1978

# Effects Of Fluoride On Mineral Uptake And Acid Resistance Of Enamel.

Luis Antonio Aponte-Merced
*University of Alabama at Birmingham*

## Recommended Citation

Aponte-Merced, Luis Antonio, "Effects Of Fluoride On Mineral Uptake And Acid Resistance Of Enamel." (1978). *All ETDs from UAB*. 4058.
https://digitalcommons.library.uab.edu/etd-collection/4058

INFORMATION TO USERS

This material was produced from a microfilm copy of the original document. While the most advanced technological means to photograph and reproduce this document have been used, the quality is heavily dependent upon the quality of the original submitted.

The following explanation of techniques is provided to help you understand markings or patterns which may appear on this reproduction.

1. The sign or "target" for pages apparently lacking from the document photographed is "Missing Page(s)". If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting thru an image and duplicating adjacent pages to insure you complete continuity.
2. When an image on the film is obliterated with a large round black mark, it is an indication that the photographer suspected that the copy may have moved during exposure and thus cause a blurred image. You will find a good image of the page in the adjacent frame.
3. When a map, drawing or chart, etc., was part of the material being photographed the photographer followed a definite method in "sectioning" the material. It is customary to begin photoing at the upper left hand corner of a large sheet and to continue photoing from left to right in equal sections with a small overlap. If necessary, sectioning is continued again — beginning below the first row and continuing on until complete.
4. The majority of users indicate that the textual content is of greatest value, however, a somewhat higher quality reproduction could be made from "photographs" if essential to the understanding of the dissertation. Silver prints of "photographs" may be ordered at additional charge by writing the Order Department, giving the catalog number, title, author and specific pages you wish reproduced.
5. PLEASE NOTE: Some pages may have indistinct print. Filmed as received.

University Microfilms International
300 North Zeeb Road
Ann Arbor, Michigan 48106 USA
St. John's Road, Tyler's Green
High Wycombe, Bucks, England HP10 8HR

7904266

APONTE-MERCED, LUIS ANTONIO
EFFECTS OF FLUORIDE ON MINERAL UPTAKE AND
ACID RESISTANCE OF ENAMEL.

THE UNIVERSITY OF ALABAMA IN BIRMINGHAM,
PH.D., 1978

University
Microfilms
International 300 N. ZEEB ROAD, ANN ARBOR, MI 48106

# EFFECTS OF FLUORIDE ON MINERAL UPTAKE AND ACID RESISTANCE OF ENAMEL.

by

LUIS ANTONIO APONTE-MERCED

A DISSERTATION

Submitted in partial fulfillment of the requirements for the
degree of Doctor of Philosophy in the Department of
Physiology and Biophysics in the Graduate School,
University of Alabama in Birmingham

BIRMINGHAM, ALABAMA

1978

# ACKNOWLEDGEMENTS

I express my sincere appreciation and gratitude to Dr. Frederick F. Feagin, my graduate faculty advisor, for his encouragement, friendship, understanding and guidance during my graduate studies.

I extend my appreciation to Drs. S. B. Barker, E. Bradley, F. F. Feagin, J. Navia, K. Pruitt and R. L. Shoemaker for serving as members of my graduate committee.

I also wish to express my gratitude to Dr. E. Bradley for the help in the statistical analysis of this work and Dr. S. Thiradilok for his friendship, direct interest and assistance in my research.

As a gesture of my most sincere appreciation and gratitude, I wish to dedicate my accomplishments of this work to my family and friends in Alabama. Without their understanding, moral support and encouragement a successful completion of this work would have been impossible.

I am greatly appreciative of the financial support from the School of Dentistry of the University of Alabama in Birmingham during my graduate studies and research (Training Fellowship No. T01 DE 00007). Part of this research was also made possible by assistance from N.I.H. Grant No. 1R01 DE 04379. I also wish to express my appreciation to the

Medical Sciences Campus of the University of Puerto Rico for its assistance and encouragement.

## TABLE OF CONTENTS

Page

# LIST OF TABLES

Page

## LIST OF FIGURES

Page

# INTRODUCTION

## 1. Mineralized Tissues

Most of the body tissues may be mineralized under abnormal conditions. The bones and teeth comprise the normally mineralized tissues. Ectopic deposits of minerals are frequently found on tooth surfaces as mineralized dental plaque. Renal stones, another type of ectopic mineral formation, are also found in the urinary system. The small particles of calcium carbonate found in the inner ear, otoliths, provide a unique example of a normal ectopic mineralization. Possibly with the exception of the otoliths, theories of the molecular events of mineralization seem to be similar for the various types of bodily mineralization, both normal and abnormal. Mineralized tissues share the common feature of an organic matrix that in some way initiates the deposition of minerals (37,79).

The mineralized portion of bones and teeth are composed of organic matrices which are secreted by specialized cells. The organic materials are oriented and organized, each in a unique manner, so that mineralization is initiated and controlled specifically in an organized manner in each of the different portions of the tissues. The originating cells persist in bone, dentin and cementum throughout mineralization and thereafter for the entire life of the tissues. These embedded cells continue to function in homeostasis of the tissues. The cells in bones also function in the homeostasis of the entire body (37,66).

The enamel of the teeth is unique among the normal mineralized tissues. The formative cells, ameloblasts are lost soon after completion of the enamel. The enamel is then acellular and enclosed in an epithelial cell lined membranous capsule until it is fully mineralized and erupted into the mouth. Once the tooth crown erupts into the oral cavity, the surface enamel contacts oral fluids which differ markedly in composition compared to the interstitial fluids. The surface enamel is then subjected to the rigorous demands of functioning in interaction with the external environment.

## 2. Tooth Enamel

Enamel is of ectodermal origin rather than mesodermal as for bone, dentin and cementum. It is the most dense tissue of the body. Enamel is composed of about 96 percent minerals and 4 percent organic matrix on a weight basis (59). The minerals of enamel are almost entirely calcium phosphates with only trace amounts of other inorganic components and carbonates. The organic matrix of enamel consists of unique types of keratin rather than collagen of most other mineralized tissues. Glimcher (31) calls the principal organic components of the matrix of enamel "enamelins or amelogens". The molecular and structural relationships of matrix and minerals of enamel are completely unknown.

The enamel rods are oriented so that they are almost perpendicular to the underlying dentin junction and the surface of the tooth crown. Evidence has been presented that indicated that the rods bend as they approach the surfaces in some teeth (59). The rods appear hexagonal in the light microscope when viewed in cross section (69). Meckel _et al_. (54) found that the enamel rods appeared to have the shape of a "keyhole" when viewed in various planes with the electron microscope. These investigators described the enamel rods as having a head and tail. The tail portions of the enamel rods are oriented toward the cervical regions of the tooth crown while the head portions are directed toward the cusps or incisal edge of the tooth (53). In the head regions the crystallites are described as being oriented almost parallel to the direction of the rod, while in the tail regions the crystallites generally assumed an orientation perpendicular to the direction of the rod. The features described as the rod sheath contained crystallites which were oriented differently than in the head tail regions of the enamel rod. The interrod substance is hardly distinguishable from the rod sheath.

Enamel has been described in the following three structural levels for purpose of clinical considerations: a) The crystallites and matrix constituents, b) The rods or organizing units; and c) Differences in structural zones from the dentinal junction to the outer surface (69). The crystallites make up approximately 90 percent of the volume of enamel (about 96 percent of the weight). The crystals appear as ribbon like structures, but their shape is hexagonal when viewed in cross section (69). As stated previously, the relationships of the organic matrix and minerals are unknown. However, the matrix has been assumed to initiate crystal formation and orientation (31). The orientation of the crystals within the enamel rods affects the rates of crystal dissolution (71). Zones have been reported of structural differences in enamel from the dentin to the surface of the tooth. The significance and degree of variability of structural differences between areas of the same tooth and between teeth have not been clearly demonstrated. The outer surfaces of the enamel, those in contact with oral fluids for lengthy periods of time, may not have a definite structural arrangement. The composition and structure of the outer enamel depends on its history of reaction with oral fluids, dental plaque, and the behavior of the individual.

The surface minerals of enamel are in a state of constant reaction with the surrounding environment. Much like the chemistry of bone minerals, the composition of the surface minerals of enamel reflect to a large degree the composition of the bathing fluids (74). A major problem in this respect is to define the surface minerals of enamel. Simply stated, they are the portions of enamel that contact the oral fluids. The penetration of the organic pellicle, i.e., organic substance derived from saliva, within the outer surface of the tooth crown shows that oral fluids penetrate at least 3 to 5 microns into sound enamel (18).

The oral fluids at tooth surfaces are heterogeneous mixtures of saliva, crevicular fluids, and ingesta. The fluid portion of dental plaque, which provides most of the contact with enamel, may be considered as a mixture of the oral fluids which are altered by bacterial metabolism. In the absence of dental plaque the enamel minerals generally extract calcium and phosphate from the oral fluids until the surface exhibits a small degree of hypermineralization compared to the underlying or subsurface minerals. In the presence of dental plaque the products of microbial metabolism may dominate the interactions of the surface minerals of the enamel and the fluid medium. Acids produced by the plaque bacteria dissolve the minerals and lead to caries.

The deposition of minerals in the surface enamel of sound erupted teeth has been termed maturation (28). Similar deposition of calcium phosphates in enamel after periodic bursts of acid activity in dental plaque has been described as remineralization (44). These phenomena of maturation and remineralization have been well documented.

However, the mechanisms of these processes have not been defined. It has been almost impossible even to define the nature of the reactions that must take place because of the complexity and heterogeneity of the oral environment and the surface minerals of the enamel. Discussion of these processes in the literature invariably fall back on what has been described as the chemistry of bone mineral (58). Seldom has the role of the organic pellicle and dental plaque been considered in the discussions of enamel remineralization in situ.

### 3. The Principal Minerals of Enamel

The principal mineral of enamel, dentin, cementum and bone is hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$. Almost all of the calcium phosphates in these tissues belong to the apatite family of minerals. The apatites have been difficult to study because of their small crystal size. In fact certain fractions of the apatites in mineralized tissues are of such small size that they are considered to be nonstructured or amorphous (18). However, the apatite in mature enamel is considered to be well crystallized compared to that of bone (14).

Many studies of mineralization have indicated that the initially formed minerals were poorly crystalline or amorphous and of relatively low Ca/P ratios (75). The studies of calcium phosphate precipitations from simple aqueous solutions, and the early minerals deposited in bone under natural conditions, indicated that the first minerals deposited were similar in composition to dicalcium phosphate and tricalcium phosphate (18,76). These initially deposited minerals on ageing change to apatites closely resembling hydroxyapatite (76). Brown has suggested that octacalcium phosphate, $Ca_8H_2(PO_4)_6 \cdot 5H_2O$, is an intermediate phase in the transformation of calcium phosphates to hydroxyapatite (8). The variable Ca/P ratios reported for bone and tooth minerals are attributed to the existence of the transforming phases of the calcium phosphates and to the adsorption of various ions including $Ca^{+2}$, $H_2PO_4^{-1}$, $HPO_4^{-2}$ and $PO_4^{-3}$ on the existing minerals (29).

Apatites of variable composition give almost the same x-ray diffraction pattern (14). Since the mineralized tissues must be altered and in effect degraded for crystallographic analysis, these studies only provide indications of the structure of the minerals as they existed in a tissue.

Thus the Ca/P ratios of the minerals have frequently been used as an indication of chemical composition of the mineralized tissues (57). The Ca/P ratios and formulas of the possible mineral phases of calcium phosphates in tooth and bone include the following:

| | | |
|---|---|---|
| Monocalcium phosphate | 0.50 | $Ca(H_2PO_4)_2$; |
| Dicalcium phosphate | 1.00 | $CaHPO_4$; |
| Octacalcium phosphate | 1.33 | $Ca_8H_2(PO_4)_6$; |
| Tricalcium phosphate | 1.50 | $Ca_3(PO_4)_2$; |

| | | |
|---|---|---|
| Hydroxyapatite | 1.67 | $Ca_{10}(PO)_6OH_2$; |
| Fluorhydroxyapatite | 1.67 | $Ca_{10}(PO_4)_6FOH$; |
| Fluorapatite | 1.67 | $Ca_{10}(PO_4)_6F_2$. |

It is important to note that the Ca/P or Ca/F ratios have not been associated with the structure of the minerals. However, the rate of reaction of the minerals may be a function of their physical state as well as their composition.

### 4. Reactions of Posteruptive Enamel

Deviations in structure and composition of the hydroxyapatite minerals in bones and teeth have often been attributed to substitution of "foreign ions" in the crystal lattice of hydroxyapatite and excessive accumulation of extraneous ions on the mineral surfaces (39). The ionic substitutions and adsorptions occur during mineralization of the enamel and later by exchange and recrystallization. These "foreign ions" have been classified into three groups: 1) higher concentrations on the surface of enamel than within (F, Pb, Zn, Fe, Sb); 2) lower concentrations on the surface than within (Na, Mg, $Co_3$); and 3) an approximately uniform distribution (Sn, Cu, Al, K).

The status of bone and tooth mineral in terms of nucleation, growth, maturation and dissolution involves primarily the dynamic surface chemistry of hydroxyapatite and its surrounding environment. The main ionic reactions of this interface have been described as adsorption, exchange, recrystallization and precipitation.

Adsorption in mineral crystals is not well understood. It is a rather nonspecific uptake of a substance in the hydration shells or on crystal surfaces involving weak electrostatic forces. Adsorption to hydroxyapatite is usually considered to be a rapid and reversible process.

Isoionic exchange is a dynamic process whereby an ion is lost and replaced by another identical ion, such as phosphate for phosphate in hydroxyapatite. Heteroionic exchange causes either minor or major alterations of some characteristics of hydroxyapatite.

Recrystallization is the dynamic process of crystal destruction and reformation. In theory it means the dissolution and reformation of crystals in identical or different structure. This term has been used to describe the growth of small particles, possibly nonstructured calcium phosphates, to crystals of hydroxyapatite (58). In this presentation, recrystallization will mean the dissolution and reprecipitation of calcium phosphates and apatites to hydroxyapatite and fluorapatite. This means that the minerals may show a net gain or loss of either calcium, phosphate or fluoride, but not a combination of calcium and phosphate. Net gains of calcium and phosphate by the surface minerals in enamel will be considered as remineralization. Net losses of calcium and phosphate are considered as demineralization.

Remineralization of enamel by definition means that the surface minerals were previously demineralized to some extent (44). In this respect remineralization is differentiated from maturation. Acid dissolution is not a prerequisite for maturation. Adsorption, ionic exchange, recrystallization, demineralization, and remineralization actually occur simultaneously within the enamel structure particularly on its reactive surfaces.

During the early part of this century observations were made of mottled enamel in certain locations of the United States (51). Although the first observations were not directly related to fluoride, some factor in the drinking water was suspected to be responsible for the mottling of enamel. Extensive studies were made by McKay and Black (5,52) in different areas of the southwestern United States. In the 1930's Smith and Smith (73) showed that relatively high levels of fluoride in the drinking water caused mottling of tooth enamel. Thus a relationship between fluoride and enamel was established. Dean in 1939 (16) associated the effect of fluoride in drinking water and reduction of caries. Volker in 1939 (80) showed that fluoride reacted with enamel and dentin to make them resistant to acid dissolution. Studies by Galagan in 1953 (29) determined safe fluoride levels in drinking water, which would not cause enamel fluorosis (mottled enamel) but still inhibit caries. About 1 ppm (0.05 mM) fluoride in drinking water usually reduces the caries incidence about 60 percent without causing enamel fluorosis (35). Thus research has been directed toward determining the mechanism of action of fluoride in caries prevention.

In 1912 Head (34) presented evidence for remineralization of enamel. He found that enamel softened in weak acid exposure later rehardened in saliva. His work was followed by others [Pickerell, 1912 (66), Andresen, 1926 (1), Ruthrauff, 1923 (67), Brodecker, 1942 (7)]. Pigman's group at the University of Alabama in Birmingham confirmed and extended the findings of Head and other investigators concerning the remineralization of enamel (21). Brudevold _et_ _al_. (11) found that the low concentration of fluoride used in fluoridation increased the amounts of precipitates recovered from mineralizing solutions. The work of Koulourides, Cueto and Pigman (1961) showed that 1 ppm $F^-$ in synthetic saliva (mineralizing solutions) increased the rehardening of enamel (46). That study gave credence for the concept of remineralization as one of the more plausible explanations of fluoride ion action of caries prevention.

Evidence that the surface minerals of enamel can be changed by remineralization is based on different types of experimental methods. These include: 1) hardness tests (26) 2) permeability tests (41); 3) examination of histologic sections of teeth (72); 4) kinetic studies of mineral uptake and chemical analysis (24).

Reactions between fluoride and enamel that may prevent dental caries have been extensively studied. The primary reaction products according to Brudevold (10) are (each based on the presence of hydroxyapatite in enamel:

1. Heteroionic exchange of $F^-$ and $OH^-$ ions in the lattice structure of hydroxyapatite to yield fluorapatite and hydroxyfluorapatite; i.e., for solid-solution reactions in the presence of about 1 to 10 ppm $F^-$ (0.05-0.50 mM; low concentration).
2. (a) Decomposition of hydroxyapatite in relative concentrated $F^-$ ion solutions to yield a surface phase of $CaF_2$ and readily soluble calcium phosphates.

   (b) Since the surface limited $CaF_2$ and $CaHPO_4$ were found to be readily soluble in saliva, Brudevold further proposed as they always dissolve and thereby increase the concentrations of $Ca^{+2}$, $PO_4^{-3}$ and $F^-$ ions sufficiently to cause the precipitation of fluorapatite on tooth minerals.

The Ca/P ratios of the reactions indicate what product, or possible combinations of reaction products, form in the enamel surface. The possible Ca/P ratios of the minerals to be considered were previously presented. In the same manner the Ca/F ratios of the reactions also indicate the products formed in the surface enamel. The Ca/F ratios of the products to be considered are:

| | | |
|---|---|---|
| hydroxyfluorapatite | 10.0 | $Ca_{10}(PO_4)_6OHF$; |
| fluorapatite | 5.0 | $Ca_{10}(PO_4)_6F_2$; |
| calcium fluoride | 0.5 | $CaF_2$. |

The major contribution of the concepts of maturation and remineralization is that they provide a rational explanation for the fluoride ion action of caries prevention. Remineralization or maturation generally causes an increase of fluoride and a decrease in carbonate in surface enamel. This accumulation of fluoride and loss of carbonate increases the resistance of surface minerals to decay processes (33). In theory, remineralization may cause fluoride uptake to greater distance in the enamel surface than maturation, i.e., to the depths of previous acid actions. Thus one may view maturation as a defensive mechanism and remineralization as both a reparative and a defensive mechanism. The exact role of the organic components of surface enamel and oral fluids is not as well known as that of the accumulated fluoride.

### 5. Theories of Fluoride Reactions with Enamel for Caries Prevention

Dental caries has been defined as a multifactorial microbial disease characterized by early loss of surface and subsurface minerals and latter destruction of the tooth (83). The exact mechanisms of the process are not completely known. Nevertheless caries may be arrested, and apparently reversed to some extent, by careful control of dental plaque and diet and by optimal utilization of fluoridation (19). Fluoridation alone may reduce caries without the control of diet or oral hygiene (84). Direct application of fluoride ions on tooth surface also inhibits the carious processes, but the results are less predictable than fluoridation (17).

Caries prevention by fluoride is believed to occur by various possible mechanisms. The contribution of each may vary at different stages of the carious processes. As a general concept fluoride ions inhibit acid production of plaque bacteria, enhance reprecipitation of minerals in the tooth, increase the acid resistance of the minerals and inhibit the adsorption of organic substances on the surface minerals of the tooth (43). The concentrations and frequencies of fluoride ion release at the tooth surface seem to control its effectiveness (84).

Historically the anti-caries action of fluoride has most often been considered to be caused by the formation of sparingly soluble fluorapatites in tooth minerals rather than hydroxyapatite (81). The differences in solubility of hydroxyapatite and fluorapatite are small (8). However, their rates of dissolution are quite different in almost all acids (13).

Bacterial metabolic products such as acid and chelators (39) cause demineralization of enamel. Preferential dissolution of the minerals in the central region of enamel rods by weak acids has been observed with the scanning and transmission electron microscopes (39,77). Calcium chelators at basic pH preferentially dissolve the sheath or peripheral regions of the rods (63). Studies on early carious lesions of enamel indicated the acid-type dissolution of enamel (70). This preferential acid dissolution may be caused by crystal orientation, differences in mineralization of individual crystals, and the relationship between organic components and the crystals (62,71).

Arrestment and reversal of carious lesions by remineralization have been indicated by clinical and laboratory evidence (6). Remineralization is enhanced by low fluoride concentrations, as used in water fluoridation, and levels of fluoride found in the dental plaque (40). Each level of fluoride ions in solutions up to about 10 ppm (0.5 mM) increased the rates of remineralization (25). The fluoride in remineralized enamel reduced the rates of enamel dissolution in acid (27). The remineralizing effect of fluoride and subsequent acid resistance of enamel minerals can be considered as a logical explanation for the mechanism of action of caries prevention. Nevertheless, remineralization is dependent on the total environment of the enamel surface. Thus the potentials of remineralization, as any of the surface-reactions of enamel, are dependent on the sets of conditions under which they are studied.

## 6. Objective of this Study

The purpose of this study was to evaluate the effects of fluoride on the rates of remineralization, ion exchange and acid resistance of enamel. The objective was to determine whether an increase in rates of remineralization,

ion exchange, and acid resistance could be a possible explanation of the anticariogenic effect of fluoride.

# EFFECT OF FLUORIDE ON RATES OF ENAMEL REMINERALIZATION

## INTRODUCTION

Remineralization is the uptake of minerals in the enamel of erupted teeth (45). It occurs in the mouth in hypomineralized areas of newly erupted teeth and requires selective acid dissolution of minerals in surface and subsurface enamel. In vitro evidence of the phenomenon was obtained by Head in 1912 (34). Pigman and associates continued the work of Head and found that the level of fluoride ions used in water fluoridation greatly increased the rates of remineralization (46). Brudevold and coworkers (11) also found that similar concentrations of fluoride increased the remineralization of enamel. Widespread use of the term remineralization originated from these studies.

Brudevold _et_ _al_. studied the seeded growth of minerals on particles of enamel or apatites (11), whereas, Koulourides _et_ _al_. initially assumed that remineralization occurred within carious surfaces of enamel (46). Obviously the word remineralization is subject to interpretation rather than having a definite meaning. The term persists because few investigators have determined the exact physical state of the minerals deposited in remineralized enamel. Electron micrographs of surfaces remineralized in solutions that contained low levels of fluoride and which were supersaturated with hydroxyapatite, showed the growth of crystals in and on the enamel (69). Surfaces remineralized in solutions that were supersaturated with dicalcium phosphate in the presence of carbonates contained amorphous or nonstructured minerals (56). Thus the nature of the remineralized components of enamel depend on the composition of the surrounding solutions. The effects of the organic constituents of oral fluids on remineralization have not been sufficiently studied. The acid resistant organic substance found in mature enamel apparently precipitated from the oral fluids (54).

The studies of Koulourides and of Brudevold cited above indicated that the effect of fluoride on remineralization would be expected to add greatly to the caries resistance of enamel. The remineralization effect of fluoride along with the acid resistance of fluoridated enamel provided one of the most likely mechanisms of the caries prevention of fluoridation. In this regard, it was found that each level of fluoride ions up to 0.5 mM increased the rates of enamel remineralization (24). The enamel remineralized in the presence of fluoride exhibited greater acid resistance than intact enamel or surfaces remineralized in the absence of fluoride (77).

The exposure of enamel to relatively concentrated solutions of fluoride results in the formation of $CaF_2$ on the mineral surfaces. Brudevold found that the $CaF_2$ dissolved in oral fluids (10). He concluded that the fluoride ions were then taken up in the enamel as fluorapatites by exchange, recrystallization and remineralization. The formation of fluorapatite in enamel is the basis for the classical concept of the effect of fluoride on caries prevention (80). However, recent evidence indicated that fluoride in acid solutions almost immediately reduced the rates of enamel dissolution well in advance of the time required for the formation of significant amounts of fluorapatites (77).

The purpose of this study was to determine the rates of calcium, phosphate and fluoride uptake during the remineralization of enamel surfaces in solutions of 0.00 to 4.00 mM NaF. The objective of this study was to determine the level of fluoride at pH 7.0 that caused maximum rates of mineral uptake in the enamel.

# METHODS

## 1. Preparation of Enamel Surfaces:

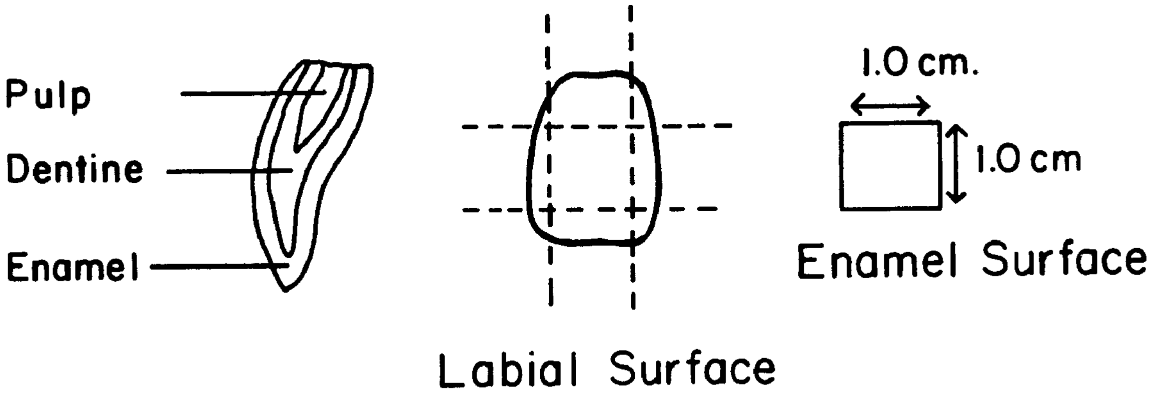
Square surfaces of labial enamel 1 $cm^2$ were sectioned from bovine incisors. The teeth were extracted at the time of slaughter and stored in deionized water at 4°C with thymol as a preservative. The enamel surfaces were sectioned using a circular rotatory diamond blade under a spray of deionized water (22). Using a micrometer to move the blade into proper positions, cuts were made on the cervical, incisal, mesial and distal sides of the teeth, such that a 1 $cm^2$ square of labial enamel was obtained from the crown of the tooth. A final cut was made of the lingual side of the crown to obtain 1 $cm^2$ square surface of labial enamel (Figure 1).

