1.). The latter were fitted with 2.5 cm lengths of polyethylene tubing which carried 3 cm x 2 cm pieces of filter paper (Whatman No. 541) rolled into cylinders. These projected 2 cm from the tubes, into the bottles, when the caps were in place. One drop of 3N potassium hydroxide - phenolphthalein solution was placed on each filter paper. By pipette, 2 ml of the perchloric acid-silver sulphate reagent was added to each of the fluoride solutions in the diffusion bottles, without allowing it to contact the necks of the bottles. The stoppers carrying the tubes and

Figure 3.1

The diffusion cell used in the preliminary experiments. A short length of polyethylene tubing is fitted into the cap of a 20 ml polyethylene bottle. The tube carries a piece of filter paper rolled into a cylinder.

filter papers were screwed into place at once and tightened with pliers. To prevent losses during diffusion the junctions between bottles and stoppers were sealed with a mixture of equal parts of carnauba and ceresin wax. Four diffusion blanks, each containing 1 ml of water instead of the fluoride solution, were set up with every batch. To accelerate diffusion the bottles were placed in a thermostatically controlled oven at 60°C.

Recovery of Fluoride. After 24 hours in the oven the bottles were opened and the filter papers removed. Fluoride was extracted from each paper by 3 ml of water contained in 5 ml graduated tubes placed in boiling water for 2 minutes. When cool the solutions were made just acid to phenolphthalein with 0.1 N perchloric acid and then diluted to 5 ml with water. The filter papers from the diffusion blanks were treated in a similar way and their extracts were combined.

Adjustment of pH. The pH of 2 ml of the diluted extract was adjusted to 3 with 0.04 N perchloric acid using 0.04 per cent w/v bromophenol blue in aqueous solution as the indicator. A colour standard for this titration was prepared by adding the indicator to distilled water which had been acidified to pH 3 with 0.04 N perchloric acid. The volume of acid added to the 2 ml portion of the extract was noted and a similar volume was added to a second 2 ml portion without the indicator.

Titration. Titration standards containing 1, 3, 5 and 10 µg fluoride were prepared by adding appropriate volumes of the standard fluoride solution to 2 ml portions of the combined diffusion blanks

after adjustment of the pH to 3. A colour blank containing 2 ml water, .02 ml of .04 N perchloric acid and 2 ml buffered alizarin sulphonate was also prepared. Buffered sodium alizarin sulphonate solution (2 ml) was added to each of the test, standard and blank solutions and these were allowed to develop their yellow colour for at least 5 minutes. Each solution was then titrated with .001 N thorium nitrate, added from a 1 ml microburette, until the yellow colour changed to shell-pink which matched the colour blank. The thorium nitrate titration values for the test and standard solutions were obtained by subtracting the mean value for the titrations of the diffusion blanks. The fluoride present in the test solutions was read from the line obtained when the thorium nitrate values for the standards were plotted against their known fluoride content. This relationship was a linear one in the range 1 - 10 µg. The amount of soluble fluoride released to 2 ml of water or saliva by 100 mg of dentifrice was then calculated.

Investigation of the Analytical Method.

The accuracy and reproducibility of the method was tested by analysing a series of aliquots from two solutions containing different amounts of fluoride as sodium fluoride. Multiple analyses were also performed upon aqueous and saliva extracts prepared from the same samples of dentifrice.

To test whether soluble components of the dentifrice interfered with the diffusion and recovery of fluoride a series of water

supernatants, prepared simultaneously from the same sample of dentifrice, were pooled. Fluoride (5 or 10 μg), in solution as sodium fluoride, was added to 5 ml portions of the combined supernatants. Aliquots of these solutions and the pooled supernatants without additional fluoride were analysed and the recovery of added fluoride determined by difference. By a similar method the recoveries of known amounts of fluoride, added to saliva extracts, were also measured.

3.3. RESULTS

3.3.1. Investigation of the Method of Extraction and Analysis of Soluble Fluoride.

The results obtained by using this metod of analysis for fluoride in aqueous solutions and dentifrice extracts are reproducible (Table 3.1). The coefficient of variation for a series of estimations on similarly prepared extracts was not greater than 7.6 per cent. In addition, satisfactory recoveries of fluoride, added to the supernatants prepared from the slurries, were achieved (Table 3.2).

Centrifuging for 15, 30, 45 and 60 minutes did not alter the amount of soluble fluoride released from the dentifrices (Table 3.3) but, after centrifuging for 15 minutes, saliva supernatants were still contaminated with insoluble material. At 30 minutes the saliva supernatants appeared to be as clear as control saliva samples which had not been mixed with toothpaste. Therefore, all samples were centrifuged for 30 minutes.

Exposure of dentifrice samples to the air, before extraction, did not influence the amount of soluble fluoride released (Table 3.4).

The effect of varying the concentration of the dentifrice in the slurries is shown in Table 3.5 and Figure 3.2. If it is assumed that the fluoride content of these dentifrices is 1 mg per g, as stated by the manufacturers, the soluble fluoride can be expressed

TABLE 3.1.

A. Repeated estimations of fluoride in solutions containing known amounts of sodium fluoride.

F Content in µg/ml	Number of Estimations	Mean F Titrated(µg)	Mean F Estimated(µg)	S.D.	Coefficient of Variation
2.5	17	1.02	2.55	± 0.11	± 4.3
5.0	10	2.03	5.08	± 0.16	± 3.2

B. Repeated estimations of soluble fluoride in water and saliva extracts of dentifrice samples.

Dentifrice Extract	Number of Estimations	Mean F Titrated(µg)	Mean F µg per 100mg paste	S.D.	Coefficient of Variation
A.10(saliva)	15	4.6	46.3	± 2.3	± 5.0
B.16(water)7	12	7.0	70.6	± 3.5	± 5.0
C.21(water)	13	4.8	47.8	± 3.65	± 7.6

S.D. = Standard Deviation

TABLE 3.2.

Recoveries of fluoride added to extracts of the dentifrices.

Dentifrice	Slurry with	Added F in µg	Recovered F in µg	Per cent Recovery
A.16	Water	5	4.85	97.0
	Saliva	5	4.8	96.0
B.20	Water	10	9.65	96.5
	Saliva	10	9.75	97.5
C.12	Water	10	10.2	102.0
	Saliva	10	10.4	104.0

Recovered F in µg is the mean of the recoveries in 4 samples.

TABLE 3.3.

The effect of varying the centrifuging time upon the amount of soluble fluoride released by dentifrice samples.

Dentifrice	Slurry with	Centrifuging time in min	Soluble F in μg
A.8	Water	15	80.0
		30	80.7
		45	81.6
		60	79.0
	Saliva	15	45.5
		30	45.9
		45	46.3
		60	47.6
C.38	Water	15	48.3
		30	47.3
		45	45.8
		60	47.8
	Saliva	15	20.4
		30	20.2
		45	22.4
		60	20.7

TABLE 3.4.

The effect upon the amount of soluble fluoride of exposing dentifrice samples to the air before preparation of the slurries.

Dentifrice	Time of exposure to air in min	Soluble F in μg
A.19	12	71.1
	65	70.4
	143	71.3
	217	72.3
	278	73.1
B.14	12	77.2
	50	79.0
	100	76.7
	197	79.2
	246	78.7
C.16	19	64.4
	50	65.2
	100	64.2
	195	62.0
	246	64.4

TABLE 3.5.

Soluble fluoride at different concentrations of dentifrice in the slurry.

Dentifrice	Slurry with	Paste in mg per 2 ml	Soluble F in µg	Soluble F % Total F
A.18	Water	50	39.6	79.2
		100	70.0	70.0
		200	135.6	67.8
		300	200.7	66.9
		500	303.4	60.7
	Saliva	50	23.8	47.6
		100	45.0	45.0
		200	85.8	42.9
		300	128.0	42.6
		500	204.5	40.9
C.38	Water	50	34.0	68.0
		100	47.3	47.3
		200	73.2	36.6
		300	88.0	29.3
		500	97.2	19.4
	Saliva	50	12.4	24.8
		100	20.2	20.2
		200	37.6	18.8
		300	44.8	14.9
		500	68.8	13.8

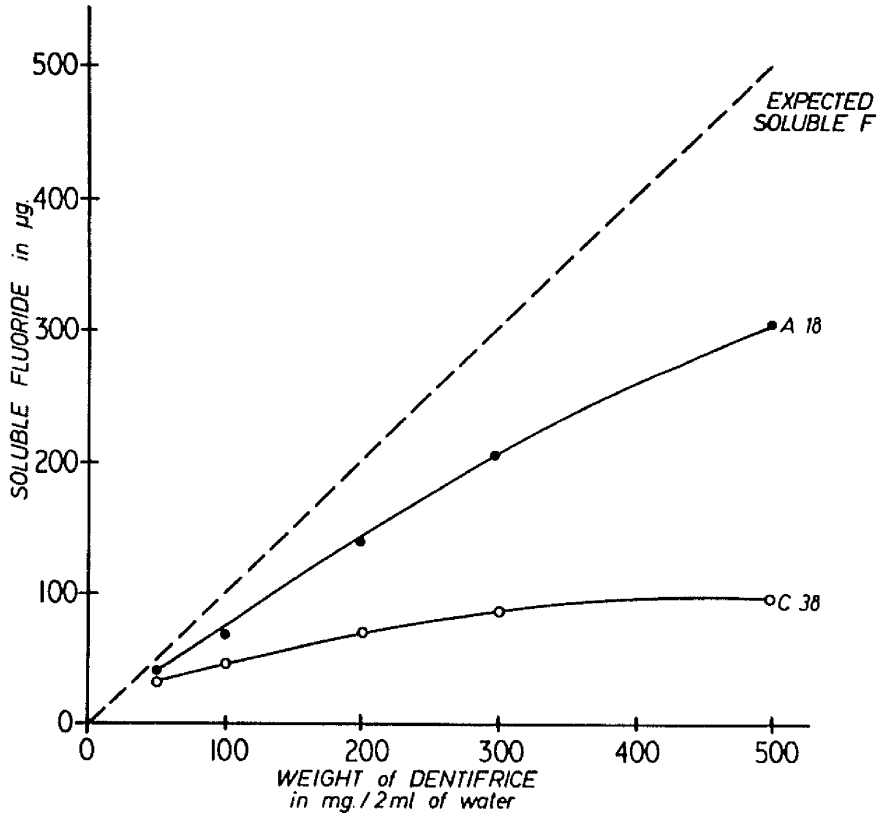


Figure 3.2.

The release of soluble fluoride in aqueous slurries containing different concentrations of Dentifrices A 18 and C 38. Expected soluble fluoride is the amount which would have been released if all the fluoride had been soluble.

as a percentage of the total fluoride in the paste. The percentage of fluoride which was soluble fell progressively as the concentration of dentifrice in the slurry was raised (Table 3.5).

3.3.2. Soluble Fluoride Released by the Dentifrices.

Although it has been shown that the percentage of soluble fluoride released from a dentifrice varies with the concentration of the slurry (Fig. 3.2) a linear correction of the amount determined was adequate for the small variations (100 ± 5 mg) in the weight of the dentifrice samples.

The amounts of soluble fluoride released in 2 ml of water or 2 ml of saliva by 100 mg of Dentifrices A, B and C, are shown in Tables 3.6 to 3.8 and Figures 3.3 to 3.5. In all three pastes there was a reduction in the amount of fluoride soluble in water and saliva as the dentifrices aged. At all ages smaller quantities of fluoride were soluble in saliva than in water.

These results will be discussed at the end of Section B together with the results obtained in the experiments described in that section.

TABLE 3.6.

The relationship between soluble fluoride in both water and saliva slurries and the age of Dentifrice A.

Age of Dentifrice in Weeks	Soluble F in µg	
	Aqueous Slurry	Saliva Slurry
8	85.2	71.9
12	-	61.2
15	77.8	46.0
16	70.2	-
18	85.0	60.4
20	71.1	-
23	67.8	-
24	-	49.6
29	66.0	-
37	65.1	42.3
48	63.2	40.9

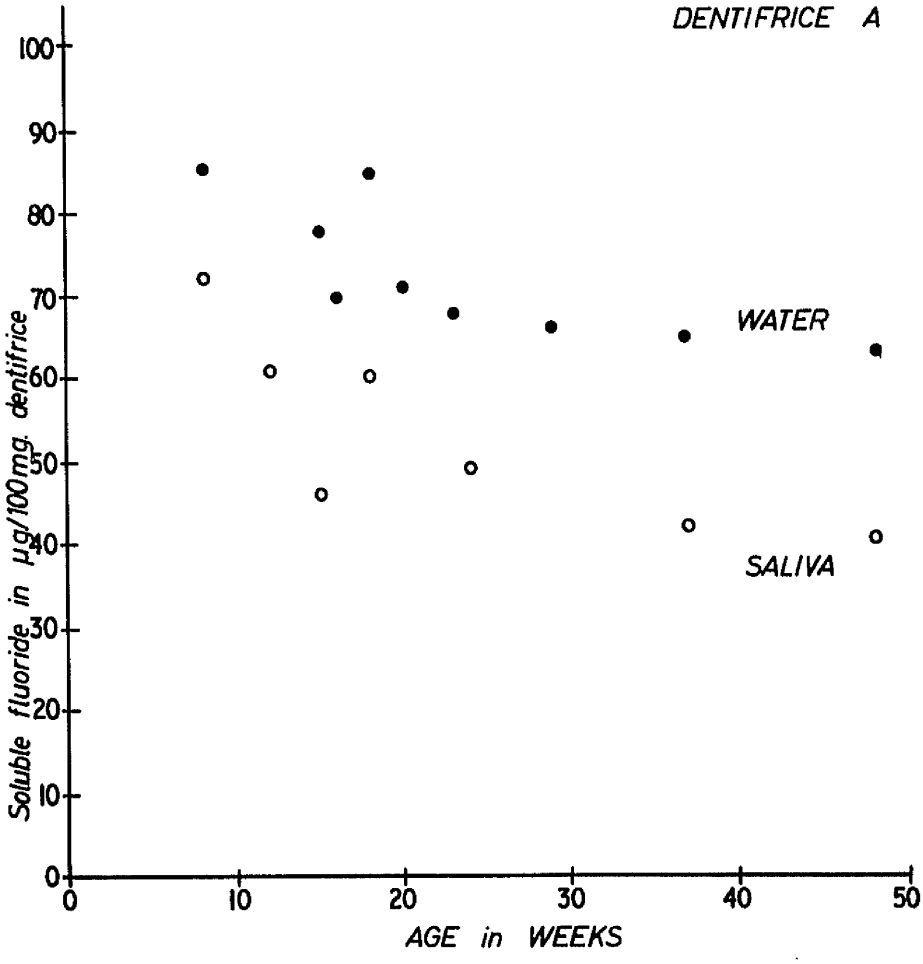


Figure 3.3.

The release of soluble fluoride in aqueous and saliva slurries (5 per cent w/v) prepared from samples of Dentifrice A stored for different periods after manufacture.

TABLE 3.7.

The relationship between soluble fluoride in both water and saliva slurries and the age of Dentifrice B.

Age of Dentifrice in Weeks	Soluble F in µg	
	Aqueous Slurry	Saliva Slurry
1	87.0	81.2
2	82.3	72.7
4	79.6	-
6	80.4	68.4
9	80.0	68.1
14	77.6	69.0
20	73.1	64.5
24	71.8	60.6

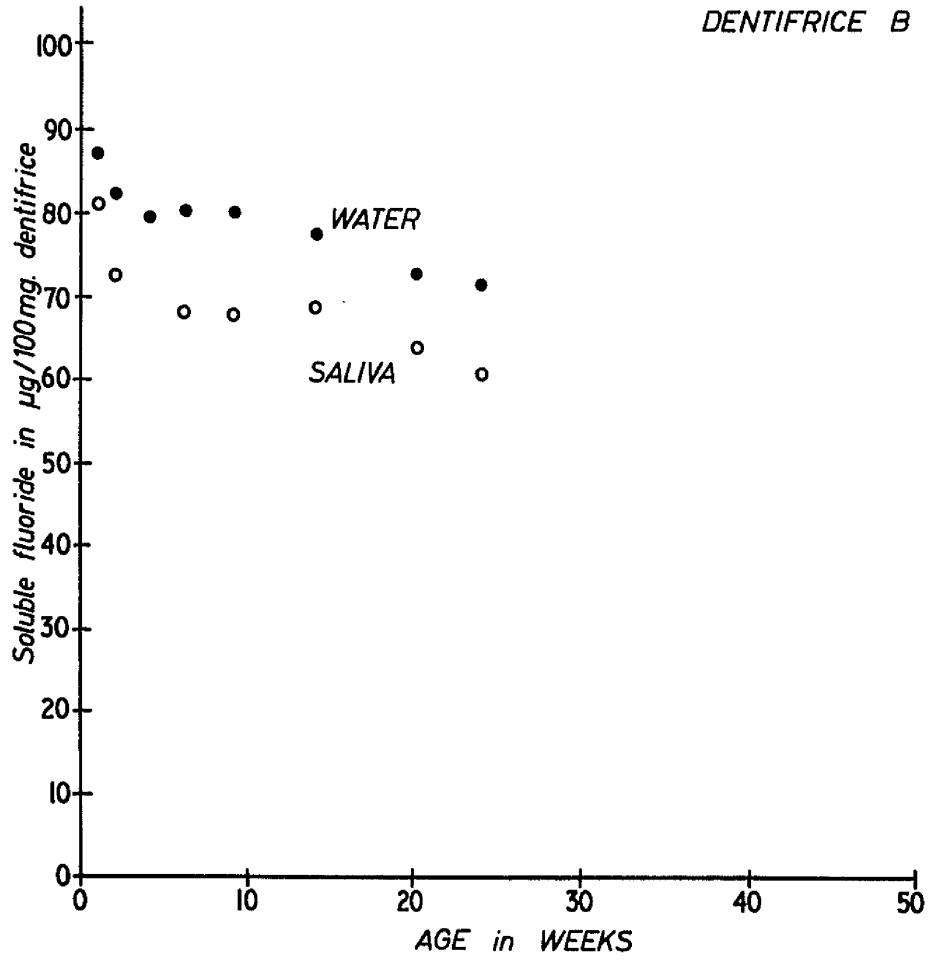


Figure 3.4.

The release of soluble fluoride in aqueous and saliva slurries (5 per cent w/v) prepared from samples of Dentifrice B stored for different periods after manufacture.

TABLE 3.8.

The relationship between soluble fluoride in both water and saliva slurries and the age of Dentifrice C.

Age of Dentifrice in Weeks	Soluble F in µg	
	Aqueous Slurry	Saliva Slurry
9	81.6	-
12	70.4	-
14	65.1	-
16	63.0	40.5
18	66.4	-
20	63.6	-
24	56.1	31.2
36	55.7	22.3
37	55.6	-
39	48.9	19.8
48	46.3	19.2

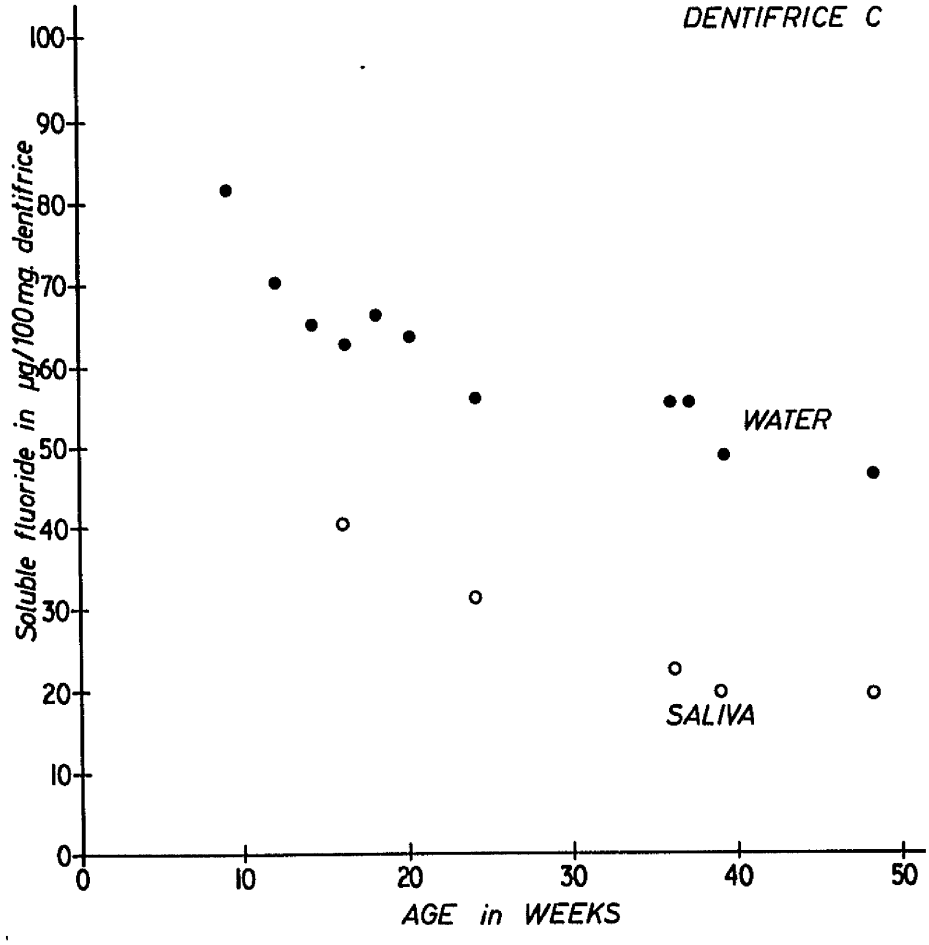


Figure 3.5.

The release of soluble fluoride in aqueous and saliva slurries (5 per cent w/v) prepared from samples of Dentifrice C stored for different periods after manufacture.

SECTION B

A FURTHER INVESTIGATION INTO THE RELEASE OF SOLUBLE FLUORIDE BY DENTIFRICES CONTAINING STANNOUS FLUORIDE

3.4. MATERIALS AND METHODS

3.4.1. The Stannous Fluoride Dentifrices.

The brands of dentifrice used in the experiments described in Section A were also used in this investigation. Standard size tubes, 60 of each brand (A, B and C), were purchased through normal retail channels. These supplies were taken from the same production batch for each of the three brands of dentifrice. This was checked by examination of the serial numbers on the boxes and the crimped ends of the individual tubes. It was one of the aims of this investigation to assess the effect of ageing after the dentifrice had been purchased from the retailer. The exact dates of production were not known. In an attempt to avoid the use of old stock the tubes were purchased from large retail stores where the turnover was rapid. Recall dates were stated for two of the brands. These were 6 months (Dentifrice B) and 7 months (Dentifrice C) after the date of purchase.

Upon receipt of each brand of dentifrice samples were extracted with water and saliva, as described below, to determine the amount of soluble fluoride released initially. The tubes of each brand were then divided into 3 equal groups for storage as follows.

Group 1. In a refrigerator at 4 to 5°C.

Group 2. In the laboratory at 19 to 22°C.

Group 3. In an oven at 35 to 37°C after each tube had been weighed.

A sample of dentifrice to be analysed was removed from the same tube on one occasion only. A 6 inch length of paste was expressed from the tube by compression at its crimped end before taking the sample. This gave some similarity in the site of sampling within each tube. After the initial analysis further samples of each dentifrice were analysed at weekly intervals during the first month of storage, at fortnightly intervals for the next two months, and at monthly intervals thereafter for a total period of 8 months.

3.4.2. Extraction of Soluble Fluoride from the Dentifrices.

Fluoride was eluted from the dentifrice samples in water and saliva slurries prepared in polyethylene vessels. The concentrations of paste (w/v) were the same as in the experiments described in Section A, although the amounts of dentifrice and water or saliva were larger. 500 ± 5 mg of paste were shaken with 10 ml of distilled-deionized water, or a similar volume of freshly expectorated human saliva collected from the same subject throughout. When the insoluble dentifrice particles had been suspended uniformly the slurries were centrifuged as described in Section A. The supernatants thus obtained were diluted (4 ml in 25 or 50 ml of water) to bring the fluoride concentration within the range of the analytical method (1 to 4 µg).

Prior to sampling the dentifrice which had been stored at 37°C the tubes were re-weighed and any weight loss was noted. Two weighed samples were taken from each of the tubes stored at 5 and 37°C and these were eluted with water. Four samples were taken from each of the tubes stored at laboratory temperature; two were eluted with water and two with saliva. Each supernatant was then analysed in duplicate.

Visual comparison of centrifuged water and saliva with the supernatants suggested that the latter were free of insoluble dentifrice particles. However, nephelometric measurements showed slight contamination. To determine its importance some supernatants were pooled and divided. Half was centrifuged again at 40,000 rpm for one hour before analysis and half was analysed without further centrifuging.

To test the effect of certain constituents of saliva upon the release of soluble fluoride some dentifrice samples were extracted with aqueous solutions of calcium chloride containing 5 mg and 20 mg per cent Ca^{++} and with a M/15 phosphate buffer solution at pH 7.4. The pH of the slurry prepared with the buffer was measured as was the pH of the water and saliva slurries prepared from the same sample of paste.

3.4.3. Analysis of Fluoride.

The supernatant solutions were analysed by the micro-diffusion method of Wharton (1962 and 1963).

Reagents.

SPADNS [4,5 - dihydroxy - 3 - (p - sulphophenylazo)- 2, 7 - naphthalene - disulphonic acid, trisodium salt]

3.16 g dissolved in 550 ml of deionized water.

Zirconyl chloride octa hydrate, 0.133 g dissolved in 500 ml of deionized water.

Reference solution - prepared by adding 50 ml of SPADNS solution to 500 ml of deionized water, 35 ml of concentrated hydrochloric acid was then added.

Colorimetric reagent - prepared by mixing equal volumes of the SPADNS and zirconyl chloride solutions.

Standard fluoride solution. A stock solution of sodium fluoride was prepared as described in Section A and diluted for use as appropriate.

Analytical Procedure.

Three-chamber polypropylene micro-diffusion cells (Size 44, Coleman) and similar cells, turned on a lathe from a polypropylene rod, were used (Fig. 3.6). The lids were disposable methacrylate Petri dishes.

By pipette, 0.8 ml of diluted supernatant was transferred to the inner annular chamber of the diffusion cell. The central chamber contained 0.3 ml of the trapping agent, 1.3 N sodium hydroxide. Perchloric acid-silver sulphate solution (0.5 ml), prepared as described earlier (see 3.2.3.), was added to the fluoride solution. The lid was at once sealed to the middle rim of the diffusion cell with silicone grease. Eight diffusion standards containing known amounts of fluoride in water in the 1 to 4 µg range were set up in a similar manner. All the cells were heated to 60°C for at least 20 hours.

Figure 3.6.

The diffusion cell used in the experiments described in Section B. It consists of a 3-chamber polypropylene unit with a disposable Petri dish as the lid.

The fluoride which had diffused into the central chamber was then transferred, with a Pasteur pipette, to a 10 ml volumetric flask. The chamber was washed with 4 changes of deionized water and these washings were also transferred to the flask. Zr-SPADNS colorimetric reagent (1 ml) was added to the washings which were then diluted to 10 ml. The absorption was read in 1 cm cells in a Spekker Absorptiometer (Hilger and Watts) against the reference solution using an Ilford filter 626 which transmits in the region of 560 - 610 mu with a peak at 570 mμ. The fluoride content of the unknowns was read from a standard line constructed from the absorption values for the diffused standards. The fluoride released by 100 mg of paste in 2 ml of supernatant was then calculated. A linear correction was made for variations in the weight of the paste samples within the range 500 ± 5 mg.

Investigation of the Analytical Method.

The accuracy and reproducibility of the analytical method were tested by analysing aliquots of fluoride solutions and dentifrice supernatants. The recoveries of fluoride added to both water and saliva supernatants were also determined by this method of analysis. These experiments were similar in design to those described in Section A of this chapter.

3.5. RESULTS

3.5.1. Investigation of the Method of Extraction and Analysis.

When the slight turbidity of the water and saliva supernatants was removed by ultra-centrifuging the amount of fluoride analysed was not affected (Table 3.9). Therefore, the supernatants were not ultra-centrifuged routinely. The reproducibility of the analytical method and the recoveries of fluoride added to supernatants prepared from dentifrice slurries were satisfactory (Table 3.9).