The surface enamel was slightly abraded with silicone carbide then wet pumiced to remove surface pellicles. All sides, except the labial surface of the enamel squares were painted with acid resistant fingernail varnish. Three of these surfaces were then attached with dental sticky wax on a rectangular plastic plate as shown in Figure 1.

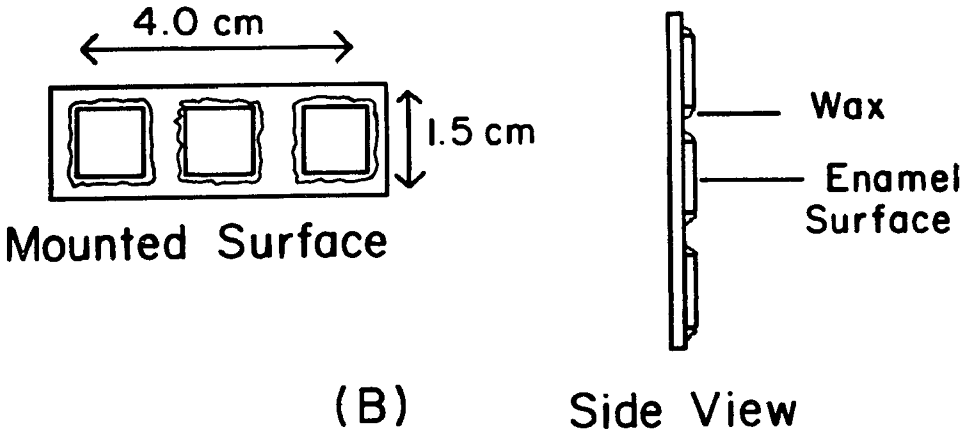
The enamel surfaces were etched 24 hours in 10 mM acetic acid of pH 5.5. Two of the plastic plates, 6 surfaces, were attached with wax on the side wall of a 600 ml beaker.

Figure 1.

(A) Preparation of surfaces of labial enamel ($1cm^2$) of bovine incisor teeth; (B) Illustration of surfaces mounted with wax on rectangular plastic plates.


Pulp
Dentine
Enamel
Labial Surface
1.0 cm.
1.0 cm
Enamel Surface

(A)


4.0 cm
1.5 cm
Mounted Surface
Wax
Enamel Surface
Side View

(B)

The 6 surfaces were then exposed to 500 ml of the acid with constant slow rate stirring by a plastic coated magnetic bar. The etched surfaces were briefly rinsed with deionized water then left to wash in 500 ml of deionized water for 24 hours.

## 2. Procedures of Remineralization of Enamel Surfaces:

Six of the acid etched surfaces of enamel were sealed in a small flow chamber as illustrated in Figure 2. Dental sticky wax was used to seal the plastic plates tightly to the chamber. Figure 3 shows the assembled system which was used to remineralize the surfaces. Small bore plastic tubing was used to connect the chamber to the solution reservoir through a roller pump. The solution was pumped from the mixing reservoir through the chamber to immerse the 6 surfaces of enamel and then back to the reservoir at a rate of 1.0 ml per minute. The system contained 8.0 ml solution of which about 1 ml was in continuous contact with the enamel. The reservoir contained $H^+$, $Ca^{+2}$, and $F^-$ electrodes for detection of changes in activity of the respective ions in the solution. The pH was maintained constant at 7.0 by automatic titration with 0.25 mM NaOH. The fluoride ion activities in the solutions were maintained at selected levels by automatic titration with 1.0 to 10.0 mM NaF.

Figure 2.

Method of enclosing six 1 $cm^2$ surfaces of enamel in the flow chamber. Dental sticky wax was used to seal the plastic plates tightly to the side of the chamber.

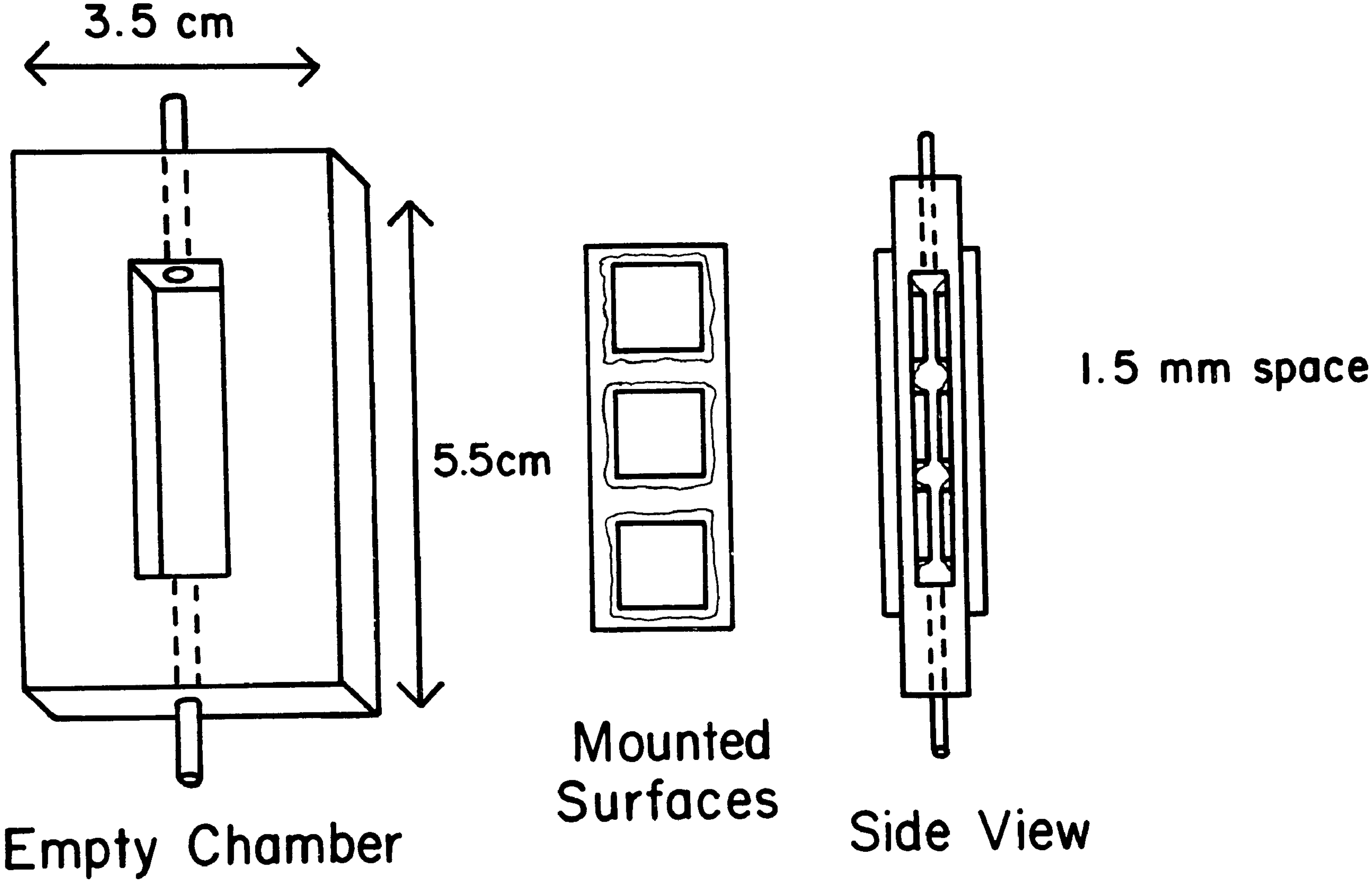
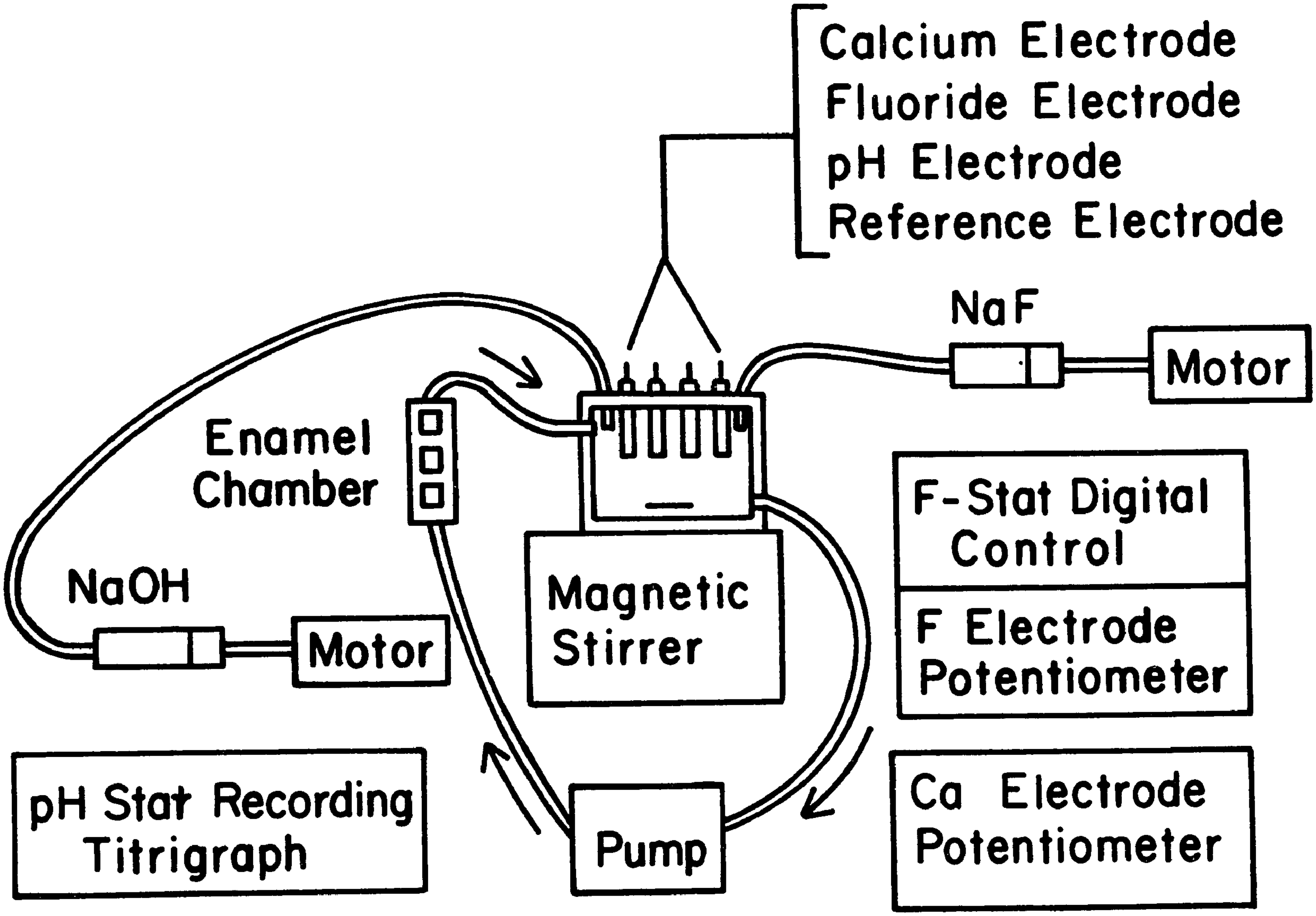

3.5 cm
5.5cm
1.5 mm space
Empty Chamber
Mounted Surfaces
Side View

Figure 3.

Diagram of the system used to contact enamel surfaces and solutions during control of pH, $F^-$, and $Ca^{+2}$ in solution.


Calcium Electrode
Fluoride Electrode
pH Electrode
Reference Electrode
NaF
Motor
Enamel Chamber
Magnetic Stirrer
F-Stat Digital Control
F Electrode Potentiometer
NaOH
Motor
Pump
Ca Electrode Potentiometer
pH Stat Recording Titrigraph

## 3. Preparation of Mineralizing Solutions:

The following stock solutions were prepared using reagent grade chemicals: 50 mM $CaCl_2$; 50 mM $NaH_2PO_4$; 25 mM NaF; 1.0 M KCl; and 100 mM NaOH. Mineralizing solutions were prepared from these stock solutions to contain: 1.5 mM $CaCl_2$; 1.0 mM $NaH_2PO_4$; 40 mM KCl; and 0.00, 0.05, 0.25, 0.50, 1.00, 2.00, 3.00 and 4.00 mM NaF. The solutions were prepared starting with a relatively large volume of deionized water, then adding in order KCl, $CaCl_2$, $NaH_2PO_4$ and NaF. The pH of the solution was slowly adjusted to pH 7.0 with NaOH before dilution to exact volume.

## 4. Experimental Procedures:

Experiments were performed with eight different mineralizing solutions that contained 1.5 mM $Ca^{+2}$; 1.0 mM $PO_4^{-3}$, 40 mM KCl and 0.00, 0.05, 0.25, 0.50, 1.00, 2.00, 3.00 or 4.00 mM NaF, each at pH 7.0. Experimental variables were evaluated in five separate experiments; i.e. five sets of six surfaces were interfaced separately with each of the different mineralizing solutions for three hours.

Recordings were made at 15 minute intervals of the millivolt response of the calcium ion electrode as a measure of the calcium ion activity (60). The change in calcium ion activity was calculated for each 15 minute time of remineralization relative to the electrode response in standard solutions. These measurements indicated the rate of calcium ion uptake in the enamel surfaces.

A 200 µl sample was removed from the solution reservoir each half hour for chemical analyses and partially to compensate for volume additions of base and NaF titrations. The 200 µl samples were diluted by a factor of 10 with deionized water. Samples were taken for spectrophotometric determination of calcium (68), and phosphate (15). These measurements along with calculations of the small volume alterations caused by titration and sampling were used to estimate mineral uptake in the enamel surfaces.

The amounts of NaOH and NaF titrants added to the solutions were recorded each half hour of the experiments. These additions to maintain constant pH and fluoride ion activity in the solutions were used to estimate the rates of $F^-$ uptake and phosphate ion reactions in the enamel.

The mean data for each of the five replicate experiments for each of the levels of NaF in the solutions were statistically compared for significant differences. The results were subjected to a computer program for curvi-linear analysis (4).

# RESULTS

Table 1 shows the change of calcium ion activity ($Ca^{+2}$) in solutions as the differences of electrode potential (mv). The electrode potential was constant in solutions before contact with the enamel surfaces. An increase of electrical potential difference reflected a decrease of $Ca^{+2}$ in solution. Table 2 shows statistical comparisons of the effect of fluoride ion concentrations on the rates of $Ca^{+2}$ losses from the solutions, i.e. rates of $Ca^{+2}$ uptake in the enamel surfaces. The $Ca^{+2}$ uptake increased with time of reaction. The uptake of $Ca^{+2}$ increased with each higher level of $F^-$ in the solution up to 2.0 mM NaF. Concentrations of NaF above 2.0 mM reduced the rates of $Ca^{+2}$ uptake to about the same uptake as found for 0.25 mM NaF. The rates of $Ca^{+2}$ uptake were similar for 0.50, 1.00, and 2.00 mM NaF.

Figure 4 shows the linear relationships between the changes of electrode potential for $Ca^{+2}$ (uptake by the enamel) and the time of reaction. Table 3 shows the linear regression equations derived from Table 1 and Figure 4. The equations indicated that the rates of $Ca^{+2}$ uptake by the enamel approached a maximum for concentrations of $F^-$ between 0.50 and 2.00 mM NaF. Statistical differences between the rates of $Ca^{+2}$ uptake by the electrode method were determined using Duncan's Multiple Range Test at the 0.05 level of

TABLE 1

Effects of NaF on Potential Differences of the $Ca^{+2}$ Specific Electrode During 3 Hour Remineralization of Enamel Surfaces.

| Solution NaF (mM) | Potential Difference* at 15 Minute Intervals (mv X $10^{-1}$ and S.D.) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| 0.00 | 6(1) | 13(2) | 20(3) | 25(4) | 31(3) | 38(4) | 41(4) | 46(4) | 51(4) | 56(4) | 62(6) | 65(5) |
| 0.05 | 10(2) | 17(5) | 26(3) | 34(4) | 41(6) | 49(5) | 57(7) | 65(6) | 73(8) | 81(10) | 88(9) | 98(10) |
| 0.25 | 11(3) | 21(5) | 32(6) | 43(6) | 54(6) | 66(7) | 77(9) | 89(9) | 100(9) | 112(11) | 124(12) | 134(13) |
| 0.50 | 17(1) | 32(2) | 47(3) | 61(4) | 76(6) | 90(7) | 104(9) | 118(10) | 131(12) | 145(13) | 159(14) | 172(16) |
| 1.00 | 19(4) | 36(6) | 51(8) | 66(10) | 81(12) | 96(14) | 111(15) | 125(16) | 133(.5) | 153(18) | 167(18) | 180(18) |
| 2.00 | 22(3) | 42(4) | 56(7) | 71(8) | 85(7) | 101(8) | 117(8) | 132(10) | 148(11) | 164(12) | 179(13) | 195(13) |
| 3.00 | 19(4) | 31(4) | 44(5) | 57(6) | 67(10) | 80(13) | 91(15) | 105(18) | 115(22) | 129(2) | 138(20) | 149(22) |
| 4.00 | 19(4) | 32(5) | 45(3) | 57(3) | 63(4) | 72(4) | 81(5) | 89(6) | 96(7) | 105(7) | 112(7) | 121(6) |

* Electrode potentials were compared at 15 minute intervals with the potential of the solutions at the initial time of contact with enamel and presented as means and standard deviations of 6 experiments. In each experiment six-1cm$^2$ surfaces of enamel were contacted with 8 ml solution that contained 1.5 mM $CaCl_2$, 1.0 mM $NaH_2PO_4$, and the indicated concentration of NaF at pH 7.0 for 3 hours.

TABLE 2

Statistical Comparisons Between the Effects of NaF Concentrations on Calcium Ion Activities (Electrode Potential Differences) During Remineralization of Enamel Surfaces.

| Concentrations (mM NaF) | t-value | p value | Conclusion |
|---|---|---|---|
| 0.05 vs. 0.25, 0.50, 1.0, 2.0, 3.0 | 6.01 | $(p < 0.001)$ | S.D. |
| 0.05 vs. 4.0 | 4.18 | $(0.002 < p < 0.005)$ | S.D. |
| 0.25 vs. 0.50, 1.00, 2.00 | 5.05 | $(p < 0.001)$ | S.D. |
| 0.50 vs. 3.00 | 2.70 | $(0.02 < p < 0.05)$ | S.D. |
| 0.50 vs. 4.00 | 7.87 | $(p < 0.001)$ | S.D. |
| 1.00 vs. 3.00 | 3.5 | $(0.01 < p < 0.05)$ | S.D. |
| 1.00 vs. 4.00 | 8.6 | $(p < 0.001)$ | S.D. |
| 2.00 vs. 3.00, 4.00 | 5.48 | $(p < 0.001)$ | S.D. |
| 3.00 vs. 4.00 | 3.72 | $(0.01 < p < 0.05)$ | S.D. |
| 0.25 vs. 3.00 | 1.76 | $(.10 < p < .20)$ | N.D. |
| 0.25 vs. 4.00 | 2.13 | $(.05 < p < .10)$ | N.D. |
| 0.50 vs. 1.00, 2.00 | 0.98 | $(p > 0.05)$ | N.D. |

Results of Students t test performed between the respective fluoride concentrations. t value given for the lowest value observed compared with the indicated concentration. S.D. = Significantly Different, N.D. = Not Significantly Different.

Figure 4.

The effects of NaF concentrations in solutions that contained 1.5 mM $CaCl_2$ and 1.0 mM $NaH_2PO_4$ at pH 7.0 on the rates of $Ca^{+2}$ uptake in enamel. The $Ca^{+2}$ uptake was measured as the change of electrical potential of the $Ca^{+2}$ electrode.

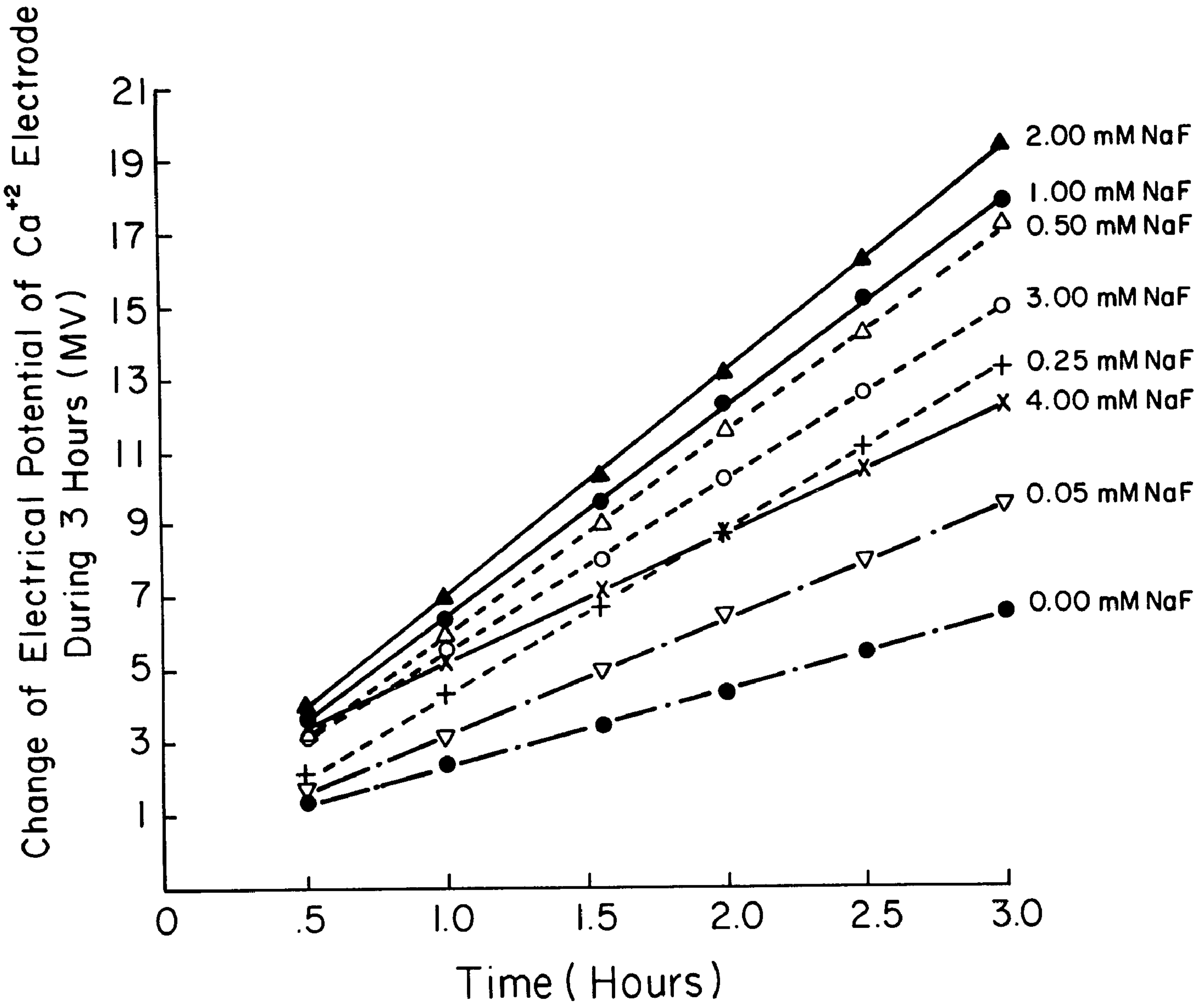

Change of Electrical Potential of Ca+2 Electrode During 3 Hours (MV)
21
19
17
15
13
11
9
7
5
3
1
0
.5
1.0
1.5
2.0
2.5
3.0
Time ( Hours )
2.00 mM NaF
1.00 mM NaF
0.50 mM NaF
3.00 mM NaF
0.25 mM NaF
4.00 mM NaF
0.05 mM NaF
0.00 mM NaF

TABLE 3

Linear Regression Analysis of the Effects of NaF Concentrations on Rates of Changes of Calcium Electrode Potential Differences During Remineralization of Enamel.

| No. | Concentration (mM) NaF | Equation | Correlation Coefficient:r |
|---|---|---|---|
| 1 | 0.00 | $y = 2.13t + 0.32$ | $r = 0.99$ |
| 2 | 0.05 | $y = 3.18t + 0.14$ | $r = 0.99$ |
| 3 | 0.25 | $y = 4.54t - 0.18$ | $r = 0.99$ |
| 4 | 0.50 | $y = 5.62t + 0.46$ | $r = 0.99$ |
| 5 | 1.00 | $y = 5.80t + 0.70$ | $r = 0.99$ |
| 6 | 2.00 | $y = 6.21t + 0.86$ | $r = 0.99$ |
| 7 | 3.00 | $y = 4.73t + 0.87$ | $r = 0.99$ |
| 8 | 4.00 | $y = 3.56t + 1.66$ | $r = 0.99$ |

Linear equations and correlation coefficients were obtained by linear regression analysis of the lines in Fig. 4 which show the effect of different fluoride concentrations (0.00, 0.05, 0.25, 0.50, 1.00, 2.00, 3.00 and 4.0 mM NaF) on the calcium ion activity change during 3 hours of experimentation. $y = bt + a$, $a$ = intercept, $b$ = slope: change of calcium ion activity per time (mV/hrs.), $t$ = time, $y$ = change in calcium ion activity (mv).

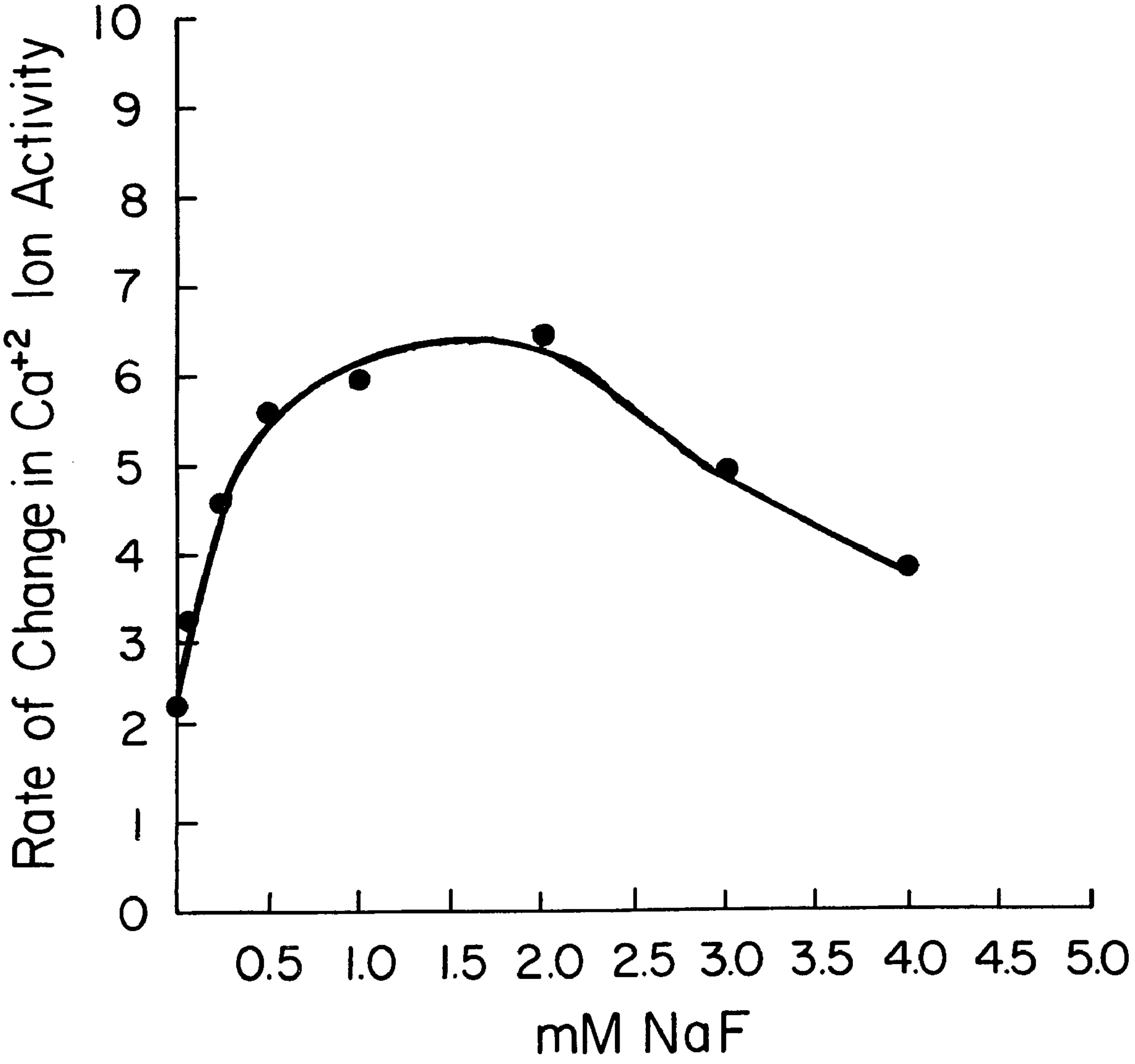
confidence (86). The 3.00 and 4.00 mM NaF levels reduced the rates of calcium uptake as noted above. The concentration of 0.05 mM NaF significantly increased the rate of $Ca^{+2}$ uptake compared to the absence of NaF in solution.

Figure 5 shows the relationship between the rates of $Ca^{+2}$ uptake by the remineralized surfaces of enamel and the concentration of NaF in the solutions. The apparent rates of $Ca^{+2}$ uptake increased rapidly for each higher concentration of NaF up to 0.50 mM, then almost flattened between 0.5 and 2.00 mM NaF, and slowly decreased for the higher concentrations of NaF.

Table 4 shows the uptake of $Ca^{+2}$, $PO_4^{-3}$, $F^-$ and $OH^-$ by the remineralized enamel during each half hour contact with the solutions. As described in the methods, samples were withdrawn from the solutions at half-hour intervals for chemical measurement of $Ca^{+2}$ and $PO_4^{-3}$. The $F^-$ uptake was estimated at these intervals by calculation of NaF titrant additions to maintain constant $F^-$ in the solutions. The $OH^-$ reactions were determined by the base additions to maintain constant pH 7.0. Table 5 shows the statistical comparisons of the effects of NaF concentrations on the reaction of $Ca^{+2}$ as determined by chemical analysis. Figure 6 illustrates the curves obtained by this method. The $Ca^{+2}$ uptake increased with time of reaction. NaF up to 1.00 mM increased the rates of $Ca^{+2}$ uptake. However, $Ca^{+2}$ uptake was not significantly different for levels of NaF between 0.5 and

Figure 5.

The effects of NaF on the 3 hour rates of $Ca^{+2}$ uptake in remineralized enamel. The data were derived from Figure 4.



Rate of Change in Ca+2 Ion Activity
0
1
2
3
4
5
6
7
8
9
10
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
mM NaF

TABLE 4

Effect of NaF Concentrations on Rates of $Ca^{+2}$, $PO_4^{-3}$, $F^-$ and $OH^-$ Uptake During Remineralization of Enamel Surfaces as Determined by Chemical Analysis.

| Time Hr. | Uptake $\mu mol/cm^2 \times 10^{-2}$ (S.D.) | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0.5 | | | | 1.0 | | | | 1.5 | | | | 2.0 | | | | 3.0 | | | |
| NaF (mM) | $Ca^{+2}$ | $PO_4^{-3}$ | $F^-$ | $OH^-$ | $Ca^{+2}$ | $PO_4^{-3}$ | $F^-$ | $OH^-$ | $Ca^{+2}$ | $PO_4^{-3}$ | $F^-$ | $OH^-$ | $Ca^{+2}$ | $PO_4^{-3}$ | $F^-$ | $OH^-$ | $Ca^{+2}$ | $PO_4^{-3}$ | $F^-$ | $OH^-$ |
| 0.00 | 20 | 26 | - | 24 | 46 | 47 | - | 45 | 69 | 61 | - | 63 | 92 | 74 | - | 77 | 111 | 87 | - | 97 |
| | (6) | (6) | - | (3) | (9) | (5) | - | (5) | (10) | (7) | - | (6) | (10) | (8) | - | (7) | (8) | (6) | - | (9) |
| 0.05 | 25 | 28 | 4 | 23 | 53 | 54 | 9 | 44 | 79 | 71 | 13 | 62 | 99 | 84 | 18 | 78 | 129 | 95 | 26 | 102 |
| | (3) | (7) | (1) | (5) | (9) | (12) | (1) | (8) | (8) | (8) | (1) | (10) | (6) | (8) | (1) | (10) | (7) | (6) | (1) | (13) |
| 0.25 | 33 | 35 | 18 | 23 | 70 | 56 | 34 | 46 | 97 | 71 | 47 | 65 | 122 | 87 | 59 | 80 | 150 | 100 | 77 | 104 |
| | (9) | (10) | (4) | (6) | (8) | (7) | (1) | (10) | (12) | (8) | (3) | (12) | (10) | (5) | (3) | (13) | (6) | (6) | (2) | (13) |
| 0.50 | 46 | 37 | 36 | 23 | 84 | 56 | 65 | 47 | 110 | 74 | 86 | 65 | 134 | 87 | 101 | 80 | 157 | 98 | 122 | 99 |
| | (4) | (4) | (2) | (4) | (7) | (5) | (5) | (7) | (7) | (9) | (7) | (8) | (7) | (7) | (8) | (9) | (4) | (8) | (11) | (15) |
| 1.00 | 53 | 41 | 56 | 31 | 96 | 62 | 84 | 56 | 124 | 79 | 104 | 79 | 146 | 90 | 119 | 95 | 164 | 103 | 138 | 116 |
| | (4) | (6) | (10) | (8) | (7) | (4) | (11) | (13) | (5) | (3) | (9) | (16) | (7) | (2) | (8) | (18) | (6) | (3) | (9) | (20) |
| 2.00 | 50 | 29 | 116 | 26 | 91 | 49 | 160 | 51 | 119 | 68 | 189 | 72 | 139 | 80 | 211 | 87 | 162 | 94 | 238 | 108 |
| | (14) | (10) | (10) | (2) | (14) | (13) | (8) | (4) | (14) | (7) | (7) | (5) | (10) | (11) | (7) | (6) | (7) | (13) | (7) | (8) |
| 3.00 | 51 | 25 | 59 | 22 | 74 | 44 | 93 | 43 | 95 | 60 | 118 | 62 | 113 | 74 | 135 | 76 | 133 | 84 | 164 | 95 |
| | (17) | (9) | (10) | (2) | (14) | (12) | (9) | (6) | (17) | (10) | (10) | (8) | (13) | (8) | (5) | (10) | (15) | (10) | (9) | (13) |
| 4.00 | 32 | 30 | 40 | 26 | 56 | 51 | 72 | 51 | 71 | 63 | 102 | 69 | 90 | 68 | 113 | 81 | 106 | 92 | 128 | 106 |
| | (6) | (11) | (11) | (7) | (4) | (8) | (22) | (11) | (7) | (32) | (19) | (11) | (7) | (11) | (16) | (12) | (7) | (7) | (15) | (15) |

Means (+S.D.) in micromoles per $cm^2$ ($\mu m/cm^2$) of the effect of 1.5 mM $Ca^{+2}$, 1.0 mM $PO_4^{-3}$ with 0.00, 0.05, 0.25, 0.50, 1.00, 2.00, 3.00 and 4.00 mM NaF on etched bovine enamel surfaces (6 $cm^2$/8 ml of solution) on the subsequence of calcium, phosphate, fluoride and of uptake from the solution, while maintaining the fluoride levels constant at a constant pH of 7.0.

TABLE 5

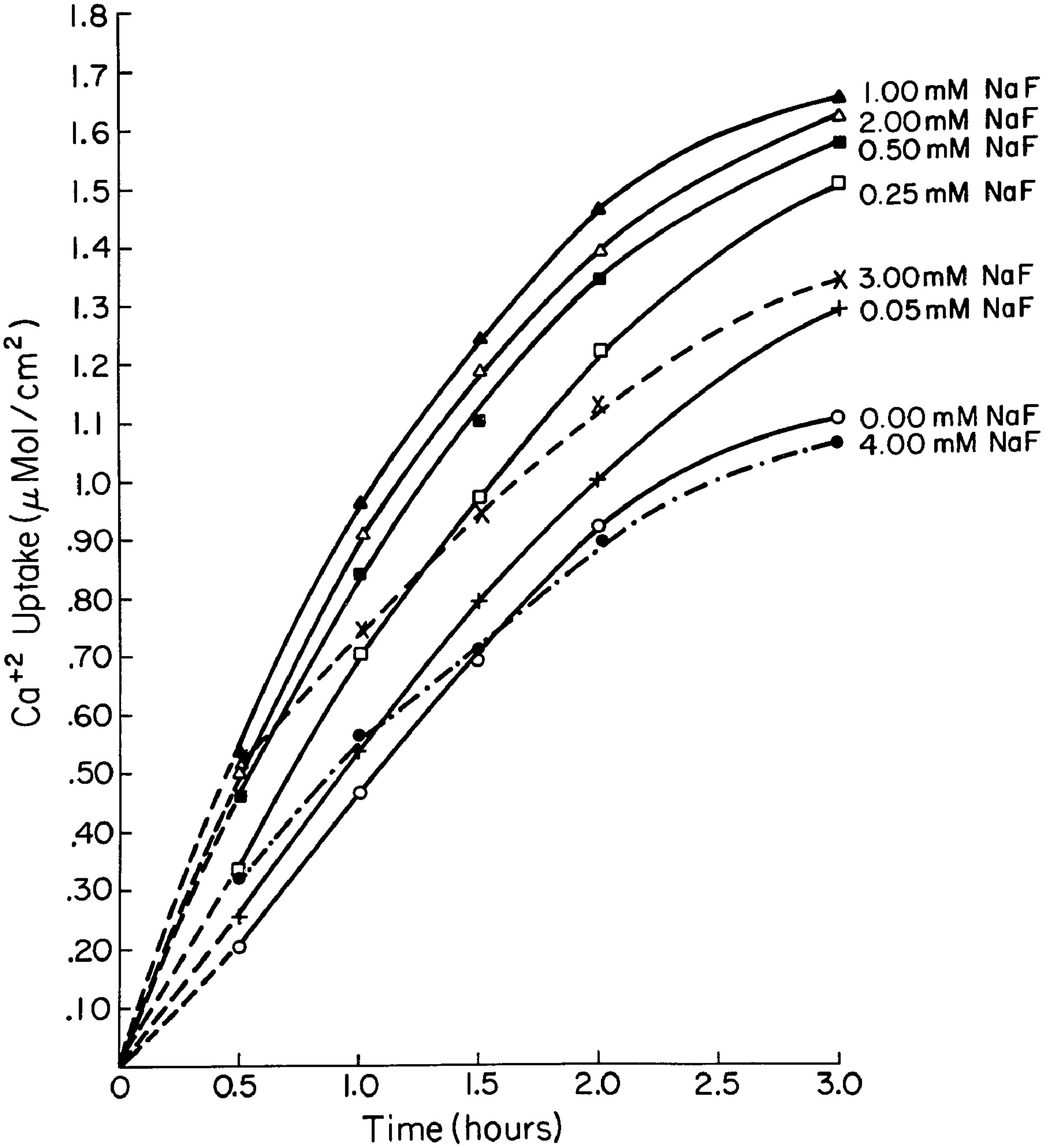
Statistical Comparisons of the Effects of NaF Concentrations on the Uptake of $Ca^{+2}$ During Remineralization of Enamel Surfaces.

| Levels Tested (mM) NaF | t-value | Conclusion |
|---|---|---|
| 0 vs. 0.05 | 3.3868* | S.D. $(0.02<p<0.05)$ |
| 0.05 vs. 0.25 | 4.5558* | S.D. $(0.01<p<0.02)$ |
| 0.25 vs. 0.50 | 1.9415 | N.D. $(p>0.10)$ |
| 0.25 vs. 1.00 | 3.2998* | S.D. $(0.02<p<0.05)$ |
| 0.50 vs. 1.00 | 1.9415 | N.D. $(p>0.10)$ |
| 0.50 vs. 2.00 | 1.2404 | N.D. $(p>0.20)$ |
| 0.50 vs. 3.00 | 3.0920* | S.D. $(0.02<p<0.05)$ |
| 1.00 vs. 2.00 | 0.4339 | N.D. $(p>0.50)$ |
| 1.00 vs. 3.00 | 3.8378* | S.D. $(0.01<p<0.02)$ |
| 2.00 vs. 3.00 | 3.5039* | S.D. $(0.02<p<0.05)$ |
| 3.00 vs. 4.00 | 3.2623* | S.D. $(0.02<p<0.05)$ |

Significant differences at the 0.05 confidence level of the uptake of calcium ($\mu m/cm^2$) between the different fluoride levels, after three hours of experiments.

Figure 6.

The effects of NaF concentrations in solutions on rates of $Ca^{+2}$ uptake in enamel surfaces as determined by chemical analysis. The data were obtained from the same experiments shown in Figure 4.



1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
.90
.80
.70
.60
.50
.40
.30
.20
.10
Ca+2 Uptake (μMol/cm2)
0
0.5
1.0
1.5
2.0
2.5
3.0
Time (hours)
1.00 mM NaF
2.00 mM NaF
0.50 mM NaF
0.25 mM NaF
3.00 mM NaF
0.05 mM NaF
0.00 mM NaF
4.00 mM NaF

2.00 mM. NaF concentrations above 1.00 mM decreased $Ca^{+2}$ uptake by the enamel.

Figure 7 shows the curves derived from the data in Table 4 for the relationship among $Ca^{+2}$, $PO_4^{-3}$, $F^-$ uptake and reactions of $OH^-$ and time of remineralization for enamel surfaces which were remineralized in the solution that contained 0.50 mM NaF. As noted from Table 4 the rates of these reactions increased with higher concentrations of NaF up to 1.00 mM. The rates of these reactions in the presence of 0.50 mM NaF were statistically the same as for solutions that contained 0.50 and 1.00 mM NaF.

The data shown in Table 4 were submitted to a computer program for curvilinear analysis (4). The mathematical model used was: $Y_t = K\ (1-e^{C-\beta^t})$. With $T = C/\beta$, the model was rearranged to $Y_t = K[1-e^{-\beta(t-T)}]$. The parameters were defined as follows:

$Y_t$ = Substance uptake at a given time (t) in $\mu$moles/cm$^2$,

K = Maximum uptake ($\mu$moles/cm$^2$),

$\beta$ = Relative Rate of uptake ($\mu$moles/cm$^2$/hr.),

C = Constant,

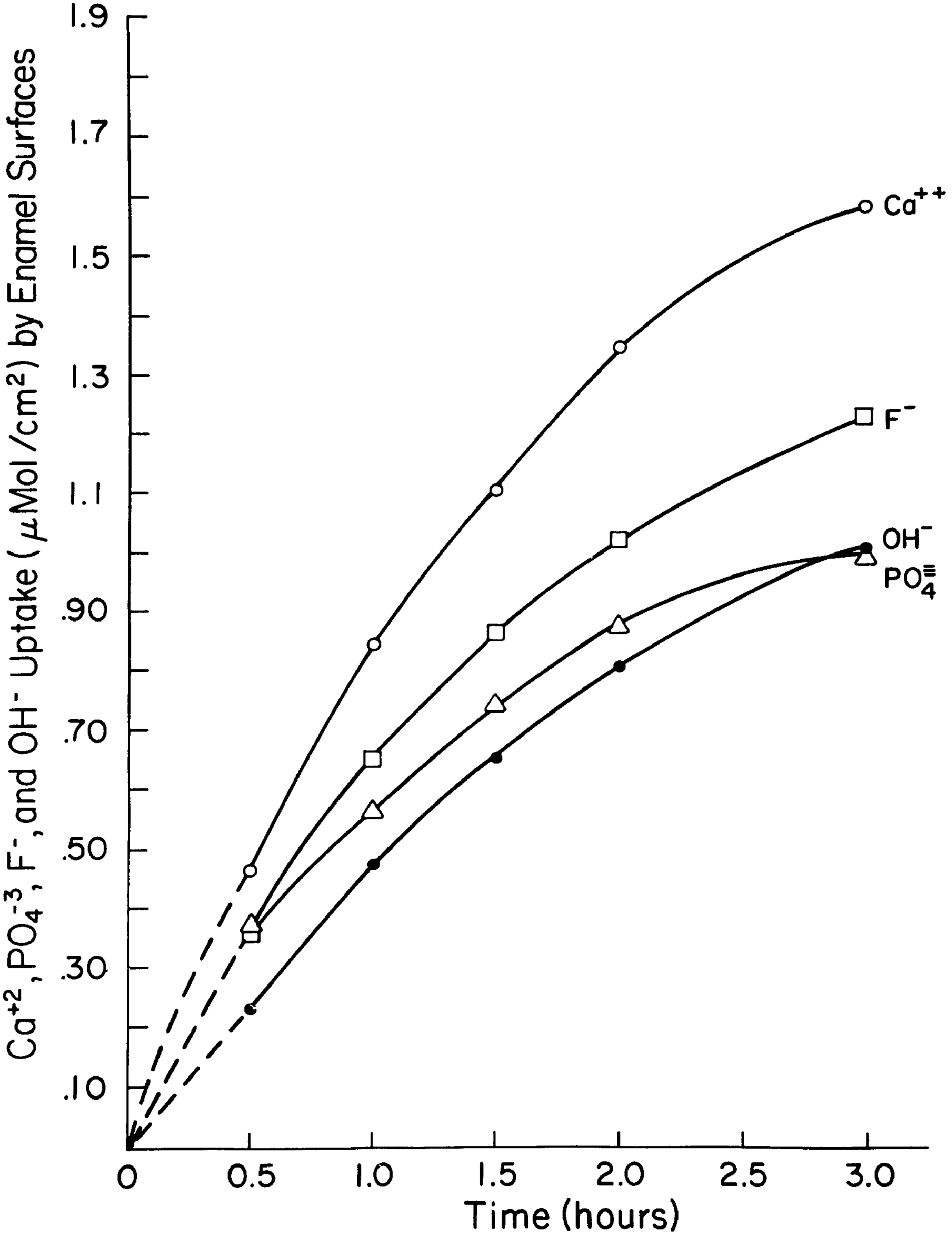
T = Lag.

Differentiation of this equation provides the relative rate of uptake. Then $\beta$ is inversely proportional to the amount of reactants remaining in solution $(K-Y_t)$.

These parameters were determined for $Ca^{+2}$, $PO_4^{-3}$, $F^-$ and $OH^-$ for each of the concentrations of NaF in the solutions using the described equation (see Appendix).

Figure 7.

Curves of the uptake of $Ca^{+2}$, $PO_4^{-3}$, $F^-$, and reactions of $OH^-$ with surfaces of enamel in solutions that contained a constant level of 0.5 mM NaF.



$Ca^{+2}, PO_4^{-3}, F^-$, and $OH^-$ Uptake ($\mu$Mol/cm$^2$) by Enamel Surfaces
1.9
1.7
1.5
1.3
1.1
.90
.70
.50
.30
.10
0
0.5
1.0
1.5
2.0
2.5
3.0
Time (hours)
$Ca^{++}$
$F^-$
$OH^-$
$PO_4^{\equiv}$

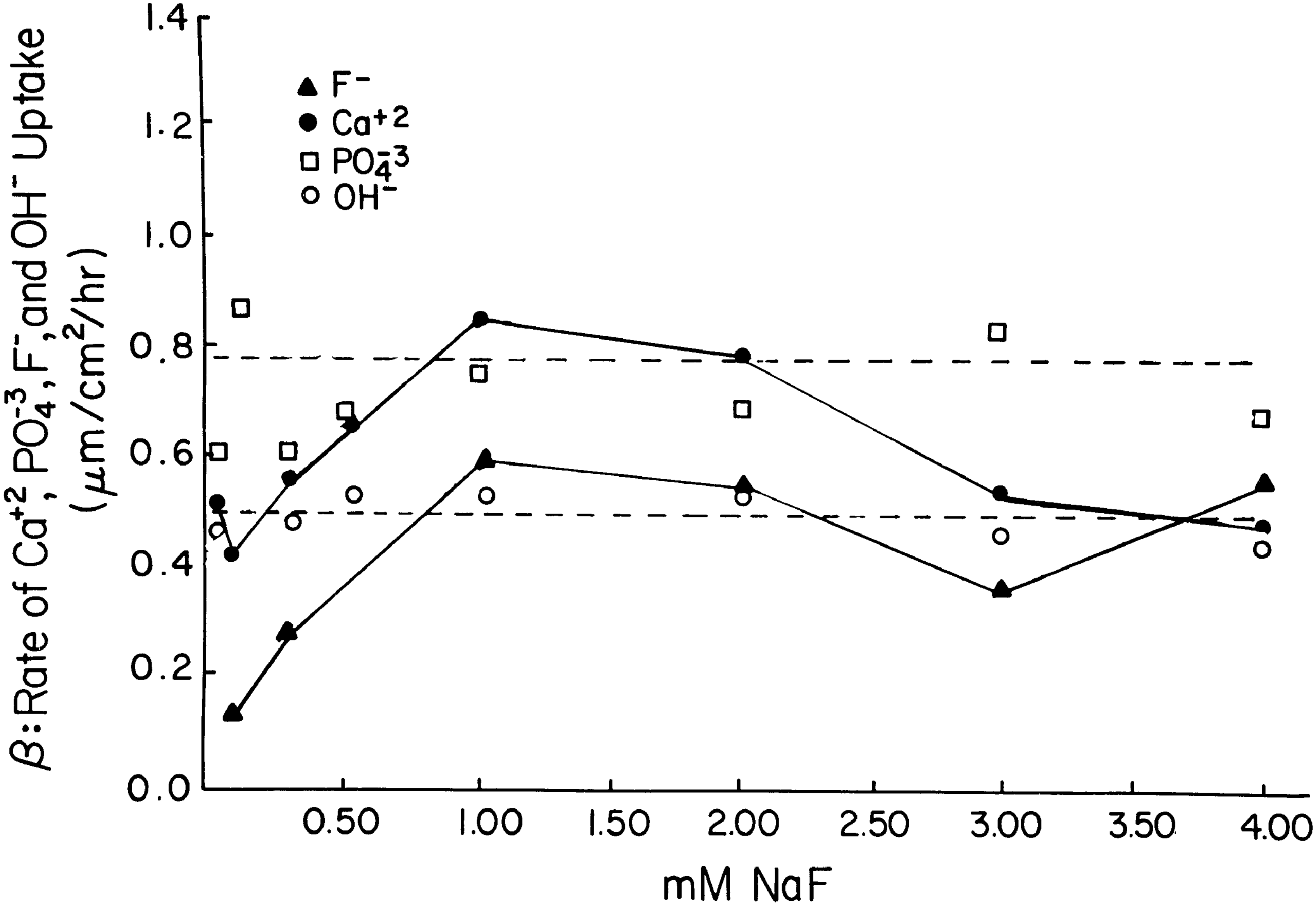
Correlations between the relative rate of uptake of $Ca^{+2}$, $PO_4^{-3}$, $F^-$, and $OH^-$ and the concentrations of NaF were then determined. These determinations showed that there was no correlation between the maximum uptakes and the levels of NaF. The rate of $Ca^{+2}$ and $F^-$ uptake ($\beta$ values) correlated with the concentrations of NaF, but $PO_4^{-3}$ and $OH^-$ uptake did not correlate with NaF.