3.5.2. Soluble Fluoride Released by the Dentifrices.

The dentifrice slurries which were used in the experiments described in this section were of the same concentration as the slurries used in the experiments in Section A. To facilitate comparison of the results in the two sections the amounts of soluble fluoride released have been expressed in similar terms. Dentifrice samples stored at $37^{\circ}C$ lost weight (Fig. 3.7). Therefore, the soluble fluoride released by samples stored at this temperature was calculated in terms of their original weight.

It was anticipated that the amount of soluble fluoride released by the dentifrices would bear an exponential relationship to the time of storage. Therefore, the results for Dentifrices A, B and C, recorded in Tables 3.10 to 3.12, have been plotted on semi-logarithmic scales. Figures 3.8 to 3.10 demonstrate the anticipated exponential relationship between soluble fluoride and time.

TABLE 3.9.

A. Fluoride analyses on 8 supernatants before and after ultra-centrifuging.

Dentifrice	A 42	B 58	C 56	C 56
Slurry with	Water	Saliva	Water	Saliva
Fluoride before ultra-centrifuging	84.4 ± 2.3	89.1 ± 2.9	71.0 ± 1.8	36.7 ± 1.2
Fluoride after ultra-centrifuging	82.9 ± 1.7	89.9 ± 3.1	72.2 ± 2.7	35.8 ± 2.1

Results expressed as µg F/100 mg paste, means and standard deviations.

B. Repeated estimations of fluoride in a solution containing a known amount of sodium fluoride and in supernatants prepared from dentifrice samples taken from the same tube.

	Number of Estimations	Mean F µg Determined	Mean F µg Estimated	S.D.	Coefficient of Variation
Solution 2 µg F/ml	15	1.59	1.99	± 0.05	± 2.7
Dentifrice C.70	8	2.41	76.2	± 3.1	± 4.2

C. Recoveries of fluoride added to extracts of the dentifrices.

Dentifrice	Slurry with	Added F in µg	Recovered F in µg	Per cent Recovery
A.71	Saliva	50	48	96
B.60	Water	50	51.1	102.2
C.67	Water	100	103.5	103.5

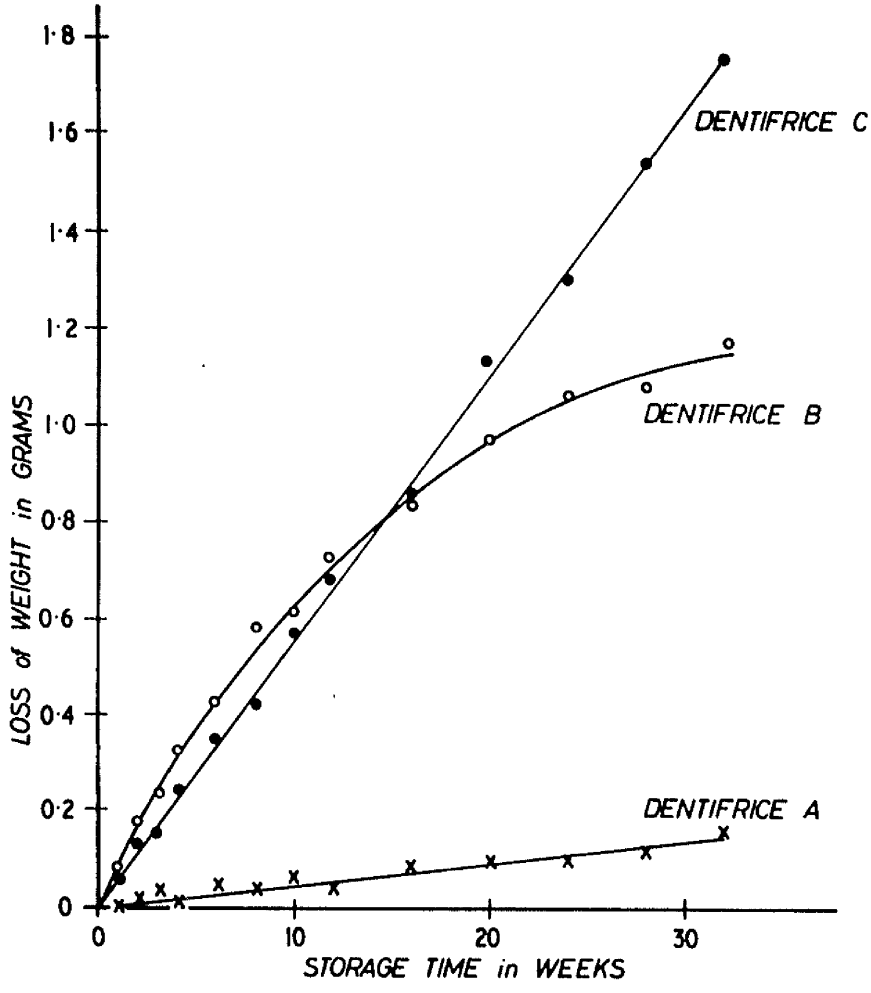


Figure 3.7.

The dentifrices lost weight during storage at 35-37°C. The loss of weight is the reduction in weight of the unopened tube after storage for the period stated.

TABLE 3.10.

The relationship between soluble fluoride in both water and saliva slurries and the time and temperature of storage of Dentifrice A. Soluble fluoride is expressed in μg/100 mg paste.

Storage Time in Weeks	Eluted with Water After storage at			Eluted with Saliva After Storage at
	4-5°C	19-22°C	35-37°C	19-22°C
0	-	103.1	-	73.0
1	105.0	98.4	95.9	70.9
2	102.3	97.5	89.3	71.2
3	103.0	96.8	84.7	66.6
4	103.0	94.0	85.4	61.0
6	98.0	90.6	75.0	55.3
8	98.5	85.3	67.7	60.3
10	93.5	84.7	61.8	60.5
12	92.8	88.8	52.2	57.2
16	89.7	86.0	42.2	53.4
20	89.6	83.0	38.4	53.8
24	87.0	82.5	29.1	48.5
28	86.1	72.2	26.5	42.3
32	80.3	73.4	20.7	40.1

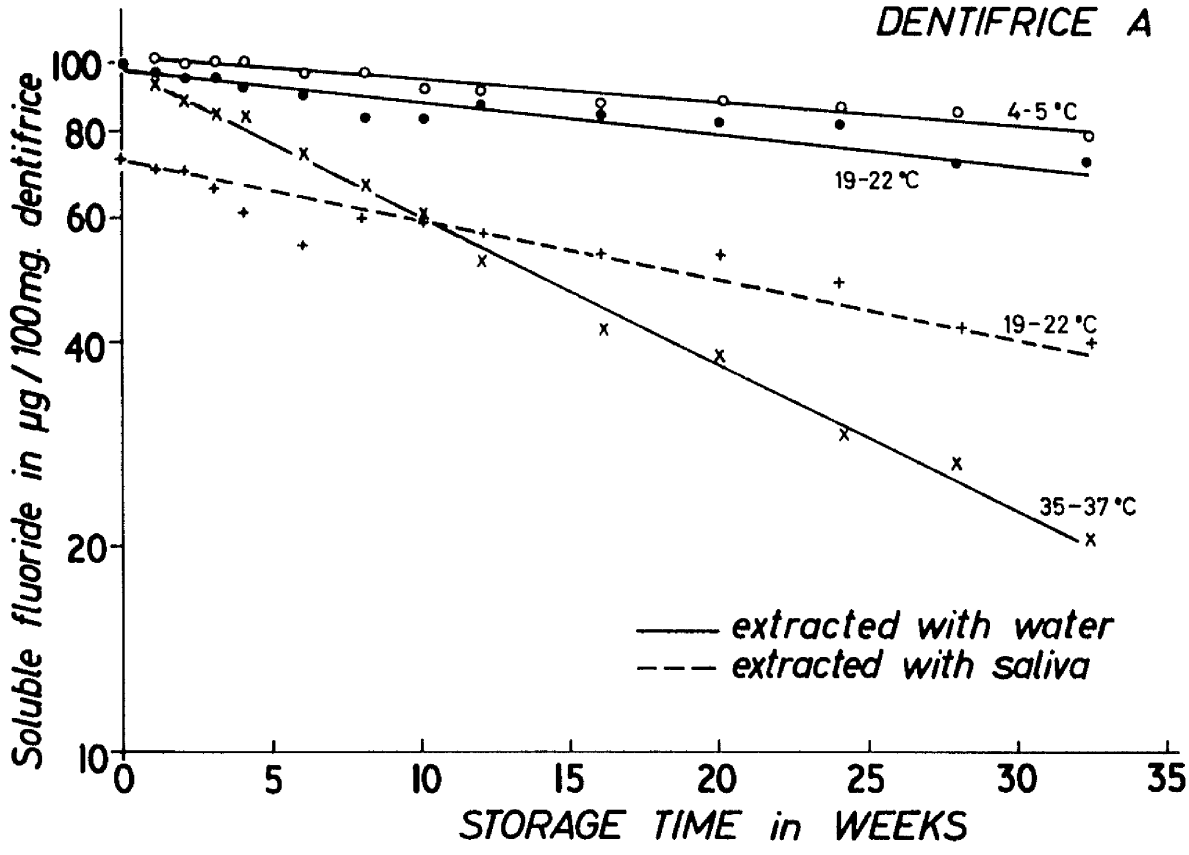


Figure 3.8.

The release of soluble fluoride in aqueous and saliva slurries (5 per cent w/v) prepared from samples of Dentifrice A stored at different temperatures for different periods after purchase.

TABLE 3.11.

The relationship between soluble fluoride in both water and saliva slurries and the time and temperature of storage of Dentifrice B. Soluble fluoride is expressed in µg/100 mg paste.

Storage Time in Weeks	Eluted with Water After Storage at			Eluted with Saliva After Storage at
	4-5°C	19-22°C	35-37°C	19-22°C
0	-	94.0	-	75.3
1	90.6	89.1	79.0	70.0
2	89.5	88.2	77.5	68.2
3	88.1	83.5	82.0	73.5
4	86.2	85.4	78.7	73.0
6	87.0	80.0	78.1	68.4
8	83.8	76.5	77.2	65.6
10	81.5	77.5	72.2	63.0
12	85.3	80.9	71.4	59.4
16	82.7	78.2	70.0	60.3
20	80.8	74.6	67.1	62.4
24	74.9	68.2	67.9	60.9
28	75.1	68.0	64.2	56.1
32	72.2	63.3	59.4	49.4

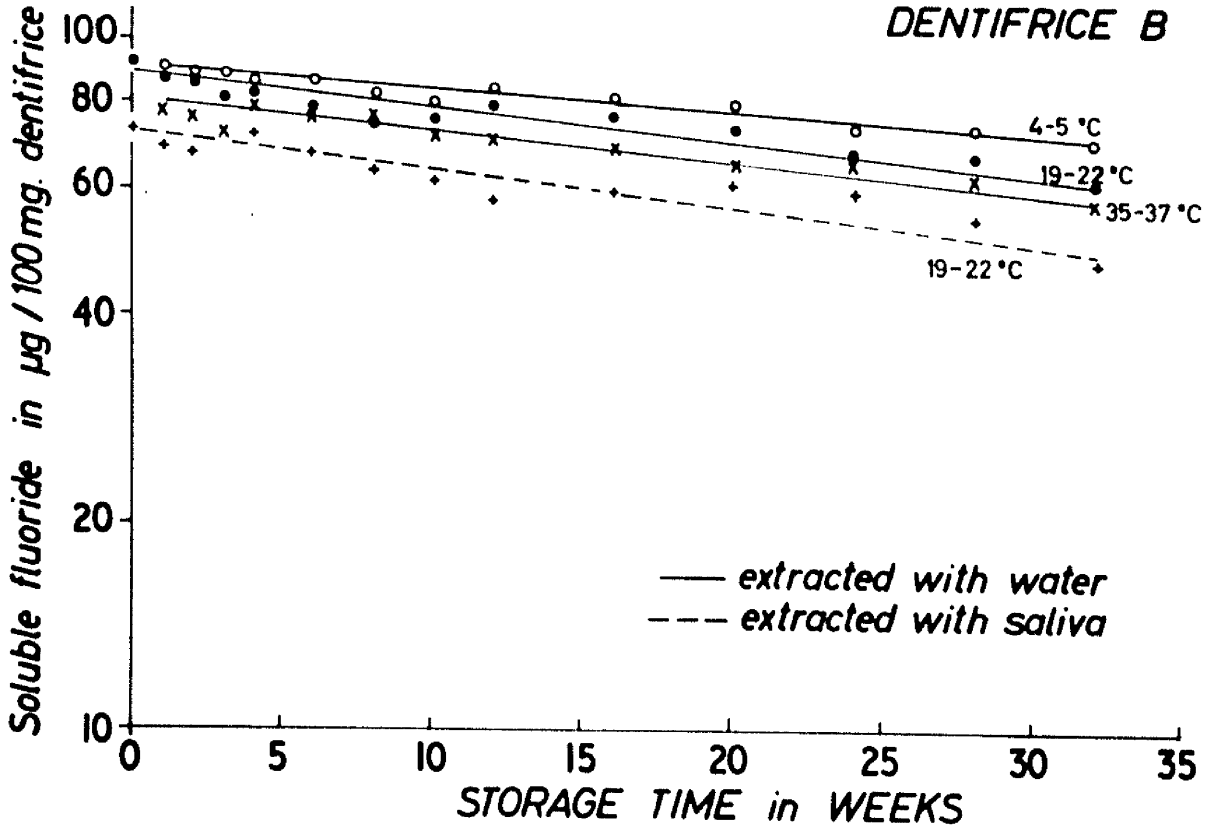


Figure 3.9.

The release of soluble fluoride in aqueous and saliva slurries (5 per cent w/v) prepared from samples of Dentifrice B stored at different temperatures for different periods after purchase.

TABLE 3.12.

The relationship between soluble fluoride in both water and saliva slurries and the time and temperature of storage of Dentifrice C. Soluble fluoride is expressed in µg/100 mg paste.

Storage Time in Weeks	Eluted with Water After Storage at			Eluted with Saliva After Storage at
	4-5°C	19-22°C	35-37°C	19-22°C
0	-	95.3	-	45.4
1	92.8	91.4	81.5	44.4
2	91.0	89.5	78.8	45.3
3	90.0	89.3	80.3	45.8
4	89.1	88.0	75.3	41.0
6	89.0	88.1	71.0	40.3
8	90.0	88.5	64.7	42.2
10	91.5	89.6	66.3	43.8
12	89.2	80.5	59.2	41.6
16	90.6	80.6	50.4	36.9
20	83.2	79.7	41.8	35.6
24	88.0	75.3	37.9	34.9
28	82.3	73.5	37.0	30.8
32	80.4	76.5	28.0	26.0

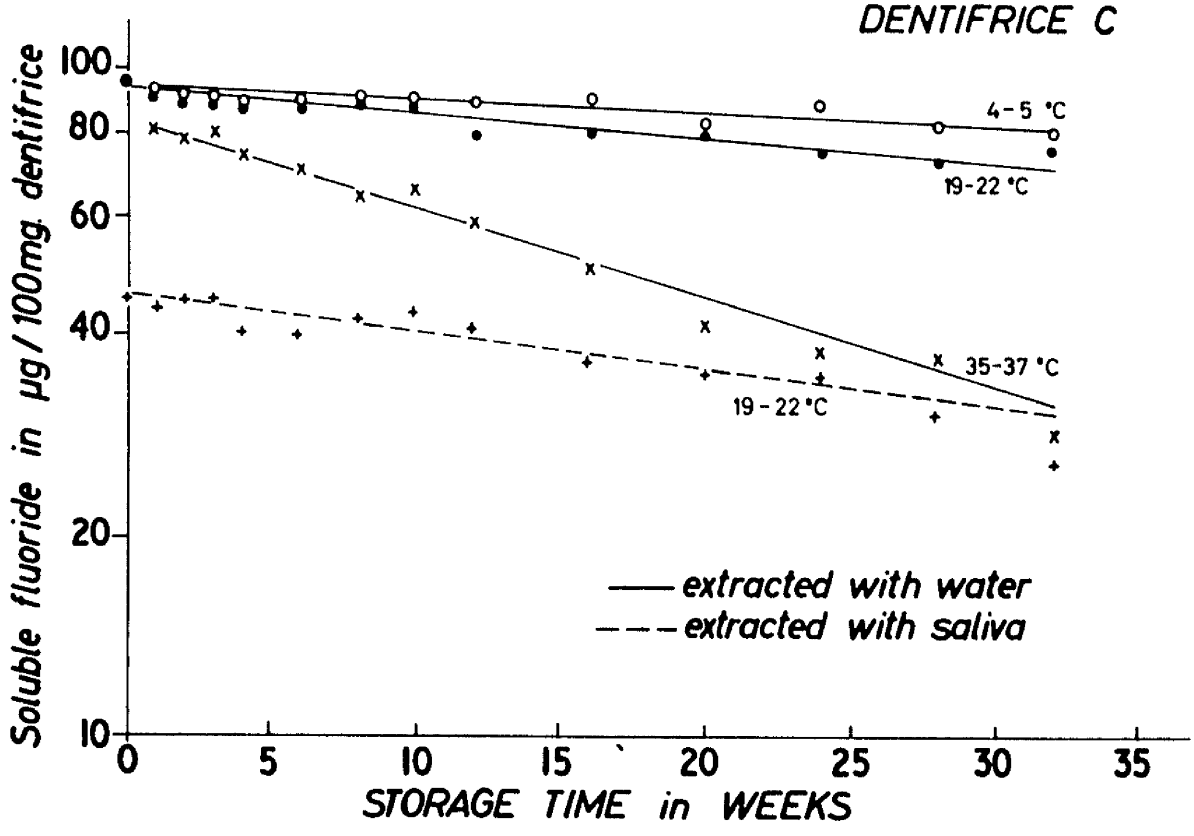


Figure 3.10.

The release of soluble fluoride in aqueous and saliva slurries (5 per cent w/v) prepared from samples of Dentifrice C stored at different temperatures for different periods after purchase.

The results show that:

1) The amount of soluble fluoride decreased as the dentifrices aged. This applied to samples stored at all three temperatures.
2) The loss of soluble fluoride with time was temperature dependent. Less soluble fluoride was extracted by water from dentifrices stored at laboratory-temperature than from dentifrices stored at 5°C. Storage at 37°C further reduced the amount of soluble fluoride.
3) From all three dentifrices less soluble fluoride was extracted by saliva than by water.
4) There were differences in the amounts of soluble fluoride released from the dentifrices under equivalent conditions of storage and extraction. These differences were most marked with water extraction after storage at 37°C and with saliva extraction after storage at laboratory-temperature (Figs. 3.11 and 3.12).
5) The lowest release of soluble fluoride occurred when Dentifrice C was extracted with saliva. With this dentifrice in M/15 phosphate buffer, which maintained the pH of the slurry at 7.3 to 7.4, the release of fluoride was similar to the release of fluoride in saliva. With water, which gave a pH in the slurry of about 5, more fluoride was released. On the other hand the release of soluble fluoride into phosphate buffer from Dentifrices A and B was comparable to the release into water (Table 3.13.A).

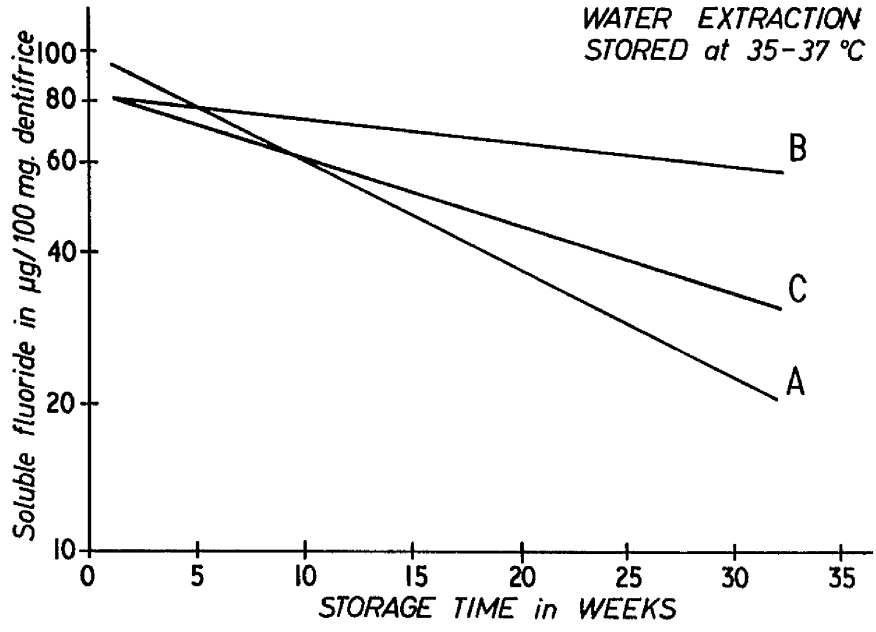
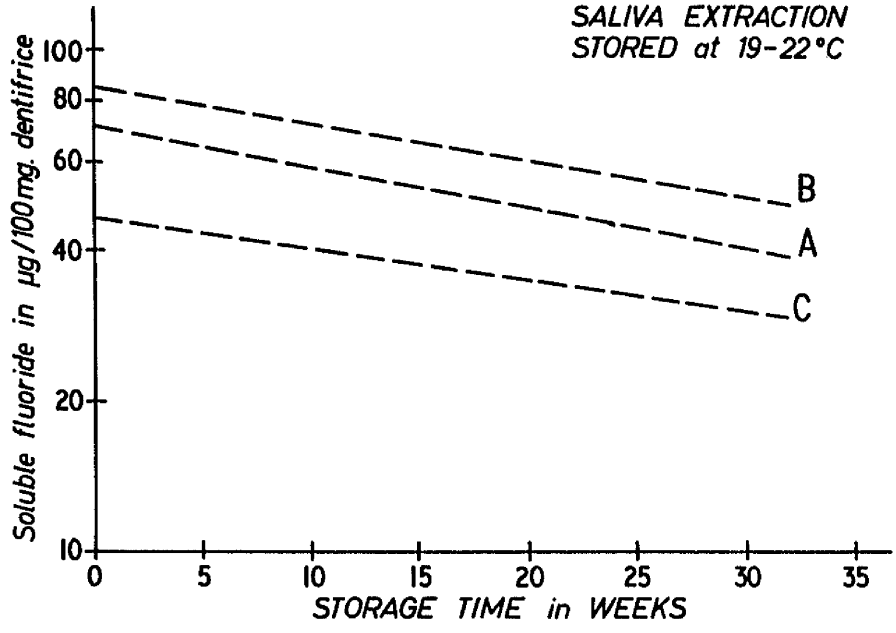


Figure 3.11.

The release of soluble fluoride to water by Dentifrices A, B and C after storage at 35-37°C. Data re-drawn from Figures 3.8, 3.9 and 3.10.

Figure 3.12.



The release of soluble fluoride to saliva by Dentifrices A, B and C after storage at 19-22°C. Data re-drawn from Figures 3.8, 3.9 and 3.10.

6) Extraction of the dentifrices with solutions containing calcium showed that this reduced the release of soluble fluoride from Dentifrice C. The release of soluble fluoride from Dentifrice A was less affected and the release from Dentifrice B was unaffected (Table 3.13.B).

TABLE 3.13

A. Release of soluble fluoride from dentifrices to water, saliva and phosphate buffer.

Slurry with	pH of Slurry 5 per cent w/v	Soluble F in µg/100 mg Dentifrice			
		A.16	B.12	C.38	C.41
Water	4.95 - 5.35	80.1	77.8	48.9	85.5
Saliva	6.8 - 7.0	61.2	64.5	22.3	26.0
M/15 Phosphate Buffer	7.3 - 7.4	75.1	79.2	30.2	30.7

B. Release of soluble fluoride from dentifrices to water and aqueous solutions of calcium chloride.

Ca^{++}mg per cent	Soluble F in µg/100 mg Dentifrice			
	A.16	B.12	C.38	C.41
0	80.1	77.8	48.9	85.5
5	67.1	78.2	29.9	57.7
20	35.4	78.9	21.8	-

3.6. DISCUSSION OF THE RESULTS RECORDED IN SECTIONS A AND B

3.6.1 Method of Extraction and Analysis of Soluble Fluoride.

In the determination of soluble fluoride and tin (II) in two dentifrices Manly (1961) used extraction and analytical procedures which were designed to minimize oxidation. To reduce the risk of losing soluble tin (II) the dentifrices were eluted with oxygen-free water in an atmosphere of nitrogen. In the experiments described here no attempt was made to determine the amount of tin(II). However, as the loss of fluoride ions from a solution of stannous fluoride exposed to air has been reported (Hatton et al., 1955) the effect, upon the release of soluble fluoride, of exposing the dentifrice to air during the preparation of the samples was determined. Deliberate exposure of samples to the air did not alter the level of soluble fluoride (Table 3.4). Therefore, a nitrogen atmosphere was not used during the preparation of aqueous extracts. Saliva extracts were prepared and analysed for fluoride in order to obtain an indication of available fluoride when the dentifrices were used in the mouth. Therefore, samples were extracted with freshly expectorated saliva under normal atmospheric conditions.

Ideally, the diffusion standards used in the analysis of dentifrice fluoride should have been prepared in extracts from fluoride-free dentifrices of otherwise similar formulation to the preparation

being analysed. Unfortunately, such dentifrices could not be obtained. However, it has been shown that fluoride added to dentifrice extracts is recovered quantitatively (Table 3.9.C). Therefore, diffusion of fluoride during analysis is not inhibited by soluble dentifrice constituents. Hence, the use of aqueous standards is permissible.

The concentration of dentifrice in the slurry appears to affect the percentage of total fluoride released from a sample (Table 3.5 and Fig. 3.2). The relative increase in soluble fluoride which is found in slurries as they become more dilute suggests that hydrolysis favours the release of fluoride.

3.6.2. The Preliminary Experiments.

In the preliminary experiments described in Section A the amounts of soluble fluoride in aged dentifrice samples were determined. However, these results were not obtained on ideal material. Aged samples of each of the three dentifrices were taken from different production batches and the temperatures at which these samples were stored for most of the period since manufacture were unknown. Although the samples of dentifrice were heterogeneous these preliminary results did suggest that ageing is accompanied by a fall in the availability of soluble fluoride and that saliva constituents hinder the release of this fluoride.

3.6.3. Aqueous and Saliva Extraction of Soluble Fluoride.

In the experiments recorded in Section B each brand of dentifrice was represented by one production batch and the storage conditions after purchase were known. The analytical method (Wharton 1962 and 1963) was simpler than the one used in the earlier experiments and the coefficients of variation for series of simultaneous analyses with this method were smaller (Tables 3.1 and 3.9). Therefore, it is considered that greater reliance can be placed upon the results recorded in Section B than upon the results of the preliminary experiments. Heterogeneous dentifrice samples, stored under largely unknown conditions, and analytical error may have contributed to the finding of lower levels of soluble fluoride in the experiments described in Section A.

The findings with aqueous extracts are in general agreement with those of Manly (1961), for he also found a reduction in soluble fluoride as the dentifrices aged. However, it is not possible to compare the results recorded here with Manly's for a much higher paste/water concentration was used in his experiments (approximately 23 per cent w/v against 5 per cent w/v in these experiments). Even if allowance is made for this higher concentration, which would cause a reduction in the percentage of soluble fluoride released, some of the low levels found by Manly (1961) are surprising. It seems unlikely that 5 weeks after production some dentifrice samples released only 2 per cent of their original fluoride content.

With saliva extraction the error due to its fluoride content is insignificant for according to Jenkins (1960) saliva contains

about 0.2 µg of fluoride per ml. Of the many differences between water and saliva the ones which appear most likely to produce the observed reduction in the release of soluble fluoride from the dentifrices are:

1) calcium, which may precipitate fluoride,
2) protein, which may bind fluoride and
3) salivary buffering which may favour the formation of insoluble fluoride complexes by keeping the pH of the slurry close to neutrality.

The varying influence of calcium upon fluoride release may be related to variations in the formulation of the dentifrices (see 3.6.4) but the inconsistent influence of extraction with a buffered solution of phosphate is not readily explained (Table 3.13.A). However, it appears that buffering may be a factor in the reduced release of fluoride to saliva by Dentifrice C.