Figure 8 shows the relationships, determined using the mathematical method, between the relative rates ($\beta$ values) of $Ca^{+2}$, $PO_4^{-3}$, $F^-$ and $OH^-$ uptake in the remineralized enamel and concentrations of NaF in the solutions. The rates of $Ca^{+2}$ and $F^-$ uptake correlated well with the levels of NaF. The rates of these two increased with increased levels of NaF up to 1.00 mM, plateaued between 1.00 and 2.00 mM NaF, then decreased with higher levels of NaF. It was obvious that the rates of $PO_4^{-3}$ and $OH^-$ reactions paralleled each other.

Table 6 shows the ratios of Ca/P and Ca/F for the apparent uptake of minerals by the enamel surfaces. The ratios of Ca/P increased with each higher concentration of NaF in solution up to about 2.00 mM. These ratios of Ca/P generally increased with time of remineralization (up to 2.00 mM NaF), but were almost constant with time for the solution that contained 2.00 mM NaF. The Ca/P ratios of mineral uptake from this particular solution (2.00 mM NaF) were similar to the 1.67 ratio of hydroxy or fluorapatite. The ratios of Ca/P decreased with levels of NaF

Figure 8.

The effects of NaF concentrations on rates of $Ca^{+2}$, $PO_4^{-3}$, $F^-$, and $OH^-$ reactions with enamel surfaces.


β: Rate of Ca⁺², PO₄⁻³, F⁻, and OH⁻ Uptake (µm/cm²/hr)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
F⁻
Ca⁺²
PO₄⁻³
OH⁻
0.50
1.00
1.50
2.00
2.50
3.00
3.50
4.00
mM NaF

## TABLE 6

Effects of NaF Concentrations in Solutions on the Ratios of Ca/P and Ca/F for the Uptake of Minerals by the Enamel Surfaces During Remineralization.

| | Time of Remineralization Reactions (Hr.) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0.5 | | 1.0 | | 1.5 | | 2.0 | | 3.0 | |
| NaF (mM) | Ca/P | Ca/F | Ca/P | Ca/F | Ca/P | Ca/F | Ca/P | Ca/F | Ca/P | Ca/F |
| 0.00 | 0.78 | - | 0.99 | - | 1.14 | - | 1.25 | - | 1.28 | - |
| 0.05 | 0.88 | 5.98 | 0.98 | 5.66 | 1.12 | 6.04 | 1.18 | 5.66 | 1.35 | 4.93 |
| 0.25 | 0.93 | 1.85 | 1.27 | 2.06 | 1.37 | 2.06 | 1.41 | 2.07 | 1.51 | 1.94 |
| 0.50 | 1.24 | 1.27 | 1.50 | 1.29 | 1.50 | 1.28 | 1.54 | 1.32 | 1.61 | 1.30 |
| 1.00 | 1.31 | 0.95 | 1.54 | 1.14 | 1.56 | 1.19 | 1.61 | 1.22 | 1.59 | 1.19 |
| 2.00 | 1.72 | 0.43 | 1.83 | 0.57 | 1.75 | 0.63 | 1.73 | 0.66 | 1.73 | 0.68 |
| 3.00 | 2.06 | 0.85 | 1.70 | 0.79 | 1.59 | 0.80 | 1.54 | 0.84 | 1.58 | 0.81 |
| 4.00 | 1.12 | 0.82 | 1.11 | 1.02 | 1.14 | 0.70 | 1.32 | 0.79 | 1.15 | 0.82 |

The effects of solutions containing 1.5 mM $Ca^{+2}$, 1 mM $PO_4^{-3}$ with 0.00, 0.05, 0.25, 0.50, 1.00, 2.00, 3.00 and 4.00 mM NaF on the Ca/P and Ca/F molar ratios of minerals taken up by etched bovine enamel surfaces. The fluoride level and pH (7.0) were maintained constant.

above 2.00 mM with only one exception.

The ratios of Ca/F for mineral uptake by the enamel surfaces from solution that contained 0.05 mM NaF ranged between 4.9 and 6.0 (the Ca/F ratio for pure fluorapatite is 5.0). The ratios of Ca/F decreased with each higher level of NaF in the solutions. These ratios derived from solutions that contained more than 2.00 mM NaF ranged between 0.4 and 0.9 (the Ca/F ratio for pure $CaF_2$ is 0.5).

Table 7 shows the ratios of P/F, OH/F, and P/OH for the reactions between the remineralizing solutions and the enamel surfaces at the 3 hour time of experimentation. As expected, the ratios of P/F and OH/F decreased for each higher level of NaF in the solutions up to 2.00 mM NaF. The small increases of these ratios for concentrations of NaF above 2.00 mM reflected the decreased uptake of $F^-$ in the enamel from the more concentrated solutions of NaF. The P/OH ratios of 0.87 to 0.99 were remarkably constant for the uptake of minerals in the enamel surface, regardless of the concentrations of NaF in the solutions. These P/OH ratios of remineralization showed that the deposition of calcium phosphate in the enamel was independent of the uptake of $F^-$.

TABLE 7

Effects of NaF Concentrations on the Molar Ratios of P/F, OH/F and P/OH for the Reactions of Enamel Surfaces Remineralization.

| NaF (mM) | Molar Ratios for the 3 Hour Time of Remineralization | | |
|---|---|---|---|
| | P/F | OH/F | P/OH |
| 0.00 | - | - | 0.90 |
| 0.05 | 3.65 | 3.92 | 0.90 |
| 0.25 | 1.30 | 1.35 | 0.93 |
| 0.50 | 0.80 | 0.81 | 0.96 |
| 1.00 | 0.75 | 0.84 | 0.99 |
| 2.00 | 0.40 | 0.85 | 0.89 |
| 3.00 | 0.51 | 0.58 | 0.87 |
| 4.00 | 0.72 | 0.83 | 0.88 |

Means of the effect of 1.5 mM $Ca^{+2}$, 1.0 mM $PO_4^{-3}$ with 0.05, 0.25, 0.50, 1.00, 2.00, 3.00 and 4.00 mM NaF on the P/F, OH/F, P/OH molar ratios for 3 hours of remineralization of etched bovine enamel surfaces maintaining the fluoride levels constant at pH 7.0.

# DISCUSSION

The $Ca^{+2}$ ion electrode used in this study responded rapidly to changes of $Ca^{+2}$ activity in solutions. The electrode responds logarithmically to changes of $Ca^{+2}$ (60). However, the changes of electrode potential, compared to zero time, provided linear relationships $Ca^{+2}$ uptake and time of remineralization. Changes of $Ca^{+2}$ in solutions determined by the spectrometric method provided hyperbolic curves for $Ca^{+2}$ uptake. The two methods gave the same results when the potentials were converted concentrations. The electrode method afforded the advantage of determination of $Ca^{+2}$ loss from the solutions without the necessity of sample removal from the system. Advantage was taken of the $F^-$ electrode and the $H^+$ electrode in this study both to maintain the activity of these ions at constant levels and to record the rates of their uptake in the enamel.

The significance of the $F^-$ effect on acid resistance of enamel was demonstrated in 1939 (80). Its effect on remineralization was reported in 1961 (11,46). These remarkable effects of $F^-$ probably cause the caries preventive actions of both fluoridation and surface applied fluoride agents. This study confirmed earlier reports that increased levels of fluoride up to about 0.50 mM NaF maximally increased the rates of enamel remineralization. Slightly less than the 0.50 mM concentration of $F^-$ in water supplies of rural schools effectively reduced caries in school aged children (36). The amounts of fluoride in dental plaque from members of fluoridated communities often exceeded concentrations equivalent to 0.5 mM NaF. This study indicated that the release of enamel and plaque $F^-$ would be expected to increase the rates of enamel remineralization after a brief period of acid production by bacteria in dental plaque.

Enamel remineralization <u>in vivo</u>, and <u>in vitro</u> under conditions similar to this study, has been well demonstrated. Most investigators have concluded that final products of remineralization in the absence of $F^-$ were octacalcium phosphate, $Ca_8H_2(PO_4)_6$, and hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$. In the presence of $F^-$, fluorapatite, $Ca_{10}(PO_4)_6F_2$, has been assumed to be one of the major products of enamel remineralization (12). The products deposited in enamel in the initial and early periods of remineralization have been assumed to be dicalcium phosphate and octacalcium phosphate (56). These acid calcium phosphates assumedly recrystallized to apatites by preferential uptake of $Ca^{+2}$ from solution and hydrolysis of $HPO_4^{-2}$ in the surface minerals (release of $H^+$ to the solution phase). This sequence has been conclusively established for the precipitation and growth of hydroxyapatite from highly concentrated solutions of calcium and phosphate (13). The Ca/P ratios for the minerals deposited in the enamel surfaces in this study indicated the initial formation of acid calcium phosphates unless the solutions contained exactly 2.00 mM NaF. Each of the NaF concentrations studied up to 2.00 mM NaF increased both the rates of remineralization and the apparent rates of conversion of the initial mineral phases to apatites. However, only the 2.00 mM NaF concentration caused the formation of minerals with almost constant Ca/P ratios approximately those of apatites throughout the time of remineralization.

The Ca/F ratios for the reactions between the solutions and the enamel surfaces were informative relative to the deposition of calcium phosphates. However, the Ca/P and Ca/F ratios could be misleading for interpretation of the chemistry of remineralized fractions of the surface minerals. The rates of $Ca^{+2}$ and $F^-$ uptake correlated to each other and to the concentrations of NaF, but not to the rates of $PO_4^{-3}$ and $OH^-$ reactions. The relationships of $Ca^{+2}$ and $F^-$ were practically constant throughout the time of experimentation for a given concentration of NaF in the solution. However, the net uptake of each of the studied ions represented the summation of adsorption, ion exchange, and remineralization in the enamel. The steady decreases of the Ca/F ratios with increased concentrations of NaF up to 2.00 mM apparently indicated that the deposited minerals contained progressively greater amounts of $CaF_2$.

The Ca/F ratios increased and $Ca^{+2}$ uptake by the enamel decreased in the presence of 3.00 and 4.00 mM NaF compared to 2.00 mM NaF. This possibly indicated that these higher levels of $F^-$ reduced the activity of $Ca^{+2}$ in the solutions. Alternatively, the rapid initial formation of $CaF_2$ could have blocked sites for the uptake of $Ca^{+2}$ and $F^-$ by exchange and remineralization in the enamel. Although small amounts were taken up by adsorption, $PO_4^{-3}$ uptake obviously represented remineralization. This was confirmed by the rates of $OH^-$ reactions. The inhibitory levels of NaF had little or no effect on $PO_4^{-3}$ and $OH^-$ reactions in terms of the ratios of P/OH for mineral deposit. This showed that the 3.00 and 4.00 mM NaF interfered with exchange of $Ca^{+2}$ and $F^-$ in the surface minerals of the enamel more so than the deposition of calcium phosphates.

Contrary to the conclusions of other investigators, using similar conditions of remineralization, the results of this study did not show the direct deposition of fluorapatite in the remineralized enamel (23,25). It was doubtful that fluorapatites were formed in the enamel except by exchange of $F^-$ from the solutions for $OH^-$ in the enamel minerals. Significant amounts of the $F^-$ uptake during remineralization probably occurred by adsorption (82) and by the formation of $CaF_2$ (24). The adsorbed $F^-$ and $CaF_2$ possibly would have been converted to fluorapatites if the reactions had been allowed to proceed further toward equilibrium. In this regard the general increase of the Ca/P ratios with increased time of reaction indicated the initial uptake of $HPO_4^{-2}$ and transformation of acid to less acid calcium phosphates in addition to the formation of $CaF_2$ in the surfaces. Subtraction of the amounts of $Ca^{+2}$ involved in $CaF_2$ formation indicated that the deposited calcium phosphate did not exceed the Ca/P ratio of octacalcium phosphate (Ca/P of 1.33) in any of the remineralized surfaces.

The data for the uptake of $Ca^{+2}$, $PO_4^{-3}$, $F^-$ and $OH^-$ by the enamel surfaces were submitted to various Growth Curves on the assumption that mineral uptake occurred by a growth process which could be described by these curves (65). However, the computerized model described previously seemed the best to represent the results obtained in this study. The model confirmed that the rates of $Ca^{+2}$ and $F^-$ uptake by the enamel increased with increased concentrations of NaF up to 1.00 mM in the solutions. No correlation was found for maximum uptake and the levels of NaF for good reason. The solutions contained limited amounts of $Ca^{+2}$ (2.00 μmole/cm$^2$) and $PO_4^{-3}$ (1.33 μmole/cm$^2$) available for uptake by the enamel, but unlimited amounts of $F^-$ and $OH^-$.

The maximum uptakes used in the chosen model were incorrectly estimated using the collected data for 3 hours as the maximum possible uptake. The total amount of $Ca^{+2}$ and $PO_4^{-3}$ in the solutions at the beginning of remineralization should have been used in the equation as the maximum possible uptake value (K). Despite this discrepancy the data clearly showed that the rates of remineralization and $F^-$ uptake were directly dependent on the concentration of NaF up to 0.50 to 2.00 mM. Earlier studies showed linear rates of $Ca^{+2}$, $PO_4^{-3}$ and $F^-$ uptake by enamel surfaces when the concentrations of each of the reactants were maintained constant (25).

The conditions of these experiments were purposefully established so that the extent of remineralization would be limited by the availability of $Ca^{+2}$ and $PO_4^{-3}$. The purpose was to restrict mineral deposition to sites within the surface of the enamel under as sensitive conditions as possible. It was believed that too rapid rates of mineral uptake would allow growth on as well as in the surfaces. Thus as remineralization with calcium phosphate proceeded, the solutions became progressively less saturated in the composition of the depositing minerals. In these conditions of limited availability of $Ca^{+2}$ and $PO_4^{-3}$, and unlimited $OH^-$ and $F^-$, the rates of $F^-$ uptake correlated to the uptake of $Ca^{+2}$. The rates of $OH^-$ reactions were directly related to $PO_4^{-3}$ uptake in the enamel even though $OH^-$ reactions involved exchange for $F^-$ and neutralization of $HPO_4^{-2}$ in the surfaces, as a result of the initial acid demineralization.

The acid demineralization of the surfaces, before the remineralization reactions, has been shown preferentially to dissolve crystallites in the central region of the enamel prisms (42,71). Surfaces remineralized 24 hours in solutions that contained relatively unlimited amounts of $Ca^{+2}$ and $PO_4^{-3}$, and 0.40 mM NaF, exhibited growth of crystals in the acid demineralized enamel prisms (77). Electron micrographs of surfaces demineralized exactly as in this study showed that sites for reactions of remineralization in the enamel were not restrictive relative to the maximum amounts of $Ca^{+2}$ and $PO_4^{-3}$ available in this study. Mild acid etching of intact surfaces of enamel dissolves the central portions of enamel crystallites and also dissolves some of the minerals of the outer surfaces of the crystallites. Therefore, the acid demineralization possibly created reactive sites for remineralization within as well as on the crystals of enamel (71).

The numbers and characteristics of the sites of $Ca^{+2}$, $PO_4^{-3}$ and $F^-$ reaction in the enamel are unknown for any conditions. The reactivity of the sites probably depends on several factors such as acid strength, type of acid, and extent of demineralization. Studies have also indicated that the initial reactions in the surface of enamel depended on the composition of the solutions used for remineralization and whether the acid etched surfaces were rinsed extensively or slightly rinsed (77). The results herein suggested that the surfaces initially adsorbed $PO_4^{-3}$ (as $HPO_4^{-2}$) from the solutions which later participated in remineralization. There was no doubt that most of the $F^-$ uptake occurred by adsorption and exchange in the presence of 0.05 and 0.25 mM NaF and additionally as $CaF_2$ in the presence of more than

0.25 mM NaF. The deposition of calcium phosphates during $CaF_2$ formation from solutions of 3.0 and 4.0 mM NaF indicated the possibility of competition for reactive sites. The competition reduced the enamel uptake of both calcium phosphates and $CaF_2$. The concurrent uptake of each of the reactants by adsorption and ionic exchange during remineralization prevented more exact interpretation of the results. However, submission of data to the Freundlich (49) and Pruitt models (64) failed to show that adsorption accounted for significant uptake of mineral constituents in the enamel.

The conditions of the experiments in this study were not intended to reproduce all of the variables involved in remineralization in the mouth. However, the model system demonstrated the reactions to be expected between minerals of early carious enamel and $F^-$, $Ca^{+2}$ and $PO_4^{-3}$ in an aqueous environment at pH 7.0. The lowest level of $F^-$ studied, which was the same as the amount in fluoridated water, significantly increased the rates of enamel remineralization. The concentration of 0.50 mM NaF caused maximum rates of remineralization. Previous evidence indicated that remineralization in the presence of 0.50 mM NaF, or additions of this level of NaF in acids, caused almost maximum acid resistance of enamel (27). Comparable levels of fluoride may be readily attained in dental plaque by fluoridation or by other direct means of application of fluoride and thereby prevent caries.

# EFFECTS OF FLUORIDE ON INITIAL RATES OF ION EXCHANGE, REMINERALIZATION, AND ACID RESISTANCE OF SURFACE ENAMEL

## INTRODUCTION

Maturation of enamel has been defined as the compositional changes of intact surface enamel of erupted teeth (28). The degree of maturation depends on unknown factors including the post-eruptive age of the tooth. Conceptually it includes all aspects of acid demineralization and remineralization of the surface enamel. However, maturation of newly erupted teeth, as remineralization, may occur without prior acid demineralization of the enamel (45). One major problem of dealing with these conceptual terms, which are used to describe the surface changes of enamel, is that they do not have a definite meaning (45, 72,77). The processes of maturation and remineralization obviously depend on ionic exchange, recrystallization, and inclusion of organic substances of oral fluids and dental plaque.

The mechanisms of maturation and remineralization seem to be similar if not identical. Thus the term remineralization is used herein to encompass maturation and recrystallization of enamel. Remineralization probably best describes the chemical reactions of surface enamel relative to net gain of minerals. Recrystallization should not be used to describe ion exchange or recrystallization unless the crystalline character of the reactants of surface enamel are determined. Regardless of the terminology, the study of post-eruptive reactions of minerals of surface enamel provides one of the most rational mechanisms of $F^-$ action for caries prevention. The formation of sparingly soluble fluorapatites in surface enamel undoubtedly plays a significant role in caries prevention (10).

Mild acid demineralization of enamel, as in the first stage of caries, provides reactive surface sites for the rapid uptake of $F^-$ by adsorption, exchange and remineralization. Repetitive episodes of demineralization and remineralization of a dynamically balanced nature might lead to caries resistant tooth surfaces. Balanced slightly in favor of remineralization, which seems to occur in the presence of $F^-$, remineralization is both reparative and defensive (45). Despite the fact that most evidence is circumstantial, it is assumed that the primary action of $F^-$ on caries prevention is the formation of fluorapatite in tooth minerals (80).

Changes can be produced rapidly in enamel by addition of fluoride to the surrounding fluid medium. Increased acid resistance of the enamel is the first change noted by the action of $F^-$ in solutions. Feagin et al. showed that some of the fluoride of remineralized enamel when released in weak acids, or simply the addition of fluoride ions in the weak acids, reduced the rates of enamel dissolution (27). The exact mechanism of this effect is still unknown. However, the fact that the composition of the environment at the enamel-solution interface has an effect on its action is well known (20).

In recent studies, it was found that 0.50 mM $F^-$ (10 ppm) caused maximum rates of enamel surface remineralization (2). The purpose of this study was to determine the effects of $F^-$ in solution on the rates of $^{45}Ca$ uptake in enamel by ion exchange and remineralization and on the subsequent acid resistance of these surfaces. The aim was to determine the initial effects of $F^-$ on these reactions within a time frame that could be utilized in the practice of preventive dentistry.

## METHODS

### 1. Preparation of Enamel Surfaces

The crowns of bovine incisor teeth were cut to provide a 1 $cm^2$ surface of intact labial enamel as recently described (2). The pieces of tooth were painted with fingernail polish and mounted on plastic plates with wax to leave the labial surface of enamel exposed as illustrated in figure 9. The exposed enamel was lightly pumiced using an aqueous slurry and dental prophylaxis procedure. In groups of 5 the surfaces were exposed for 24 hours in 500 ml of 10 mM acetic acid of pH 5.5, with slow rate stirring. The acid exposed surfaces were briefly rinsed in 500 ml of deionized water and either given an additional 24 hour rinse in deionized water or immediately subjected to exposure in experimental solutions. The different rinse procedures were used to determine the possible effects of residual acid products in the surfaces on subsequent reactions.

### 2. Experimental Solutions

The experimental solutions for ion exchange and remineralization were prepared from the following separate stock solutions: 50 mM $CaCl_2$, 50 mM $NaH_2PO_4$, 25 mM NaF, 1.0 M KCl and 17 mCi $^{45}Ca$ as 1 mg of $CaCl_2$. The isotope was diluted with distilled water to provide a stock solution that contained 1.0 μCi/ml, which was further diluted in the experimental solutions so that each contained 0.04 μCi/ml. The solutions used to study ion exchange in the absence of remineralization contained: 0.04 μCi $^{45}$Ca/ml, 40 mM KCl, and 0.00, 0.05, 0.50 or 5.00 mM NaF, each at pH 7.0. The solutions used to study remineralization were prepared to contain 1.5 mM $CaCl_2$, 1.0 mM $NaH_2PO_4$, 80 mM KCl and 0.00, 0.05, 0.50 or 5.00 mM NaF. These solutions also contained 0.04 μCi $^{45}$Ca/ml and were made to pH 7.0.

## 3. Procedure of Experimentation

### A. Ion Exchange and Remineralization

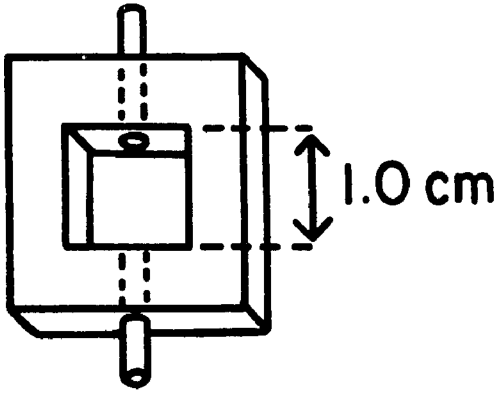
Two of the acid exposed surfaces of enamel were enclosed in a small flow chamber as shown in figure 9. Thus two of the 1 $cm^2$ surfaces were exposed to a continuous flow of solution for one-half hour using constant flow rate of 0.5 ml per minute. The flow-off from the chamber was collected in plastic tubes (Fig. 10) at 10-minute intervals (5 ml per collection) for chemical analyses. Five experimental runs of two surfaces each were performed for each level of $F^-$ in the solutions. Each group of 5 experiments contained surfaces which were either briefly rinsed or surfaces that were rinsed 24 hours after the 24 hour acid demineralization.

### B. Determination of Acid Resistance of Ion Exchanged and Remineralized Surfaces of Enamel

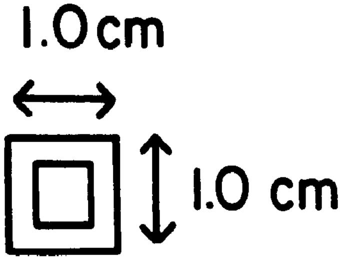
One of the two enamel surfaces was removed from the flow chamber at the 0.5 hour time of exposure to solutions of pH 7.0. The surface remaining in the chamber was briefly

Figure 9.

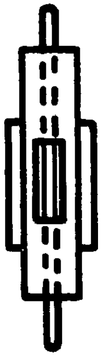
Illustration of the methods of attachement of enamel surfaces on plastic plates and enclosure in small flow chamber for solution exposure at pH 7.0 and 4.5. Each piece of enamel was attached with wax on the plastic plate. The plate was sealed with wax and used as a side of the flow chamber as indicated.



Empty Chamber



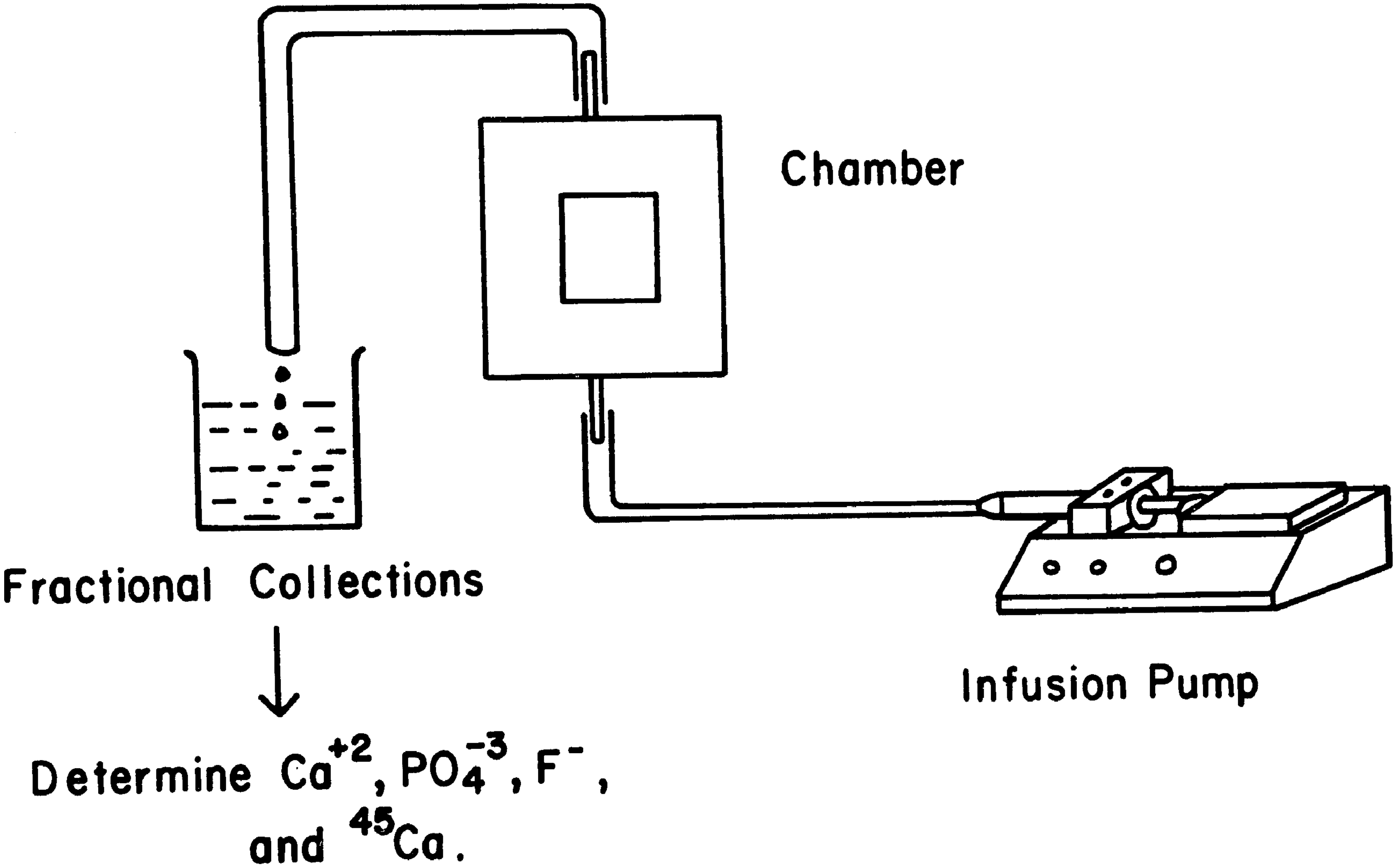
Enamel Surface



Side View

Figure 10.

Schematic diagram of the system used to expose surfaces of enamel to experimental solutions. The solutions at pH 7.0 and 4.5 were flowed at constant rates through the chamber to contact the enamel surfaces. The off-flow was collected at 10-minute intervals in plastic tubes for chemical analyses. In this way the surfaces were exposed to a continuous flow of solution in constant volume.


Chamber
Infusion Pump
Fractional Collections
Determine $Ca^{+2}$, $PO_4^{-3}$, $F^-$, and $^{45}Ca$.

rinsed with deionized water before resealing the chamber with a blank plate of plastic (Fig. 9). Ten mM acetic acid at pH 4.5 was pumped through the chamber at a constant rate of 1.0 ml per minute to immerse the enamel surface. The flow from the chamber was collected in plastic tubes in 3 sequential 10-minute intervals. The amounts of $^{45}Ca$, $Ca^{+2}$, $PO_4^{-3}$, and $F^-$ that dissolved from the surface of enamel were measured in the collections of acid. Groups of 5 surfaces were used to determine acid resistance for each of the studied levels of NaF in the ion exchange solutions and the remineralization solutions. Separate groups of 5 surfaces each were used from the briefly rinsed and 24 hour rinsed surfaces for each of the studied levels of NaF in the solutions.

## 4. Chemical Analyses

The $Ca^{+2}$ (68) and $PO_4^{-3}$ (15) were determined in each of the solutions after contact with the surfaces. The fluoride ion specific electrode was used to measure $F^-$ in the solutions (50). The differences in the amounts of $^{45}Ca$, $Ca^{+2}$, $PO_4^{-3}$ and $F^-$ in the solutions before and after contact with the enamel were calculated on the basis of uptake or dissolution from the surfaces. The $^{45}Ca$ in solutions was determined by liquid scintillation. The mean data of each set of 5 experimental surfaces of enamel were compared for statistical differences at the 0.05 level of confidence.

## RESULTS

### 1. Acid Demineralization of Enamel Surfaces

Table 8 shows the amounts of $Ca^{+2}$ and $PO_4^{-3}$ that dissolved from the pumiced surfaces of enamel in the 24 hour exposure to 500 ml of the 10 mM acetic acid. The minerals dissolved in Ca/P ratio of 1.7 when analyzed at the 24 hour time of acid exposure. The initial pH of the acid of 5.5 changed to 6.1 during the dissolution of the surface minerals. The table also shows the small amounts of $Ca^{+2}$ and $PO_4^{-3}$ that dissolved in the 24 hour rinse of the acid demineralized surfaces. These water soluble minerals dissolved in a mean Ca/P ratio of 1.5 without affecting the pH of the water. The 24 hour rinsed and other groups of briefly rinsed surfaces were used to study ion exchange reactions and remineralization of enamel.

### 2. Studies of Ion Exchange Reactions Between Demineralized Surfaces of Enamel and Solutions that Contained Trace Amounts of $Ca^{+2}$ as $^{45}Ca$, 40 mM KCl, 0.00, 0.05, 0.50 and 5.00 mM NaF at pH 7.0

Table 9 shows the levels of $^{45}Ca$ in the ion exchange solutions at the 0, 10, 20 and 30 minute intervals of contact with the enamel surfaces. The solutions were pumped through a small chamber to contact two of the $cm^2$ surfaces of enamel

TABLE 8

Relationships of $Ca^{+2}$, $PO_4^{-3}$, and pH for 24 Hour Demineralization and Subsequent 24 Hour Water Rinse of Labial Surfaces of Bovine Enamel.

| | Minerals Dissolved, μMol/$cm^2$ (S.D.) | | | |
|---|---|---|---|---|
| Conditions* | $Ca^{+2}$ | $PO_4^{-3}$ | Ca/P | Final pH |
| 10 mM Acetic Acid, pH 4.5 | 29.4(1.5) | 16.9(0.9) | 1.7(0.2) | 6.1(0.2) |
| Deionized $H_2O$, pH 7.0 | 1.3(0.2) | 0.9(0.2) | 1.5(0.2) | 7.0(0.0) |

* In each of 7 experiments, five surfaces of enamel were exposed in 500 ml of 10 mM acetic acid of pH 5.5 with constant stirring for 24 hours. Each group of 5 surfaces were then briefly rinsed in a flow of water, then stirred 24 hours in 500 ml of deionized water.

TABLE 9

The Effects of NaF on the Uptake of $^{45}$Ca in Enamel During 0.5 Hour Exposure to Solutions Under Conditions of Ion Exchange at pH 7.0.

| | | $^{45}$Ca in Solution at Minute: CPM/ml X $10^4$ (S.D.) | | | | |
|---|---|---|---|---|---|---|
| Surface Rinse* | Solution NaF (mM) | 0 | 10 | 20 | 30 | Total Uptake ** |
| Brief | - | 11.3(.4) | 10.4(.5) | 10.2(.4) | 10.4(.5) | 26(2) |
| 24 Hrs. | - | 11.9(.4) | 10.8(.4) | 10.6(.5) | 10.6(.5) | 30(5) |
| Brief | 0.05 | 12.5(1) | 11.2(.1) | 11.3(.4) | 11.5(1) | 26(1) |
| 24 Hrs. | 0.05 | 12.1(.4) | 10.7(1) | 10.8(.1) | 11.0(.1) | 30(3) |
| Brief | 0.50 | 12.2(.4) | 10.9(.4) | 11.1(.2) | 10.9(.1) | 32(2) |
| 24 Hrs. | 0.50 | 13.3(.3) | 11.9(.4) | 11.6(.2) | 11.3(.1) | 38(4) |
| Brief | 5.00 | 12.5(.1) | 11.0(.2) | 11.3(.1) | 11.3(.1) | 30(4) |
| 24 Hrs. | 5.00 | 12.4(.2) | 10.8(.1) | 11.1(.1) | 11.2(.2) | 33(2) |

* Acid demineralized surfaces were either briefly rinsed in a flow of deionized water or briefly rinsed and then again rinsed 24 hours. The surfaces in group of two each were then exposed to a flow of solution at pH 7.0 that contained 40 mM KCl, the isotope and the indicated concentrations of NaF. The 2 surfaces were exposed to the solution at a flow rate of 0.5 ml per minute. The solution was collected at 10-minute intervals.

**Total uptake of $^{45}$Ca in 0.5 hour was calculated as the percentage of the amounts of isotope lost from the solutions during contact with enamel.

at a flow rate of 0.5 ml per minute so that the two surfaces were constantly in contact with 1 ml of the solution. The levels of $^{45}Ca$ were determined in successive 5 ml (10-minute) collections of the solution after contact with the enamel. The $^{45}Ca$ in each of the collections of the solution were compared to the level of $^{45}Ca$ in the solution at time zero.

Changes in amounts of $^{45}Ca$ in solution were attributed to uptake by the enamel. The uptake of $^{45}Ca$ was shown in Table 9 as the percentage of the amounts available in the solutions. The differences in amounts of $^{45}Ca$ in the collections of solution after contact with the enamel and the amounts in solutions at zero time were almost the same at each time interval and for each experimental group of surfaces. There was a trend for slightly greater uptake of $^{45}Ca$ in 24 hour rinsed than briefly rinsed surfaces. This trend was not significant except for solutions that contained 0.50 mM NaF. The presence of 0.05 mM NaF in solutions had no remarkable effects on the uptake of $^{45}Ca$ under these conditions. However, the presence of 0.50 mM NaF in the solutions significantly increased the uptake of $^{45}Ca$ particularly in the 24 hour rinsed surfaces.

Table 10A shows the concentrations of $F^-$ in the solutions at the 0, 10, 20 and 30 minute intervals of enamel-solution contact. The uptake of $F^-$ in the enamel was calculated by difference of fluoride content of the solution at the zero time and $F^-$ content in each collection

## TABLE 10A

The Effects of NaF on the Uptake of $F^-$ in Enamel During 0.5 Hour Exposure to Solutions Under Conditions of Ion Exchange at pH 7.0: Changes in Concentrations of $F^-$ in Solutions.

| | | $F^-$ in Solution at Minute: mM X $10^{-2}$ (S.D.) | | | |
|---|---|---|---|---|---|
| Surface Rinse* | Solution NaF (mM) | 0 | 10 | 20 | 30 |
| Brief | - | - | - | - | - |
| 24 Hrs. | - | - | - | - | - |
| Brief | 0.05 | 5.0(0) | 4.2(0) | 4.7(0) | 4.4(0) |
| 24 Hrs. | 0.05 | 5.0(.2) | 4.2(.2) | 4.7(.1) | 4.4(.1) |
| Brief | 0.50 | 5.0(.3) | 4.3(.1) | 4.3(.2) | 4.4(.2) |
| 24 Hrs. | 0.50 | 43(1) | 41(1) | 42(1) | 42(1) |
| Brief | 5.00 | 432(11) | 400(10) | 396(10) | 396(10) |
| 24 Hrs. | 5.00 | 421(10) | 394(11) | 395(10) | 400(10) |

* Conditions and surfaces were the same as described for Table 9. The levels of NaF in each 10 minute collection of solution after contact with surface of enamel were compared to the levels at 0 time.

of solution after contact with the enamel. Simply multiplying the $F^-$ concentration µmol/ml by 5 ml and dividing the product by 2 (two-1 $cm^2$ surfaces in each chamber) and subtracting this value from the $F^-$ content of the solution at the zero time gives the uptake of $F^-$ in the enamel as µmol/$cm^2$ (see Table 10B). The uptake of $F^-$ in the enamel was almost the same at each time interval for a group of surfaces in a given solution. The uptake of $F^-$ increased for each higher level of $F^-$. The $F^-$ uptake was significantly greater with increasing fluoride levels, except between 0.05 and 0.50 mM NaF for 24 rinsed surfaces. The 24 hour rinsed surfaces took up significantly less $F^-$ than briefly rinsed surfaces except for solutions that contained 0.05 mM NaF.

Table 11 shows the concentrations of $Ca^{+2}$ found in the solutions after contact with the enamel surfaces. Obviously these concentrations of $Ca^{+2}$ resulted from dissolution of surface minerals of the enamel. The solutions contained no $Ca^{+2}$ or $PO_4^{-3}$ before contact with the enamel. The concentrations of $Ca^{+2}$ attained in the solutions did not exceed 0.03 mM. The total amounts of $Ca^{+2}$ that dissolved from the surfaces under these conditions were very small, but accurately measured. Apparently smaller amounts of $Ca^{+2}$ dissolved from the 24 hour rinsed than briefly rinsed surfaces, but only in the absence of NaF. The presence of NaF in the solutions reduced the amounts of $Ca^{+2}$ that dissolved from the surfaces, but the reduction was not

Table 10B

Effects of NaF in Solutions on the Uptake of $F^-$ in Enamel Under Conditions of Ion Exchange at pH 7.0: Calculated Uptake of $F^-$ in the Enamel.

| | | $F^-$ Uptake at 10 Minute Intervals: $\mu mol/cm^2 \times 10^{-2}$ (S.D.) | | | |
|---|---|---|---|---|---|
| Surface Rinse | Solution NaF (mM) | 10 | 20 | 30 | Total |
| Brief | - | - | - | - | - |
| 24 Hrs. | - | - | - | - | - |
| Brief | 0.05 | 3(.1) | 2(.1) | 2(.1) | 7(1) |
| 24 Hrs. | 0.05 | 2(.1) | 2(.1) | 2(.2) | 6(1) |
| Brief | 0.50 | 11(4) | 7(3) | 9(3) | 27(9) |
| 24 Hrs. | 0.50 | 5(1) | 4(1) | 4(1) | 13(13) |
| Brief | 5.00 | 79(2) | 89(14) | 89(14) | 257(22) |
| 24 Hrs. | 5.00 | 64(13) | 62(12) | 53(10) | 175(21) |

* Conditions and surfaces were the same as described for Table 9.

## Table 11

Effects of NaF in Solutions Under Conditions of Ion Exchange on $Ca^{+2}$ Dissolution from Enamel Surfaces at pH 7.0: Concentrations of $Ca^{+2}$ in Solutions.

| | | $Ca^{+2}$ Dissolved at Minute: mM X $10^{-3}$ (S.D.) | | | |
|---|---|---|---|---|---|
| Surface Rinse | Solution NaF (mM) | 10 | 20 | 30 | Total |
| Brief | - | 30(1) | 20(9) | 10(7) | 13(9) |
| 24 Hrs. | - | 10(1) | 10(1) | 10(1) | 6(1) |
| Brief | 0.05 | 20(0) | 20(5) | 10(4) | 12(1) |
| 24 Hrs. | 0.05 | 20(4) | 10(1) | 1(0) | 11(2) |
| Brief | 0.50 | 11(0) | 8(1) | 1(0) | 7(0) |
| 24 Hrs. | 0.50 | 8(1) | 7(1) | 8(1) | 5(1) |
| Brief | 5.00 | 6(1) | 5(1) | 5(1) | 4(2) |
| 24 Hrs. | 5.00 | 8(1) | 7(1) | 7(1) | 6(1) |

Conditions and surfaces were the same as described for Table 9. The solutions used for ion exchange contained no $Ca^{+2}$ or $PO_4^{-3}$ at the initial time of contact with the enamel surfaces. The table values show the concentrations of $Ca^{+2}$ that dissolved in each 10 minutes flow of solution after contact with two 1-$cm^2$ surfaces of enamel.

* Total dissolved in 0.5 hr. as $\mu mol/cm^2$ X $10^{-2}$ (S.D.).

significantly greater with increasing concentrations of NaF levels.

Table 12 shows the concentrations of $PO_4^{-3}$ found in the solutions that corresponded to the $Ca^{+2}$ shown in Table 11. The amounts of $PO_4^{-3}$ that dissolved from the surfaces were also small under these conditions. Except for the 24 hour rinsed surfaces in the absence of NaF, the amounts of $PO_4^{-3}$ that dissolved were greater than the amounts of $Ca^{+2}$ that dissolved from the surfaces. This was particularly noted in the presence of 5 mM NaF.

### 3. Effects of 0.5 Hour Exposure of Enamel in Solutions of NaF Under Conditions of Ion Exchange on Acid Dissolution of Surface Minerals.

After 0.5 hour contact with solutions of pH 7.0 that contained 40 mM KCl and 0.00, 0.05, 0.50 and 5.00 mM NaF, the enamel surfaces were each enclosed in a separate flow chamber. Acetic acid (10 mM) at pH 4.5 was pumped through the chamber at a rate of 1.0 ml per minute so that the surface was constantly immersed in about 1 ml of the acetic acid. The flow from the chamber was collected in 3 sequential 10 ml (10-minutes) fractions. The amounts of $^{45}Ca$, $Ca^{+2}$, $PO_4^{-3}$ and $F^-$ in the acid were determined and calculated as the amount that dissolved from the 1 $cm^2$ surface.

Table 13 shows the amounts of $^{45}Ca$ that dissolved in the acid from the exchanged surfaces. The amounts of $^{45}Ca$ in the collections (c.p.m./ml) were changed to represent the

Table 12

Effects of NaF in Solutions Under Conditions of Ionic Exchange on $PO_4^{-3}$ Dissolution from Enamel Surfaces at pH 7.0: Concentrations of $PO_4^{-3}$ in Solutions.

| Surface Rinse | Solution NaF (mM) | $PO_4^{-3}$ Dissolved at Minute: mM X $10^{-3}$ (S.D.) | | | |
|---|---|---|---|---|---|
| | | 10 | 20 | 30 | Total* |
| Brief | - | 20(5) | 20(5) | 20(6) | 19(3) |
| 24 Hrs. | - | 3(1) | 5(2) | 3(1) | 3(1) |
| Brief | 0.05 | 10(5) | 10(1) | 10(0) | 10(2) |
| 24 Hrs. | 0.05 | 20(0) | 20(0) | 20(0) | 17(1) |
| Brief | 0.50 | 20(0) | 20(0) | 10(1) | 13(0) |
| 24 Hrs. | 0.50 | 10(0) | 10(0) | 10(0) | 10(1) |
| Brief | 5.00 | 40(5) | 30(30 | 20(2) | 23(2) |
| 24 Hrs. | 5.00 | 50(1) | 40(1) | 30(1) | 28(1) |

Conditions and surfaces were the same as described for Table 9. The solutions used for ion exchange contained no $Ca^{+2}$ or $PO_4^{-3}$ at the initial time of contact with the enamel surfaces. The table values show the concentrations of $PO_4^{-3}$ in each 10 minutes flow of solution after contact two 1-$cm^2$ surfaces of enamel.

* Total uptake at 0.5 hr.: $\mu mol/cm^2 \times 10^{-2}$ (S.D.).

## TABLE 13

Effects of 0.5 Hour Exposure in Solutions of NaF on the Subsequent Recovery of Exchanged $^{45}$Ca from Surfaces of Enamel in Acid*.

| Surface Rinse | Solution NaF, mM | Percentage Recovery of $^{45}$Ca in 10 Minute Collections of Acid (S.D.) | | | Total** |
|---|---|---|---|---|---|
| | | 10 | 20 | 30 | |
| Brief | - | 20(3) | 6(1) | 4(1) | 30(5) |
| 24 Hrs. | - | 15(2) | 5(1) | 3(1) | 23(4) |
| Brief | 0.05 | 9(1) | 4(1) | 2(1) | 15(2) |
| 24 Hrs. | 0.05 | 8(1) | 4(1) | 3(1) | 15(1) |
| Brief | 0.50 | 4(1) | 2(1) | 1(0) | 7(2) |
| 24 Hrs. | 0.50 | 6(2) | 2(1) | 2(1) | 10(3) |
| Brief | 5.00 | 9(0) | 4(0) | 3(0) | 16(1) |
| 24 Hrs. | 5.00 | 12(1) | 4(1) | 3(1) | 19(1) |

* Surfaces of enamel were demineralized 24 hours in weak acid then either briefly rinsed or rinsed 24 hours. The surfaces were then exposed 30 minutes in a flow of solution at pH 7.0 that contained 40 mM KCl, isotope and the indicated levels of NaF. The surfaces were then each exposed 30 minutes in a small flow chamber to 10 mM acetic acid at pH 4.5 at flow rate of 1.0 ml per minute. The acid flow was collected in 10 minute fractions for determination of dissolved $^{45}$Ca, $F^-$, $Ca^{+2}$, and $PO_4^{-3}$.

** Total uptake at 0.5 hr.: Percentage (S.D.).

percentage of the amounts of $^{45}Ca$ which were taken up by the enamel from the ion exchange solutions, i.e. the percentage recovery of $^{45}Ca$. The recovery of $^{45}Ca$ was much greater in the first than in succeeding collections of the acid from the surfaces. The recovery of $^{45}Ca$ was significantly greater from briefly rinsed than 24 hour rinsed surfaces, but only in the absence of NaF. Significantly smaller recoveries of $^{45}Ca$ were found for surfaces that were exposed to 0.50 mM NaF than 0.05 or 5.00 mM NaF. Exposure in solutions of NaF generally reduced the amounts of $^{45}Ca$ recovered in the acid.

Table 14 shows the amounts of $F^-$ that dissolved in the acid from the exchanged surfaces of enamel. The $F^-$ dissolved in almost constant rates from surfaces exposed to 0.05 and 0.50 mM NaF. However, slightly greater amounts of $F^-$ were dissolved from surfaces exposed in solutions of 0.50 than 0.05 mM NaF. Significantly greater amounts of $F^-$ were dissolved from the surfaces exposed in 5 mM than 0.50 mM NaF. More than twice the amounts of $F^-$ dissolved in the first than the second and third collections of acid from surfaces exposed in solutions of 5.00 mM NaF. There were no meaningful differences of $F^-$ dissolution from the briefly rinsed and 24 hour rinsed surfaces.

Table 15 shows the amounts of $Ca^{+2}$ that dissolved in the acid from the ion exchanged surfaces. The rates of $Ca^{+2}$ dissolution were reasonably constant within a group of experimental surfaces. Thus the total amounts of $Ca^{+2}$ that

## TABLE 14

Effects of 0.5 Hour Exposure in Solutions of NaF at pH 7.0 on the Subsequent Acid Recovery of Exchanged $F^-$ from Enamel Surfaces*.

| | | $F^-$ Dissolved in 10 Minute Collections of Acid, $\mu mol/cm^2 \times 10^{-3}$ (S.D.) | | | |
|---|---|---|---|---|---|
| Surface Rinse | Solution NaF, mM | 10 | 20 | 30 | Total** |
| Brief | - | - | - | - | - |
| 24 Hrs. | - | - | - | - | - |
| Brief | 0.05 | 1.4(0.2) | 2.0(0.1) | 2.1(0.2) | 6.0(0.1) |
| 24 Hrs. | 0.05 | 2.7(0.4) | 2.3(0.2) | 2.3(0.0) | 7.3(0.5) |
| Brief | 0.50 | 2.5(0.4) | 2.6(0.5) | 2.9(0.3) | 8.0(0.4) |
| 24 Hrs. | 0.50 | 4.6(0.3) | 2.5(0.4) | 3.1(0.4) | 10.2(0.6) |
| Brief | 5.00 | 103.2(8.0) | 42.1(4.4) | 38.7(4.4) | 184.0(5.6) |
| 24 Hrs. | 5.00 | 107.3(13.2) | 39.2(2.2) | 38.5(3.1) | 187.6(13.0) |

* Conditions and surfaces were the same as described for Table 13.

** Total dissolved at 0.5 hr.

## TABLE 15.

Effects of 0.5 Hour Exposure in Solutions of NaF at pH 7.0 on the Subsequent Acid Dissolution of $Ca^{+2}$ from Enamel Surfaces*.

| | | $Ca^{+2}$ Dissolved in 10 Minute Collections of Acid, $\mu mol/cm^2 \times 10^{-2}$ (S.D.) | | | |
|---|---|---|---|---|---|
| Surface Rinse | Solution NaF, mM | 10 | 20 | 30 | Total** |
| Brief | - | 31(2) | 34(14) | 30(5) | 95(18) |
| 24 Hrs. | - | 37(5) | 42(5) | 41(6) | 120(12) |
| Brief | 0.05 | 22(9) | 23(8) | 22(7) | 67(24) |
| 24 Hrs. | 0.05 | 34(8) | 31(2) | 32(5) | 97(17) |
| Brief | 0.50 | 16(2) | 14(2) | 15(1) | 45(5) |
| 24 Hrs. | 0.50 | 18(2) | 15(3) | 14(3) | 47(8) |
| Brief | 5.00 | 23(6) | 22(5) | 21(4) | 66(15) |
| 24 Hrs. | 5.00 | 28(4) | 28(3) | 21(3) | 77(9) |

* Conditions and surfaces were the same as described for Table 13.

** Total dissolved at 0.5 hr.

dissolved in the 0.5 hour acid exposure reflected the effects of ion exchange in solutions of NaF and the prior rinse procedures. There was a trend of slightly greater amounts of $Ca^{+2}$ dissolution from 24 hour than briefly rinsed surfaces. The studied levels of NaF in the solutions significantly reduced the amounts of $Ca^{+2}$ that dissolved in the acid when compared to the absence of NaF. The 0.50 mM level of NaF produced the maximum effect of decreased $Ca^{+2}$ dissolution. The levels of 0.05 and 5.00 mM NaF produced about the same effect on $Ca^{+2}$ dissolution from the surfaces.