3.6.4. Differences Between the Dentifrices.

The principal differences between the dentifrices are shown in the two graphical comparisons of the amounts of soluble fluoride released to water after storage at 35 - 37°C (Fig. 3.11) and to saliva after storage at laboratory-temperature (Fig. 3.12). Thus, heating to 35 - 37°C accelerates the ageing of Dentifrices A and C more than Dentifrice B and less fluoride is extracted with saliva from Dentifrice C than from the other two preparations.

These differences should be related to differences in the composition of the dentifrices, but, because of the limited data which are available upon their formulation, it is difficult to establish such a relationship. However, it is known that Dentifrice C has a large calcium content (Muhler, 1961a) whereas Dentifrice B appears to be free of added calcium, the polishing agent being sodium metaphosphate (Slack and Martin, 1964). Dentifrice A contains both sodium metaphosphate and a calcium compound but the exact amounts do not appear to have been published. Notwithstanding this lack of detail it seems probable that the calcium content of Dentifrice C $\gg$ Dentifrice A $>$ Dentifrice B.

The influence of calcium upon fluoride release (Table 3.13.B) suggests that the sequestering action of sodium metaphosphate in the dentifrice is an important factor favouring the release of fluoride when calcium is present. The release of fluoride from Dentifrice B, with the highest sodium metaphosphate content, was unaffected whereas the release from Dentifrice C, which contains no sodium metaphosphate, was much reduced. Dentifrice A, which contains some sodium metaphosphate, released much less fluoride when the amount of calcium in the elutant was increased to 20 mg per cent (Table 3.13.B).

Table 3.14 shows a grading of the release of soluble fluoride to water, after storage of the dentifrices at 37°C, and to saliva, after storage at laboratory-temperature, together with a grading of the probable relative calcium and sodium metaphosphate content of the dentifrices. The release of soluble fluoride is graded approximately in proportion to the areas below the lines plotted in Figures 3.11 and 3.12.

TABLE 3.14

The possible relationship between the calcium content of the dentifrices and the release of soluble fluoride.

Dentifrice	Probable Content of		Release of Soluble Fluoride	
	Calcium	Na metaphosphate	To water Storage at 37°C	To saliva Storage at 20°C
A	+	+	++	++
B	-	++	+++ +	+++
C	++	-	++	+

This representation of the data does suggest the possibility that the release of soluble fluoride from a stannous fluoride dentifrice, under the conditions described, is inversely related to its free calcium content. However, the results obtained with water extraction after storage of the dentifrices at 5 and 20°C show only slight differences and do not appear to conform to this suggestion.

3.6.5. Clinical Significance of the Findings In Vitro.

The clinical significance of the observations in this chapter is problematical. The data suggest that the shelf-life of a stannous fluoride dentifrice could have a bearing upon its effectiveness and that for maximum effect the manufacturer should get his product to the consumer as quickly as possible. However, when a stannous fluoride dentifrice is tested in a clinical trial the time elapsing between manufacture and supply to the subjects should not be unrealistically related to the probable rate

of supply to the ordinary consumer. Unless it can be shown in clinical trials that prolonged storage of stannous fluoride dentifrices is not accompanied by reduced effectiveness, the tubes containing these dentifrices should be date-stamped to permit the consumer to reject old stock.

In several clinical trials Muhler and his colleagues have claimed to show that Dentifrice C exerts a caries-preventive effect, yet under the conditions of the experiments described here the release of soluble fluoride to saliva by this dentifrice appears to be relatively low. Conversely, Dentifrice B, which had the greatest release of soluble fluoride to saliva, was not shown to be effective in the only clinical trial so far reported (Slack and Martin, 1964). Results of clinical trials with Dentifrice A, for comparison with the findings here, are not yet available.

Thus, on the available evidence the release in vitro of soluble fluoride to saliva, by a stannous fluoride dentifrice, does not appear to be a useful indicator of its clinical performance. It could be that the soluble fluoride which has been measured is not ionic fluoride but a soluble fluoride complex inactive in the prevention of dental caries. One important difference between the method of testing in vitro and a clinical trial is the difference in the concentrations of the paste/saliva slurries. As salivation is stimulated during toothbrushing this concentration will fall but it probably remains several times higher than the concentration of the dilute slurries used in the experiments in vitro. In vivo the release of soluble fluoride would be expected to be less than shown here because of the lower dilution. It is possible that this would be accompanied by a change in the relative availability of fluoride from the dentifrices and this could be related to differences in their clinical effect.

3.7. CONCLUSIONS

Under the conditions of the experiments described in this chapter it has been shown that:

1) The amount of soluble fluoride extracted from 3 commercial brands of stannous fluoride dentifrice decreased during a period of storage lasting 8 months.

2) The temperature at which the dentifrices were stored influenced the release of soluble fluoride. This was greatest after storage at 4 - 5°C and least after storage at 35 - 37°C.

3) Extraction of the dentifrices with saliva, instead of with water, showed that the presence of unidentified saliva constituents was unfavourable to the release of soluble fluoride.

4) Extraction with water after storage at 35 - 37°C and extraction with saliva after storage at 19 - 22°C demonstrated that the different brands of dentifrice differed in their ability to release soluble fluoride.

It is tentatively suggested that the differences between the dentifrices are related to differences in their formulation. When sodium metaphosphate is the only abrasive it appears that fluoride release is favoured, but this is reduced when the dentifrice contains both sodium metaphosphate and a calcium compound. The release of fluoride is reduced still further when the preparation contains a calcium compound as the sole polishing agent.

CHAPTER 4

UPTAKE OF FLUORIDE AS FLUORINE-18 BY PREMOLAR TEETH IN VIVO FROM DENTIFRICES CONTAINING STANNOUS FLUORIDE

4.1. INTRODUCTION

There is evidence that the enamel of a fully erupted tooth can take up fluoride from its environment. Thus, it has been shown that the fluoride content of enamel increases progressively after eruption (Jackson and Weidmann, 1959) and that most of this increase occurs in the superficial layers (Isaac, Brudevold, Smith and Gardner, 1958). This preferential uptake by surface enamel suggests that fluoride is directly transferred to it from drinking water, food and saliva. Additional evidence of the uptake of fluoride from solutions applied to the teeth in situ has been obtained by Syrrist (1949); Adler, Straub and Popovics (1950) and Sundvall-Hagland, Brudevold, Armstrong, Gardner and Smith (1959).

The results obtained by these investigators suggest the possibility that soluble fluoride, contained in a dentifrice, could be transferred to the enamel surface during toothbrushing and thereby increase its fluoride content. Measurement of this increase in the fluoride content of enamel in vivo could provide another method of testing the availability of fluoride in a dentifrice. Therefore, the experiments described in this chapter were designed to measure and compare the uptake of fluoride by enamel from 3 brands of dentifrice, each of which contained stannous fluoride.

Controls for chemical methods of investigating fluoride uptake give rise to difficulties because the fluoride content of surface enamel varies widely, even in homologous teeth from the same mouth. In addition it was anticipated that any increase in the fluoride content of the teeth, produced by brushing with these dentifrices, would be less than the lowest levels detectable by the available chemical methods. Therefore, quantitative data have had to be obtained by labelling the fluoride of the dentifrices with fluorine-18.

4.2. MATERIALS AND METHODS

4.2.1. Preparation of Fluorine-18.

Fluorine-18 was prepared according to the nuclear reactions

$$^{16}O(\alpha, pn)^{18}F \quad \text{and} \quad ^{16}O(\alpha, d)^{18}F$$

by bombardment of water with alpha particles in the 30 MeV external beam of the cyclotron at the Cyclotron Unit of the Medical Research Council.

The target and the vessel containing it were altered at various times during the course of this study. Initially aluminium was used but this proved unsuitable because adsorption of fluorine-18 upon the walls of the container led to low yields. Such losses were reduced by using a sodium bicarbonate buffer solution at pH 8, as the target, but this dissolved aluminium (about 50 µg/ml) from the vessel.

Recently "Water for injection B.P." has been employed as the target material and this has been contained in a specially designed titanium cell (Fig. 4.1). The latter was fitted with a syringe for handling the liquid, a splash trap to reduce losses during bombardment and a cooling plate to carry away thermal energy. Under these conditions a one-hour bombardment at a beam current of 18 µA yielded about 10 mC of fluorine-18 in 7 ml of solution. Titanium contamination was less than 1 µg per ml.

Other radioisotopes produced during bombardment were several short-lived species, of which oxygen-15 had the longest half-life (2 minutes). These short-lived contaminants were allowed to decay for about 20 minutes before the active solution was removed from the target vessel.

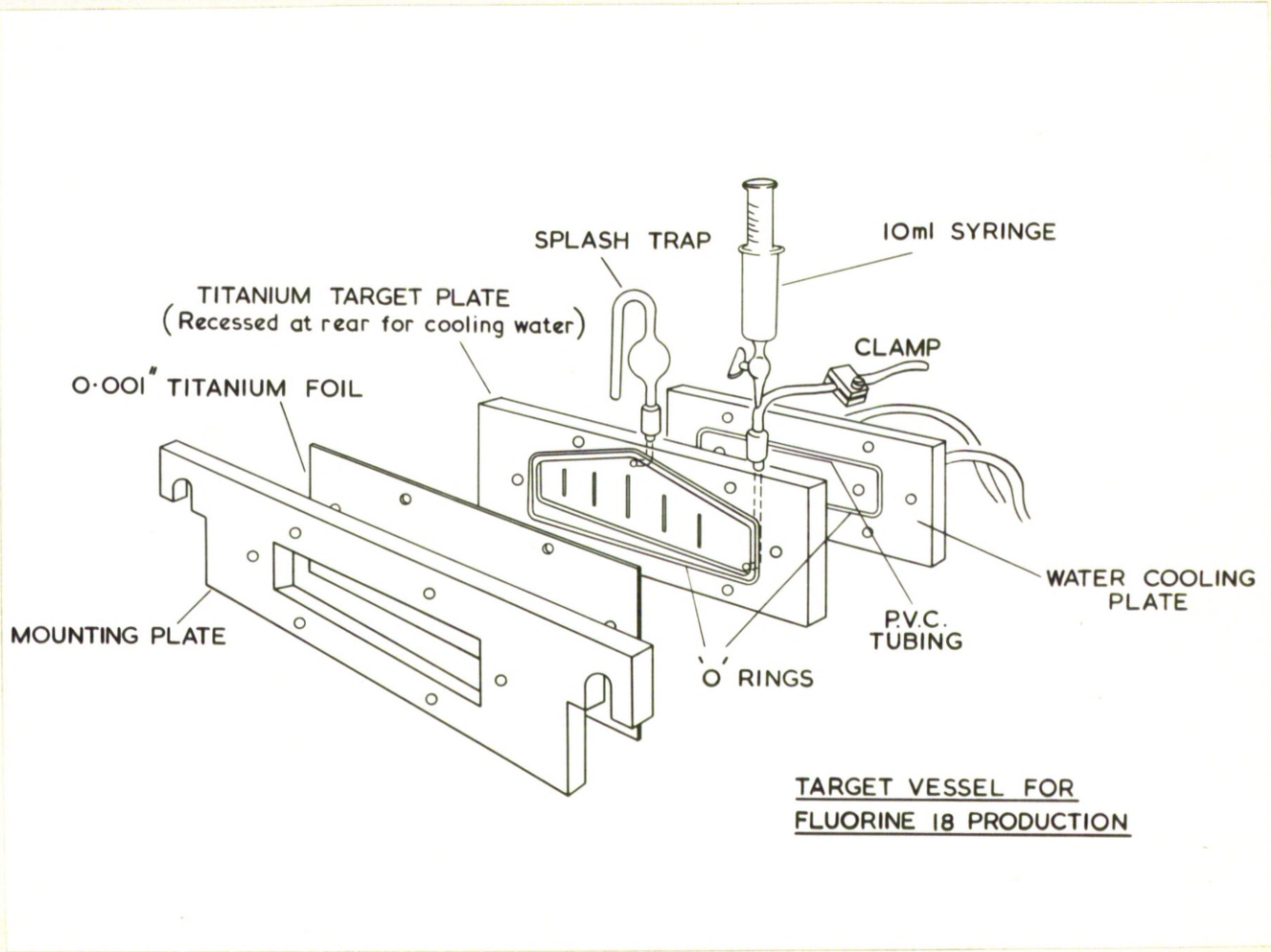


Figure 4.1.

An exploded view of the target vessel used in the production of fluorine-18.

Traces of vanadium-48 (16.1 days) and chromium-51 (27.8 days) were also produced. The total activity of these contaminants was less than 1 μC at the end of the bombardment. It was calculated that any error in subsequent measurements of fluorine-18, due to the presence of these contaminating radioisotopes, was never more than 0.2 per cent.

Fluorine-18 produced in this way is carrier-free but chromatographic studies show it to be complexed in some form, which, as yet, has not been identified. Experiments have shown, however, that the complexed fluorine-18 will readily exchange with and label non-radioactive fluoride ions in solution (Silvester, 1964, personal communication).

4.2.2. Labelling of Dentifrices with Fluorine-18.

The three proprietary brands of stannous fluoride containing dentifrices used in the experiments described in Chapter 3 were employed in these uptake studies. The tubes of dentifrice were purchased through normal retail channels, not more than two months before use, and were stored at room temperature.

Approximately 5 g of the dentifrice was placed in one-half of a disposable methacrylate Petri dish. Sufficient carrier-free fluorine-18 solution (usually 2 or 3 drops) was added to the paste with a Pasteur pipette to give an activity of 1-2 μC/g of paste at the time of use. The dentifrice and radioactive fluorine solution were mixed for 5 minutes with a polyethylene rod. The labelled paste was then allowed to stand for at least 30 minutes to permit the fluorine-18 to come into equilibrium with the non-radioactive fluoride.

About 1 g of the labelled dentifrice was loaded on a previously weighed dry toothbrush and the weight of the paste on the brush was accurately determined by re-weighing. To permit calculation of the results in terms of the mass of fluoride taken up by the crowns of the teeth standards were prepared by suspending six accurately weighed samples of fluorine-18 labelled paste in 1 ml of water in capped polyethylene tubes.

It was necessary to show that the added fluorine-18 was uniformly distributed in the dentifrice and that it had reached equilibrium with the non-radioactive fluoride. Therefore, radioactivity measurements were made on weighed samples of paste (20 to 300 mg) taken from various parts of the mix. After labelling the dentifrices with fluorine-18, duplicate 500 mg samples were allowed to stand for up to 2.5 hours before elution with water by the method described previously (see 3.4.2). Aliquots of each supernatant (1 ml), and standards of the labelled dentifrice, prepared as described above, were counted. From these data the mean percentage of fluorine-18 extracted from samples of the same dentifrice, at intervals during an equilibration period of 2.5 hours, was calculated.

In addition, 500 $\pm$ 5 mg samples of each dentifrice, labelled with fluorine-18, were eluted with water and saliva. Two 1 ml aliquots of each supernatant were pipetted into polyethylene tubes and counted with six accurately weighed standards of each labelled dentifrice.

A fluoride content of 1 mg per gram of dentifrice, as given by the manufacturers, was assumed and the data obtained from the procedure described above were used to calculate the amount of soluble fluoride in

100 mg of paste. Thus,

Soluble fluoride (µg)

$$= \frac{\text{Activity of supernatant (cpm/ml)} \times 10 \times 100}{\text{Activity of paste (cpm/mg)} \times \text{Wt of eluted paste (mg)}}$$

The amount of soluble fluoride determined in this way was then compared with the amount of soluble fluoride shown by chemical analysis of the same supernatant by the method already described (see 3.4.3).

4.2.3. Toothbrushing with Fluorine-18 Labelled Dentifrices.

Preliminary experiments showed that the uptake of fluorine-18 by the crowns of teeth was greatly increased in the presence of caries or a restoration. Therefore, subjects aged 10 to 15 years, with clinically sound, caries-free premolar teeth which were to be extracted for orthodontic reasons, were chosen for these experiments. Because variations in the surface areas of the crowns was one of the factors expected to influence the uptake of fluoride, teeth which were obviously incompletely erupted were not included in the series. Teeth which, after extraction, were shown to have early approximal carious lesions, recognisable with the naked eye, were also excluded.

There were 30 subjects arranged in 3 groups of 10. The subjects in each group used a different brand of dentifrice. With a weighed amount of fluorine-18 labelled paste on the brush the subject cleaned the quadrant of the jaw, containing the tooth to be extracted, for one minute. Where a second tooth was to be extracted from the same patient both

quadrants were brushed with the same brushful of paste for a total time of 2 minutes. On completion of toothbrushing the subject rinsed with a mouthful of water. Not more than two teeth were extracted from a subject at one sitting. Seventy-five premolar teeth (25 in each group) were extracted from the subjects. After extraction the amount of fluorine-18 taken up by each crown was measured.

4.2.4. Radioactivity Measurements.

Fluorine-18 decays by emission of a positron according to the following scheme:

$$^{18}_{9}F \longrightarrow {}^{18}_{8}O + \beta^{+}$$

Two gamma quanta with energies of 0.51 MeV are emitted when a positron is annihilated.

Counting Apparatus.

Radioactivity measurements were made in a well-type scintillation counter (E.R.D. Engineering Co.Ltd.)fitted with a thallium-activated sodium iodide crystal. A power unit of high stability (Dynatron type N.103) energized the equipment. Experiments showed that the greatest stability of the apparatus was obtained when working with an EHT of 1700 volts. After amplification, counts were recorded upon a scaler (Dynatron type 1009E) fitted with an automatic timer (Dynatron type N.108). A discriminator voltage of 20 volts gave the maximum counting sensitivity. Where count rates were low, as was sometimes the case with extracted teeth, pulses were fed into a single channel pulse amplitude analyser

(Dynatron type N.101) and then to the scaler. The analyser was adjusted to record the annihilation gamma radiation from the fluorine-18 positrons. This reduced the background count rate and improved the sensitivity of fluorine-18 determinations at low counting rates. The stability of the counting apparatus was checked from time to time with a radium reference source.

Counting Errors and Correction Factors.

In the experiments described in this and subsequent chapters counting errors were minimized by applying the techniques recommended by Veall and Vetter (1958). Thus, the levels of fluorine-18 activity were selected to permit:

1) The recording of at least 10,000 counts, thus reducing the standard error of the observed count to ± 1 per cent.
2) A sample count rate at least 5 to 10 times higher than the background rate, thus reducing to negligible levels the contribution of the error in the background count to the error in the count of the sample.

However, the counting rates provided by the disintegration of fluorine-18, taken up by the crowns of teeth in vivo, were too low to meet these requirements. For reasons of safety it was not possible to increase the amount of radiofluorine in the dentifrice. Therefore, when measuring the radioactivities of the teeth larger counting errors had to be accepted. In these instances it was calculated that the standard error in a count of the radioactivity of a crown did not exceed ± 5 per cent.

The contribution of background radiation to the observed counting rate was determined at the beginning, during and at the end of every series of measurements. Radioactivity measurements on samples were then corrected for this background activity. Corrections for the radioactive decay of fluorine-18 were made. A correction factor appropriate to the interval between zero time and the mid-time of the counting period was calculated for each sample on the basis of a half-life of 112 minutes (Allen et al., 1961).

Counting of Teeth and Standards.

Before measuring the radioactivities of the extracted teeth they were scraped free of soft tissue and scrubbed with a soft nailbrush under running tap water for 5 minutes. The teeth were then dried with paper tissues and counted crown downwards in 5 x 1 cm polyethylene tubes which fitted the well of the scintillation counter.

Similar tubes containing the labelled dentifrice standards and water were re-shaken before counting to ensure suspension of insoluble particles. From measurements of the radioactivities and weights of 6 standards the number of counts/min/mg of paste was determined.

The uptake of stable fluoride by each tooth from 1 g of dentifrice, containing 1 mg of fluoride, was calculated as follows:

Uptake of fluoride (mμg) =

$$\frac{\text{Activity of tooth (cpm)} \times 1000}{\text{Activity of paste (cpm/mg)} \times \text{Wt of paste (mg)}} \times 1000$$

4.3. RESULTS

Measurements of the radioactivities of weighed, labelled samples of paste, taken from different parts of the mix, showed that the fluorine-18 was distributed evenly, its activity being directly proportional to the weight of dentifrice in the sample (Fig. 4.2). Table 4.1 shows that the amount of fluorine-18 extracted from labelled Dentifrice B remained constant from 20 to 150 minutes after mixing with the radioisotope. Similar results were obtained with Dentifrices A and C. These results indicate that after 20 minutes the added fluorine-18 is in equilibrium with the fluoride in the dentifrice. The results obtained when soluble fluoride was extracted from the dentifrices and analysed by both chemical and fluorine-18 methods are shown in Table 4.2. It is evident that there is close agreement between the two sets of data.

The uptake of fluoride, as fluorine-18, from 3 different dentifrices by the crowns of premolar teeth in vivo is shown in Tables 4.3 to 4.5. The summary of the results in Table 4.6 reveals only small differences in mean uptake from the different pastes. Student's t test was applied and none of these differences was found to be statistically significant (p was always greater than .05).

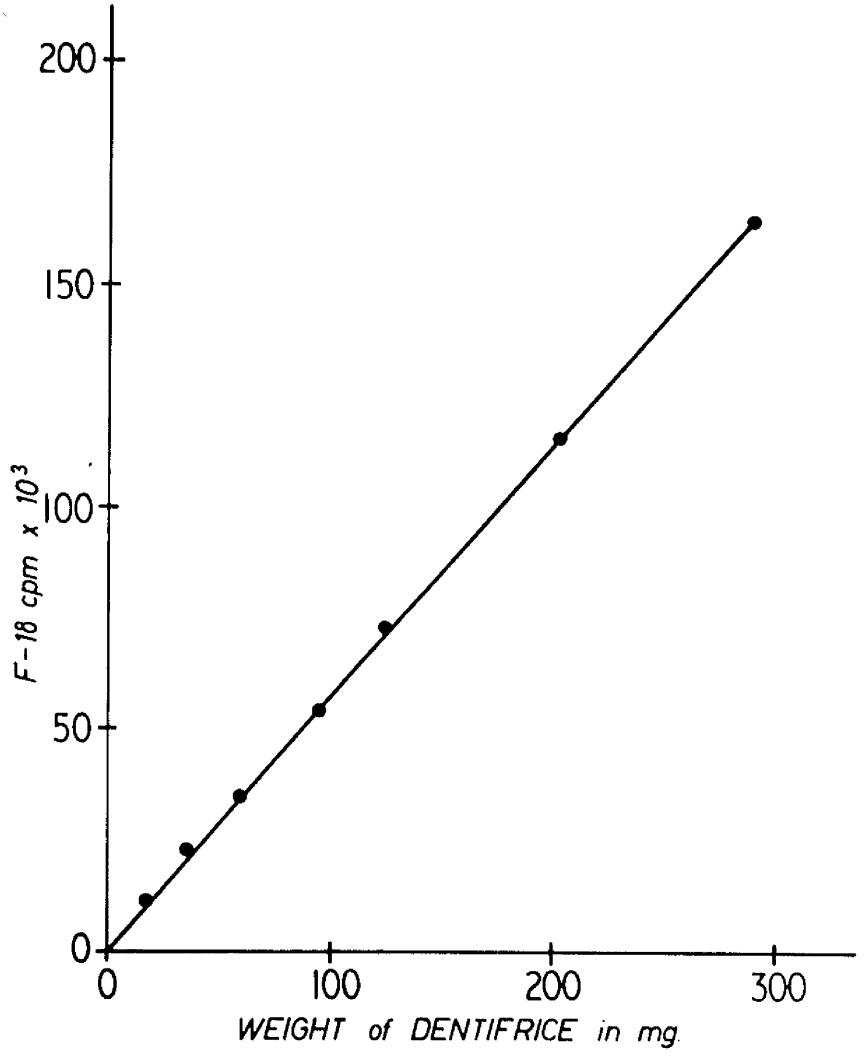


Figure 4.2.

The activity of fluorine-18 is proportional to the weight of the dentifrice sample.

TABLE 4.1.

Extraction of fluorine-18 with water from Dentifrice B at intervals after labelling.

Time mins	F-18 cpm Paste	F-18 cpm Extract	F-18 per cent Extracted	Mean F-18 per cent Extracted
20	51,200	41,200	80.3	80.0
	52,500	41,800	79.7	
47	52,700	40,900	77.6	78.7
	53,700	48,800	79.8	
100	54,500	43,800	80.5	78.3
	53,100	40,400	76.1	
130	52,700	42,200	80.1	79.0
	52,300	40,600	77.9	
150	52,400	43,000	80.5	81.5
	52,500	43,300	82.5	

TABLE 4.2.

Soluble fluoride in stannous fluoride dentifrices determined by chemical and fluorine-18 methods of analysis applied to the same supernatants.

Dentifrice	Eluted with	Soluble Fluoride in μg Determined Chemically	Soluble Fluoride in μg Determined with F-18
A	Water	79.2	85.9
	Saliva	48.2	46.8
B	Water	69.4	72.1
	Saliva	60.2	58.7
C	Water	75.0	76.4
	Saliva	39.1	42.6

TABLE 4.3.

Uptake of F-18 by the crowns of premolar teeth in vivo during brushing with F-18 labelled Dentifrice A.

Subject	Age in Years	Tooth	F Uptake in mμg per g paste
R.W.	13	$\underline{/4}$	96
		$\underline{4/}$	108
L.S.	12	$\underline{/5}$	321
		$\underline{5/}$	204
		$\overline{/5}$	119
		$\overline{5/}$	137
T.T.	14	$\overline{5/}$	48
S.J.	12	$\underline{/4}$	106
		$\underline{4/}$	152
R.B.	11	$\underline{/5}$	101
		$\overline{/5}$	79
S.F.	14	$\underline{/4}$	130
		$\underline{4/}$	119
		$\overline{/4}$	105
		$\overline{4/}$	38
R.C.	12	$\overline{/5}$	196
R.T.	13	$\underline{/4}$	184
		$\underline{4/}$	182
		$\overline{4/}$	140
		$\overline{/4}$	164
A.D.	12	$\underline{/5}$	103
		$\overline{/5}$	96
T.L.	11	$\underline{/5}$	215
		$\underline{5/}$	204
		$\overline{/5}$	92

TABLE 4.4.

Uptake of F-18 by the crowns of premolar teeth in vivo during brushing with F-18 labelled Dentifrice B.

Subject	Age in Years	Tooth	F Uptake in mμg per g paste
W.H.	14	$\frac{/5}{}$	458
		$\frac{/5}{}$	209
S.W.	10	$\frac{/4}{}$	138
		$\frac{4/}{}$	142
E.J.	12	$\frac{/5}{}$	108
		$\frac{5/}{}$	127
R.L.	12	$\frac{4/}{}$	324
		$\frac{/4}{}$	283
S.S.	11	$\frac{4/}{}$	87
		$\frac{/4}{}$	109
		$\frac{/4}{}$	121
		$\frac{4/}{}$	98
W.F.	13	$\frac{5/}{}$	134
		$\frac{/5}{}$	112
		$\frac{5/}{}$	94
R.D.	13	$\frac{4/}{}$	90
		$\frac{4/}{}$	111
M.D.	11	$\frac{/4}{}$	160
		$\frac{4/}{}$	143
		$\frac{/4}{}$	72
K.R.	12	$\frac{5/}{}$	101
		$\frac{5/}{}$	89
C.M.	10	$\frac{4/}{}$	133
		$\frac{/4}{}$	121
		$\frac{5/}{}$	77

TABLE 4.5.

Uptake of F-18 by the crowns of premolar teeth in vivo during brushing with F-18 labelled Dentifrice C.

Subject	Age in Years	Tooth	F Uptake in mμg per g paste
F.H.	12	$\underline{5/}$	168
		$\overline{5/}$	159
S.T.	13	$/\underline{4}$	106
		$\overline{4/}$	99
		$\underline{4/}$	127
		$/\overline{4}$	79
R.Y.	11	$/\underline{4}$	298
		$\overline{4/}$	119
S.M.	14	$/\overline{5}$	171
		$\overline{5/}$	123
F.H.	12	$/\underline{4}$	92
		$\underline{4/}$	104
		$/\overline{4}$	72
		$\overline{4/}$	111
P.S.	11	$\overline{5/}$	124
C.B.	13	$/\underline{4}$	263
		$/\overline{4}$	147
S.B.	12	$\underline{5/}$	151
		$/\underline{5}$	139
		$/\overline{4}$	107
		$\overline{4/}$	84
R.T.	13	$/\underline{5}$	143
		$\underline{5/}$	119
P.G.	10	$\underline{4/}$	129
		$/\underline{4}$	172

TABLE 4.6.

Uptake of F-18 by the crowns of premolar teeth in vivo during brushing with F-18 labelled dentifrices. Summary of results shown in Tables 4.3 to 4.5.

A. All teeth.

Dentifrice	Number of Teeth	Mean Uptake of F in mμg per g paste	Standard Deviation
A	25	137.6	± 61.2
B	25	145.6	± 88.1
C	25	136.2	± 51.9

B. Upper and lower teeth considered separately.

Dentifrice	Number of Teeth		Mean Uptake of F in mμg per g Paste	Standard Deviation
	Upper	Lower		
A	14		158.9	± 55.3
		11	110.4	± 57.2
B	15		169.0	± 97.1
		10	110.6	± 61.2
C	13		154.7	± 52.2
		12	116.2	± 31.3

4.4. DISCUSSION

4.4.1. Introduction.

The experiments described in this chapter have shown that measurable quantities of fluoride, as fluorine-18, are transferred to the crowns of sound premolar teeth in vivo during toothbrushing with dentifrices containing stannous fluoride.

Fluorine-18 has been used to determine the quantity of stable fluoride taken up by the teeth when solutions were applied topically (Myers et al., 1952), when mouth washes were used (Hellstrom, 1960) and when fluoridated gum was chewed (Emslie et al., 1961). Therefore, it appeared permissible to express the present results in a similar manner and this has been done in Tables 4.3 to 4.5. With the results in this form, the mean uptake of fluoride per gram of paste was determined for the three groups of teeth, each brushed with a different dentifrice (Table 4.6). However, before measurements made with fluorine-18 are accepted as indicators of the uptake of stable fluoride by enamel, and therefore a basis for comparison between dentifrices, several factors affecting the uptake of radiofluorine from a dentifrice must be considered. Firstly, if calculations of fluoride uptake are to be made in terms of the total amount of fluoride present in the paste, it has to be shown that all the fluoride in the dentifrice is labelled with fluorine-18. Secondly, the mechanism of uptake of fluorine-18, by the crowns of the teeth, must be known and this should be shown to represent the transfer of non-radioactive

fluoride to the enamel. Thirdly, variations in the uptake of fluorine-18 by individual teeth, and the differences between the means of the uptake by the groups of teeth, must be due in part to the dentifrice, and not entirely due to variations in the surface areas of the crowns. These factors will be considered in turn.

4.4.2. Labelling of Fluoride in the Dentifrice with Fluorine-18.

Although fluorine-18 exists in a complexed form it exchanges with and labels fluoride ions in a simple aqueous solution (Silvester, 1964, personal communication). This probably occurs when radiofluorine is added to a stannous fluoride dentifrice but, some of the ingredients of the dentifrice may retard the attainment of equilibrium between fluorine-18 and non-radioactive fluoride.

Uniform mixing of fluorine-18 with the dentifrice has been demonstrated (Fig. 4.2) but this gives no information upon the distribution of radiofluorine between the soluble and insoluble fractions of fluoride contained in the paste. However, fluorine-18 appears to equilibrate with dentifrice fluoride in less than 20 minutes (Table 4.1) and the results of parallel analyses to determine soluble fluoride by chemical methods, and by the use of fluorine-18, show close agreement (Table 4.2). Thus, under the conditions described, radiofluorine is an accurate indicator of the behaviour of the fluoride ion in a dentifrice.

4.4.3. Fluorine-18 as an Indicator of the Uptake of Stable Fluoride.

The reactions of fluoride with powdered enamel have been investigated by McCann and Bullock (1955). They suggest five mechanisms for the entry of fluoride into enamel. Three of these, namely the formation of fluorapatite by exchange with hydroxyl ions, direct adsorption of fluoride by enamel and double decomposition to form calcium fluoride were considered to play a part in the reaction of fluoride with surface enamel in vivo. Clearly, the chemical reactions of fluorine-18 should be identical with the reactions of non-radioactive fluoride. However, an additional mode of uptake of fluorine-18 by enamel is possible.

When fluorine-18 is introduced into a fluoride containing solution, bathing the crown of a tooth, it should enter the enamel, in part, by a process of iso-ionic exchange. Iso-ionic exchange is defined by Neuman and Neuman (1953) as a process by which ions from the solution phase exchange with similar ions in the surface of the solid phase with no net change in the composition of the two phases. This probably occurs in addition to the entry of fluorine-18 by the other mechanisms referred to above. Entry of radioactive fluoride by iso-ionic exchange alone would render this isotope valueless in uptake studies. Conversely, uptake of fluorine-18 by processes other than iso-ionic exchange would make this radioisotope a useful marker in the measurement of the uptake of fluoride by enamel.

The possible role of iso-ionic exchange appears to have been recognised by the earliest observers to study the uptake of fluorine-18

by the hard dental tissues (Volker, Hodge, Wilson and Van Voorhis,1940). They comment upon the fact that although powdered dentine has a higher fluoride content than powdered enamel this did not appear to favour fluorine-18 uptake by the dentine. Brudevold et al.(1957) have noted the ready uptake of fluorine-18 by fluorosed surface enamel and by surface enamel with a high fluoride content from the teeth of older subjects. However, the figure in their paper which illustrates this point also shows that unaltered surface enamel from a young subject takes up fluorine-18 twice as quickly as fluorosed enamel from a subject of similar age. These observations suggest that iso-ionic exchange of fluorine-18, with non-radioactive fluoride in mineralised dental tissues, is not the sole method of uptake of the radioisotope.

This is supported by the experiments of McCann and Bullock (1955) and Leach (1959) which make it clear that the fluoride content of powdered enamel can be raised by exposing it to dilute solutions of sodium fluoride. After chemical studies in vitro on surface enamel (Brudevold et al., 1956) and sub-surface enamel (Cooley, 1961) the same conclusion was reached. Clearly, the uptake of fluoride by enamel which has been demonstrated by such chemical analyses cannot be due to iso-ionic exchange.

In an attempt to decide the relative importance of hetero-ionic and iso-ionic exchange, Fremlin et al.(1959) carried out experiments which, they claimed, demonstrated some important limitations inherent in the use of fluorine-18 in the study of mineralised tissues. These authors concluded that most of the fluorine-18, taken up by enamel from its environment, was taken up by a process of iso-ionic exchange. Nevertheless,

some hetero-ionic exchange was thought to occur.

Thus the role of fluorine-18 in uptake studies remains uncertain. The available evidence suggests that it enters enamel by a combination of iso-ionic exchange, hetero-ionic exchange, adsorption and by the formation of calcium fluoride. The amount of radiofluorine which enters by iso-ionic exchange probably leads to an over-estimate of the uptake of non-radioactive fluoride by enamel. For this reason the uptake of fluoride recorded in Tables 4.3 to 4.6 is probably in excess of the true uptake.

This problem will be returned to in Chapter 5 where an investigation in vitro of the uptake of fluorine-18 will be described.

4.4.4. The Relationship Between the Surface Area of the Crown and its Uptake of Fluorine-18.

Among the factors which will have contributed to the wide variation in the uptake of fluorine-18 by the crowns of the teeth is the area of the enamel surface. However, it is unlikely that the extremes of uptake by an upper premolar (87 and 458 mug of fluoride) can be explained solely by variations in crown surface area, for the tooth with the low uptake would have been so incompletely erupted that it would not have been accepted for the series. Smaller variations in the uptake of fluorine-18 could, however, be explained by this area factor. Reference to Table 4.6 will show that the mean uptake by the larger upper premolars was, as expected, greater than the mean uptake by the smaller lower premolars when they were brushed with the same brand of labelled dentifrice.

However, although these differences in uptake show the anticipated trend they are not statistically significant (for the 3 groups of teeth p lies between 0.05 and 0.2).

4.4.5. Other Factors Influencing the Uptake of Fluorine-18.

These include the ability of the dentifrice to provide soluble fluoride to the saliva-enamel environment; the fluoride content of the surface enamel; the presence of organic debris upon the tooth surfaces which would retain fluorine-18; the presence of pits, fissures and lamellae in the enamel surfaces and the existence of early carious lesions not visible to the naked eye.

The differences between the means of the uptake of fluoride, as fluorine-18, from Dentifrices A, B and C by all teeth, by upper teeth and by lower teeth (Table 4.6) are small and in any case not statistically significant. Therefore, no differences in the ability of these 3 brands of dentifrices to transfer soluble fluoride in saliva to the crowns of the teeth has been demonstrated.

The teeth of the subjects taking part in this investigation were not polished before the labelled dentifrices were used. Therefore, it is probable that there were differing amounts of organic debris upon the tooth surfaces. The uptake of fluorine-18 by the crowns may have been prevented by such debris. The removal of this debris when washing the teeth, prior to making the radioactivity measurements, may have left relatively small and variable amounts of radiofluorine in the underlying enamel. Conversely, crowns which were free of debris at the outset

may have taken up much more fluorine-18.

Autoradiographic studies have shown that fluorine-18 is concentrated in pits and fissures and in enamel imperfections, including early carious lesions (Myers et al., 1952; Hardwick et al., 1958). Hence, the uptake of fluorine-18 by the crowns in vivo would probably have been influenced by the presence of imperfections in the enamel.

Thus, in these experiments many variables were uncontrolled but apart from polishing the teeth of the subjects before they used the labelled dentifrices the control of these variables did not seem possible.

4.5. CONCLUSIONS

An uptake of fluoride, as fluorine-18, by premolar teeth in situ, from dentifrices containing stannous fluoride, has been demonstrated. Under the conditions of the experiment there were no detectable differences in the ability of 3 different brands of dentifrice to transfer fluorine-18 to the enamel. To what extent this uptake of fluorine-18 indicates a true uptake of non-radioactive fluoride remains uncertain.

This uncertainty and the existence of so many uncontrolled variables, which can influence the uptake of fluorine-18 by the enamel of crowns in situ, lead to the conclusion that the procedures described in this chapter are unsuitable for determining the availability of fluoride contained in a stannous fluoride dentifrice.

CHAPTER 5

THE UPTAKE AND RELEASE OF FLUORINE-18 BY INTACT SURFACE ENAMEL IN VITRO

5.1. INTRODUCTION

A knowledge of the mechanism of fluorine-18 uptake by surface enamel is essential for the proper consideration of the results recorded in Chapter 4. Fremlin et al. (1959) have presented data which indicate that the uptake of fluorine-18, from aqueous solutions of sodium fluoride in vitro, is largely by a process of iso-ionic exchange. Thus, it would seem probable that radiofluorine would be taken up by enamel from labelled stannous fluoride dentifrices by a similar exchange process. However, this does not appear to have been demonstrated. Therefore, in addition to repeating some of the experiments described by Fremlin and his colleagues, the uptake of fluorine-18 by enamel from stannous fluoride solutions and from dentifrices containing stannous fluoride has been studied.

In this investigation intact surface enamel has been immersed for one week, in fluoride solutions or dentifrices. Both were labelled with fluorine-18 at the beginning of the experiment and were re-labelled with the radioisotope one week later. After each labelling the uptake of radiofluorine by the enamel was measured and a comparison of the rates of the first and second uptake was made.

The hypothesis tested in these experiments is similar to the one tested by Fremlin et al. (1959). Reference to Figure 5.1 will simplify its description. In this graph the first uptake of fluorine-18 by enamel is compared with four possible uptake curves which might be

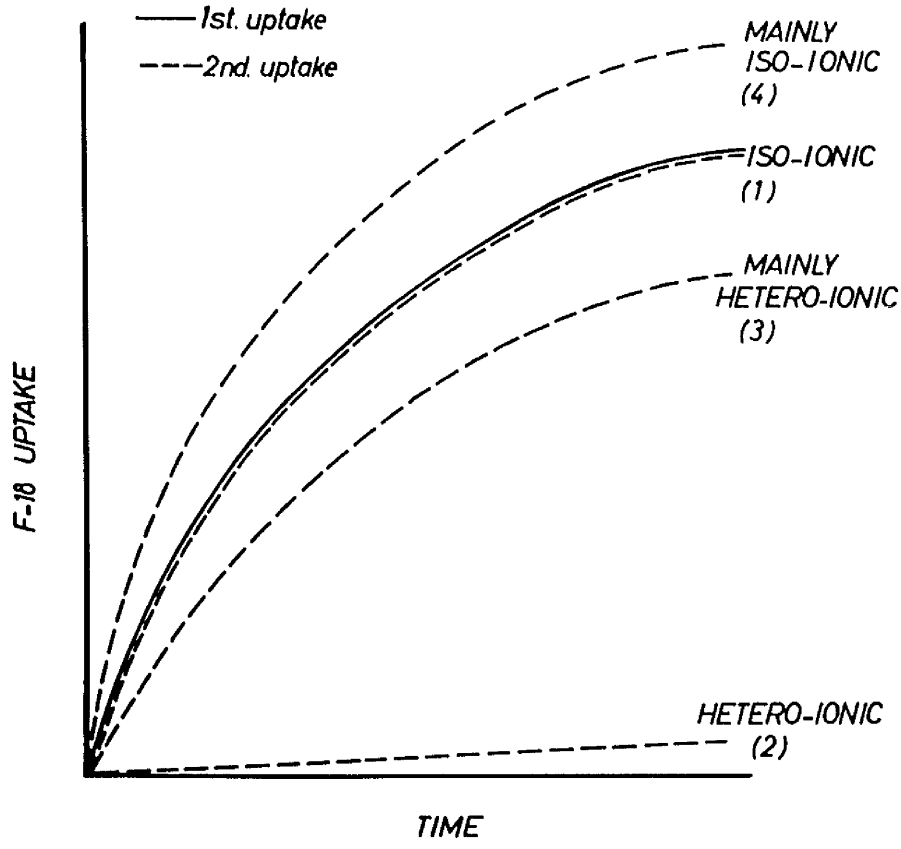


Figure 5.1.

Four hypothetical curves showing the second uptake of fluorine-18 by enamel. Each curve suggests a different mechanism of uptake (Fremlin et al., 1959).

obtained after the second labelling. Each of the hypothetical curves showing the second uptake suggests a different mechanism for the entry of fluoride into enamel.

1. If this is entirely by iso-ionic exchange the second uptake, after re-labelling with fluorine-18, would be identical with the first, there having been no net change in the amount of fluoride in the enamel surface.
2. Conversely, if the uptake is entirely by hetero-ionic exchange the second uptake would be much slower and probably less than the first. Most of the sites for hetero-ionic exchange near the surface of the enamel would have been utilised already and this slower second uptake of fluorine-18, if found, would probably represent penetration of fluoride ions into deeper layers of the enamel and into enamel faults.
3. If uptake is preferentially hetero-ionic, yet partly by iso-ionic exchange, the second uptake of fluorine-18 would be slower than the first. During the second uptake the rate of entry by limited hetero-ionic exchange in the deeper layers of enamel, as in 2., and some iso-ionic exchange, would be less than the initial rate of hetero-ionic exchange in the superficial layers.
4. The possibility remains that surface enamel takes up fluorine-18 preferentially by iso-ionic exchange but that limited hetero-ionic exchange also occurs. Hetero-ionic exchange of

fluoride would raise the fluoride content of the surface enamel when first immersed in the solution and this would permit an increase in iso-ionic exchange when the solution was re-labelled. Therefore, the second uptake would proceed at a greater rate than the first.

The exchange reactions of fluorine-18 with surface enamel may also be studied by reversing the above procedures. This is achieved, with teeth which have already taken up fluorine-18, by washing the crowns with water and fluoride solutions of varying concentration (Fremlin et al., 1959). If hetero-ionic exchange is more important than iso-ionic exchange quicker removal of fluorine-18 would be expected when washing with water than when washing with fluoride solutions. If the converse were true, fluoride solutions would be more effective in removing radio-fluorine from the crowns of the teeth. This hypothesis will also be tested in the experiments described below.

5.2. MATERIALS AND METHODS

5.2.1. Preparation of Fluorine-18 Labelled Solutions and Dentifrices.

Fluoride in the following solutions and dentifrices was labelled with carrier-free fluorine-18 prepared as described previously (see 4.2.1).

1. 1 and 100 ppm fluoride as sodium fluoride in distilled-deionised water at the natural pH of the solutions (pH 6.0-6.2).
2. 1 ppm fluoride as sodium fluoride and as stannous fluoride in 0.2 M acetate buffer at pH 4.5.
3. The 3 brands of dentifrice used in the experiments described in Chapter 3. The tubes containing these pastes were supplied as described previously and stored at room temperature for not more than 3 months before use.

A buffer pH of 4.5 was chosen because this is close to the pH of the fluoride dentifrices but sufficiently acid to prevent precipitation of stannous fluoride in dilute solutions (10 ppm F or less) for at least one week. More concentrated solutions of stannous fluoride were not used because heavy white precipitates, of uncertain composition, formed within minutes of their preparation.

Volumetric work apart, all the solutions were handled in polyethylene vessels. After adding fluorine-18, at the rate of about 2 μC per ml at the time of labelling, the solutions were stirred with polyethylene rods. Dentifrices were labelled as described in the previous chapter (see 4.2.2).

To permit calculation of the results in terms of the fluorine-18 activity of 1 ml of solution or dentifrice, counting standards were prepared. Triplicate 1 ml aliquots were taken from each labelled solution and at least four samples of labelled dentifrice were weighed in polyethylene tubes. To reduce possible counting errors, due to differences in volume, these dentifrice standards were suspended in 1 ml of water. Counts per minute per mg of paste were converted to counts per minute per ml after the densities of the dentifrices had been determined by comparing the weights of equal volumes of water and paste.

5.2.2. Preparation of Teeth.

Caries-free premolar teeth, extracted from children receiving orthodontic treatment, were used in these experiments. Extractions could not usually be integrated with the production of fluorine-18, therefore, after extraction and washing, most teeth were stored in distilled water at 5°C for periods not in excess of 2 weeks. Before use adherent soft tissue was cleaned from the roots and necks of the teeth with a knife and debris was removed from the crowns by polishing with a new softened bristle-brush in a dental handpiece. The brush was kept moist with distilled water; no abrasive was used.

The roots of the teeth and strips of cervical enamel approximately 1 mm wide, were covered with molten blue inlay wax (Kerr). Care was taken to secure a close seal at the junction of wax with enamel. In a few teeth a small, white but still glazed zone was present on the approximal surfaces of the crowns. Such areas were sealed off by small

amounts of wax. For convenience in handling, one end of a 6" length of suture silk, or fine linen thread, was embedded in the wax at the apex of each tooth which was to be immersed in a fluoride solution. A tooth to be exposed to labelled dentifrice was fixed to a 2" length of thin glass rod by blue wax. To prevent uptake of fluorine-18 by the glass, in case of its accidental immersion in the paste, the length of rod close to the apex of the tooth was also covered with wax.

Some of the crowns to be immersed in solutions of sodium fluoride were dipped momentarily into N hydrochloric acid to etch lightly the enamel surface.

5.2.3. Measurement of the Uptake of Fluorine-18 by the Crowns of Teeth Immersed in Labelled Fluoride Solutions and in Dentifrices Containing Labelled Stannous Fluoride.

Each tooth was suspended by its thread in 20 ml of a fluoride solution in a 3" x 1" capped polyethylene tube, or was pushed, on its glass rod, into a similar volume of paste. In both cases the solution or paste just covered the exposed crown. Each tooth was removed for radioactivity measurements at intervals during a total immersion time of 300 or 350 minutes.

All teeth were returned to the solutions or pastes, on completion of the last count, and were stored at room temperature. To control evaporation during storage the caps of the tubes were sealed with paraffin wax. After one week the teeth were removed from the solutions or

pastes which were then re-labelled with carrier-free fluorine-18. New counting standards were prepared as already described. The teeth were replaced and their uptake of radioactivity was determined at intervals over a further period of 300 or 350 minutes.

On removal from the fluoride solutions the teeth were washed under running tap-water for 1 minute and dried on a paper tissue. Teeth removed from labelled dentifrices were first wiped with a tissue, lightly scrubbed with a clean nail brush to remove adherent paste, washed under tap-water for 2 minutes and dried. Teeth, fluoride solution standards (1 ml) and dentifrice standards suspended in 1 ml water, were counted in polyethylene tubes as described already (see 4.2.4).

In the earlier experiments in the series the efficiency of the wax seal was determined by separating the roots of 12 teeth from their crowns after measurements of the second uptake had been completed. The activity of each separated root together with its wax covering and the attached thread or glass rod, was measured and expressed as a percentage of the activity of the intact preparation.

5.2.4. Measurement of the Removal of Fluorine-18 from the Crowns of Teeth by Washing with Water and Fluoride Solutions.

The exposed crowns of waxed premolar teeth were immersed in either a fluorine-18 labelled solution of stannous fluoride at pH 4.5, containing 1 ppm fluoride, or a labelled stannous fluoride dentifrice. After immersion for two hours the teeth were washed for two minutes under

running tap-water, dried on paper tissues and counted. Teeth so prepared were then placed individually in polyethylene beakers and were washed with one of the following:

1. Tap-water containing approximately 0.05 ppm fluoride.
2. An aqueous solution of sodium fluoride containing 1 ppm fluoride at its natural pH.
3. An aqueous solution of sodium fluoride containing 100 ppm fluoride at its natural pH.

Irrigating tap-water and fluoride solutions were supplied by gravity flow from 10 litre polyethylene bottles at a constant rate (50 ml per minute). At intervals, during a total washing time of up to nearly 3 hours, the teeth were removed from the beakers, dried and their radioactivities measured.

5.3. RESULTS

The densities of the toothpastes, which were required for calculation of the radioactivity of 1 ml of each dentifrice from the measured activity per mg, are shown in Table 5.1. The amount of fluorine-18 found in the separated root, covering wax and attached thread or rod of each of 12 teeth was always less than 1 per cent of the activity of the whole preparation (Table 5.2). Therefore, wax covering the roots of the teeth prevented the uptake of radiofluorine by cementum and dentine.

Tables 5.3 to 5.10 show the uptake of fluorine-18 by the exposed crowns of 32 premolar teeth at intervals during immersion in the various solutions and pastes. The uptake was calculated as a function of the radioactivity of unit volume (1 ml) of the solutions or dentifrices. Expressing the results in this way eliminates apparent variations in uptake due to labelling the solutions or pastes with different activities of fluorine-18. Thus, comparisons can be made between the uptake of fluorine-18 by the enamel of a crown when first immersed and the uptake by the same crown when the solution or paste is re-labelled with radiofluorine one week later.

5.3.1. Uptake of Fluorine-18 from Solutions Containing Sodium Fluoride. 1 ppm fluoride at its natural pH. Crowns not etched.

The uptake of fluoride, as fluorine-18, by the crowns of four premolars (teeth numbered 1 to 4) is shown in Table 5.3. The uptake

TABLE 5.1.

Densities of the pastes used in the calculation of fluorine-18 activity per ml of dentifrice.

Dentifrice	A	B	C
Density of Samples	1.580	1.605	1.465
	1.570	1.603	1.455
	1.575	1.595	1.463
Mean Density	1.575	1.601	1.461

TABLE 5.2.

Fluorine-18 activity of the severed root, covering wax and attached thread or glass rod as a percentage of the activity of the whole preparation.

Tooth	Percentage Activity	Tooth	Percentage Activity
1	0.45	7	0.63
2	0.57	8	0.31
3	0.43	9	0.28
4	0.59	10	0.69
5	0.17	11	0.42
6	0.44	12	0.38

TABLE 5.3.

Uptake of F-18 by the crowns of premolar teeth immersed in solutions containing 1 ppm F as $Na^{18}F$.

Uptake expressed as $\frac{\text{cpm per crown}}{\text{cpm per ml of } Na^{18}F \text{ soln}} \times 10^2$

Time of Immersion in mins	Uptake by Tooth 1		Uptake by Tooth 2	
	1st	2nd	1st	2nd
5	.425	1.18	.428	1.17
10	.576	1.68	.645	1.80
25	.910	2.88	1.02	2.91
50	1.34	4.06	1.40	4.09
100	1.85	5.71	2.01	5.68
200	2.79	7.84	2.62	7.66
350	3.48	9.51	3.62	10.2
	Uptake by Tooth 3		Uptake by Tooth 4	
	1st	2nd	1st	2nd
5	.334	.885	.455	1.32
10	.514	1.43	.691	1.64
25	.862	2.55	1.01	2.61
50	1.16	3.75	1.50	3.58
100	1.74	5.22	2.02	4.86
200	2.83	7.04	2.88	7.10
350	3.76	10.1	3.76	9.02

2nd uptake = uptake after 1 week from the same solution re-labelled with F-18.

by the crown of Tooth 1 is plotted against time in Figure 5.2. This shows that the second uptake of fluorine-18, after the tooth had been immersed in the solution for one week, was faster than the first. Similar results were obtained for Teeth 2 to 4. Plotting the data logarithmically shows a linear relationship between the logarithm of the uptake of fluorine-18 and the logarithm of the time of immersion in the labelled or re-labelled fluoride solution (Fig. 5.3). This form of graphic representation will be used to illustrate the subsequent results of similar uptake experiments.

100 ppm fluoride at its natural pH. Crowns not etched.

The uptake of fluorine-18 by the crowns of teeth numbered 5 to 8 is shown in Table 5.4 and in Figure 5.4. In each case the second uptake proceeded at a greater rate than the first.

1 ppm fluoride at its natural pH. Crowns etched.

The results of uptake measurements on four teeth (numbered 9 to 12) are shown in Table 5.5 and Figure 5.5. The rate of the second uptake was slightly greater than the first. On their first immersion these etched crowns took up about 25 times more fluorine-18 than the unetched crowns of teeth immersed in fluoride solutions of similar concentration for a similar period of time.

1 ppm fluoride buffered to pH 4.5

The uptake of fluorine-18 by each of the crowns of teeth numbered 13 to 16, immersed in acid sodium fluoride solutions is shown in Table 5.6 and Figure 5.6. The rates of uptake were greater than those of etched or unetched crowns immersed in solutions containing 1 ppm fluoride at their natural pH.

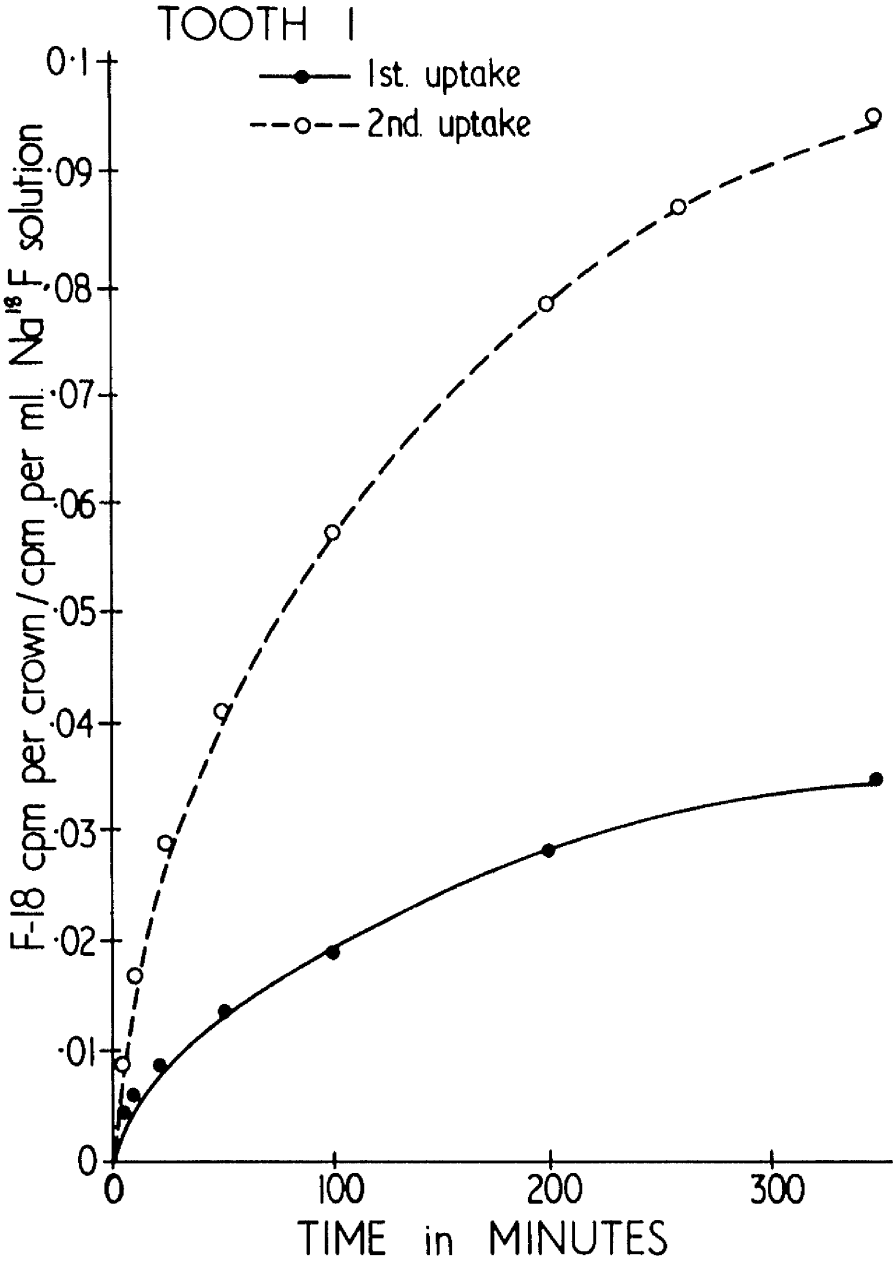


Figure 5.2.