Table 16 shows the amounts of $PO_4^{-3}$ that dissolved in the acid from the surfaces of enamel after the ion exchange reactions. Generally smaller amounts of $PO_4^{-3}$ were dissolved in the first than the second or third collections of the acid. Significantly more $PO_4^{-3}$ dissolved from 24 hour rinsed than briefly rinsed surfaces that were exposed to 0.05 and 0.50 mM NaF. The amounts of $PO_4^{-3}$ that dissolved from surfaces that were exposed in solutions in the absence of NaF and with 5.00 mM in solutions were slightly greater for 24 hour than briefly rinsed surfaces, but the differences were not significant. NaF in the ion exchange solutions significantly reduced the amounts of $PO_4^{-3}$ that dissolved from the surfaces when compared to the absence of NaF. For briefly rinsed surfaces, 0.50 and 5.00 mM NaF caused almost the same reduction of $PO_4^{-3}$ dissolution in the acid.

TABLE 16

Effects of 0.5 Hour Exposure in Solutions of NaF at pH 7.0 on the Subsequent Acid Dissolution of $PO_4^{-3}$ from Enamel Surfaces*.

| | | $PO_4^{-3}$ Dissolved in 10 Minute Collections of Acid, $\mu mol/cm^2 \times 10^{-2}$ (S.D.) | | | |
|---|---|---|---|---|---|
| Surface Rinse | Solution NaF, mM | 10 | 20 | 30 | Total** |
| Brief | - | 9(4) | 12(2) | 14(8) | 36(6) |
| 24 Hrs. | - | 9(1) | 18(1) | 20(1) | 47(2) |
| Brief | 0.05 | 7(4) | 10(4) | 11(5) | 28(13) |
| 24 Hrs. | 0.05 | 12(5) | 15(3) | 17(2) | 44(14) |
| Brief | 0.50 | 7(1) | 7(1) | 7(1) | 21(3) |
| 24 Hrs. | 0.50 | 4(1) | 5(1) | 7(1) | 16(2) |
| Brief | 5.00 | 6(2) | 8(3) | 10(2) | 24(7) |
| 24 Hrs. | 5.00 | 6(1) | 9(1) | 11(2) | 26(4) |

* Conditions and surfaces were the same as described for Table 13.

** Total dissolved at 0.5 hr.

However, for 24 rinsed surfaces the 0.50 mM NaF level reduced the amounts of $PO_4^{-3}$ that dissolved more than 5.00 mM NaF. The Ca/P ratios for the minerals that dissolved in the acid from these surfaces ranged between 2.1 and 2.9.

### 4. Studies of Reactions Between Demineralized Surfaces of Enamel and Solutions that Contained 1.5 mM $Ca^{+2}$, 1.0 mM $PO_4^{-3}$, and 0.00, 0.05, 0.50, and 5.00 mM NaF at pH 7.0

Groups of acid demineralized surfaces of enamel, after brief or 24 hour rinse, were exposed to remineralizing solutions using the same procedure as for the ion exchange studies. Table 17 shows the levels of $^{45}Ca$ in the sequential 10 minute (5 ml) collections of the solution after contact with the surfaces. The rates of $^{45}Ca$ uptake were almost constant in each of 10 minute time intervals. The total amounts of $^{45}Ca$ taken up, on percentage basis of the amounts available, were similar except for briefly rinsed surfaces. Significantly smaller amounts of $^{45}Ca$ were taken up in briefly rinsed than 24 hour rinsed surfaces which were remineralized in solutions of 0.05 and 0.50 mM NaF.

Table 18 shows the concentrations of $F^-$ in the remineralizing solutions after contact with the enamel surfaces. The rates of $F^-$ uptake were the same for each 10-minute flow of solution within an experimental group. The total uptake of $F^-$ was significantly greater for each higher level of NaF in the solutions. The uptake of $F^-$ was similar for briefly rinsed and 24 hour rinsed surfaces.

## TABLE 17

Effects of NaF in Solutions of 1.5 mM $Ca^{+2}$ and 1.0 mM $PO_4^{-3}$ at pH 7.0 on the Uptake of $^{45}Ca$ in Remineralized Surfaces of Enamel: Changes in Concentrations of $^{45}Ca$ in Solutions.

| Surface* Rinse | Solution NaF (mM) | $^{45}Ca$ in Solutions at Minute: CPM/ml X $10^5$ (S.D.<0.1) 0 | 10 | 20 | 30 | Total** (S.D.) |
|---|---|---|---|---|---|---|
| Brief | - | 9.8 | 9.2 | 9.2 | 9.2 | 18(1) |
| 24 Hrs. | - | 9.6 | 8.8 | 9.0 | 9.2 | 17(3) |
| Brief | 0.05 | 13.9 | 13.2 | 13.3 | 13.3 | 12(2) |
| 24 Hrs. | 0.05 | 13.8 | 12.9 | 13.5 | 13.3 | 17(5) |
| Brief | 0.50 | 13.3 | 12.7 | 12.5 | 12.7 | 14(1) |
| 24 Hrs. | 0.50 | 13.5 | 12.5 | 12.6 | 12.7 | 21(2) |
| Brief | 5.00 | 13.3 | 12.5 | 12.5 | 12.3 | 17(4) |
| 24 Hrs. | 5.00 | 13.1 | 12.2 | 12.2 | 12.3 | 20(2) |

* Acid demineralized surfaces of enamel were either briefly rinsed or rinsed 24 hours. The surfaces in pairs (two-1 $cm^2$) were exposed to 1 ml solution which was flowed at a rate of 0.5 ml per minute to contact the surfaces. The off-flow was collected in 10 minute fractions for 30 minutes. Solutions were prepared to contain 1.5 mM $CaCl_2$, 1.0 mM $NaH_2PO_4$, 40 mM KCl and the indicated levels of NaF at pH 7.0. The levels of $^{45}Ca$ in the solutions before were determined and after contact with the enamel at each 10 minute interval in separate collections.

**The total uptake of $^{45}Ca$ by the remineralized surfaces was calculated as the percentage of the amounts of isotope available in the solutions before contact with enamel.

Table 18

Effects of NaF in Solutions of 1.5 mM $Ca^{+2}$ and 1.0 mM $PO_4^{-3}$ at pH 7.0 on the Uptake of $F^-$ in Remineralized Surfaces of Enamel: Changes in Concentrations of $F^-$ in Solutions.

| | | $F^-$ in Solution at Minute mM X $10^{-2}$ (S.D.) | | | | |
|---|---|---|---|---|---|---|
| Surface* Rinse | Solution NaF (mM) | 0 | 10 | 20 | 30 | Total** |
| Brief | - | - | - | - | - | |
| 24 Hrs. | - | - | - | - | - | |
| Brief | 0.05 | 4(0) | 3.9(0) | 3.8(0) | 3.7(0) | 3(1) |
| 24 Hrs. | 0.05 | 5(0) | 4.0(0) | 4.1(0) | 4.1(0) | 3(1) |
| Brief | 0.50 | 43(1) | 41(1) | 40(1) | 42(1) | 15(4) |
| 24 Hrs. | 0.50 | 44(1) | 42(1) | 43(1) | 42(1) | 13(3) |
| Brief | 5.00 | 421(10) | 387(9) | 394(11) | 396(11) | 218(10) |
| 24 Hrs. | 5.00 | 453(10) | 423(10) | 425(9) | 429(11) | 197(15) |

* Conditions and surfaces were the same as described for Table 17. The levels of $F^-$ in the solutions were compared before and after contact with the enamel at each 10 minute interval in separate collections.

**Uptake of $F^-$ at the 0.5 hour time of enamel-solution contact was calculated as $\mu mol/cm^2$ X $10^{-2}$ with standard deviation shown in parenthesis.

However, briefly rinsed surfaces showed a significantly greater total uptake of $F^-$ from solutions of 5.00 mM NaF than 24 hour rinsed surfaces.

Table 19 shows the concentrations of $Ca^{+2}$ in the solutions before (zero time) and at the 10-minute intervals of contact with the enamel surfaces. The changes of $Ca^{+2}$ concentrations were very small after each of the 10 minute intervals of contact with the enamel. The levels of 0.50 and 5.00 mM NaF significantly increased the uptake of $Ca^{+2}$ in the enamel compared to 0.05 mM NaF or the absence of NaF in solutions. There were insignificant differences in the $Ca^{+2}$ uptake in briefly rinsed and in 24 hours rinsed surfaces. However, trends of greater $Ca^{+2}$ uptake in briefly rinsed than 24 hours rinsed surfaces were found, except in the absence of NaF where the opposite trend occurred.

Table 20 shows the concentrations of $PO_4^{-3}$ in the solutions initially and at 10 minute intervals of contact with the enamel surfaces. The changes of $PO_4^{-3}$ concentrations in the solutions were extremely small. However, these changes indicated consistent trends for the 10 minute collections of the solutions. Except for solutions of 0.05 mM NaF, the apparent total uptake of $PO_4^{-3}$ in the enamel was greater for 24 hour rinsed than briefly rinsed surfaces.

TABLE 19

Effects of NaF in Solutions of 1.5 mM $Ca^{+2}$ and 1.0 mM $PO_4^{-3}$ at pH 7.0 on the Uptake of $Ca^{+2}$ in Remineralized Enamel: Changes in Concentration of $Ca^{+2}$ in Solutions.

| Surface* Rinse | Solution NaF (mM) | $Ca^{+2}$ in Solution at Min. mM X $10^{-2}$ (S.D.) 0 | 10 | 20 | 30 | Total** |
|---|---|---|---|---|---|---|
| Brief | - | 149(1) | 142(1) | 143(1) | 143(1) | 41(6) |
| 24 Hrs. | - | 153(2) | 145(1) | 148(1) | 147(1) | 59(14) |
| Brief | 0.05 | 151(1) | 143(1) | 146(1) | 146(1) | 44(26) |
| 24 Hrs. | 0.05 | 150(1) | 143(4) | 148(3) | 145(4) | 39(16) |
| Brief | 0.50 | 153(2) | 144(3) | 144(3) | 144(5) | 76(23) |
| 24 Hrs. | 0.50 | 157(2) | 146(4) | 148(4) | 148(4) | 69(21) |
| Brief | 5.00 | 153(2) | 140(3) | 143(2) | 144(3) | 85(6) |
| 24 Hrs. | 5.00 | 152(1) | 141(2) | 141(2) | 144(2) | 75(15) |

* Conditions and surfaces were the same as described for Table 17.

** Total uptake at 0.5 hr. as $\mu mol/cm^2$ (S.D.).

TABLE 20

Effects of NaF in Solutions of 1.5 mM $Ca^{+2}$ and 1.0 mM $PO_4^{-3}$ at pH 7.0 on the Uptake of $PO_4^{-3}$ in Remineralized Enamel: Changes in Concentrations of $PO_4^{-3}$ in Solutions.

| Surface* Rinse | Solution NaF (mM) | $PO_4^{-3}$ in Solution at Minute: mM X $10^{-2}$ (S.D.) 0 | 10 | 20 | 30 | Total** |
|---|---|---|---|---|---|---|
| Brief | - | 104(1) | 98(1) | 98(1) | 99(1) | 39(11) |
| 24 Hrs. | - | 106(1) | 96(1) | 96(1) | 96(1) | 77(9) |
| Brief | 0.05 | 99(1) | 92(6) | 95(6) | 95(4) | 32(18) |
| 24 Hrs. | 0.05 | 99(1) | 93(3) | 95(5) | 94(3) | 35(12) |
| Brief | 0.50 | 99(1) | 94(1) | 93(2) | 94(3) | 39(15) |
| 24 Hrs. | 0.50 | 101(1) | 93(3) | 95(2) | 95(3) | 58(8) |
| Brief | 5.00 | 99(1) | 96(4) | 95(4) | 96(5) | 22(9) |
| 24 Hrs. | 5.00 | 100(1) | 95(3) | 94(2) | 95(3) | 56(15) |

* Conditions and surfaces were the same as described for Table 17.

** Total uptake at 0.5 hr. as $\mu mol/cm^2 x 10^{-2}$ (S.D.).

### 5. Effects of 0.5 Hour Exposure of Enamel in Solutions of NaF Under Conditions of Remineralization on Dissolution of Surface Minerals.

Table 21 shows the recovery of $^{45}$Ca from the remineralized surfaces of enamel in the 10-minute (10 ml) collections of acid as previously described. In general, greater amounts of $^{45}$Ca were recovered in the first than in the successive collections of acid. Except for surfaces remineralized in solutions of 5.00 mM NaF, significantly greater total amounts of the isotope were recovered from briefly rinsed than 24 hour rinsed surfaces. NaF had no meaningful effects on the recovery of $^{45}$Ca from the remineralized surfaces.

Table 22 shows the amounts of $F^-$ that dissolved in the acid collections from remineralized surfaces. Similar amounts of $F^-$ were dissolved from 24 hour rinsed and briefly rinsed surfaces. The amounts of $F^-$ that dissolved from surfaces remineralized in the presence of 0.05 mM NaF were the same in successive collections of acid. However, greater amounts of $F^-$ were dissolved in the first than in succeeding collections of acid from surfaces which were remineralized in solutions of 0.50 and 5.00 mM NaF. Significantly greater amounts of total $F^-$ were dissolved from surfaces which were remineralized in solutions of each higher level of NaF.

TABLE 21

Effects of 0.5 Hour Remineralization in Solutions of NaF on the Acid Recovery of $^{45}$Ca from Enamel Surfaces*.

| Surface Rinse | Solution NaF, mM | Percentage Recovery of $^{45}$Ca in 10 Minute Collections of Acid (S.D.) 10 | 20 | 30 | Total** |
|---|---|---|---|---|---|
| Brief | | 12(2) | 6(2) | 4(1) | 22(4) |
| 24 Hours | - | 8(2) | 5(1) | 3(1) | 16(3) |
| Brief | 0.05 | 12(3) | 5(1) | 5(2) | 22(5) |
| 24 Hrs. | 0.05 | 9(3) | 5(2) | 4(1) | 18(4) |
| Brief | 0.50 | 11(1) | 6(1) | 4(1) | 21(2) |
| 24 Hrs. | 0.50 | 7(1) | 4(1) | 3(1) | 14(4) |
| Brief | 5.00 | 8(2) | 5(1) | 3(1) | 16(3) |
| 24 Hrs. | 5.00 | 8(2) | 5(1) | 4(1) | 17(4) |

---

* Surfaces of enamel were demineralized 24 hours in weak acid then either briefly rinsed or rinsed 24 hours. The surfaces were then remineralized 30 minutes in flowing solution at pH 7.0 that contained 1.5 mM $CaCl_2$, 1.0 mM $NaH_2PO_4$, and the indicated levels of NaF. The remineralized surfaces were each separately exposed for 30 minutes to 10 mM acetic acid at pH 4.5. The acid contacted the surface at a flow of 1.0 ml per minute in 1.0 ml volume. The acid was collected in 10-minute fractions for determination of dissolved $^{45}$Ca, $F^-$, $Ca^{+2}$, and $PO_4^{-3}$.

**Total recovered at 0.5 hr.

TABLE 22

Effects of 0.5 Hour Remineralization in Solutions of NaF on the Acid Recovery of $F^-$ from Enamel Surfaces*.

| Surface Rinse | Solution NaF, mM | $F^-$ Dissolved in 10 Minute Collections of Acid, $\mu mol/cm^2 \times 10^{-3}$ (S.D.) | | | |
|---|---|---|---|---|---|
| | | 10 | 20 | 30 | Total** |
| Brief | - | - | - | - | - |
| 24 Hrs. | - | - | - | - | - |
| Brief | 0.05 | 2.3(0.2) | 2.3(0.0) | 2.5(0.0) | 7.1(0.2) |
| 24 Hrs. | 0.05 | 2.0(0.1) | 2.1(0.2) | 2.3(0.2) | 6.4(0.2) |
| Brief | 0.50 | 14.9(3.6) | 8.1(0.8) | 6.7(0.7) | 29.8(0.6) |
| 24 Hrs. | 0.50 | 14.9(1.1) | 6.3(0.5) | 6.4(0.4) | 27.6(1.8) |
| Brief | 5.00 | 111.6(5.8) | 47.5(4.6) | 45.8(1.8) | 202.6(7.1) |
| 24 Hrs. | 5.00 | 104.2(2.4) | 49.7(4.8) | 33.7(3.2) | 187.2(2.7) |

* Conditions and surfaces were the same as described for Table 21.

** Total dissolved at 0.5 hr.

Table 23 shows the amounts of $Ca^{+2}$ that dissolved from the remineralized surfaces in the 10 minutes collections of acid. In general more $Ca^{+2}$ was dissolved in the first than in subsequent collections of acid in each group of surfaces. There were trends for greater dissolution of $Ca^{+2}$ from 24 hour rinsed than briefly rinsed surfaces, but no significant differences were found. Similar amounts of $Ca^{+2}$ dissolved from surfaces remineralized in the absence of NaF and in the presence of 0.05 mM NaF. Significantly smaller amounts of $Ca^{+2}$ were dissolved from surfaces remineralized in solutions of 5.00 mM NaF than other surfaces.

Table 24 shows the amounts of $PO_4^{-3}$ that dissolved in the acid from the remineralized surfaces of enamel. Contrary to $Ca^{+2}$, similar amounts of $PO_4^{-3}$ were dissolved in successive collections of acid for surfaces within an experimental group. The amounts of $PO_4^{-3}$ that dissolved from 24 hour rinsed surfaces were smaller than from briefly rinsed surfaces for surfaces remineralized in the absence of NaF and those remineralized in 0.05 mM NaF. There were trends for greater amounts of $PO_4^{-3}$ to dissolve from 24 hour rinsed than briefly rinsed surfaces for surfaces remineralized in solutions of 0.50 and 5.00 mM NaF. Only the level of 5.00 mM NaF caused a significant reduction in the acid dissolution of $PO_4^{-3}$ from the remineralized surfaces of enamel.

## TABLE 23

Effects of 0.5 Hour Remineralization in Solutions of NaF on the Acid Dissolution of $Ca^{+2}$ from Enamel Surfaces*.

| | | $Ca^{+2}$ Dissolved in 10 Minute Collections of Acid, $\mu mol/cm^2 \times 10^{-2}$ (S.D.) | | | |
|---|---|---|---|---|---|
| Surface Rinse | Solution NaF, mM | 10 | 20 | 30 | Total** |
| Brief | - | 37(3) | 34(6) | 31(5) | 103(8) |
| 24 Hours | - | 43(9) | 39(9) | 39(9) | 121(27) |
| Brief | 0.05 | 37(7) | 30(7) | 29(7) | 96(21) |
| 24 Hrs. | 0.05 | 37(3) | 33(2) | 34(4) | 104(8) |
| Brief | 0.50 | 36(5) | 26(4) | 23(2) | 85(6) |
| 24 Hrs. | 0.50 | 42(9) | 29(4) | 27(4) | 98(16) |
| Brief | 5.00 | 29(8) | 20(4) | 18(3) | 67(15) |
| 24 Hrs. | 5.00 | 30(8) | 21(2) | 21(1) | 75(10) |

* Conditions and surfaces were the same as described for Table 21.

** Total dissolved at 0.5 hr.

TABLE 24

Effects of 0.5 Hour Remineralization in Solutions of NaF on the Acid Dissolution of $PO_4^{-3}$ from Enamel Surfaces*.

| | | $PO_4^{-3}$ Dissolved in 10 Minute Collections of Acid, $\mu mol/cm^2 \times 10^{-2}$ (S.D.) | | | |
|---|---|---|---|---|---|
| Surface Rinse | Solution NaF, mM | 10 | 20 | 30 | Total** |
| Brief | - | 19(2) | 17(4) | 18(1) | 53(4) |
| 24 Hrs. | - | 13(2) | 15(3) | 16(4) | 44(9) |
| Brief | 0.05 | 16(4) | 17(4) | 17(4) | 51(12) |
| 24 Hrs. | 0.05 | 16(3) | 17(2) | 18(1) | 50(6) |
| Brief | 0.50 | 17(3) | 14(3) | 13(3) | 44(7) |
| 24 Hrs. | 0.50 | 21(7) | 16(3) | 15(2) | 52(11) |
| Brief | 5.00 | 10(3) | 9(3) | 9(2) | 28(8) |
| 24 Hrs. | 5.00 | 12(4) | 11(3) | 11(1) | 34(7) |

* Conditions and surfaces were the same as described for Table 21.

** Total dissolved at 0.5 hr.

## 6. Comparison of the Effects of Brief and 24 Hour Rinse of Acid Demineralized Surfaces of Enamel.

The $^{45}Ca$ uptake of the two groups of surfaces, brief and 24 hour rinsed, were approximately the same from solutions used for ion exchange and remineralization. The uptake of $^{45}Ca$ was slightly smaller for brief than 24 hour rinsed surfaces which were remineralized in the absence of NaF. The uptake of $F^-$ was slightly smaller for 24 hour rinsed than briefly rinsed surfaces, particularly for 0.50 mM NaF in ion exchange solutions. The uptake of $F^-$ was about the same for brief and 24 hour rinsed surfaces after remineralization. The amounts of $Ca^{+2}$ that dissolved in the ion exchange solutions (pH 7.0) were similar, except smaller amounts dissolved from 24 hour rinsed than briefly rinsed surfaces in the absence of NaF. Slightly more $Ca^{+2}$ was apparently taken up in the absence of NaF in briefly rinsed than 24 hour rinsed surfaces during remineralization. However, greater amounts of $PO_4^{-3}$ were deposited in 24 hour rinsed than briefly rinsed surfaces except those remineralized in the presence of 0.05 mM NaF. In the absence of NaF in ion exchange solutions smaller amounts of $PO_4^{-3}$ dissolved from 24 hour than briefly rinsed surfaces. However, with 0.05 mM NaF in the solutions smaller amounts of $PO_4^{-3}$ dissolved from the brief than 24 hour rinsed surfaces.

## TABLE 25

Effects of NaF in Solutions Used for Ion Exchange and Remineralization at pH 7.0 on the Ratios of Ca/P, Ca/F, and P/F of the Minerals Subsequently Dissolved in Acid from Enamel Surfaces*.

| | | Ratios for Minerals Dissolved in 30 Minute Acid Exposure | | | | | |
|---|---|---|---|---|---|---|---|
| Surface Rinse | Solution NaF, mM | Ca/P | | Ca/F | | P/F | |
| | | E | R | E | R | E | R |
| Brief | - | 2.5 | 1.9 | - | - | - | - |
| 24 Hrs. | - | 2.6 | 2.8 | - | - | - | - |
| Brief | 0.05 | 2.4 | 1.9 | 1.1 | 1.4 | 0.5 | 0.7 |
| 24 Hrs. | 0.05 | 2.8 | 2.1 | 1.3 | 1.6 | 0.5 | 0.8 |
| Brief | 0.50 | 2.1 | 1.9 | 1.5 | 0.3 | 0.7 | 0.2 |
| 24 Hrs. | 0.50 | 2.8 | 1.9 | 0.4 | 0.4 | 0.3 | 0.2 |
| Brief | 5.00 | 2.5 | 2.4 | Less than 0.1 | | | |
| 24 Hrs. | 5.00 | 2.9 | 2.2 | (0.01 to 0.04) | | | |

---

* Conditions and surfaces were the same as described for Tables 13 through 21. Also E represents exchanged surfaces and R represents remineralized surfaces.

Table 25 shows the calculated ratios of Ca/P, Ca/F, and P/F for the total amounts of minerals that dissolved in the flowing acid from the ion exchanged and the remineralized surfaces of enamel. The Ca/P ratios of the minerals that dissolved from the briefly rinsed surfaces after the ion exchange reactions varied between 2.1 and 2.5 for each level of NaF. The Ca/P ratios ranged between 2.6 and 2.9 for the minerals that dissolved from the 24 hour rinsed surfaces after ion exchange reactions. The Ca/P ratios for minerals that dissolved from remineralized surfaces were about the same, 1.9 to 2.4, except for 24 hour rinsed surfaces remineralized in the absence of NaF (2.8). In general, smaller recoveries of $^{45}Ca$ were indicated for 24 hour than briefly rinsed surfaces.

The amounts of $F^-$ that dissolved from the briefly and 24 hour rinsed surfaces were the same for any one of the levels of NaF in both ion exchange and remineralizing solutions. The Ca/F ratios of the minerals that dissolved in the acid generally decreased with each higher level of NaF from all groups of surfaces, as did the P/F ratios. These ratios of Ca/F fell in the range of 0.01 to 0.04 for minerals that dissolved from surfaces exposed to solutions of 5.00 mM NaF.

# DISCUSSION

The remineralization of enamel has been studied almost exclusively using acid demineralized surfaces. In most studies the surfaces were exposed to acids to simulate carious attack of the enamel. The surfaces were subjected to acid demineralization in this study to simulate acid reactions of caries. Perhaps more importantly the acid increased the reactive surface area and thereby increased the rates of ion exchange and remineralization of the enamel.

The surface minerals dissolved in the 24 hour acid demineralization in the Ca/P ratio expected for the apatite of enamel (39). Previous studies showed that such acid demineralized surfaces retained adsorbed $Ca^{+2}$ and $HPO_4^{-2}$ even after brief rinsing in water (77). The demineralized surfaces were briefly rinsed to remove any adherent acid. Groups of the surfaces were additionally rinsed 24 hours to remove readily soluble $Ca^{+2}$ and $HPO_4^{-2}$. The Ca/P ratios of the small amounts of minerals that dissolved in the 24 hour rinse indicated that indeed the surfaces contained small amounts of $HPO_4^{-2}$. Apparently $HPO_4^{-2}$ dissolved in a manner slightly preferential to $Ca^{+2}$, leaving the 24 hour rinsed surfaces deficient in total $PO_4^{-3}$ relative to the Ca/P composition of apatites. The two groups of surfaces, briefly rinsed and 24 hour rinsed, were subjected to the same conditions of study to determine effects of the surface adsorbed $Ca^{+2}$ and $HPO_4^{-2}$ on reactivity of the surfaces.