The uptake of fluorine-18 when the crown was first immersed in a labelled solution of NaF and the uptake after re-labelling the solution one week later.

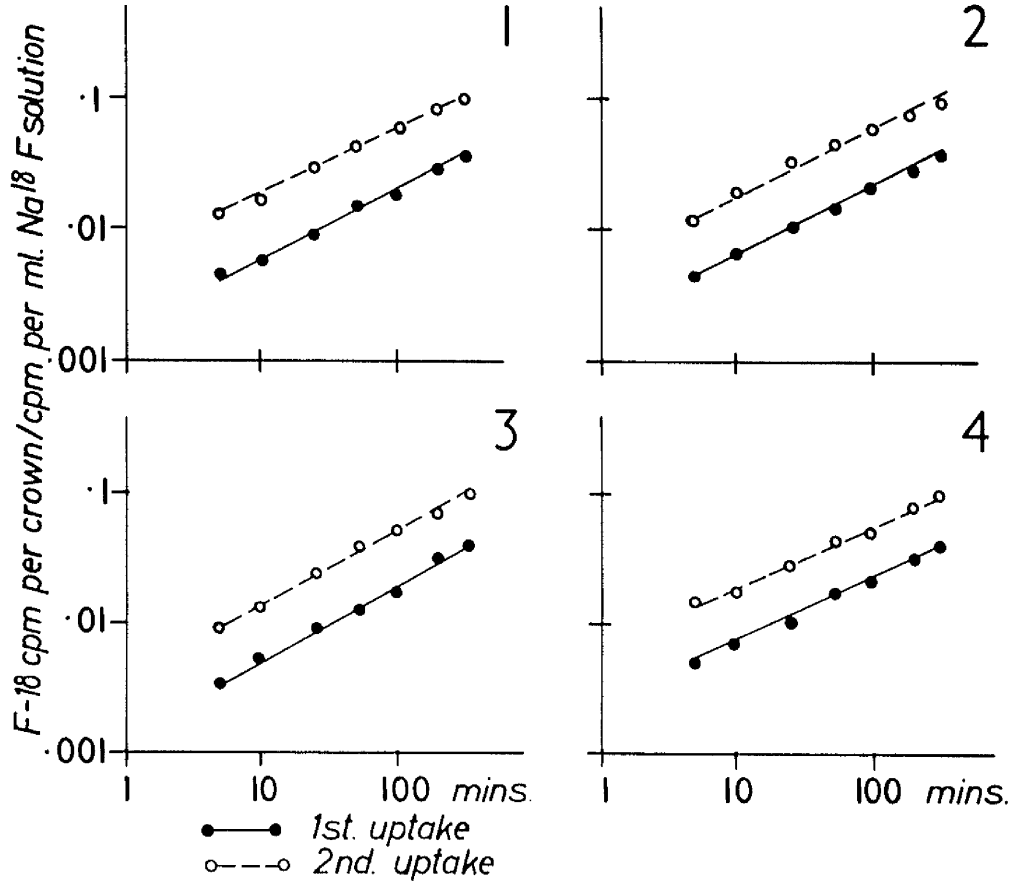


Figure 5.3.

Uptake of fluorine-18 by crowns 1-4 from solutions containing 1 ppm F as NaF plotted on logarithmic scales.

TABLE 5.4.

Uptake of F-18 by the crowns of premolar teeth immersed in solutions containing 100 ppm F as $Na^{18}F$.

$$\text{Uptake expressed as } \frac{\text{cpm per crown}}{\text{cpm per ml of } Na^{18}F \text{ soln}} \times 10^2$$

Time of Immersion in mins	Uptake by Tooth 5		Uptake by Tooth 6	
	1st	2nd	1st	2nd
5	.102	.361	.082	.283
10	.135	.592	.124	.433
25	.218	.914	.192	.571
50	.315	1.20	.285	.764
100	.401	1.92	.433	.989
200	.542	2.81	.648	1.38
350	.740	3.50	.912	1.83
	Uptake by Tooth 7		Uptake by Tooth 8	
	1st	2nd	1st	2nd
5	.063	.253	.086	.348
10	.090	.334	.126	.488
25	.148	.512	.201	.742
50	.230	.708	.296	.994
100	.335	1.06	.404	1.35
200	.583	1.45	.537	1.93
350	.705	1.86	.713	2.45

2nd uptake = uptake after 1 week from the same solution re-labelled with F-18.

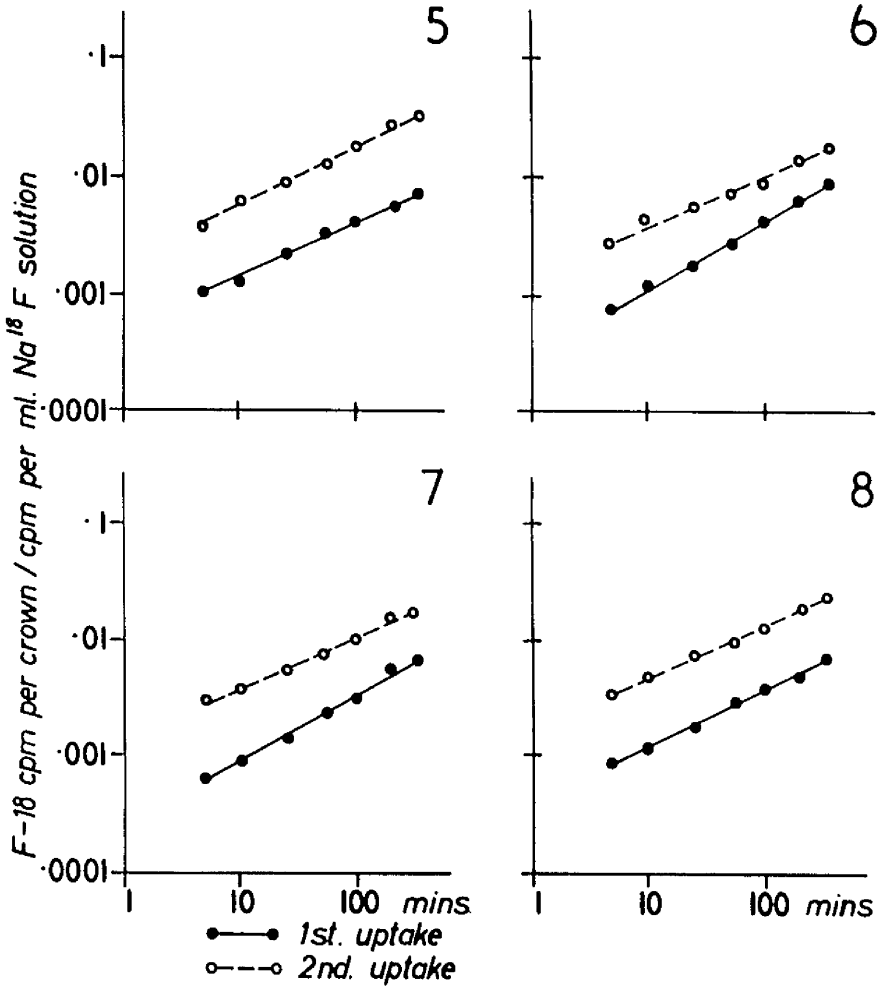


Figure 5.4.

Uptake of fluorine-18 by crowns 5-8 from solutions containing 100 ppm F as NaF.

TABLE 5.5.

Uptake of F-18 by the crowns of premolar teeth immersed in solutions containing 1 ppm F as $Na^{18}F$.

Uptake expressed as $\frac{\text{cpm per crown}}{\text{cpm per ml of } Na^{18}F \text{ soln}}$

Crowns etched in N HCl

Time of Immersion in mins	Uptake by Tooth 9		Uptake by Tooth 10	
	1st	2nd	1st	2nd
5	.102	.121	.096	.160
10	.151	.191	.142	.218
25	.238	.380	.228	.335
50	.380	.520	.351	.518
100	.518	.620	.603	.718
200	.808	.914	.902	1.06
300	.943	1.26	1.09	1.31
	Uptake by Tooth 11		Uptake by Tooth 12	
	1st	2nd	1st	2nd
5	.092	.101	.129	.142
10	.129	.145	.162	.215
25	.251	.290	.290	.355
50	.350	.412	.425	.512
100	.524	.624	.571	.760
200	.765	.903	.930	1.08
300	1.07	1.36	1.11	1.35

2nd uptake = uptake after 1 week from the same solution re-labelled with F-18

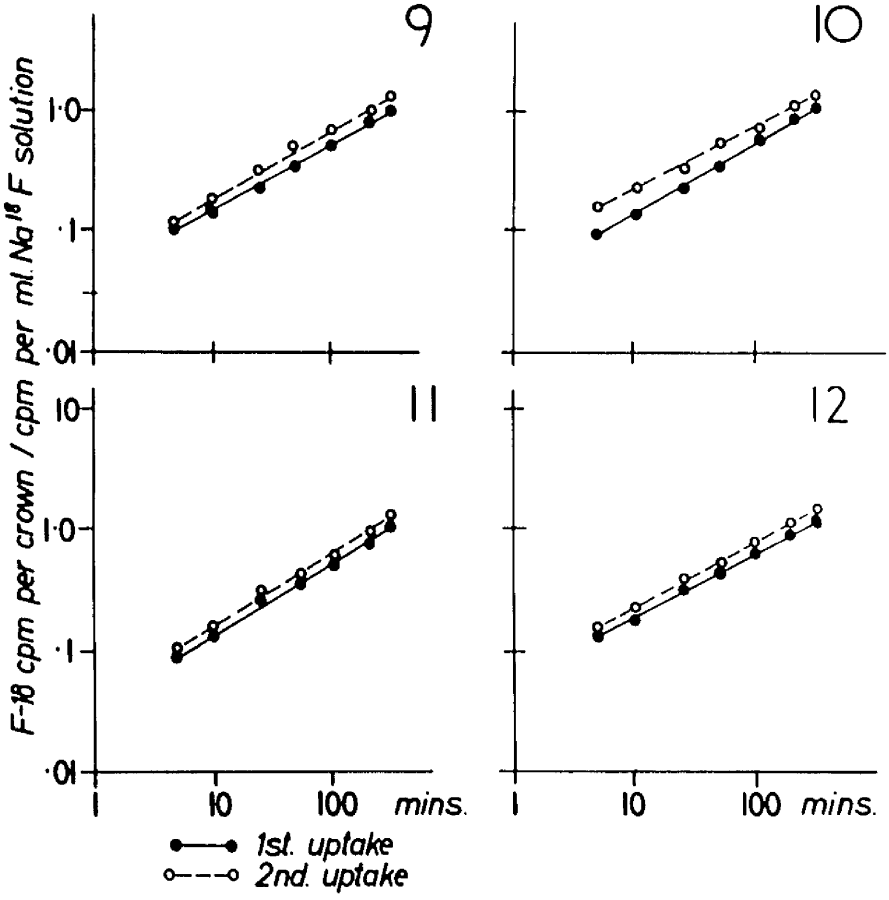


Figure 5.5.

Uptake of fluorine-18 by crowns 9-12 from solutions containing 1 ppm F as NaF.

The crowns of these teeth were etched.

TABLE 5.6.

Uptake of F-18 by the crowns of premolar teeth immersed in solutions containing 1 ppm F as $Na^{18}F$ at pH 4.5.

Uptake is expressed as $\frac{\text{cpm per crown}}{\text{cpm per ml of } Na^{18}F \text{ soln}}$

Time of Immersion in mins	Uptake by Tooth 13		Uptake by Tooth 14	
	1st	2nd	1st	2nd
5	.052	.102	.066	.133
10	.092	.186	.113	.232
25	.211	.472	.261	.610
50	.448	.911	.488	1.08
100	.896	1.63	1.00	2.05
200	1.78	2.82	1.93	3.62
300	2.54	4.56	2.59	5.81
	Uptake by Tooth 15		Uptake by Tooth 16	
	1st	2nd	1st	2nd
5	.080	.162	.065	.131
10	.128	.280	.120	.251
25	.278	.661	.252	.480
50	.469	1.04	.458	.920
100	.874	2.18	.845	1.44
200	1.67	3.87	1.69	2.84
300	2.32	4.84	2.36	3.79

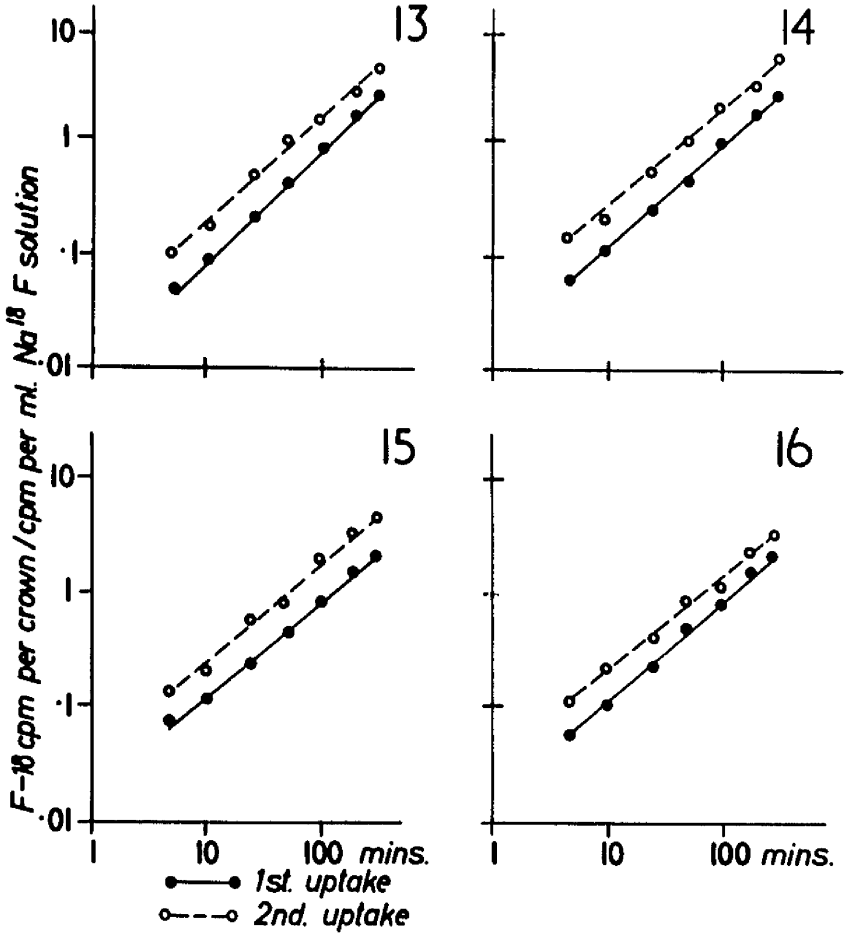


Figure 5.6.

Uptake of fluorine-18 by crowns 13-16 from solutions containing 1 ppm F as NaF at pH 4.5.

5.3.2. Uptake of Fluorine-18 from Solutions Containing 1 ppm Fluoride as Stannous Fluoride Buffered to pH 4.5.

The uptake of fluorine-18 by the crowns of teeth numbered 17 to 20, which were immersed in solutions of stannous fluoride buffered to pH 4.5, is shown in Table 5.7 and Figure 5.7. The second uptake of fluorine-18 by all four teeth occurred at a greater rate than the first. The initial uptake, measured after 5 minutes, was similar to the uptake by the crowns which had been etched before immersion in solutions containing the same concentration of fluoride, as sodium fluoride, at the natural pH. However, the rate of uptake was greater from the solutions containing stannous fluoride at pH 4.5.

5.3.3. Uptake of Fluorine-18 from Dentifrices Containing Stannous Fluoride.

The uptake of fluorine-18 from the labelled dentifrices by 12 crowns is shown in Tables 5.8 to 5.10 and Figures 5.8 to 5.10.

After the second labelling of the pastes the crowns took up from 10 to 40 per cent more fluorine-18, during 300 or 350 minutes, than on their first exposure to the radioisotope for similar periods of time.

5.3.4. Removal of Fluorine-18 from the Crowns of Teeth by Washing with Water and Fluoride Solutions.

Radioactivity measurements made upon six teeth (numbered 33 to 38), at intervals during the washing process, were calculated as a percentage of the initial activity of the crowns before washing commenced.

The results shown in Table 5.11 are represented graphically in Figure 5.11. The rate of removal of fluorine-18 was greatest when the crowns were washed with solutions containing 100 ppm fluoride and least when tap-water was used.

TABLE 5.7.

Uptake of F-18 by the crowns of premolar teeth immersed in solutions containing 1 ppm F as $Sn^{18}F_2$ at pH 4.5.

Uptake is expressed as $\frac{\text{cpm per crown}}{\text{cpm per ml of } Sn^{18}F_2 \text{ soln}}$

Time of Immersion in mins	Uptake by Tooth 17		Uptake by Tooth 18	
	1st	2nd	1st	2nd
5	.141	.189	.109	.235
10	.208	.306	.161	.351
25	.472	.601	.340	.672
50	.850	1.12	.671	1.24
100	1.38	1.69	.992	1.78
200	2.49	2.88	1.81	3.05
350	3.94	4.82	2.82	4.11
	Uptake by Tooth 19		Uptake by Tooth 20	
	1st	2nd	1st	2nd
5	.081	.089	.069	.075
10	.131	.182	.131	.151
25	.327	.394	.298	.322
50	.662	.711	.621	.742
100	1.07	1.24	1.02	1.33
200	2.11	2.42	2.11	2.72
350	3.35	3.96	3.32	4.42

2nd uptake = uptake after 1 week from the same solution re-labelled with F-18.

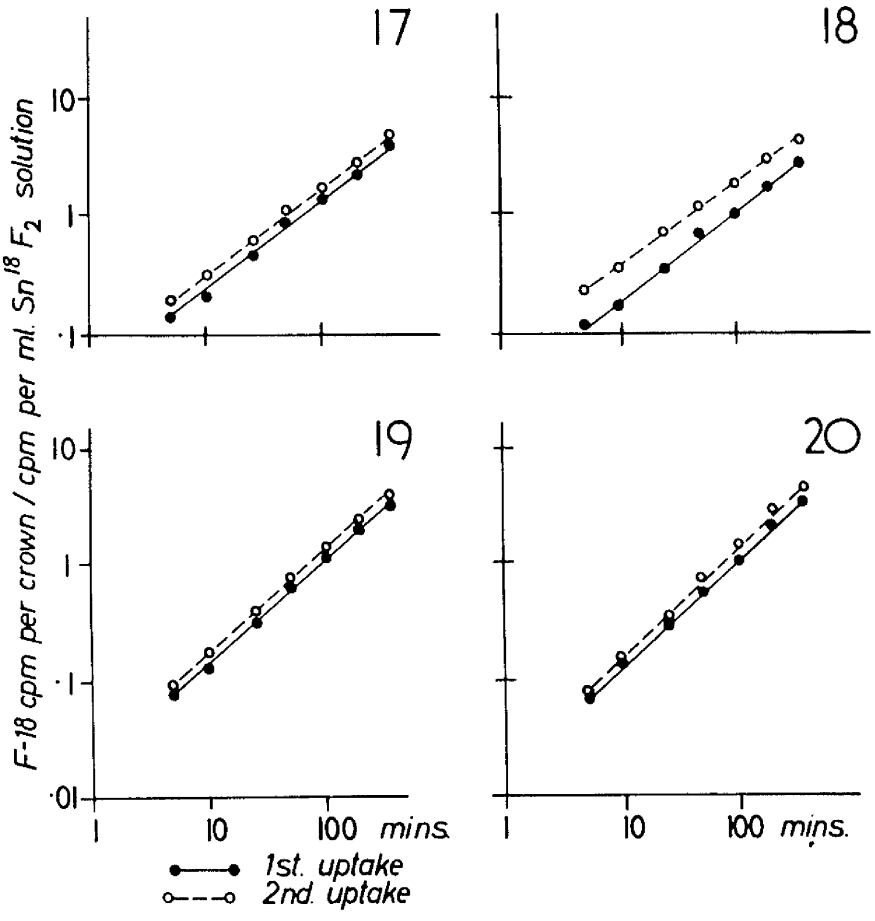


Figure 5.7.

Uptake of fluorine-18 by crowns 17-20 from solutions containing 1 ppm F as SnF_2 at pH 4.5.

TABLE 5.8.

Uptake of F-18 by the crowns of premolar teeth immersed in F-18 labelled Dentifrice A.

Uptake expressed as $\frac{\text{cpm per crown}}{\text{cpm per ml of paste}} \times 10^3$.

Time of Immersion in mins	Uptake by Tooth 21		Uptake by Tooth 22	
	1st	2nd	1st	2nd
5	.433	.845	.692	1.04
10	.921	1.38	1.20	1.42
25	1.39	1.91	1.91	2.22
50	1.89	2.53	2.55	2.98
100	2.80	3.20	3.61	4.51
200	3.89	4.16	4.92	5.93
300	4.71	5.08	5.86	7.97
	Uptake by Tooth 23		Uptake by Tooth 24	
	1st	2nd	1st	2nd
5	.473	.787	.572	.876
10	.819	1.38	1.02	1.27
25	1.40	2.12	1.45	1.99
50	2.08	2.60	2.17	2.58
100	2.89	3.88	3.18	3.21
200	4.01	4.96	4.08	5.04
350	5.57	6.30	5.27	6.53

2nd Uptake = uptake after 1 week from the same paste re-labelled with F-18

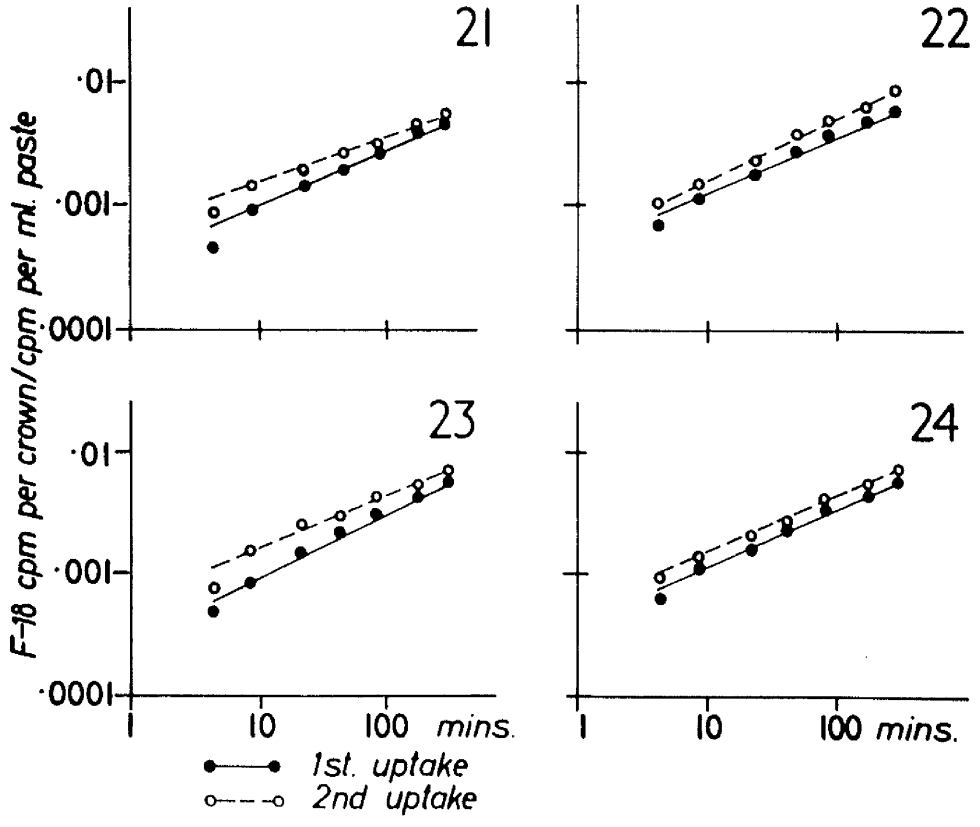


Figure 5.8.

Uptake of fluorine-18 by crowns 21-24 from Dentifrice A.

TABLE 5.9.

Uptake of F-18 by the crowns of premolar teeth immersed in F-18 labelled Dentifrice B.

Uptake expressed as $\frac{\text{cpm per crown}}{\text{cpm per ml of paste}} \times 10^3$.

Time of Immersion in mins	Uptake by Tooth 25		Uptake by Tooth 26	
	1st	2nd	1st	2nd
5	.418	.733	.347	.645
10	.618	.966	.547	.864
25	.891	1.50	.885	1.39
50	1.37	2.04	1.38	2.07
100	1.79	2.88	2.16	2.83
200	2.66	3.63	3.15	4.04
300	3.22	4.40	4.54	5.03
	Uptake by Tooth 27		Uptake by Tooth 28	
	1st	2nd	1st	2nd
5	.289	.397	.438	.631
10	.412	.585	.560	.748
25	.698	.918	.877	1.18
50	1.16	1.35	1.02	1.46
100	1.67	2.08	1.47	1.90
200	2.59	3.29	2.21	2.63
350	3.19	3.49	2.66	2.95

2nd uptake = uptake after 1 week from the same paste re-labelled with F-18

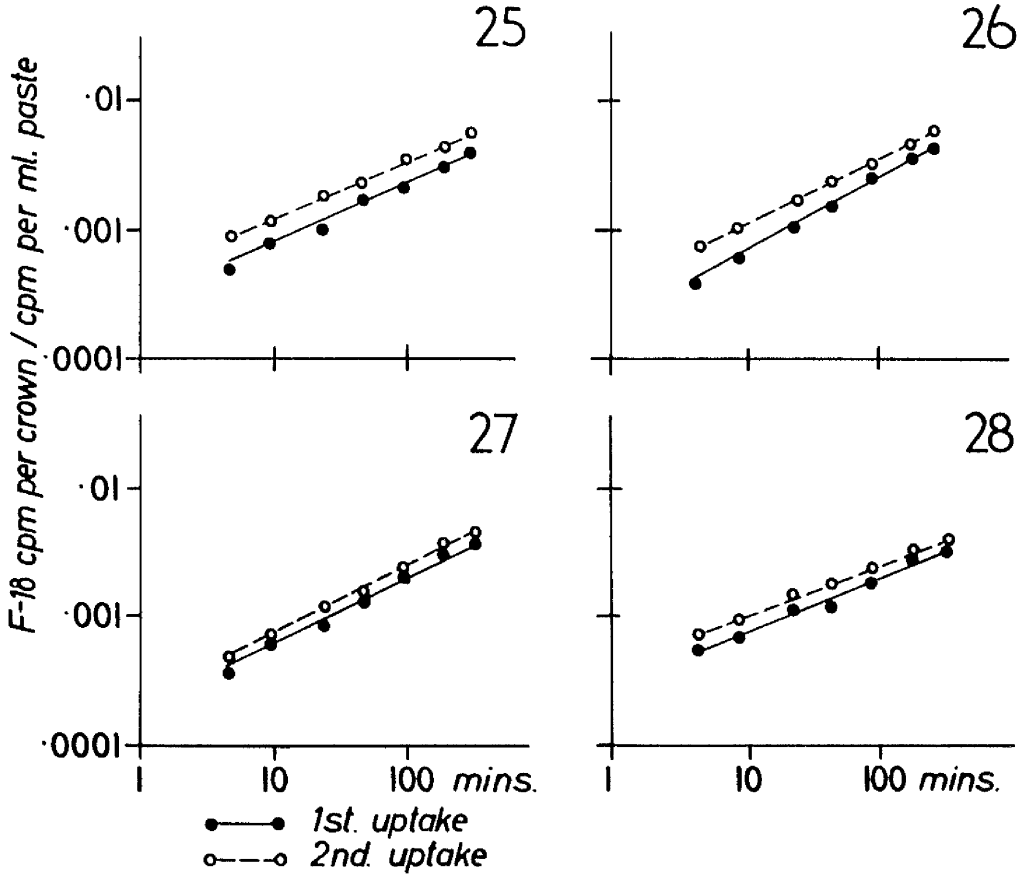


Figure 5.9.

Uptake of fluorine-18 by crowns 25-28 from Dentifrice B.

TABLE 5.10.

Uptake of F-18 by the crowns of premolar teeth immersed in F-18 labelled Dentifrice C.

Uptake expressed as $\frac{\text{cpm per crown}}{\text{cpm per ml of paste}} \times 10^3$

Time of Immersion in mins	Uptake by Tooth 29		Uptake by Tooth 30	
	1st	2nd	1st	2nd
5	.348	.562	.288	.388
10	.474	.816	.406	.498
25	.850	1.12	.712	.949
50	1.23	1.67	.938	1.26
100	1.63	2.40	1.46	1.88
200	2.50	3.12	1.99	2.52
300	3.07	3.78	2.52	3.25
	Uptake by Tooth 31		Uptake by Tooth 32	
	1st	2nd	1st	2nd
5	.400	.523	.231	.325
10	.606	.774	.363	.504
25	.818	1.25	.575	.756
50	1.24	1.61	.787	1.29
100	1.74	2.30	1.27	1.90
200	2.24	3.50	1.79	2.54
300	2.98	4.23	2.31	3.10

2nd uptake = uptake after 1 week from the same paste re-labelled with F-18

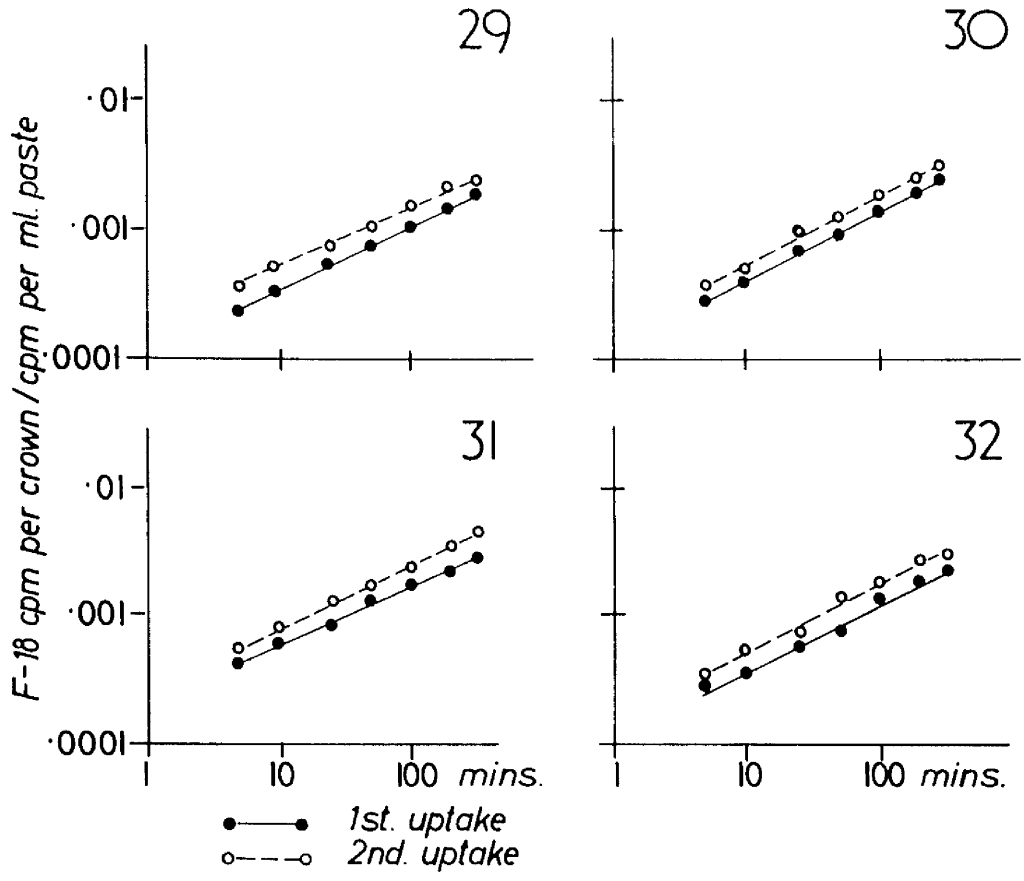


Figure 5.10.

Uptake of fluorine-18 by crowns 29-32 from Dentifrice C.

TABLE 5.11.

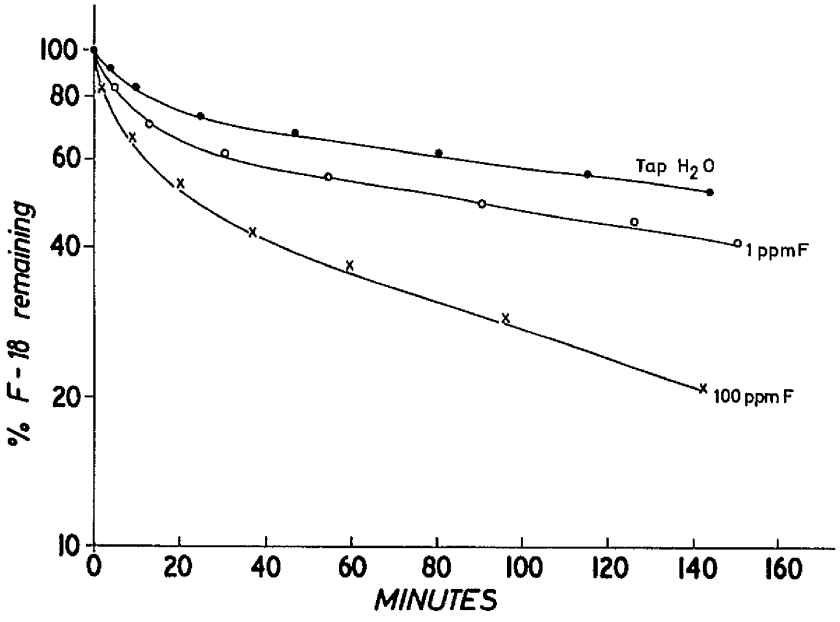
Removal of F-18 from the crowns of premolar teeth by washing with tap water and solutions containing 1 and 100 ppm F as NaF.

Tooth 33 Tap H_2O		Tooth 34 1 ppm F		Tooth 35 100 ppm F	
Washing Time in mins	Percent F-18 Remaining	Washing Time in mins	Percent F-18 Remaining	Washing Time in mins	Percent F-18 Remaining
0	100	0	100	0	100
4	92	5	84	2	84
10	84	14	70	9	67
25	73	31	61	20	54
47	68	55	55	37	43
80	62	90	49	60	37
115	56	126	45	96	29
143	52	150	41	142	21

Tooth 36 Tap H_2O		Tooth 37 1 ppm F		Tooth 38 100 ppm F	
Washing Time in mins	Percent F-18 Remaining	Washing Time in mins	Percent F-18 Remaining	Washing Time in mins	Percent F-18 Remaining
0	100	0	100	0	100
12	85	6	86	9	68
20	80	15	78	18	54
52	75	30	72	36	43
87	70	50	69	73	36
143	66	68	66	118	26
171	65	100	62	150	21
		148	55		

Teeth 33 - 35 preliminary uptake of F-18 from an aqueous solution containing 1 ppm F as $Sn^{18}F_2$ at pH 4.5

Teeth 36 - 38 preliminary uptake of F-18 from Dentifrice A containing SnF_2 labelled with F-18.



Teeth 33 to 35
Preliminary uptake of F-18 from 1 ppm F as SnF_2 at pH 4.5.

Teeth 36 to 38
Preliminary uptake of F-18 from Dentifrice A.

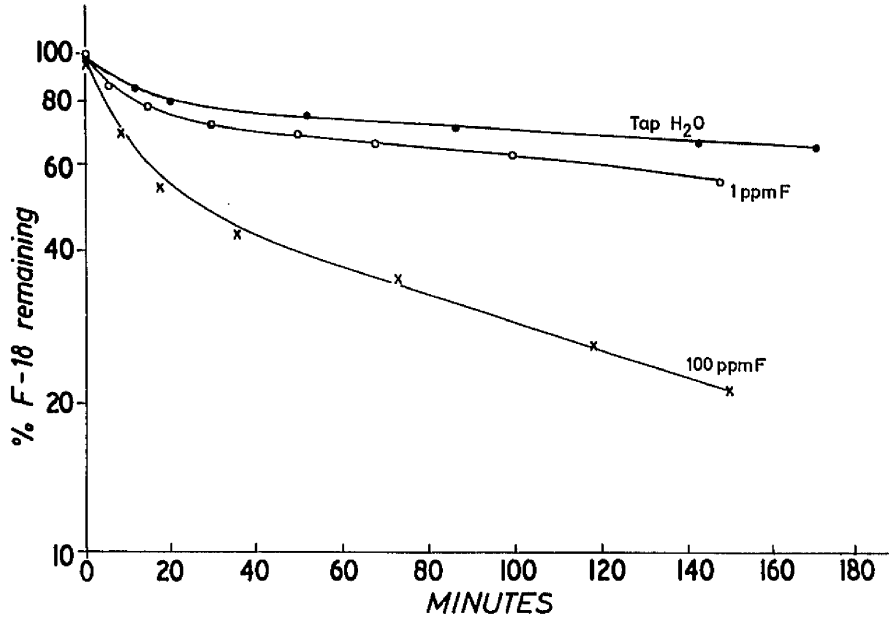


Figure 5.11.

Removal of F-18 from the crowns of premolar teeth by washing with tap water and solutions containing 1 and 100 ppm F as NaF.

5.4. DISCUSSION

The experiments described in this chapter were designed to obtain information on the relative importance of iso-ionic and hetero-ionic exchange mechanisms in the uptake of fluorine-18 by the intact enamel of individual teeth. However, the data also provide information on differences in fluorine-18 uptake when the crowns are exposed to different concentrations of non-radioactive fluoride, labelled with radiofluorine, and the possible existence of a diffusion process at the enamel-liquid interface.

5.4.1. Fluorine-18 Uptake by the Crowns of Individual Teeth.

The interval of one week between the first and second labelling with fluorine-18 is important for two reasons. Firstly, it permits the radioactivity, from the initial labelling, to decay to unmeasurable levels before the second uptake is determined. Secondly, the rapidity with which fluoride in powdered enamel equilibrates with fluoride in solution (Leach, 1959), would suggest that one week is sufficient time for fluoride in the surface of the enamel to come into equilibrium with the fluoride in the liquid phase, before this is re-labelled. This equilibrium must not be disturbed when adding more radiofluorine. Therefore, it was an essential feature of the experiment to use carrier-free fluorine-18.

The uptake of fluorine-18 from fluoride solutions or dentifrices has been recorded for individual teeth and not for groups of teeth

exposed to different treatments. There are two reasons for presenting the data in this extended form.

Firstly, the presentation of data showing differences between the first and second uptake by individual teeth demonstrates the consistency with which these differences were found. Thus, in all the double uptake experiments the second uptake of fluorine-18, by a given crown, always occurred at a greater rate than the first.

Secondly, the amount of the fluorine-18 taken up by intact surface enamel must depend, in part, upon the area of the surface. Although the reactive surface area of any one crown remained constant throughout the experiment, this area varied from tooth to tooth according to crown size and the amount of enamel at the cervical margin which was covered by wax. Therefore, in the first instance, mean values for the uptake of fluorine-18 by a group of teeth exposed to the same treatment have not been presented, although such pooled results will be considered later in this discussion and are illustrated in Figure 5.12. Use of the "window-technique", in which defined areas of surface enamel could have been exposed to labelled solutions or pastes, would have eliminated area variations, but it was decided to use the whole crown in an attempt to imitate the conditions in vivo.

Four hypothetical mechanisms of fluorine-18 uptake by surface enamel which were postulated by Fremlin et al.(1959) have been detailed at the beginning of this chapter (Fig. 5.1). The results obtained in the experiments described here are compatible with the fourth of these uptake mechanisms. That is, the uptake of fluorine-18 by the enamel

surface is preferentially an iso-ionic exchange process, with some hetero-ionic exchange occuring when the crown is first immersed in the labelled solution or paste. This is in accordance with the conclusion reached by Fremlin and his colleagues.

Within the limits of the range of variables explored, this mechanism of uptake appears to apply irrespective of the concentration of fluoride in the solution, the cation of the fluoride molecule and the pH of the solution. It also applies to enamel which has been etched and to the uptake of fluorine-18 by sound enamel from dentifrices containing stannous fluoride.

The rapidity with which fluorine-18 is removed from crowns by washing with a solution containing 100 ppm of fluoride, compared with the slower removal by a solution containing only 1 ppm fluoride, and the still slower removal by washing with tap-water, suggests that iso-ionic exchange is also important in the removal of fluorine-18 from surface enamel. This is in keeping with the results of similar experiments described by Fremlin et al.(1959) and with the conclusion arrived at above.

It would be possible to calculate the apparent uptake of non-radioactive fluoride from the data on fluorine-18 uptake and the known specific activities of the labelled solutions or dentifrices. However, this would be without meaning for the demonstration of a preferentially iso-ionic exchange process in the uptake of fluorine-18 from solutions and dentifrices in vitro indicates that the radiofluorine label is not limited to the transfer of non-radioactive fluoride. The doubts expressed earlier

(see 4.4.3) about the validity of fluorine-18 measurements in fluoride uptake studies are endorsed.

Toothbrushing with a slurry of dentifrice and saliva in vivo is very different from immersing the crowns of teeth in undiluted paste in vitro but it is probable that similar uptake mechanisms would operate. Therefore, the measured uptake of fluorine-18 in vivo, from which the uptake of fluoride recorded in the last chapter was calculated, gave an over-estimate of the true uptake. The size of this error cannot be calculated from the data available.

Thus, it seems unjustifiable to use fluorine-18 to determine the mass of fluoride taken up by the teeth in situ when labelled fluorides are applied topically. The results of fluorine-18 uptake studies should not be expressed in terms of the absolute uptake of non-radioactive fluoride. This criticism applies to the results detailed in Chapter 4 and to some of the results of other clinical experiments reported by earlier workers (Myers et al., 1952; Hellstrom, 1960, and Emslie et al., 1961).

In several studies employing fluorine-18 Ericsson (1961, 1962a and 1962b) has investigated the reactions of both surface and powdered enamel with different fluorides in various media. His results have been expressed in terms of the fluorine-18 activity of enamel after treatment and comparisons between different treatments have been made on this basis. It is submitted that the ratios which Ericsson calculates do not give valid comparisons, for they are derived from results which must have included uptake by iso-ionic exchange. Whilst it is true that the uptake of some fluorine-18 by hetero-ionic exchange would have occurred

in Ericsson's experiments, the existence of a relationship between the observed fluorine-18 uptake and the uptake of non-radioactive fluoride does not appear to have been demonstrated. Indeed, the results of experiments mentioned by Fremlin et al.(1959) suggest that the uptake of fluorine-18 by powdered enamel, dentine and hydroxyapatite is unrelated to the uptake of the non-radioactive fluoride carrier. Therefore, the results of Ericsson's experiments with fluorine-18 cannot yet be accepted as necessarily indicating the uptake by enamel of the fluoride ion.

5.4.2. Uptake of Fluorine-18 from Different Fluoride Solutions and Stannous Fluoride Dentifrices.

Differences in the areas of the crowns immersed in the fluoride solutions or dentifrices will have caused differences in the uptake of fluorine-18. However, when the uptake measurements for certain groups of teeth are compared it is clear that differences between the means are large in comparison with the ranges over which the individual values in the groups extend. This is illustrated for the second uptake of fluorine-18 in Figure 5.12. Differences between the means of uptake measurements of the order shown for teeth numbered 1-4, 5-8, 9-12 and 21-32 are too large to be due to differences in the surface areas of the crowns. Therefore, Figure 5.12 gives some indication of the relative rates of uptake of radiofluorine from solutions containing 1 and 100 ppm fluoride and dentifrices containing 1000 ppm fluoride. It also shows the effect upon uptake of etching the enamel surface and the effect of lowering the pH of the fluoride solutions.

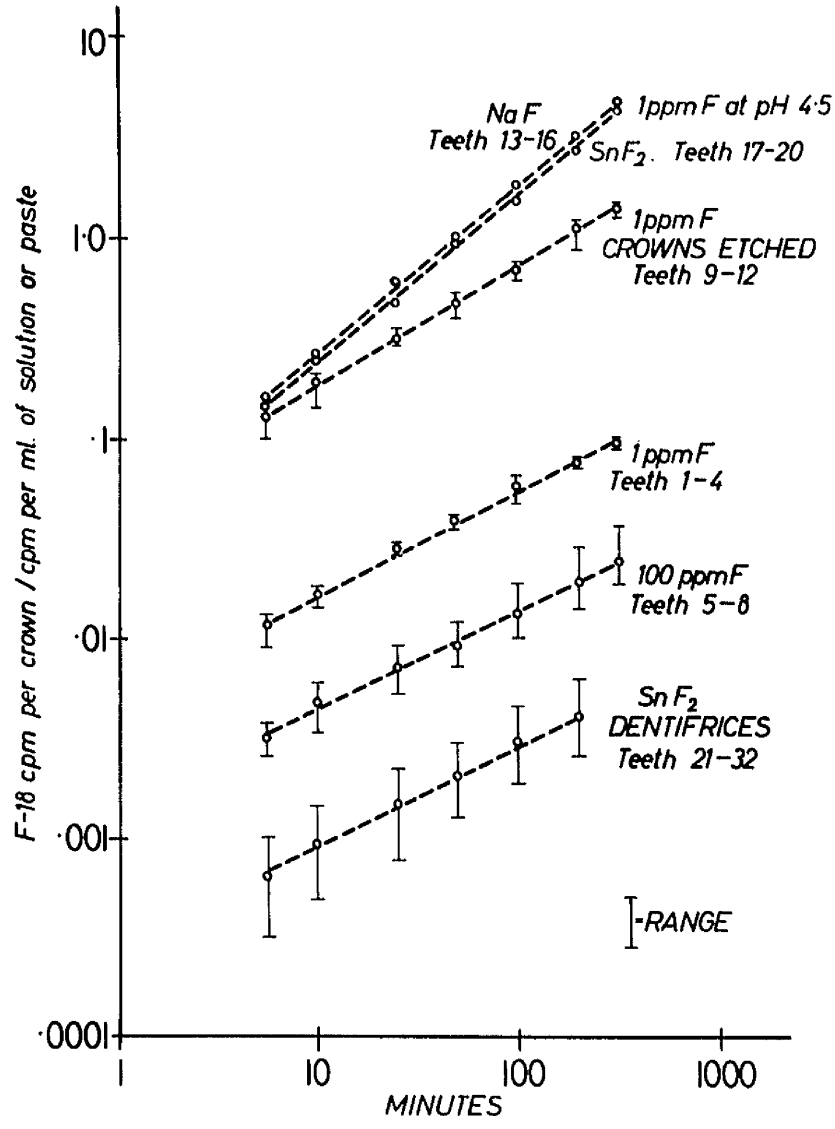


Figure 5.12

The mean second uptake of F-18 by intact enamel from solutions and dentifrices containing fluoride ions. The data shown here are taken from Tables 5.3-5.10 and Figures 5.3-5.10 which give the amounts of F-18 taken up by individual crowns.

The rates of uptake of fluorine-18 from solutions containing 1 and 100 ppm fluoride and dentifrices containing 1000 ppm fluoride fall as the concentrations of non-radioactive fluoride rise (Fig. 5.12). This apparently paradoxical behaviour of the fluorine-18 label supports the contention that radiofluorine should not be used to measure the uptake of non-radioactive fluoride by enamel. A suggested explanation for these observations will be offered in a later section (see 5.4.4).

The decrease in uptake of fluorine-18 by enamel which occurs as the concentration of fluoride in the liquid phase rises, appears, at first sight, to be contrary to the findings of Volker et al. (1940). These workers found an increase in uptake of fluorine-18 (expressed as non-radioactive fluoride) with increasing concentration of fluoride in the liquid phase. However, the limitation upon fluorine-18 uptake measurements imposed by iso-ionic exchange was not recognised by these authors and their calculation of results in terms of the uptake of non-radioactive fluoride does not appear to be valid. Thus, results reported by Volker et al. (1940) are unacceptable and therefore not to be regarded as in conflict with the results of the experiments described in this chapter.

It has already been noted that etching of the enamel, before immersion of the crowns in the labelled fluoride solutions, does not alter the pattern of fluorine-18 uptake, although it is increased in rate. This increase in the rate of uptake which follows etching (Fig. 5.12) appears to be due to an increase in the number of sites for both iso-ionic and hetero-ionic exchange. The greatest uptake is found when the etching

effect is continuous. That is, when the labelled fluoride solutions are buffered to pH 4.5 (Fig. 5.12). These findings are supported by the results of work which has shown that the uptake of fluorine-18 by apatite crystals is greater at lower concentrations of hydroxyl ions which favour intra-crystalline exchange (Myers, 1957, cited by Neuman and Neuman, 1958).

The acidity of the dentifrice would also have been expected to increase the uptake of fluorine-18. However, this effect may have been obscured by the relatively high concentration of non-radioactive fluoride in the dentifrice which may have reduced the uptake of the radioisotope.

Ericsson (1961) listed the viscosity of dentifrices as one of the factors influencing the uptake of fluoride by enamel. The relatively low uptake of fluorine-18 from dentifrices observed here (Fig. 5.12) may be attributed, in part, to their viscosity for this will be many times greater than the viscosity of the aqueous fluoride solutions. An increase in viscosity is accompanied by a decrease in the diffusion coefficient (see 5.4.3), hence a decrease in the mass of radiofluorine diffusing into the enamel would be expected. In addition, it would seem that uptake could be further reduced by various compounds in the dentifrice which interfere with the entry of fluorine-18 into enamel (Ericsson, 1961). These findings with radiofluorine are supported by the results of fluoride uptake, studied by chemical techniques, in which Brudevold et al. (1956) failed to show an increase in the fluoride content of surface enamel after treatment with a slurry of a stannous fluoride dentifrice and water.

5.4.3. Diffusion of Fluorine-18 into the Enamel Surface.

When the means of the amounts of fluorine-18 taken up from aqueous solutions of fluoride at pH 6 and from stannous fluoride dentifrices are plotted against time on logarithmic scales the lines relating these parameters have gradients very close to 0.5 (Fig. 5.12). Therefore, the uptake of fluorine-18 from these solutions and pastes is approximately related to time according to the following equation:-

$$\log \text{uptake of F-18} = \tfrac{1}{2} \log \text{time} + c$$

It follows that:-

$$\text{uptake of F-18} \propto t^{\frac{1}{2}}$$

When an ion diffuses from the solution phase to the solid phase, in the experimental system described, the mass diffusing per unit time per unit area (M_t) is related to the time of diffusion (t) by the following equation (Crank, 1957):-

$$M_t = 2\, C_o \left[\frac{Dt}{\pi}\right]^{\frac{1}{2}}$$

Where C_o = the original concentration in the solution and

D = the coefficient of diffusion.

Thus, in this diffusion process $M_t \propto t^{\frac{1}{2}}$ and $2\, C_o \left[\frac{D}{\pi}\right]^{\frac{1}{2}}$ is the slope of the linear plot obtained.

That this relationship applies to the data obtained in the experiments described here is demonstrated in the graphs in Figure 5.13. These show the means of the second uptake measurements plotted against the square roots of time. The graphs demonstrate the linearity of the relationship and the fact that the lines pass through the origin.

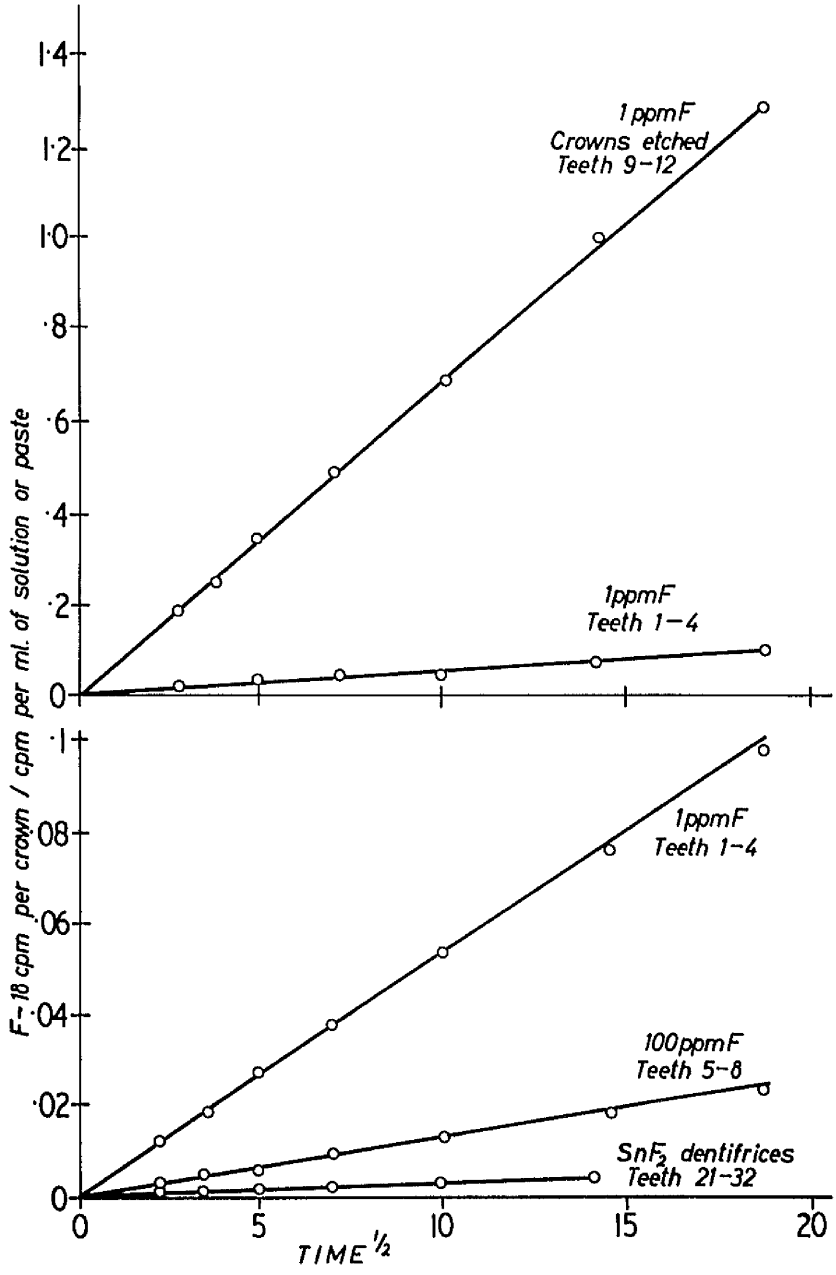


Figure 5.13.

The relationship between the second uptake of F-18 by the crowns and the square root of the time of immersion in the solutions or pastes.
Teeth 1-4 are represented on both graphs, the upper graph being drawn to a vertical scale which is different to the one used in the lower graph.

Therefore, these data are consistent with the uptake of fluorine-18 by enamel being a diffusion controlled process. Diffusion is probably accompanied by simultaneous chemical reaction of the fluoride ions with enamel constituents.