The 24 hour rinse slightly altered the reactions of ion exchange and remineralization of the enamel surfaces compared to the brief rinse. However, these effects of the rinse methods were inconsistent and often fell within the range of experimental errors. Collectively, the results indicated that the 24 hour rinsed surfaces contained slightly less $PO_4^{-3}$ ($HPO_4^{-2}$) than the briefly rinsed surfaces. This was most evident in the Ca/P ratio for mineral uptake in remineralized surfaces, i.e., greater $PO_4^{-3}$ uptake was found relative to $Ca^{+2}$ uptake in 24 hour rinsed surfaces. In ion exchange reactions relatively greater amounts of $Ca^{+2}$ dissolved from 24 hour rinsed surfaces than $PO_4^{-3}$. The generally higher Ca/P ratios of minerals that dissolved in the acid from the 24 hour rinsed than briefly rinsed surfaces (ion exchanged and remineralized) indicated that the relative $PO_4^{-3}$ deficiency, caused by the 24 hour rinse, persisted throughout the experiments. One of the most important findings of this study was that the method of rinse had no effect on the uptake of $F^-$ in the surfaces.

It was assumed that the briefly rinsed surfaces represented the reactions of enamel immediately after the acid attack of caries. The 24 hour water rinse at pH 7.0 altered to a small degree the acid residues in the surfaces in a more artificial manner than the brief rinse. Therefore, the following discussions will be confined mostly to reactions of the briefly rinsed surfaces.

Figure 11 shows the uptake of $F^-$ in the ion exchanged and remineralized surfaces (pH 7.0) and the amounts of $F^-$ that later dissolved in the flowing acid (pH 4.5) from the same surfaces. The uptake of $F^-$ was linearly related to the concentration of NaF in the solutions. The same amounts of $F^-$ uptake were found for the surfaces of enamel from ion exchange solutions and remineralizing solutions. Therefore, the uptake was independent of the concentrations of $Ca^{+2}$ and $PO_4^{-3}$ in the solutions. The rates of $F^-$ uptake were constant at each level of NaF in the solutions. These results indicated that $F^-$ uptake in the enamel surfaces occurred mostly by ion exchange and adsorption (84) rather than remineralization.

The amounts of $F^-$ that dissolved from the ion exchanged and remineralized surfaces were similar except for the 0.50 mM levels of NaF in the solutions. Slightly more than twice the amounts of $F^-$ were dissolved in the acid from surfaces which were remineralized in the presence of 0.50 mM NaF than surfaces which were subjected to ion exchange in the presence of this level of NaF. This possibly indicated that a larger percentage of the $F^-$ uptake in the enamel occurred by exchange for $OH^-$ in the presence of 0.50 mM NaF from ion exchange solutions than from remineralizing

Figure 11.

The effects* of 0.05, 0.50 and 5.00 mM NaF in solutions at pH 7.0 on the uptake of $F^-$ in enamel surfaces and subsequent acid resistance of the surface minerals at pH 4.5.

A. The uptake of $F^-$ in enamel under conditions of ion exchange from solutions that contained the NaF and 40 mM KCl at pH 7.0.

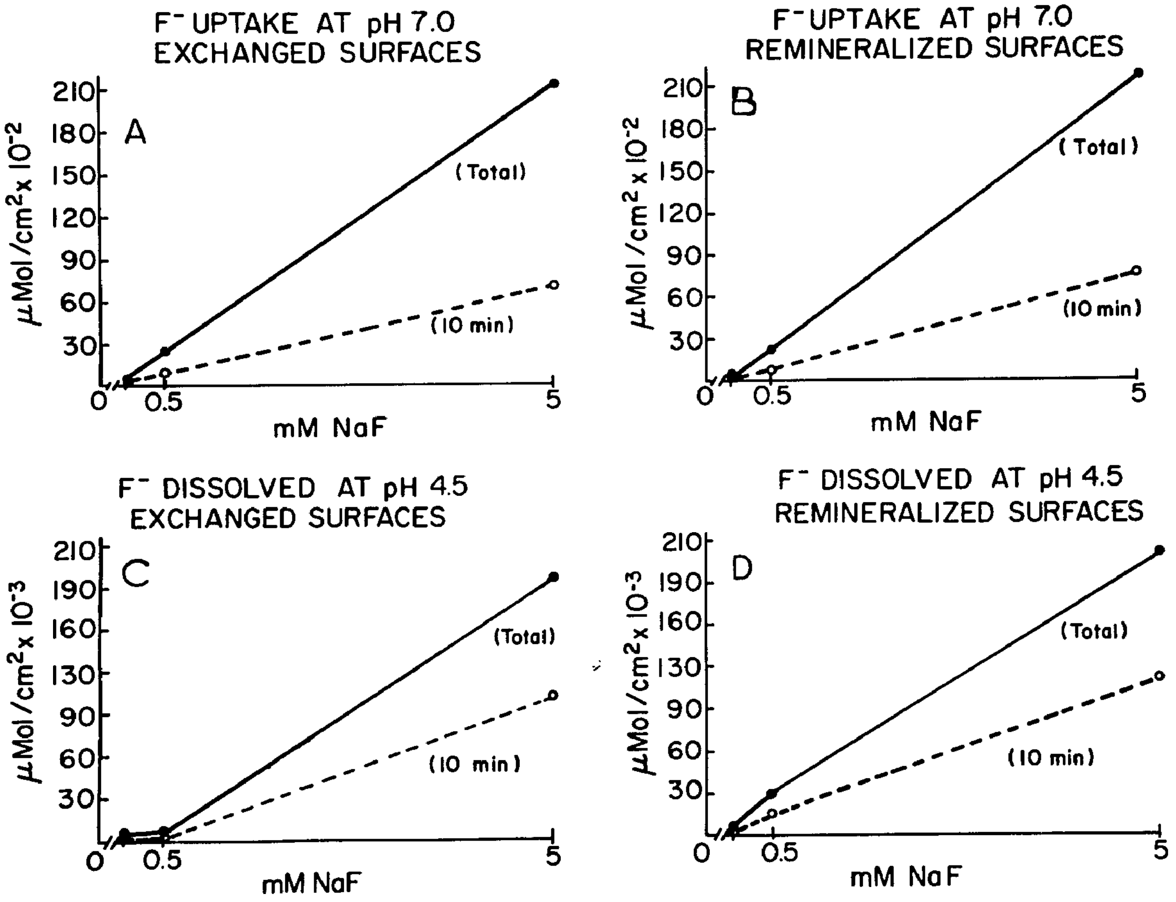
B. The uptake of $F^-$ in enamel under conditions remineralization from solutions that contained 1.5 mM $Ca^{+2}$, 1.0 mM $PO_4^{-3}$, the NaF, and 40 mM KCl at pH 7.0.

C. The amounts of $F^-$ that dissolved from the exchanged surfaces (A above) in 10 mM acetic acid at pH 4.5.

D. The amounts of $F^-$ that dissolved from the remineralized surfaces (B above) in 10 mM acetic acid at pH 4.5.

* Solid line represents total effects in 30 minutes. Dashed line represents the effects in 10 minutes.

BRIEFLY RINSED SURFACES



solutions. The $F^-$ that exchanged for $OH^-$ would be expected to be less soluble than the $F^-$ taken up by other mechanisms (13).

The amounts of $F^-$ that dissolved in the acid were only about one tenth of the amounts which were taken up in the ion exchanged and remineralized surfaces. In this regard only about 3 percent of the $F^-$ uptake in surfaces from ion exchange solutions of 0.50 mM NaF was dissolved in the acid. Thus at least 90 per cent of the $F^-$ was taken up in relatively acid insoluble minerals in the surfaces from solutions of 0.05, 0.50, and 5.00 mM NaF. The apparent acid insolubility of the $F^-$ may be explained in the following two ways: (a) The $F^-$ was taken up from the solutions at pH 7.0 in exchange for $OH^-$ in hydroxyapatite of the surfaces to form fluorapatite or (b) the $F^-$ was taken up by adsorption (82), and by $CaF_2$ formation both of which rapidly dissolved in the acid to release $F^-$ for equally as rapid readsorption on the surface minerals and exchange for $OH^-$ in hydroxyapatite. The finding that greater amounts of $F^-$ were dissolved in the first than in the second or third 10 minutes of acid exposure supported the assumption that most of the $F^-$ eventually exchanged for $OH^-$ in the surface minerals. The small amounts of $F^-$ that did dissolve in the acid probably represented mostly adsorbed $F^-$ and $CaF_2$.

Figure 12 shows the effects of NaF on the uptake of $^{45}Ca$ in ion exchanged and remineralized surfaces of enamel and the effects of the uptake of $F^-$ on the subsequent

Figure 12.

The effects* of 0.05, 0.50, and 5.00 mM NaF on the uptake of $^{45}Ca$ in enamel surfaces under conditions of ion exchange and remineralization at pH 7.0 and subsequent acid resistance of the surface minerals at pH 4.5.

A. The uptake of $^{45}Ca$ in enamel from solutions under conditions of ion exchange that contained the NaF and 40 mM KCl at pH 7.0.

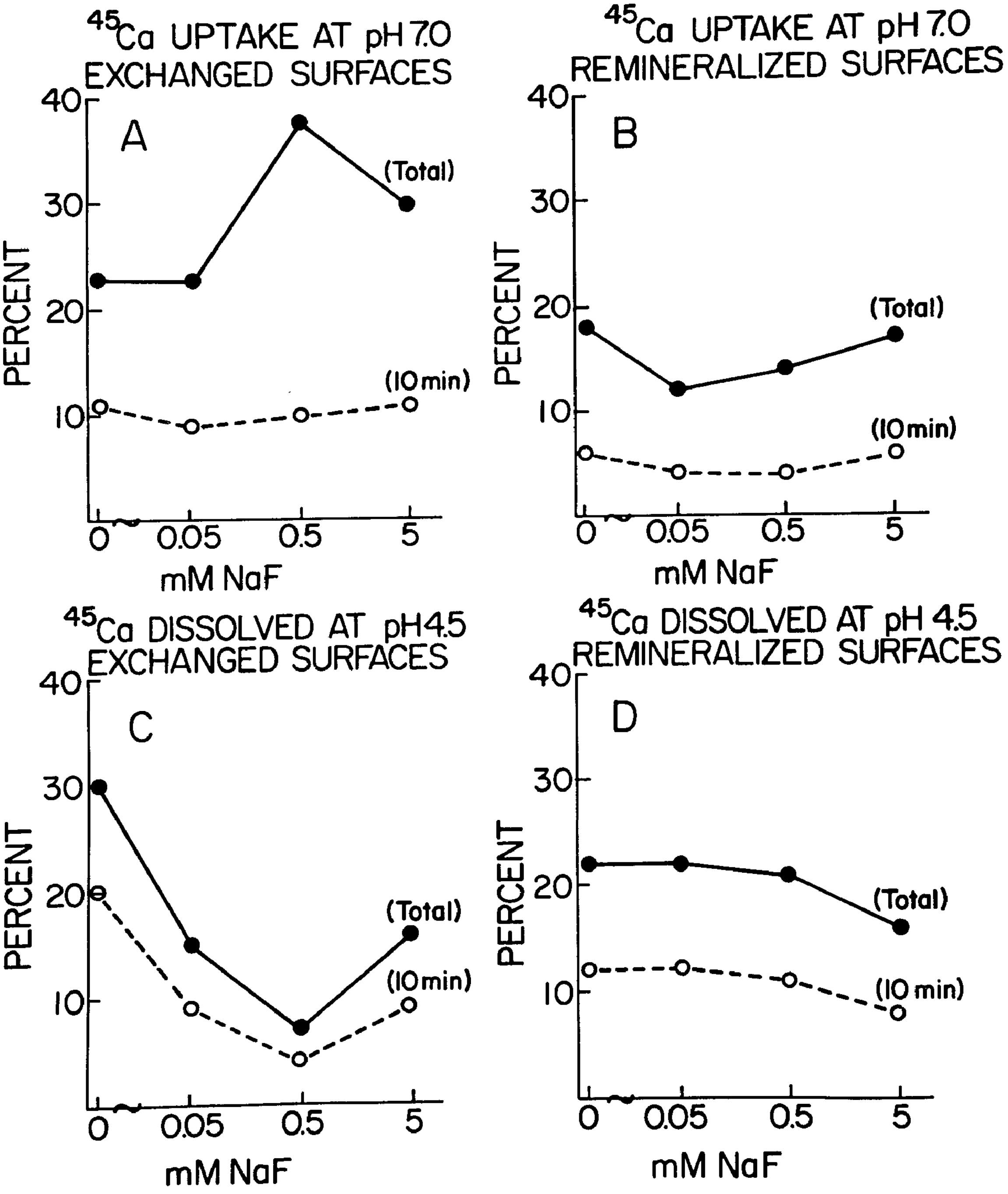
B. The uptake of $^{45}Ca$ in enamel under conditions of remineralization from solutions that contained 1.5 mM $Ca^{+2}$, 1.0 mM $PO_4^{-3}$, the NaF and 40 mM KCl at pH 7.0.

C. The recovery of $^{45}Ca$ from exchanged surfaces (A above) in 10 mM acetic acid at pH 4.5.

D. The recovery of $^{45}Ca$ from remineralized surfaces (B above) in 10 mM acetic acid at pH 4.5.

* Solid line represents total effects in 30 minutes. Dashed line represents the effects in 10 minutes.

## BRIEFLY RINSED SURFACES



recovery of $^{45}Ca$ in the acid. NaF had no effect on $^{45}Ca$ uptake in the first 10 minutes of ion exchange or remineralization. The 0.05 mM NaF had no effect on total $^{45}Ca$ uptake from ion exchange solutions, but it reduced $^{45}Ca$ uptake in remineralized surfaces. The 0.50 mM NaF greatly increased $^{45}Ca$ uptake in ion exchange surfaces, but the 5.00 mM NaF did not increase $^{45}Ca$ uptake as much as 0.50 mM NaF. The 5.00 mM NaF level had no effect on $^{45}Ca$ uptake in remineralized surfaces. The uptake of $^{45}Ca$ in the absence of NaF was slightly smaller for remineralized than exchanged surfaces because of $Ca^{+2}$ competition for isotope binding in the surfaces.

The $F^-$ in the exchanged surfaces significantly reduced the amounts of $^{45}Ca$ that were recovered in the acid. The effect of the exchanged $F^-$ was obvious even in the first of the 10 minute collections of acid. The $F^-$ in surfaces from ion exchange solutions of 0.50 mM NaF reduced $^{45}Ca$ recovery to a lower level than 0.05 and 5.00 mM NaF, both of which caused identical recoveries of $^{45}Ca$. Thus 0.50 mM NaF caused the maximum uptake and minimum recovery of $^{45}Ca$ for the ion exchange surfaces. This indicated that 0.50 mM NaF supported the exchange of $^{45}Ca$ and $F^-$ in the recrystallization of fluorapatites in enamel to a greater extent than 0.05 and 5.00 mM NaF. The $F^-$ in remineralized surfaces had no appreciable effects on acid recoveries of $^{45}Ca$ unless the solution contained 5.00 mM NaF, and even then the effect was relatively small.

Figure 13 and 14 illustrate the effects of NaF in the ion exchange and remineralizing solutions on the surface reactions of $Ca^{+2}$ and $PO_4^{-3}$ at pH 7.0 and later at pH 4.5. Small amounts of $Ca^{+2}$ and $PO_4^{-3}$ dissolved from the surfaces into the ion exchange solutions. The presence of 0.50 and 5.00 mM NaF slightly reduced the amounts of $Ca^{+2}$ that dissolved in the ion exchange solutions, but 0.05 mM NaF had no effect on the amounts of $Ca^{+2}$ that dissolved in these solutions. The presence of 0.05 mM NaF reduced $PO_4^{-3}$ dissolution more than 0.50 mM NaF, but 5.00 mM NaF had no effect on $PO_4^{-3}$ dissolution in the ion exchange solutions. It seemed as though 0.50 and 5.00 mM NaF in the ion exchange solutions actually displaced surface $PO_4^{-3}$ and caused the retention of $Ca^{+2}$.

The presence of 0.50 and 5.00 mM NaF in solutions increased the amounts of $Ca^{+2}$ that were deposited in the remineralized surfaces. However, NaF concentrations of 0.05 and 0.50 mM NaF did not affect the uptake of $PO_4^{-3}$ in the remineralized surfaces. The 5.00 mM NaF reduced the uptake of $PO_4^{-3}$ in these surfaces. The NaF in remineralizing solutions generally increased $Ca^{+2}$ uptake in a manner consistent with $CaF_2$ formation, particularly in the presence of 0.50 and 5.00 mM NaF.

The amounts of $Ca^{+2}$ and $PO_4^{-3}$ that dissolved in the acid from ion exchanged surfaces both followed the same trends as $^{45}Ca$ respective to the amounts of $F^-$ taken up

Figure 13.

The effects* of 0.05, 0.50, and 5.00 mM NaF in solutions on reactions of $Ca^{+2}$ in enamel surfaces at pH 7.0 and 4.5.

A. The amounts of $Ca^{+2}$ that dissolved from enamel surfaces under conditions of ion exchange in solutions that contained the NaF and 40 mM KCl at pH 7.0.

B. The amounts of $Ca^{+2}$ that deposited in enamel surfaces under conditions of remineralization from solutions that contained the NaF, 1.5 mM $Ca^{+2}$, 1.0 mM $PO_4^{-3}$ and 40 mM KCl at pH 7.0.

C. The amounts of $Ca^{+2}$ that dissolved from the ion exchanged surfaces (A above) in 10 mM acetic acid at pH 4.5.

D. The amounts of $Ca^{+2}$ that dissolved from the remineralized surfaces (B above) in 10 mM acetic acid at pH 4.5.

* Solid line represents the total effects in 30 minutes. Dashed line represents the effects in 10 minutes.

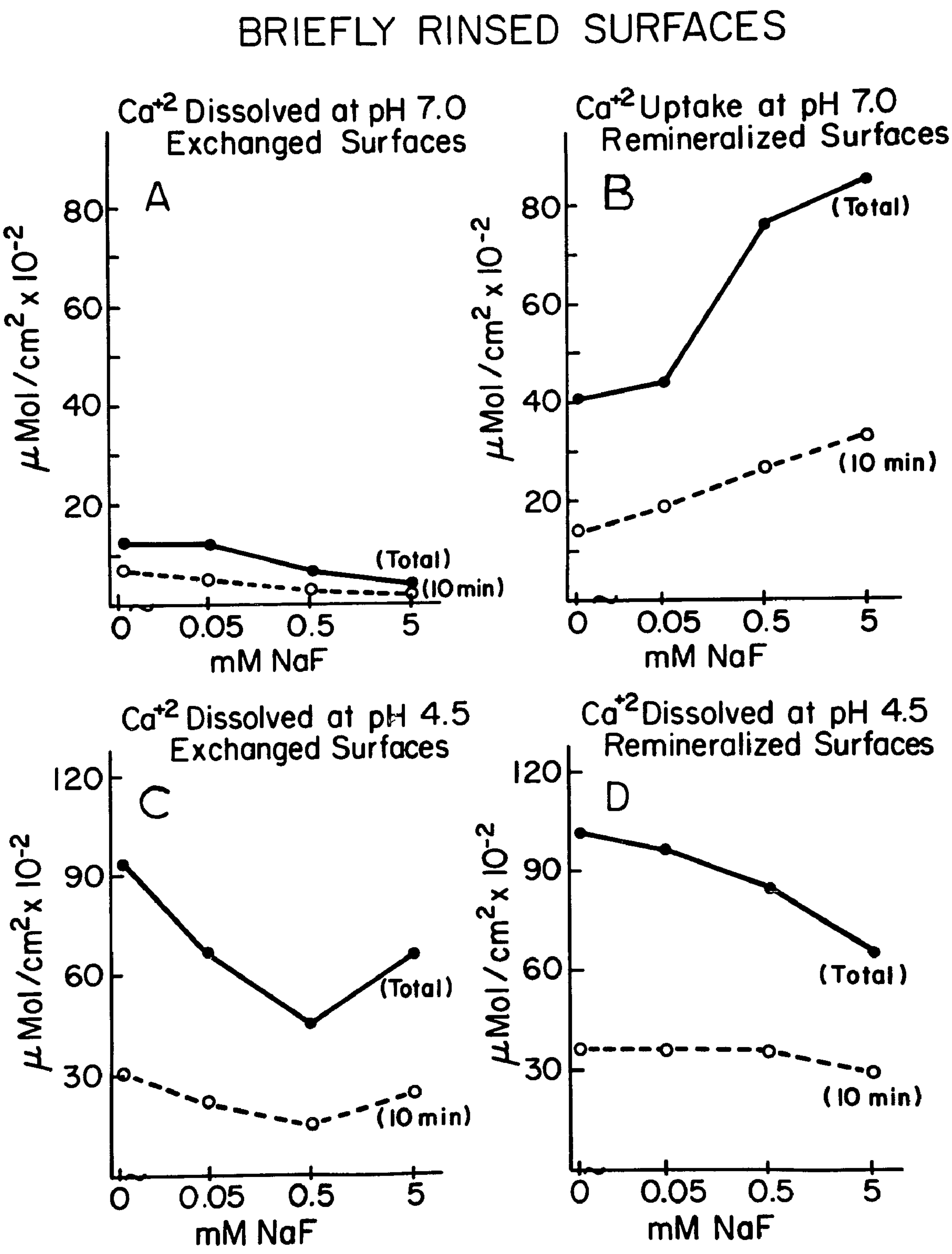

BRIEFLY RINSED SURFACES
Ca$^{+2}$ Dissolved at pH 7.0
Exchanged Surfaces
A
80
60
40
20
µMol/cm$^2$ x 10$^{-2}$
(Total)
(10 min)
0
0.05
0.5
5
mM NaF
Ca$^{+2}$ Uptake at pH 7.0
Remineralized Surfaces
B
80
60
40
20
µMol/cm$^2$ x 10$^{-2}$
(Total)
(10 min)
0
0.05
0.5
5
mM NaF
Ca$^{+2}$ Dissolved at pH 4.5
Exchanged Surfaces
C
120
90
60
30
µMol/cm$^2$ x 10$^{-2}$
(Total)
(10 min)
0
0.05
0.5
5
mM NaF
Ca$^{+2}$ Dissolved at pH 4.5
Remineralized Surfaces
D
120
90
60
30
µMol/cm$^2$ x 10$^{-2}$
(Total)
(10 min)
0
0.05
0.5
5
mM NaF

Figure 14.

The effects* of 0.05, 0.50 and 5.00 mM NaF in solutions on reactions of $PO_4^{-3}$ in enamel surfaces at pH 7.0 and 4.5.

A. The amounts of $PO_4^{-3}$ that dissolved from enamel surfaces under conditions of ion exchange in solutions that contained the NaF and 40 mM KCl at pH 7.0.

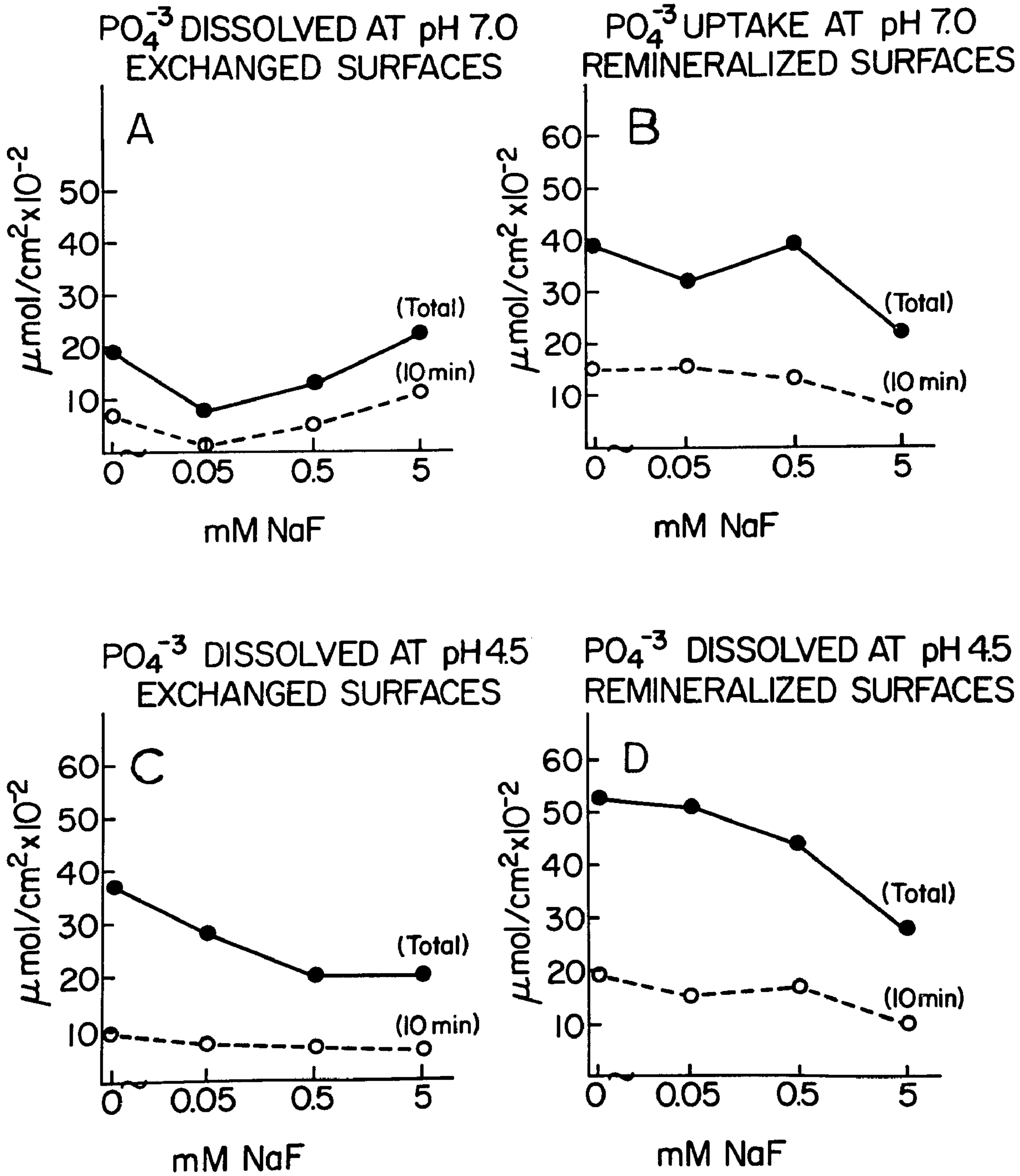
B. The amounts of $PO_4^{-3}$ that deposited in enamel surfaces under conditions of remineralization from solutions that contained the NaF, 1.5 mM $Ca^{+2}$, 1.0 mM $PO_4^{-3}$ and 40 mM KCl at pH 7.0.