The above is in agreement with the description by Neuman and Neuman (1958) of the diffusion of ions into the hydration layer of apatite crystals and the statement by Brudevold (1962) that such diffusion is the first step in the uptake of fluoride by enamel.

With sodium and stannous fluoride solutions, buffered to pH 4.5, the gradients of the fluorine-18 uptake plots are steeper (Fig. 5.12). Therefore, the $t^{\frac{1}{2}}$ relationship does not hold for these acid solutions and it would appear that some mechanism other than, or in addition to, diffusion must operate during the uptake of radiofluorine.

5.4.4. Application of Diffusion Theory to the Experimental Results.

It has been demonstrated that the uptake of fluorine-18 by enamel, from all media except the fluoride solutions acidified to pH 4.5, appears to be a diffusion controlled process. Therefore, explanations have been sought in terms of diffusion mechanics for 1) the observed increases in the rates of uptake of fluorine-18 after re-labelling the solutions or pastes and 2) the apparently paradoxical relationship between the concentration of fluoride in the solutions and fluorine-18 uptake by surface enamel.

Hermans (1947) has derived a formula for diffusion with simultaneous chemical reaction for a system where the solid phase contains a

known concentration of fixed reactive sites. The concentration of fluoride reactive sites in enamel is, of course, not known but it is thought probable that this would be much greater than the low concentration of fluoride present in the solutions in this study. It can be shown for this case that the formula derived by Hermans (1947) reduces to

$$d = \left[\frac{2DC_F}{C_h}\right]^{\frac{1}{2}} \cdot t^{\frac{1}{2}}$$

where d = the depth of penetration of the ions into the solid phase,

D = the coefficient of diffusion,

C_F = the concentration of fluoride in the liquid phase,

C_h = the concentration of fluoride reactive sites in the enamel where hetero-ionic exchange can occur.

Then the amount of fluoride taken up by enamel per unit area is

$$\triangle C_F = d \times C_h$$

$$= C_h \left[\frac{2DC_F}{C_h}\right]^{\frac{1}{2}} \cdot t^{\frac{1}{2}}$$

$$= (2DC_F C_h)^{\frac{1}{2}} \cdot t^{\frac{1}{2}}$$

In the liquid phase when fluorine-18 is added there is a mixture of fluorine-18 and non-radioactive fluoride. Let the fraction

$$\frac{\text{Fluorine-18 concentration}}{\text{Non-radioactive fluoride concentration}} = \alpha$$

It follows that the concentration of fluorine-18 in solution is αC_F and the uptake of fluorine-18 by hetero-ionic exchange is

$$\triangle C_{F\text{-}18h} = (2D\,\alpha C_F C_h)^{\frac{1}{2}} \cdot t^{\frac{1}{2}} \qquad \ldots\ldots \quad 1$$

The observed uptake of fluorine-18 also includes uptake by iso-ionic exchange with reactive sites of concentration C_i. The reaction of these sites, which can only be shown with fluorine-18, leads to an uptake

$$\triangle C_{F\text{-}18i} = (2D \alpha C_F C_i)^{\frac{1}{2}} . t^{\frac{1}{2}} \quad \ldots\ldots \quad 2$$

Therefore, the total uptake of fluorine-18 is given by adding equations 1 and 2. Thus,

$$\triangle C_{F\text{-}18} = (2D \alpha C_F)^{\frac{1}{2}} (C_h + C_i)^{\frac{1}{2}} . t^{\frac{1}{2}} \quad \ldots\ldots \quad 3$$

So far it has been assumed that during permeation of the fluoride into the enamel all the available sites for both hetero-ionic and iso-ionic exchange have been filled. This is unlikely, therefore, let the reacting fractions of C_h and C_i be p and q respectively. Equation 3 becomes

$$\triangle^1 C_{F\text{-}18} = (2D \alpha C_F)^{\frac{1}{2}} (p^{\frac{1}{2}} C_h^{\frac{1}{2}} + q^{\frac{1}{2}} C_i^{\frac{1}{2}}) . t^{\frac{1}{2}} \quad \ldots\ldots \quad 4$$

This represents the uptake of fluorine-18 by enamel when first immersed in a liquid phase containing labelled fluoride.

When the liquid phase is re-labelled with fluorine-18 one week later, there are 3 reacting species to consider.

1. The term pC_h from equation 4 now represents reactive sites in the enamel available for iso-ionic exchange, since, by definition, these sites have taken up fluoride. The number of these sites which have taken up stable fluoride is represented by $(1-\alpha)pC_h$, but since the concentration of radiofluorine in the liquid phase is very low compared with the concentration of non-radioactive fluoride α is negligible com-

pared with one. Therefore, after the second labelling a term pqC_h will represent iso-ionic exchange at sites in the enamel where fluoride has been newly acquired.

2. If p is the fraction of C_h exchanging initially, $C_h - pC_h$ i.e. $(1-p)C_h$ is still available for hetero-ionic exchange when the solution is re-labelled. Therefore, the amount taken up by hetero-ionic exchange on the second occasion is represented by $p(1-p)C_h$.

3. A concentration $C_i - qC_i$ i.e. $(1-q)C_i$ remains for iso-ionic exchange. Hence, on the second occasion the uptake of fluorine-18 by iso-ionic exchange will be $q(1-q)C_i$. Therefore, when the liquid is labelled a second time the uptake of fluorine-18 is given by the addition of the above, viz:

$$\triangle^2 C_{F-18} = (2D \alpha C_F)^{\frac{1}{2}} \left[p^{\frac{1}{2}} q^{\frac{1}{2}} C_h^{\frac{1}{2}} + p^{\frac{1}{2}}(1-p)^{\frac{1}{2}} C_h^{\frac{1}{2}} + q^{\frac{1}{2}}(1-q)^{\frac{1}{2}} C_i^{\frac{1}{2}} \right] t^{\frac{1}{2}} \quad \ldots\ 5$$

In the logarithmic form equations 4 and 5 become

$$\log \triangle^1 C_{F-18} = \tfrac{1}{2} \log t + \tfrac{1}{2} \log 2D \alpha C_F + \log(p^{\frac{1}{2}} C_h^{\frac{1}{2}} + q^{\frac{1}{2}} C_i^{\frac{1}{2}})$$

and

$$\log \triangle^2 C_{F-18} = \tfrac{1}{2} \log t + \tfrac{1}{2} \log 2D \alpha C_F + \log \left[p^{\frac{1}{2}} q^{\frac{1}{2}} C_h^{\frac{1}{2}} + p^{\frac{1}{2}}(1-p)^{\frac{1}{2}} C_h^{\frac{1}{2}} + q^{\frac{1}{2}}(1-q)^{\frac{1}{2}} C_i^{\frac{1}{2}} \right]$$

Clearly, the bracketed terms are responsible for the differences in the intercepts when the data are plotted logarithmically and hence for the differences between the lines plotting the first and second uptake of fluorine-18.

Let the ratio of these terms be X. Then,

$$X = \frac{p^{\frac{1}{2}}q^{\frac{1}{2}}C_h^{\frac{1}{2}} + p^{\frac{1}{2}}(1-p)^{\frac{1}{2}}C_h^{\frac{1}{2}} + q^{\frac{1}{2}}(1-q)^{\frac{1}{2}}C_i^{\frac{1}{2}}}{p^{\frac{1}{2}}C_h^{\frac{1}{2}} + q^{\frac{1}{2}}C_i^{\frac{1}{2}}}$$

By dividing throughout by $C_h^{\frac{1}{2}}$

$$X = \frac{p^{\frac{1}{2}}q^{\frac{1}{2}} + p^{\frac{1}{2}}(1-p)^{\frac{1}{2}} + q^{\frac{1}{2}}(1-q)^{\frac{1}{2}}C_i^{\frac{1}{2}}/C_h^{\frac{1}{2}}}{p^{\frac{1}{2}} + q^{\frac{1}{2}}\,C_i^{\frac{1}{2}}/C_h^{\frac{1}{2}}}$$

Since it is expected that the concentration of sites for iso-ionic exchange is much less than the concentration of sites for hetero-ionic exchange $C_i^{\frac{1}{2}}/C_h^{\frac{1}{2}} \ll 1$ and it is reasonable to write that

$$X = \frac{p^{\frac{1}{2}}q^{\frac{1}{2}} + p^{\frac{1}{2}}(1-p)^{\frac{1}{2}}}{p^{\frac{1}{2}}}$$

$$= q^{\frac{1}{2}} + (1-p)^{\frac{1}{2}}$$

Since $p < 1$ the binomial theorem permits the expansion of $(1-p)^{\frac{1}{2}}$ to approximately $1 - \frac{1}{2}p + \frac{1}{4}p^2$. Therefore,

$$X = 1 + q^{\frac{1}{2}} + \tfrac{1}{4}p^2 - \tfrac{1}{2}p$$

It is assumed that the iso-ionic reaction is more facile than the hetero-ionic exchange reaction, therefore $p < q$ and $\frac{1}{2}p < q$ and as p and q are both < 1, $\frac{1}{2}p < q^{\frac{1}{2}}$. Therefore,

$$X > 1$$

This means that the second uptake of fluorine-18 by enamel should be greater than the first; a conclusion in keeping with the observed facts (see 5.4.1).

To explain the influence of fluoride concentration in the liquid phase upon fluorine-18 uptake by enamel, reference is made to equations 4 and 5. Both show that the uptake of radiofluorine should fall as the concentration of non-radioactive fluoride rises, for

$$\triangle C_{F-18} \text{ is proportional to } \alpha \quad \ldots\ldots\ldots \quad 4, 5.$$

Therefore, $$\triangle C_{F-18} \propto \frac{C_{F-18}}{C_{F-19}}$$

All experimental data were normalised for the various activities of fluorine-18. Therefore,

$$\triangle C_{F-18} \propto \frac{1}{C_{F-19}}$$

i.e. as the concentration of stable fluoride in the liquid phase rises the uptake of fluorine-18 should decrease; again this conclusion is in keeping with the experimental data (see 5.4.2.).

This application of the laws of diffusion mechanics to the data recorded here has involved the making of a number of assumptions. Although they appear to be reasonable assumptions the uncertainties which are created suggest that this analysis should only be regarded as an interim explanation of the experimental results. A further study of the uptake of fluorine-18 labelled fluoride by enamel, this time powdered enamel, is envisaged.

5.5. CONCLUSIONS

The results of the experiments described in this chapter support the conclusion of Fremlin et al. (1959) that the uptake of fluorine-18 by surface enamel is preferentially by iso-ionic exchange. Therefore, clinical uptake measurements which depend upon the use of radiofluorine appear to be without value and some previously reported conclusions cannot now be accepted. It is concluded that the method of measuring the availability of dentifrice fluoride in vivo, reported in Chapter 4, is not suitable for screening future stannous fluoride dentifrices before embarking upon clinical trials.

The information provided in this chapter on the reaction of enamel with fluorine-18 is incomplete and this is worthy of further study. It has become clear that a full investigation of the conditions which influence fluorine-18 uptake by enamel should be controlled by parallel chemical determinations of fluoride uptake under identical conditions. Intact enamel is unsuitable for such experiments because of the difficulty of defining and reproducing a given reactive area of crown surface. Instead, powdered enamel of known particle size should be used. The lower fluoride content of powdered enamel and its relative freedom from gross imperfections also make it more suitable than surface enamel for such uptake experiments.

The demonstration that, under certain conditions, the uptake of fluorine-18 appears to be a diffusion controlled process permits the

analysis of the data in terms of the laws of diffusion mechanics. It is concluded that this form of analysis should be applied to the results of future experiments with powdered enamel.

It seems worth-while to speculate upon the information which might be obtained. The measurement of iso-ionic exchange could provide data upon the exchangeable fluoride in enamel in the way that calcium-45 has been used to determine the exchangeable calcium in bone (Dallemagne and Richelle, 1962). The results obtained could be compared with data on the iso-ionic exchange of fluorine-18 obtained in similar experiments with dentine, bone and synthetic apatites. Although uptake would be followed for only a limited period, because of the short half-life of radiofluorine, information might be obtained upon the location of exchangeable fluoride on and within the crystal and upon the conditions which control its iso-ionic exchange. This could lead to a kinetic analysis of fluoride exchange in vitro, similar to the analysis of calcium exchange suggested by Richelle and Bronner (1964).

CHAPTER 6

THE DISTRIBUTION OF DENTIFRICE FLUORIDE DURING AND AFTER TOOTHBRUSHING

6.1. INTRODUCTION

When the teeth are cleaned some dentifrice is swallowed during brushing, some remains in the mouth to be swallowed later and the rest is recovered on the brush, in expectorated saliva and in water used to rinse the mouth. The aim of the investigation reported here was to obtain quantitative data on this distribution when a dentifrice containing stannous fluoride was used by adult subjects. For this purpose the fluoride in the dentifrice was labelled with fluorine-18. Measurements were made of the amount of fluoride, as fluorine-18, recovered on the toothbrush and in rinsings from the mouth after brushing. From these data the retention of fluoride by the body was calculated. Some of the retained fluoride was absorbed and its excretion in the urine, during the 4 hours following toothbrushing, was determined.

To test the effect of dentifrice ingredients upon the absorption of ingested fluoride some of the subjects also drank an aqueous solution of stannous fluoride labelled with fluorine-18. The amount of fluorine-18 excreted in their urine was compared with their excretion of radiofluorine after toothbrushing with the labelled dentifrice.

6.2. MATERIALS AND METHODS

6.2.1. Preparation of Fluorine-18 Labelled Dentifrice.

Dentifrice C, which had been stored for not more than 3 months at room temperature, was used in these experiments. It was labelled with fluorine-18 at the rate of approximately 2 μC per gram of paste at the time of use and 0.5 to 1 g was loaded on a previously weighed toothbrush. The brush was weighed again to determine the weight of paste given to the subject. Six accurately weighed standards of labelled dentifrice were each suspended in 5 ml of water. The details of these procedures were similar to those given in an earlier section (see 4.2.2).

6.2.2. Procedure Followed by the Experimental Subjects.

On the days chosen for the experiment the time of supply of fluorine-18 varied widely. Therefore, eating by the subjects and fluoride intake were not related by a constant time interval. Immediately after emptying the bladder 30 adult males, ranging in age from 20 to 34 years, brushed their teeth with the fluorine-18 labelled dentifrice on a dry brush. After brushing for 1 minute, without spitting out, the subject placed his brush in a dry polyethylene beaker and rinsed his mouth with 25 ml of tap-water. The rinsings, collected in a second polyethylene beaker, and the unwashed brush, were retained for subsequent radioactivity measurements. Urine was collected at hourly intervals for 4 hours from 25 of the 30 subjects and intravenous blood (12 ml) was taken from 4 subjects 30 minutes after toothbrushing. The volumes of the

urine samples were measured.

Several weeks after using the labelled stannous fluoride dentifrice 9 of the above subjects took part in a second experiment. They drank 50 ml of a solution of stannous fluoride (1 mg fluoride per 100 ml) in distilled-deionized water. This was labelled with fluorine-18 so that each subject ingested approximately 2 μC of the radioisotope. Counting standards were prepared by pipetting four 5 ml aliquots of this solution into polyethylene tubes. Hourly urine samples were again collected from these subjects over 4 hours and the volume excreted per hour was measured.

The fluorine-18 activity in the mouths of 10 of the subjects was counted immediately after toothbrushing and rinsing, and the activity remaining was counted one hour later. To do this a wax record of the teeth of the subject in the closed position was made on a stainless steel bite-fork before the labelled dentifrice was used. The record on the bite-fork was mounted above the crystal of the scintillation counter on a locating methacrylate disc (Fig. 6.1). To count the activity of fluorine-18 in the whole mouth the subject brought his head over the counter and closed his teeth into the wax record. This record served to maintain the geometrical relationship of the mouth of the subject to the crystal of the counter during counting and it allowed this relationship to be reproduced one hour later for the second determination of radioactivity in the whole mouth.

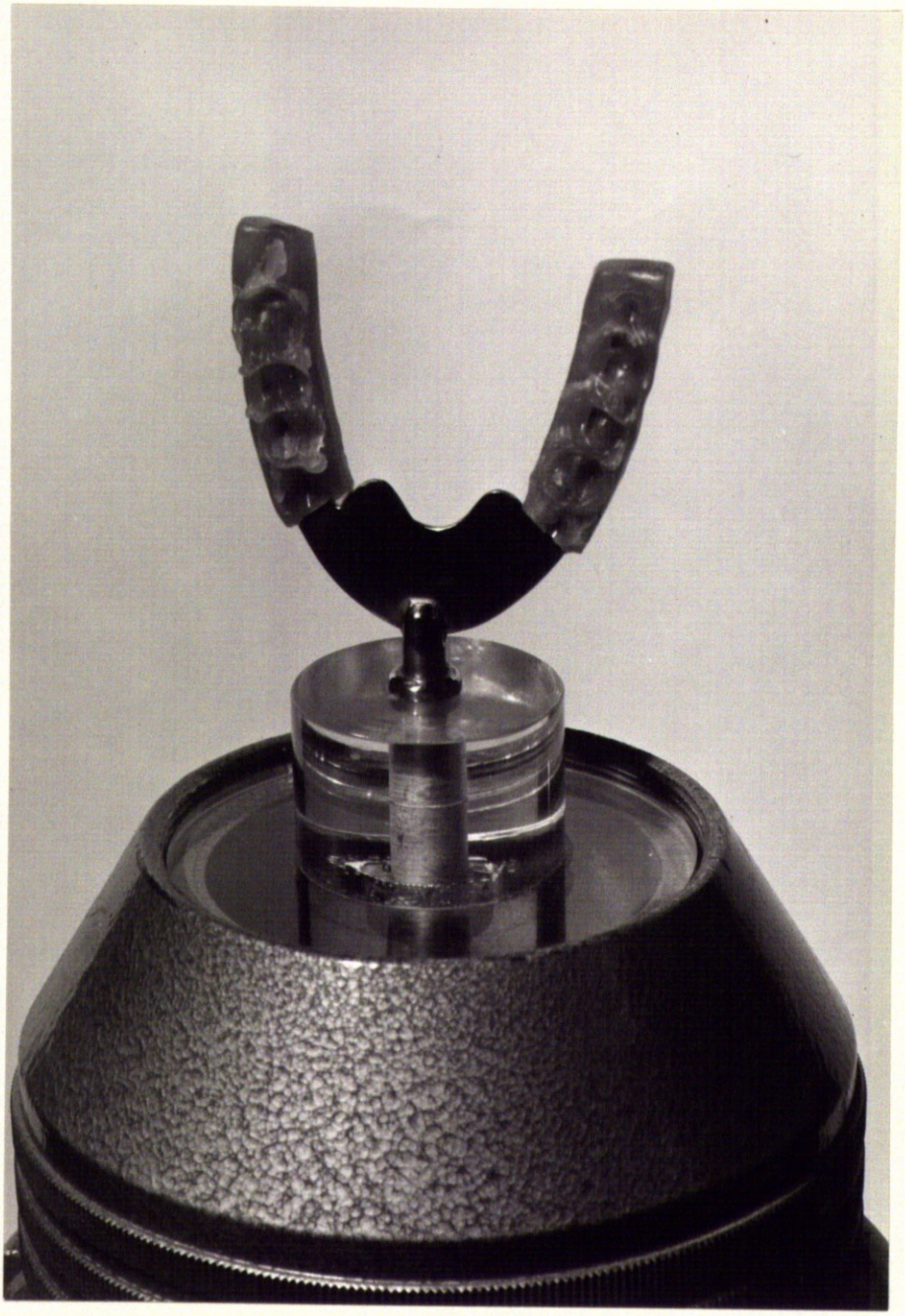


Figure 6.1.

The bite-fork and wax record used to locate the mouth of a subject over the crystal of the scintillation counter.

6.2.3. Preparation and Counting of Samples.

The residual paste was washed from the used toothbrush in 50 ml of water and the mouth rinsings were made up with water to a volume of 50 ml. After shaking to suspend dentifrice particles duplicate 5 ml aliquots of each suspension were pipetted into capped polyethylene tubes and their fluorine-18 activities were counted. The completeness of removal from the brushes of dentifrice containing fluorine-18 was checked by washing the first 10 brushes with a further 50 ml of water. Duplicate 5 ml aliquots of these washings were then counted.

Duplicate 5 ml aliquots of each of the hourly urine samples were pipetted into polyethylene tubes and counted, as were duplicate 5 ml aliquots of the 4 blood samples.

All these samples were counted in the way described previously together with dentifrice standards and stannous fluoride solution standards. All tubes containing suspended dentifrice particles were reshaken immediately before counting.

6.2.4. Counting Errors and Calculation of Results.

Counting errors were reduced to the minimum by the methods described in an earlier section (see 4.2.4). However, the counting rates of urine samples from 3 subjects were too low for accurate determination and these results had to be discarded. The radioactivity measurements of the remaining urine samples had standard errors, calculated according to the method given by Veall and Vetter (1958) which were not in excess of $\pm$ 5 per cent. A similar standard error applied to the determination of

fluorine-18 activity in the whole mouth. The counting rates of the blood samples were never more than 20 per cent above the background rate, therefore, attempts to measure fluorine-18 activity in the blood were abandoned.

The radioactivity of the fluorine-18 present in the dentifrice on the brush at the beginning of toothbrushing was calculated from the known weight of paste on the brush and the mean radioactivity per mg of the weighed standards. The recovery of fluoride, as fluorine-18, in the brush washings and in the mouth rinsings was calculated as a percentage of the amount on the brush before brushing. The percentage of fluoride retained in the body was calculated by subtracting the recovery on the brush and in the rinsings from 100 per cent. The urinary excretion of dentifrice fluoride, as fluorine-18, was expressed in two ways; as a percentage of the original amount on the brush and as a percentage of the fluorine-18 retained in the body. The excretion of fluorine-18 in the urine following ingestion of the labelled solution was expressed as a percentage of the total activity swallowed, calculated from the activity of the solution standards. The activity remaining in the whole mouth one hour after toothbrushing was expressed as a percentage of the fluorine -18 activity measured immediately after brushing and rinsing.

6.3. RESULTS

Almost all the fluorine-18 labelled dentifrice was removed from the brushes during the first washing with 50 ml of water, for, when washed for a second time the brushes released less than 1 per cent of the amount removed in the first washing. This gave a maximum error in the measurement of recovered fluorine-18 of less than 0.6 per cent of the radiofluorine on the brush initially.

The mean total recovery of dentifrice fluoride, as fluorine-18, in 30 subjects, was 82.3 ± 8.4 per cent, the quantity retained in the body was 17.7 ± 8.4 per cent and the excretion of fluorine-18 in the urine over 4 hours by 22 subjects amounted to 1.63 ± 1.24 per cent (Table 6.1). These measurements are illustrated in Figure 6.2. In Table 6.2 the hourly and total excretion of fluorine-18 at the end of 4 hours is shown as a percentage of the fluorine-18 retained in the body (Table 6.1). The mean excretion of fluorine-18 per hour and the mean output of urine per hour are illustrated in Figure 6.3.

After drinking a solution of labelled stannous fluoride 9 subjects excreted a mean amount of radiofluorine in the urine in four hours equivalent to 18.2 ± 6.8 per cent of the fluorine-18 swallowed (Table 6.3). Table 6.4 shows that the excretion of dentifrice fluoride, as fluorine-18, by the same 9 subjects was 5.4 ± 2.8 per cent of the fluoride retained. Graphs showing a comparison of the mean amounts of fluorine-18 excreted by the same subjects from the dentifrice and from the solution are drawn

TABLE 6.1.

Distribution of fluorine-18 labelled fluoride in a stannous fluoride dentifrice during toothbrushing by 30 adult subjects.

Subject	Percentage of Fluorine-18 on Brush at Beginning				
	Recovered on Brush	Recovered in Rinsings	Total Recovered	Retained in Body	Excreted in Urine
R.S.	28.0	44.1	72.1	27.9	.98
P.W.	12.1	59.0	71.1	28.9	1.20
R.W.	34.5	46.1	80.6	19.4	.64
A.W.	30.0	49.8	79.8	20.2	1.37
D.B.	12.5	65.5	78.0	22.0	.84
J.B.	20.4	66.1	86.5	13.5	.91
G.B.	18.2	63.9	82.1	17.9	1.33
B.F.	45.4	33.6	79.0	21.0	.41
A.S.	27.0	24.7	51.7	48.3	5.30
P.G.	29.3	49.6	78.9	21.1	4.82
B.P.	13.5	69.8	83.3	16.7	1.90
P.S.	25.8	64.7	90.5	9.5	-
W.N.	5.9	72.7	78.6	21.4	2.03
B.S.	28.2	54.9	83.1	16.9	1.81
M.W.	27.1	61.3	88.4	11.6	1.12
B.B.	41.6	46.2	87.8	12.2	.93
P.S.	29.7	60.7	90.4	9.6	1.20
M.H.	40.7	50.7	91.4	8.6	1.14
R.B.	47.6	41.9	89.5	10.5	1.62
R.J.	26.2	61.3	87.5	12.5	-
G.S.	37.9	49.6	87.5	12.5	2.51
J.G.	53.5	34.9	88.4	11.6	.20

(continued on next page)

TABLE 6.1. (continued)

Subject	Percentage of Fluorine-18 on Brush at Beginning				
	Recovered on Brush	Recovered in Rinsings	Total Recovered	Retained in Body	Excreted in Urine
P.R.	30.5	52.0	82.5	17.5	-
B.D.	31.1	55.5	86.6	13.4	-
P.H.	59.5	32.1	91.6	8.4	.91
R.L.	18.2	60.2	78.4	21.6	2.59
S.W.	46.3	47.8	94.1	5.9	-
R.B.	40.2	30.5	70.7	29.3	-
W.H.	19.9	56.7	76.6	23.4	-
S.W.	40.4	43.5	83.9	16.1	-
Mean	30.7	51.6	82.3	17.7	1.63
Range	5.9 - 53.5	24.7 - 72.7	51.7 - 94.1	5.9 - 48.3	.20 - 5.3
Standard Deviation	± 13.1	± 12.1	± 8.4	± 8.4	± 1.24

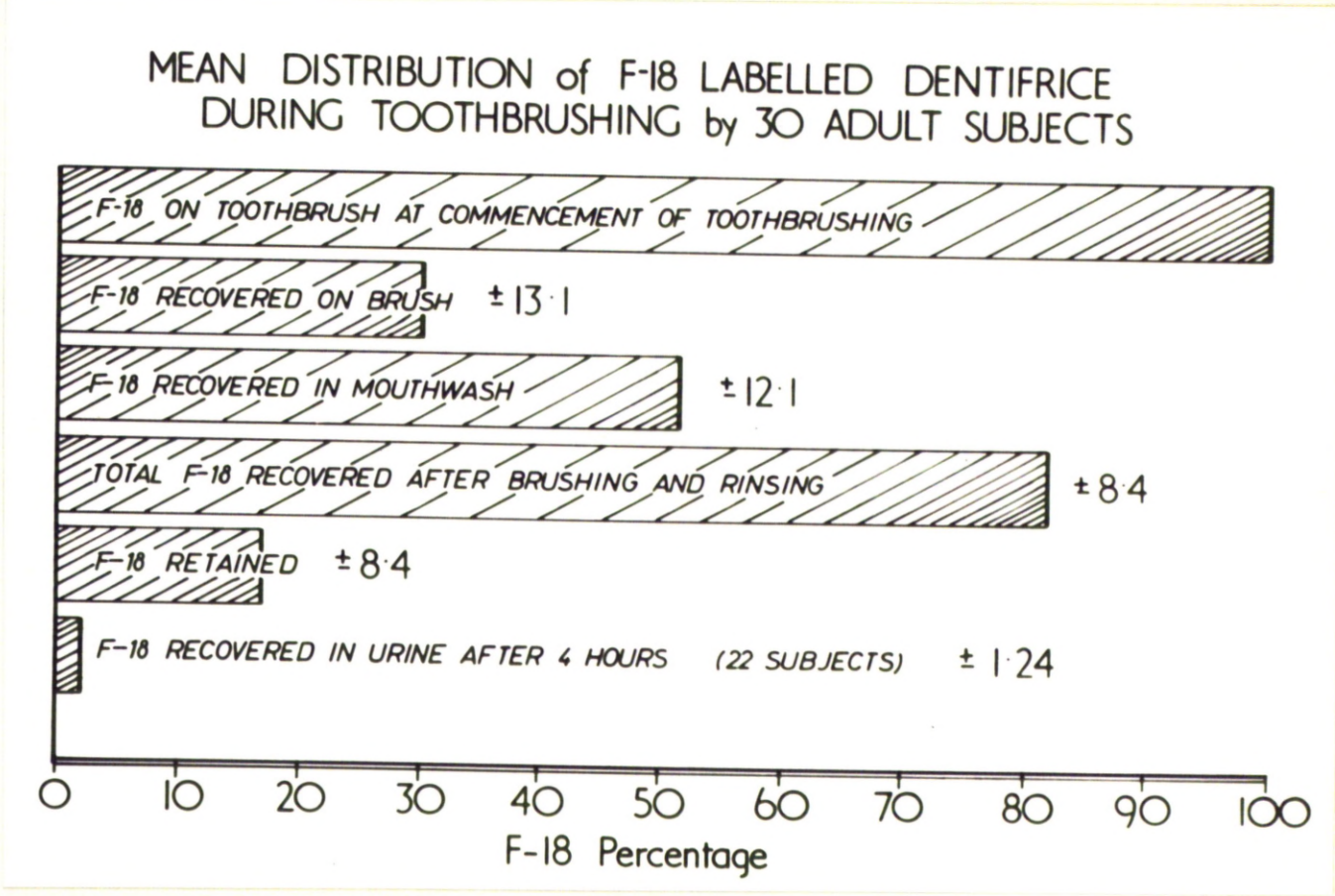


Figure 6.2.

The figures (±) indicate the standard deviations of the means of the measurements.

TABLE 6.2.

Excretion of F-18 in the urine, hourly and total, in the 4 hours following tooth brushing with labelled stannous fluoride dentifrice, expressed as a percentage of F-18 retained in the body.

Subject	Hourly Increments in Urinary F-18				Total F-18 Excretion
	1st hour	2nd hour	3rd hour	4th hour	
R.S.	1.8	.90	.53	.29	3.5
P.W.	1.14	1.74	.69	.59	4.2
A.W.	1.34	.77	.62	.57	3.3
R.W.	2.48	1.63	1.73	.94	6.8
D.B.	.64	1.55	.86	.77	3.8
J.B.	3.33	1.48	1.11	.81	6.7
G.B.	2.18	2.35	1.45	1.45	7.4
B.F.	.62	.57	.43	.33	1.9
A.S.	6.21	1.86	1.45	1.45	11.0
P.G.	5.50	7.77	5.26	4.31	22.8
B.P.	2.99	3.77	2.81	1.80	11.4
W.N.	2.94	3.04	2.10	1.40	9.5
B.S.	3.67	2.01	2.90	2.13	10.7
M.W.	2.24	4.14	2.16	1.12	9.7
B.B.	2.29	2.70	1.64	.98	7.6
P.S.	2.08	4.16	3.96	2.29	12.5
M.H.	3.72	4.65	3.26	1.63	13.3
R.B.	4.38	5.05	3.52	2.48	15.4
G.S.	6.48	6.88	4.32	2.40	20.1
J.G.	.34	.43	.43	.52	1.7
P.H.	3.33	3.33	2.26	1.90	10.8
R.L.	3.61	4.26	1.99	2.13	12.0
Mean	2.88	2.96	2.07	1.47	9.4
Range	.34-6.48	.43-7.77	.43-5.26	.29-4.31	1.7-22.7
Standard Deviation	± 1.66	± 1.93	± 1.30	± .92	± 5.4

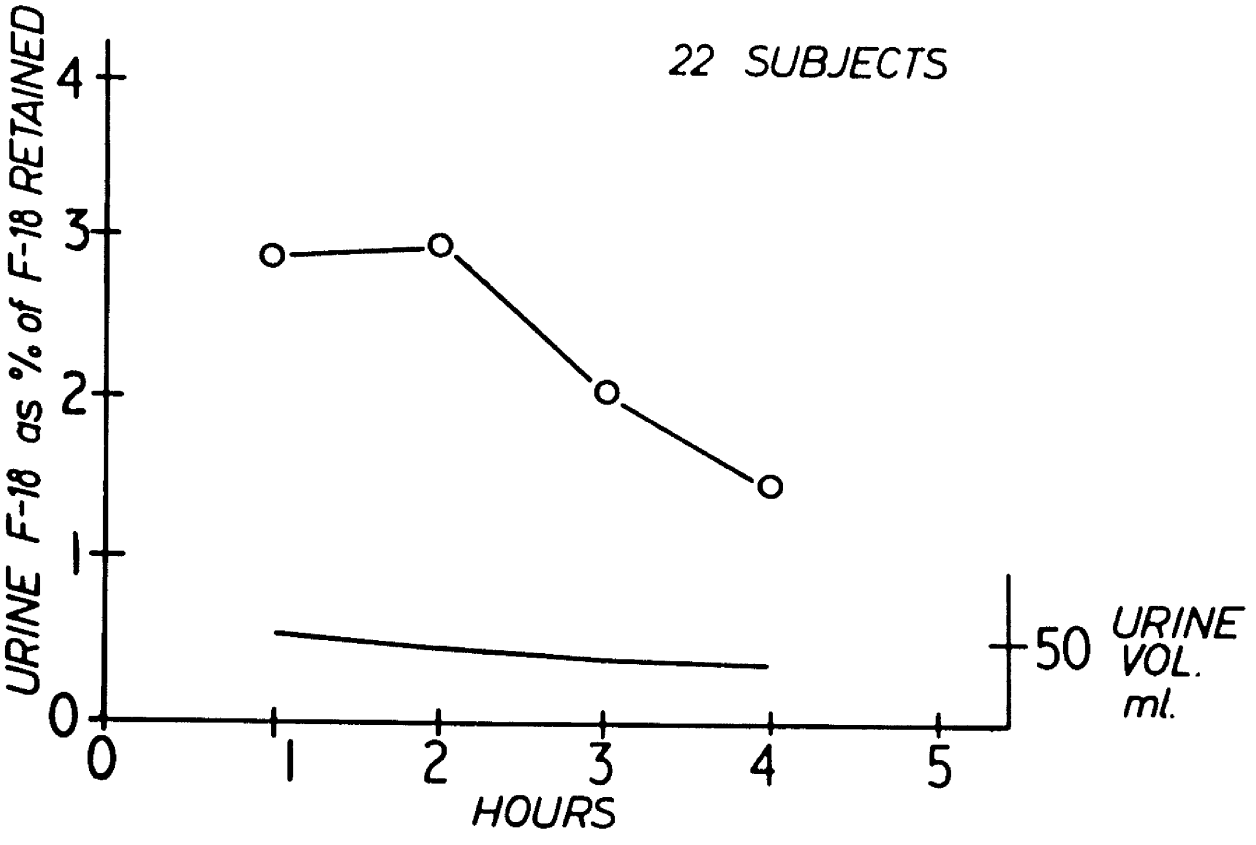


Figure 6.3.

The mean hourly excretion of F-18 and the output of urine after brushing the teeth with labelled Dentifrice C.

TABLE 6.3.

Excretion of F-18 in the urine, hourly and total, in the 4 hours after drinking an aqueous solution of labelled stannous fluoride, expressed as a percentage of F-18 swallowed.

Subject	Hourly Increments in Urinary F-18				Total F-18
	1st hour	2nd hour	3rd hour	4th hour	Excretion
R.S.	12.1	5.6	2.5	2.2	22.4
P.W.	11.1	8.5	4.0	2.7	26.3
A.W.	11.7	5.7	2.8	2.3	22.5
R.W.	4.0	2.2	1.1	3.4	10.7
D.B.	2.3	2.9	1.7	2.0	8.9
J.B.	4.3	7.1	4.5	3.7	19.6
G.B.	11.5	6.7	4.3	2.4	24.9
B.F.	10.0	4.0	2.9	2.7	19.6
A.S.	4.0	3.0	1.2	1.1	9.3
Mean	7.9	5.1	2.8	2.5	18.2
Range	2.3-12.1	2.2-8.5	1.1-4.5	1.1-3.7	8.9-26.3
Standard Deviation	± 4.1	± 2.2	± 1.2	± .78	± 6.8

TABLE 6.4.

Summary of data from Table 6.2 which applies to the 9 subjects listed in Table 6.3.

	Hourly Increments in Urinary F-18				Total F-18
	1st hour	2nd hour	3rd hour	4th hour	Excretion
Mean	2.19	1.43	.99	.80	5.4
Range	.62-6.21	.57-2.35	.43-1.73	.29-1.45	1.9-11.0
Standard Deviation	± 1.75	± .57	± .47	± .42	± 2.8

in Figure 6.4, together with the mean volumes of urine excreted on the two occasions. By applying Student's t test to the differences between the means of the hourly excretion of fluorine-18 from the dentifrice and the solution and the difference between the means of the total fluorine-18 excreted in 4 hours from the dentifrice and from the solution these differences were found to be statistically significant (p was always $< .01$).

The results in Table 6.5 indicate that appreciable quantities of fluoride, as fluorine-18, remained in the mouth for one hour after tooth-brushing, in 9 out of 10 subjects.

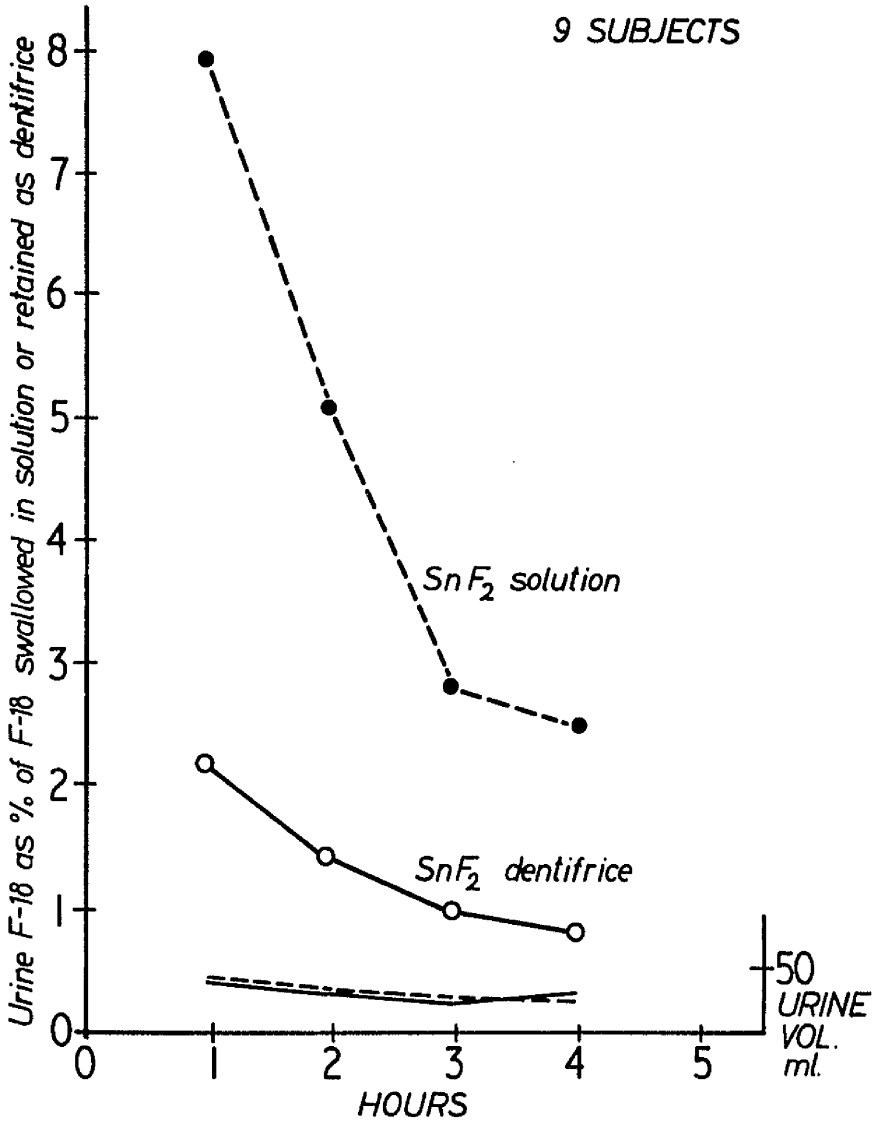


Figure 6.4.

The mean hourly excretion of F-18 and the output of urine after brushing the teeth with labelled Dentifrice C and after drinking a solution of F-18 labelled stannous fluoride.

TABLE 6.5.

Fluorine-18 present in the mouth one hour after toothbrushing with labelled paste expressed as a percentage of F-18 in the mouth immediately after brushing and rinsing.

Subject	Per cent F-18	Subject	Per cent F-18
P.G.	27.4	R.B.	-[+]
P.S.	29.2	R.J.	17.3
B.S.	15.9	B.D.	42.0
M.W.	20.5	P.H.	12.9
M.H.	46.7	S.W.	11.3

[+] Insufficient fluorine-18 for accurate counting.

6.4. DISCUSSION

In a study of the kind reported here the investigator has the choice of allowing the subject to brush and rinse according to his habitual method, or, he may impose upon the subject a standardized tooth-brushing procedure. Unsupervised brushing, which is performed in a variety of ways and for widely varying periods of time (Robinson, 1946), is often accompanied by repeated rinsing of the brush under running tap-water. This would have created difficulties in this investigation because accurate counting of very low activities of fluorine-18 in large volumes of water is not possible. Therefore, as it was essential to collect and measure all the fluoride not retained in the body, it was considered that more accurate measurements could be made if standardized brushing/rinsing procedures were used.

However, several of the subjects complained that their ingestion of dentifrice may have been greater than usual because toothbrushing for one minute seemed artificially long and during this time spitting was not allowed. One subject (A.S.) was convinced that he had swallowed much of the dentifrice and this conviction was supported by the relatively small amount of fluorine-18 recovered (56.7 per cent, compared with a mean recovery of 82.3, Table 6.1). Conversely, some subjects volunteered that it was not their habit to rinse after toothbrushing. It is possible that these subjects ingested less of the paste than when brushing according to their usual method. Clearly, the findings reported here apply

only when the teeth are cleaned in the way described.

The measurements of the recovery and retention of fluorine-18 labelled fluoride will be in error because some of the fluorine-18 will have been retained by iso-ionic exchange with stable fluoride in enamel. To calculate the order of magnitude of this error it is assumed that the reactive surface area of enamel in the full natural dentition is equivalent to the sum of the surface areas of 50 premolar teeth. It is further assumed that the mean uptake of fluorine-18 by 25 premolar teeth from Dentifrice C, which has been shown to be equivalent to 136 mμg of fluoride (Table 4.6), is entirely by iso-ionic exchange. Then, the uptake of fluorine-18 by the enamel of the full natural dentition should be equivalent to 136 x 50 mμg = 6.8 μg. Fluorine-18 uptake equivalent to approximately 7 μg of fluoride represents between 0.7 to 1.4 per cent of the fluoride present on the brush before toothbrushing began. This is probably an overestimate of the error because enamel would be expected to take up some fluorine-18 by hetero-ionic exchange. Therefore, the error in the measurements of recovery and retention, due to iso-ionic exchange in enamel, is probably equivalent to less than 1 per cent of the fluoride on the brush at the beginning of the experiment.

The amount of fluoride excreted in the urine has been used by McClure, Mitchell, Hamilton and Kinser (1945) and Largent (1954) as an indicator of the amount of fluoride absorbed, for McClure (1946) has claimed that urinary fluoride output parallels any increase in fluoride intake. Schweinsberger and Muhler (1957) and Büttner et al. (1961)

were unable to show an increase in fluoride excretion in the urine of subjects using fluoride dentifrices. Therefore, an increased absorption of fluoride due to fluoride in swallowed dentifrice was excluded by these workers.

The experiments recorded here were designed to measure the excretion of absorbed dentifrice fluoride by a more sensitive method than the chemical procedures used by the workers mentioned above. However, as Carlson et al.(1960) have pointed out fluorine-18 will probably exchange iso-ionically with stable fluoride in bone; indeed such exchange would be expected from the results of the experiments with enamel (Chapter 5). Consequently, fluorine-18 in the urine provides an underestimate of the excretion of fluoride and hence an underestimate of fluoride absorption. In addition, the use of 2 μC of fluorine-18 per subject permitted accurate counting of the radiofluorine in the urine for only 4 hours whereas the graph in Figure 6.3 suggests that excretion continues for a longer period.

Thus, it appears that the measurements of the recovery of dentifrice fluoride and its retention by the body are reasonably accurate but the mean excretion of the retained fluoride is probably somewhat below the true level by an amount which cannot be estimated from the data obtained.

The mean retention of dentifrice fluoride for 30 subjects, 17.7 ± 8.4 per cent, is similar to the retention of fluoride from a sodium fluoride dentifrice reported by Eichler et al. (1955), but 40 per cent higher than the mean retention found by Ericsson (1961).

However, to express fluoride retention as a percentage of the fluoride present on the brush is not very informative. The subjects in this study used a mean of 0.722 g of dentifrice containing, according to the manufacturer, 0.722 mg of fluoride. Therefore, the mean retention was approximately 0.13 mg fluoride and the maximum retention (by subject A.S., Table 6.2) was 48.3 per cent of .904 mg = .44 mg fluoride.

Although the results in Table 6.5 show that some of the retained fluorine-18 remains in the mouth for at least one hour it is not known whether this represents permanent fixation of fluoride by enamel, or, fluoride temporarily retained by dental plaque and oral debris. Much of it could be fluorine-18 in enamel, taken up largely by iso-ionic exchange. From the results of the experiments performed it is not possible to calculate how much of the fluorine-18 originally on the brush remains in the mouth one hour later, because there are differences in instrumentation and different geometrical factors involved in counting the external activity of the whole mouth and counting all other samples within the well of the crystal of the scintillation counter.

It seems probable that most of the retained fluoride is swallowed at the time of toothbrushing or very soon afterwards. Calcium in the dentifrice used in these experiments (Dentifrice C) would be expected to reduce fluoride absorption for this effect of calcium has been demonstrated in rats (Wagner and Muhler, 1960). That absorption of fluorine-18 from a calcium pyrophosphate-stannous fluoride dentifrice was slower than from a solution of labelled stannous fluoride of approximately

equivalent fluoride content is shown for a small number of subjects by the results reported here (Tables 6.3 and 6.4; Figure 6.4). These results do not prove that the total excretion and hence absorption of fluoride was less from the dentifrice than from the solution. However, the rate of excretion of fluorine-18 from both was falling at the end of 4 hours, therefore it seems likely that differences found over this period reflect differences in total excretion. Clearly, the observed differences were not due to differences in the mean rate of urine output because the two rates are very similar (Fig. 6.4).

Thus, the excretion in the urine of 1.63 $\pm$ 1.22 per cent of fluoride, as fluorine-18, which is equivalent to 0.012 mg of fluoride, although possibly an underestimate, does suggest that very little fluoride was absorbed from Dentifrice C when it was used in the way described. Many field studies have shown that an intake of approximately 1 mg of fluoride per day in water containing 1 ppm fluoride does not cause undesirable effects. On the evidence presented here it appears improbable that frequent use of Dentifrice C, with any technique of brushing and rinsing, would raise the amount of fluoride absorbed to a level approaching the amount absorbed from fluoridated water. Therefore, the risk that this dentifrice will cause chronic fluorosis is negligible.

CONCLUSIONS

It is concluded that under the conditions of the experiment described in this chapter less than 20 per cent of the fluoride in the stannous fluoride dentifrice was retained by the body during toothbrushing. Some of this retained fluoride was swallowed at once and some remained in the mouth to be swallowed later. It is probable that a portion of fluoride was retained by the teeth but the amount cannot be determined with the fluorine-18 label.

Some ingested fluoride was absorbed as evidenced by the excretion of small amounts of fluorine-18 in the urine after toothbrushing. However, it is concluded that the output of fluorine-18 in the urine, measured in this study, represents an underestimate of the true output of dentifrice fluoride.

The excretion and hence absorption of fluorine-18 ingested in a dentifrice was slower than the excretion of fluorine-18 ingested in a labelled stannous fluoride solution. Therefore, it is concluded that dentifrice ingredients inhibit the absorption of fluoride during the 4 hours following toothbrushing.

CHAPTER 7

CONCLUDING COMMENTARY

In a recently published evaluation of a proprietary stannous fluoride dentifrice the Council on Dental Therapeutics of the American Dental Association stated that "the demonstration by laboratory tests of the continued availability of stannous ions and of fluoride ions in a stannous fluoride dentifrice is an important prerequisite in the consideration of this product" (J. Amer. dent. Ass. 69, 197, 1964). This statement appears to suggest that the clinical effect of such a dentifrice is related to the amounts of stannous and fluoride ions available in vitro. Thus, it is important to consider whether the demonstration of soluble stannous and fluoride ions in slurries prepared from these dentifrices has any relevance to their effect in vivo.

Whatever method is used for the extraction of these ions the equilibrium conditions within the dentifrice will be disturbed by the procedure. This is supported by the demonstration that the fraction of fluoride released from a dentifrice falls as the concentration of dentifrice in the slurry rises. It seems probable that the release of stannous ions would be similarly affected by changes in the concentration of the slurry. Therefore, the results of the experiments described in Chapter 3 do not indicate the actual amounts of fluoride which would be available in the mouth from the dentifrices, although the results obtained, after saliva extraction, may suggest their relative abilities to release fluoride ions. It would have been more realistic and perhaps more valuable to have measured the soluble fluoride in saliva-dentifrice slurries expectorated after toothbrushing with stannous fluoride dentifrices.

Differences between the abilities of the 3 dentifrices to release soluble fluoride to saliva in vitro have been demonstrated. It could be argued that these differences will be reflected in their clinical performance as inhibitors of dental caries; that is, the more fluoride released the greater the preventive effect. It could also be argued that these differences are unimportant providing that the amount of available fluoride in the mouth reaches a certain level, beyond which no further reduction in caries occurs.

At the present time there are two difficulties in relating the results of tests in vitro with the results of clinical trials. Firstly, at least 15 trials of Dentifrice C appear to have been completed, whereas there is only one completed trial of Dentifrice B and none of Dentifrice A, although the results of 3 trials of a dentifrice which may be similar to Dentifrice A are cited in the above mentioned report of the Council on Dental Therapeutics. Therefore, the effectiveness of Dentifrices A and B remains uncertain until the results of many more trials are available for evaluation. Secondly, it should be remembered that comparisons between the clinical effects of different dentifrices can only be made in the same trial. To date, such trials of different dentifrices containing stannous fluoride do not appear to have been carried out.

If the probable effect of these toothpastes is to be assessed, prior to embarking upon clinical trials, it would appear that the mouth is the most realistic testing laboratory. One possible test has been mentioned, the analysis of soluble fluoride in the slurry expectorated after toothbrushing, and another, depending upon solubility measurements in vivo,

has been devised by Holmes and Middleton (1962). The approach to testing in vivo which has been described here, in Chapter 4, proved to be unrewarding because fluorine-18 does not indicate the uptake of non-radioactive fluoride by enamel. Perhaps measurements by chemical methods of the amount of fluoride in the dental plaque, before and after tooth-brushing with a stannous fluoride dentifrice, would have given a better indication of the quantity of fluoride available to prevent dental caries.

In view of the demonstration in Chapter 5 of the importance of iso-ionic exchange in the uptake of fluorine-18 by enamel acceptance of some previously published conclusions may not now be justified. However, it does seem probable that this part of the study will lead to further investigations which may add to knowledge on the dynamics of fluoride-enamel interaction.

It has been noted that the interpretation of some of the measurements of distribution, absorption and excretion of dentifrice fluoride is limited when the measurements depend upon determining the fluoride as fluorine-18. However, it is considered that even when allowance is made for errors introduced by iso-ionic exchange of fluorine-18 with fluoride in enamel and bone there is nothing in the data reported in Chapter 6 to suggest that the use of a stannous fluoride dentifrice will raise the absorption of fluoride to undesirable levels.

APPENDIX

ACKNOWLEDGEMENTS

The investigation reported in this thesis originated at Guy's Hospital in 1961. I was fortunate in being invited to join in a study of the uptake of fluoride by enamel in which Professor R. D. Emslie and Mr. N. Veall were at that time engaged. I am most grateful to these gentlemen for stimulating my interest in this field of research and for the continued interest which they have shown in my later experiments.

This collaboration was made possible by the Nuffield Foundation. I acknowledge my debt to its Trustees for granting me a Dental Fellowship and to Professor M. A. Rushton, C.B.E. of Guy's Hospital, for allowing me to work in his department during the first few months of the investigation.

The greater part of the work reported in these pages has been carried out in the Department of Dental Pathology at The London Hospital Medical College. There, I received assistance from several colleagues. Professor A. E. W. Miles has given me constant encouragement and he always ensured that I had time, space and technical facilities to pursue this investigation. I am grateful to him for his assistance in these many ways and for reading and commenting upon this manuscript. Dr. M. Braden has been a sympathetic listener and a knowledgeable adviser. I am especially grateful to him for his part in devising the mathematical treatment of some of the data reported in Chapter 5. I also wish to thank Professor G. L. Slack, T. D., O.B.E., for permitting me to use equipment in his department and to Mr. R. A. D. Williams for his advice on some chemical aspects of the investigation.

I have been fortunate in having expert assistance from the Staff

of the Medical Research Council's Cyclotron Unit at Hammersmith Hospital. In particular my thanks are due to Mr. P. C. R. Turner and Dr. D. J. Silvester who did so much to supply me with fluorine-18. Dr. Silvester was kind enough to provide me with details of the production of fluorine-18 and the illustration of the target cell (Fig. 4.1).

Radioisotope counting equipment was kindly put at my disposal by the Colgate-Palmolive Company and I am grateful to Professors R. D. Emslie and B.E.D.Cooke for arranging this on my behalf. Other equipment was purchased with an award from the Advisory Medical Research Committee of the London Hospital.

Mr. B. Rittman has provided invaluable technical assistance with many of the numerous chemical analyses. I gratefully acknowledge the fact that much of the work reported in Chapter 3 has depended upon his skill. This could not have been bettered.

I wish to record my thanks to the many children and their parents who cooperated with me in parts of this study. For similar assistance I am grateful to students and colleagues at the London Hospital Medical College who acted as subjects for some of the experiments.

The figures in this thesis were prepared by Miss M. Smith and photographed by Messrs. Broadbery and Kirk all of the London Hospital. I am grateful to Mrs. M. Pulsford who has undertaken with such efficiency the considerable task of preparing the typescript and to Miss P. Mockford who duplicated these pages.

Lastly, I acknowledge with gratitude and affection the many hours my Wife has spent in analysing my written words to ensure that they matched my spoken thoughts.

September, 1964. R.D.

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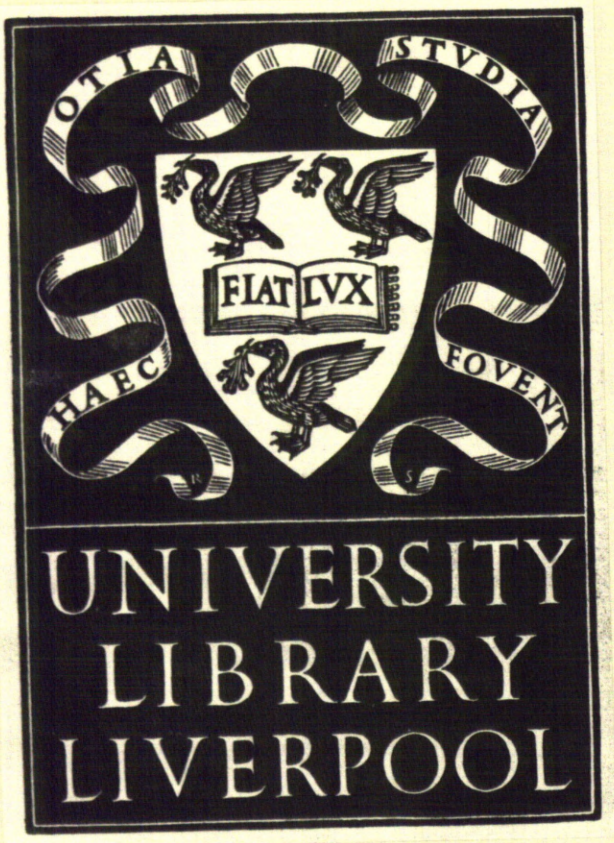
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