C. The amounts of $PO_4^{-3}$ that dissolved from the ion exchange surfaces (A above) in 10 mM acetic acid at pH 4.5.

D. The amounts of $PO_4^{-3}$ that dissolved from the remineralized surfaces (B above) in 10 mM acetic acid at pH 4.5.

* Solid line represents total effects in 30 minutes. Dashed line represents the effects in 10 minutes.

# BRIEFLY RINSED SURFACES



in the surfaces. The amounts of $Ca^{+2}$ and $PO_4^{-3}$ that dissolved in the acid were reduced from surfaces that were subjected to ion exchange in solutions of NaF. However, 5.00 mM NaF had no more effect than 0.50 mM NaF. The surface reactions indicated that 0.50 mM NaF uniquely increased the acid resistance of minerals in the enamel.

The effects of NaF on the acid resistance of remineralized surfaces were consistent with the amounts of $^{45}Ca$ recovered and the amounts of $Ca^{+2}$ and $PO_4^{-3}$ that dissolved in the acid. Surfaces remineralized in the presence of 0.50 and 5.00 mM NaF, but not 0.05 mM NaF resisted dissolution in the acid. However, the acid resistance was not as apparent in the first 10 to 20 minutes of acid exposure as for the total amounts of dissolved minerals. The Ca/P ratios of the minerals that dissolved in the acid were identical for surfaces remineralized in the absence of NaF and the presence of 0.05 and 0.50 mM NaF. The higher ratios of Ca/P for minerals dissolved from the surfaces remineralized in the presence of 5.00 mM NaF than the other surfaces indicated that the surfaces contained $CaF_2$.

The rates of $F^-$ uptake were constant for each level of NaF in the solution and almost identical for the ion exchanged and remineralized surfaces of enamel. The uptake of $F^-$ was independent of the uptake of $^{45}Ca$ and the concentrations of $Ca^{+2}$ and $PO_4^{-3}$ in the solutions. As discussed before, about 90 percent of the $F^-$ which was taken up in the enamel apparently ended up in fluorapatites. In addition, 70 to 85 percent of the $^{45}Ca$ which was taken up in the ion exchanged surfaces, along with $F^-$, was resistant to acid dissolution. The $^{45}Ca$ in the remineralized enamel was acid resistant only in the surfaces which were remineralized in the presence of 5.00 mM NaF. This showed that most of the $^{45}Ca$ was taken up in relatively soluble minerals in both the remineralized and the ion exchanged surfaces.

The minerals that dissolved from the surfaces in the acid represented extremely small fractions of the total minerals in the enamel. The fact all too often overlooked in studies of the effect of various $F^-$ treatments on the acid resistance of minerals in enamel is that the dissolved substances were soluble and the undissolved minerals comprised the acid resistance minerals. The answer sought in these studies was the character of the acid resistant minerals rather than how much $Ca^+$ and $PO_4^{-3}$ dissolved in a given time interval in the acid. In this study $F^-$ was taken up almost entirely in acid resistant minerals in both the ion exchanged and the remineralized surfaces. The acid resistance of these minerals indicated that $F^-$ was ultimately incorporated in fluorapatites in the enamel rather than in the remineralized constituents of the surfaces.

The deposition of $Ca^{+2}$ and $PO_4^{-3}$ in the remineralized surfaces masked the effect of $F^-$ on the acid resistance of $Ca^{+2}$, $PO_4^{-3}$ and $^{45}Ca$ in the enamel. The $^{45}Ca$ taken up in the remineralized surfaces was largely restricted to the relatively soluble deposits of $Ca^{+2}$ and $PO_4^{-3}$. However, the $F^-$ taken up during remineralization was either directly taken up in the enamel minerals or it was relocated in the enamel during acid exposure. Such redistribution of $F^-$ was suggested by Manly and Harrington (48). The relatively soluble fraction of the $F^-$ in the ion exchanged and the remineralized surfaces which comprised less than 10 percent of the total $F^-$ in the surfaces, was obviously adsorbed $F^-$. The Ca/F ratios of the minerals that dissolved in the acid from surfaces which were exposed in solutions of 5.00 mM NaF were much too low to have indicated the dissolution of $CaF_2$ (Ca/F of 0.5 for $CaF_2$).

Primary objectives of this study were to determine the potential effectiveness of $F^-$ on remineralization (repair of caries) and acid resistance (prevention of caries) of enamel. The intent was to accertain the effects of $F^-$ in a short enough time span for possible clinical utilization with respect to the prevention of dental caries. The results were sufficiently sensitive to predict that frequent and brief applications of low levels of $F^-$ would be expected to increase acid resistance of enamel. Furthermore, the results indicated that the concentrations of $F^-$ need not exceed 0.50 mM for maximum effectiveness. However, the degree of effectiveness would be expected to depend on the time and frequency of application.

The results for remineralization were disappointing only in regard to apparent acid resistance of the deposited minerals. Remineralization in the presence of $F^-$, given sufficient time, results in the formation of acid resistant fluorapatite in enamel (77). Thus for the remineralization effect of $F^-$ to be evident clinically, the repetitive cycles of acid production must be stopped for a lengthy period of time to allow formation of acid resistant fluorapatites in the enamel. The results of this study indicated that the same degree of acid resistance of enamel could be expected from applications of $F^-$ in the absence and presence of remineralization (remineralization in terms of mineral deposition from solutions). This effect seemingly provides the most feasible means available for obtaining the anti-carious action of $F^-$.

# CONCLUSIONS

## 1. Studies of Limited Rates of Enamel Remineralization

The minerals of intact surfaces of enamel dissolved in the Ca/P ratio of hydroxyapatite during 24 hour demineralization in the weak acid. The demineralized surfaces contained small amounts of water soluble $Ca^{+2}$ and $HPO_4^{-2}$ as residues of the acid reactions. The demineralized surfaces were remineralized 3 hours in solutions that contained 0.00 to 4.00 mM NaF at pH 7.0. The extent of remineralization was limited by the total amounts of $Ca^{+2}$ and $PO_4^{-3}$ in the solutions. Unlimited amounts of $F^-$ and $OH^-$ were available at fixed concentrations of NaF and at pH 7.0 to support the uptake of $Ca^{+2}$ and $PO_4^{-3}$ in the enamel.

Concentrations of 0.05, 0.25 and 0.50 mM NaF progressively increased the rates of enamel remineralization with respect to the uptake of both $Ca^{+2}$ and $PO_4^{-3}$. The rates of remineralization reached a maximum in solutions of 0.50, 1.00, and 2.00 mM NaF. The levels of 3.00 and 4.00 mM NaF did not support maximum rates of remineralization. Thus $F^-$ concentrations up to 0.50 mM NaF were the dominant driving force of enamel remineralization. This indicated that $F^-$ caused the deposition of fluorapatites in the remineralized enamel.

The uptake of $Ca^{+2}$, but not $PO_4^{-3}$, was closely related to the uptake of $F^-$ in the remineralized enamel over the range of NaF concentrations (0.05 to 4.00 mM NaF). The $Ca^{+2}$ and $F^-$ correlation and the Ca/F ratios of the deposited minerals showed that $CaF_2$ was a significant product of surface reactions in solutions that contained more than 0.50 mM NaF.

The uptake of $PO_4^{-3}$ related to the reactions of $OH^-$ during remineralization of the enamel over the entire range of NaF concentrations (0.00 to 4.00 mM NaF). This indicated that $HPO_4^{-2}$ was deposited in the enamel and then hydrolyzed to form octacalcium phosphate which released $H^+$ in the solutions. These reactions were envisioned to occur at constant pH 7.0 in the following sequence:

$$\underset{\text{Solution}}{\underline{8Ca^{+2}+8HPO_4^{-2}}} \longrightarrow \underset{\substack{\text{Enamel}\\ \text{Surface}}}{\underline{8CaHPO_4}} \longrightarrow \underset{\substack{\text{Enamel}\\ \text{Surface}}}{\underline{Ca_8H_2(PO_4)_6}} \longrightarrow \underset{\text{Solution}}{\underline{4H^{+}+2HPO_4^{-2}}}$$

This sequence of reactions was confirmed by the almost constant ratios of P/OH for remineralization over the range of NaF concentrations, and the Ca/P ratios of mineral uptake in the absence of NaF. The additional uptake of $Ca^{+2}$ that caused Ca/P ratios above 1.33, in the presence of NaF, indicated the deposition of $CaF_2$ in the surfaces along with octacalcium phosphate.

### 2. Studies of the Effect of $F^-$ on Ion Exchange and Remineralization

Groups of the acid demineralized surfaces of enamel were subjected 0.5 hour to reactions of ion exchange alone and other groups were subjected to remineralization. Conditions were established to maintain constant the concentration of the reactants in each of the solutions during contact with the enamel. The ion exchange solutions contained trace $Ca^{+2}$ as $^{45}Ca$, an ionic background of 40 mM KCl, and 0.00, 0.05, 0.50 or 5.00 mM NaF at pH 7.0. The remineralizing solutions contained 1.5 mM $CaCl_2$ and 1.0 mM $NaH_2PO_4$ in addition to the composition of the ion exchange solutions. The intention of these studies was to limit the rates of reactions to one variable, the concentration of $F^-$ in the solutions. Whether the demineralized surfaces were briefly rinsed or rinsed 24 hours had no significant effects on ion exchange and remineralization.

The rates of $F^-$ uptake in the enamel surfaces were almost identical under the conditions of ion exchange and the conditions of remineralization. Thus the uptake of $F^-$ was independent of the concentrations of $Ca^{+2}$ and $PO_4^{-3}$ in the solutions. The uptake of $F^-$ apparently occurred by exchange for $OH^-$ in the enamel in the presence of 0.05 mM NaF and also by adsorption and $CaF_2$ formation in the presence of 0.50 and 5.00 mM NaF. Compared to the effects of 0.05 mM NaF, 0.50 and 5.00 mM NaF actually displaced

$PO_4^{-3}$ and caused the retention of $Ca^{+2}$ in the minerals of the enamel. This effect of 0.50 and 5.00 mM NaF was most obvious for the remineralized surfaces, which removed any doubt that one of the major products of reaction was $CaF_2$.

### 3. Acid Resistance of Exchanged and Remineralized Surfaces of Enamel

The enamel surfaces were tested for acid resistance after the 0.5 hour exposure in the ion exchange and remineralizing solutions. Each surface was exposed in a weak acid in a miniature flow chamber to prevent the accumulation of dissolved products in the acid. The 3 levels of NaF in the ion exchange solutions subsequently increased the acid resistance of $^{45}Ca$, $Ca^{+2}$, $PO_4^{-3}$, and $F^-$ in the exchanged surfaces. The 0.50 mM NaF caused maximum exchange uptake of $^{45}Ca$ at pH 7.0 and maximum acid resistance of the exchanged surfaces. However, 5.00 mM NaF was required for significant acid resistance of the remineralized surfaces. The lack of effect or small effect of 0.05 and 0.50 mM NaF on acid resistance of the remineralized surfaces seemed to confirm that they contained octacalcium phosphate as earlier suggested. The presence of deposited octacalcium phosphate would be expected to mask the acid resistance effect of the $F^-$ in the surfaces. The minerals that dissolved in the acid from surfaces which were previously exposed in solutions of 0.50 and 5.00 mM NaF apparently contained $CaF_2$ and an additional source of $F^-$ other than fluorapatites.

The $F^-$ which was taken up in the enamel under conditions of ion exchange alone and under conditions of remineralization exhibited the greatest acid resistance of the surface ions. About 90 percent of the $F^-$ in the surfaces was undissolved in the acid. This suggested that the acid resistant $F^-$ was exchanged for $OH^-$ in the apatite of the enamel to form fluorapatites. The relatively small amounts of $F^-$ that dissolved in the acid probably represented surface adsorbed $F^-$ rather than $CaF_2$ as previously assumed. It is noteworthy to recall that 0.50 mM NaF had the maximum effect on ion exchange and acid resistance of enamel in the absence of remineralization.

# SUMMARY

Fluoride was taken up in enamel under conditions of ion exchange in the same amounts and at the same rates as under conditions of remineralization. The rates of $F^-$ uptake under these conditions depended only on the concentrations of NaF in the solutions. Although the uptake of $F^-$ possibly occurred by adsorption, exchange, and $CaF_2$ formation, acid resistance depended on the formation of fluorapatites by exchange of $F^-$ for $OH^-$ in the enamel at pH 7.0 and pH 4.5.

The exchanged $F^-$ in the enamel reduced the solubility of the mineral surfaces. The resulting decreased rates of release of ions to the solutions shifted the dynamic imbalance of ionic exchange at the mineral surfaces to favor the stable less soluble mineral phase, fluorapatite. This or a closely similar concept of the mechanism of action of $F^-$ on synthetic apatite was proposed by Weikel *et al.* (85). The proposed mechanism should be operative at the neutral and acid pH of this study.

The results of this study indicated that the predominant effect of $F^-$ on enamel was the formation of fluorapatites by the surface exchange of $F^-$ for $OH^-$. Most importantly this study showed that the readily and safely applicable level of 0.50 mM $F^-$ would be expected to provide in a practical way the maximum beneficial effect on caries prevention. The accumulation of similar levels of $F^-$ in dental plaque on caries prone tooth surfaces may partially explain the benefits of fluoridation. The ion exchange studies showed that brief, frequent applications of low levels of $F^-$ would be expected to increase the caries resistance of enamel.

# APPENDIX

Summary of the Parameters Determined with the Mathematical Model Used in the Computer Program Curvilinear Analysis for the Effect of Fluoride on Rates of Enamel Remineralization.

Model: $Y_t = K[1-e^{-\beta(t-T)}]$.

$Y_t$ = Substance uptake at a given time (t) in μmols/cm$^2$,
K = Maximum uptake (μmoles/cm$^2$),
β = Relative rate of uptake (μmoles/cm$^2$/hr),
C = Constant,
T = Lag = C/β.

1. Parameters Determined for $Ca^{+2}$:

| NaF | Mean Values X $10^{-2}$ (S.D.) | | | |
|---|---|---|---|---|
| (mM) | K | β | C | T |
| 0.00 | 151(1) | 51(10) | 12(5) | 23(8) |
| 0.05 | 196(27) | 40(12) | 7(5) | 15(9) |
| 0.25 | 196(14) | 54(12) | 8(1) | 16(5) |
| 0.50 | 189(13) | 64(15) | 4(3) | 6(5) |
| 1.00 | 182(11) | 83(17) | 7(1) | 7(6) |
| 2.00 | 189(16) | 74(25) | 6(1) | 5(4) |
| 3.00 | 196(71) | 56(42) | -3(2) | -28(4) |
| 4.00 | 181(11) | 51(26) | 1(.1) | -11(3) |

2. Parameters Determined for $PO_4^{-3}$:

| NaF (mM) | Mean Values X $10^{-2}$ (S.D.)<br>K | β | C | T |
|---|---|---|---|---|
| 0.00 | 106(8) | 60(20) | 2(.1) | -1(.1) |
| 0.05 | 106(6) | 84(23) | 12(1) | 12(1) |
| 0.25 | 126(22) | 59(25) | .3(1) | -13(3) |
| 0.50 | 115(10) | 67(17) | -3(1) | -9(1) |
| 1.00 | 115(5) | 74(10) | -4(1) | -6(1) |
| 2.00 | 114(19) | 64(19) | 4(1) | 3(12) |
| 3.00 | 115(53) | 82(62) | 15(2) | 8(3) |
| 4.00 | 108(5) | 70(24) | -36(10) | -66(17) |

3. Parameters Determined for $F^-$:

| NaF (mM) | Mean Values X $10^{-2}$ (S.D.)<br>K | β | C | T |
|---|---|---|---|---|
| 0.00 | - | - | - | - |
| 0.05 | 104(69) | 7(5) | 1(.1) | 9(1) |
| 0.25 | 162(90) | 30(14) | 1(.1) | -.1(.1) |
| 0.50 | 145(25) | 61(4) | 2(.3) | 2(.1) |
| 1.00 | 171(35) | 71(18) | -5(.1) | -10(2) |
| 2.00 | 266(14) | 66(11) | -24(8) | -37(15) |
| 3.00 | 169(73) | 43(28) | -14(1) | -31(2) |
| 4.00 | 158(45) | 78(39) | 12(2) | 17(3) |

4. Parameters Determined for $OH^-$:

| NaF (mM) | Mean Values X $10^{-2}$ (S.D.) K | β | C | T |
|---|---|---|---|---|
| 0.00 | 134(11) | 43(5) | 3(1) | 6(1) |
| 0.05 | 173(8) | 30(6) | 1(.1) | 3(.1) |
| 0.25 | 150(15) | 45(15) | 6(1) | 11(1) |
| 0.50 | 131(27) | 53(15) | 6(1) | 11(1) |
| 1.00 | 148(17) | 53(10) | 3(1) | 6(3) |
| 2.00 | 143(14) | 50(5) | 5(1) | 10(2) |
| 3.00 | 130(18) | 46(6) | 5(13) | 10(5) |
| 4.00 | 209(151) | 44(28) | 3(.5) | 2(.1) |

# BIBLIOGRAPHY

1. Andreson, V.: The Physiology and Artifical Mineralization of the Enamel, p. 93, Statens Tandlaegeinstitute, Oslo, 1926.

2. Aponte-Merced, L., Feagin, F. F., and Thiradilok, S.: Effect of fluoride ion activity on rates of calcium uptake by enamel. Ala. J. Med. Sci. 14(3):238-246, 1977.

3. Bachra, B. N., Trautz, O. R., and Simon, S. L.: Precipitation of calcium carbonates and phosphates - III The effect of magnesium and fluoride on the spontaneous precipitation of calcium carbonates and phosphates. Archs. Oral Biol. 10:731-738, 1965.

4. Barr, A. J. and Goodnight, J. H. Statistical Analysis System, Users Guide, Raleigh, N. C., North Carolina State University, 1972.

5. Black, G. V. and McKay, F. S.: Mottled teeth: An endemic development imperfection of the teeth, heretofore unknown in the literature of dentistry. Dental Cosmos 58:129, 1916.

6. Briner, W. W., Gray, J. A., and Francis, M. D.: Significance of enamel remineralization. J. Dent. Res. Suppl. to No. 2, 53:239-243, 1974.

7. Brodecker, C. F.: Concerning the character of the age changes in enamel and dentin and their relation to the vital pulp. J. Am. Coll. Dent. 9:380-399, 1942.

8. Brown, W. E.: Crystal growth of bone mineral. Clin. Orthop. Related Res. 44:205-220, 1966.

9. Brown, W. E., Gregory, T. M., and Chow, L. C.: Effects of fluoride on enamel solubility and cariostasis. Caries Res. 11:118-141, 1977.

10. Brudevold, F.: Interaction of fluoride with human enamel. In Symposium: Chemistry and Physiology of Enamel, pp. 73-90. The University of Michigan, Sept. 17-18, 1971.

11. Brudevold, F., Amdur, B., and Messer, A.: Factors involved in remineralization of carious lesions. Arch. Oral Biol. 6:304, 1961.

12. Brudevold, F., Grøn, P., and McCann, H. G.: Physico-chemical aspects of the enamel-saliva system. Advances Fluorine Res. 3:63-78, 1965.

13. Brudevold, F. and McCann, H. G.: Enamel solubility tests and their significance in regard to dental caries. Ann. N. Y. Acad. Sci. 153:20-51, 1968.

14. Carlstrom, D.: X-ray crystallographic studies on apatite and calcified structures. Acta Radio. Suppl. 121:1-59, 1965.

15. Chen, P. S., Torribara, T. Y., and Warner, H.: Micro-determination of phosphorus. Analyt. Chem. 28:1756-1758, 1956.

16. Dean, H. T.: Domestic water and dental caries including certain epidemiological aspects of oral L. acidophillus. Public Health Rept. (U.S.) 54:862, 1939.

17. DePaola, P. F.: A review of clinical trials utilizing acidulated phosphate fluoride topical agents. J. Am. Coll. Dent. 35:22-33, 1968.

18. Eanes, E. D. and Posner, A. S.: Intermediate phases in the basic solution preparation of alkaline earth phosphates. Calc. Tiss. Res. 2:38, 1968.

19. Ericsson, S. Y.: Cariostatic mechanisms of fluorides: Clinical observations. Caries Res. 11:2-41, 1977.

20. Ericsson, S. Y.: The chemistry of the enamel-saliva interface. Ala. J. Med. Sci. 5(3):256-266, 1968.

21. Feagin, F.: A Quantitative Method for the Measurement of Factors Affecting Calcification Processes. Ph.D. dissertation, University of Alabama in Birmingham, 1969.

22. Feagin, F.: Calcium, phosphate and fluoride deposition on enamel surfaces. Calc. Tiss. Res. 8:154-174, 1971.

23. Feagin, F. and Gonzalez, M.: Fluorapatite crystal growth during remineralization of enamel surfaces. Ala. J. Med. Sci. 10:46-51, 1973.

24. Feagin, F., Gonzalez, M., and Jeansonne, B. G.: Kinetic reactions of calcium, phosphate and fluoride

ions at the enamel surface-solution interface. Calcif. Tissue Res. 10:113-127, 1972.

25. Feagin, F. and Jeansonne, B. G.: Effective fluoride concentration to promote apatite mineralization at the enamel surface. Ala. J. Med. Sci. 10:107-114, 1973.

26. Feagin, F., Koulourides, T., and Pigman, W.: The characterization of enamel surface demineralization, remineralization and associated hardness changes in human and bovine material. Archs. Oral Biol. 14:1407-1417, 1969.

27. Feagin, F., Pope, H., and Aponte-Merced, L.: Effect of sodium fluoride in acid solution on dissolution of powdered human enamel. Ala. J. Med. Sci. 13:21-28, 1976.

28. Fehr, F. R. von der: Maturation and remineralization of enamel. Advances Fluorine Res. 3:83-99, 1965.

29. Galagan, D. J. and Lamson, G. G.: Climate and endemic dental fluorosis. Public Health Report (U.S.) 68:497, 1953.

30. Glimcher, M. J.: Studies of the proteins of embryonic and mature dental enamel: The "Enamelins". In Symposium: Chemistry and Physiology of Enamel, pp. 2-4, The University of Michigan, Sept. 1971.

31. Glimcher, M. J. and Krane, S. M.: The organization and structure of bone, and the mechanism of calcification. In Treatsie on Collagen. (ed. Gould, B. S.), p. 67, Academic Press, London and New York, 1968.

32. Gonzalez, M. and Feagin, F.: Density and X-ray diffraction studies of enamel surface reactions. Ala. J. Med. Sci. 10:83-88, 1973.

33. Hallsworth, A. S., Weatherell, J. A., and Robinson, C.: Loss of carbonate during the first stages of enamel caries. Caries Res. 7:345-348, 1973.

34. Head, J.: A study of saliva and its action on tooth enamel in reference to its hardening and softening. J. Am. Med. Assoc. 59:2118-2122, 1912.

35. Hodge, H. C. and Smith, F. A.: Some Public Health Aspects of Water Fluoridation, Fluoridation as a Public Health Measure. (ed. Shaw, J. H.), pp. 132-145, A.A.A.S., Washington, D. C., 1954.

36. Horowitz, H. S., Heiftz, S. B., and Law, F. E.: Effect of water fluoridation and dental caries: Final result in Elk Lake, Pa. after 12 years. J. Am. Dent. Assoc. 84:832-838, 1976.

37. Irving, J. T.: Calcium and Phosphorus Metabolism, Academic Press, New York, 1973.

38. Jenkins, G. N.: A critique of the proetolysis-chelation theory or caries. Bri. Dent. J. 11(9):311-330, 1961.

39. Jenkins, G. N.: The Physiology of the Mouth, 3rd ed., F. A. Davis, Co., Philadelphia, 1966.

40. Jenkins, G. N., Edgar, W. M., and Ferguson, D. B.: The distribution and metabolic effects of human dental plaque fluoride. Arch. Oral Biol. 14:105-119, 1969.

41. Johansson, B.: Remineralization of slightly etched enamel. J. Dent. Res. 44:64-70, Jan.-Feb., 1965.

42. Johnson, N. W., Poole, D. F. G., and Tyler, J. E.: Factors affecting the differential dissolution of human enamel in acid and EDTA. Scanning electron microscope study. Archs. Oral Biol. 16:385-396, 1971.

43. Keyes, P. H. and Englander, H. R.: Fluoride therapy in the treatment of dentomicrobial plaque diseases. J. Am. Soc. Prev. Dent. 5:16-40, 1975.

44. Koulourides, T.: "Remineralization of Enamel and Dentine". In Dental Clinics of North America (eds. Johansen, E. and Shapiree, M.) pp. 485-497, W. B. Saunders Co., Philadelphia, Pa., 1962.

45. Koulourides, T.: Remineralization methods. Ann. N. Y. Acad. Sci. 153:84-101, 1968.

46. Koulourides, T., Cueto, H., and Pigman, W.: Rehardening of softened enamel surfaces of human teeth by solutions of calcium phosphates. Nature 189:226-227, 1961.

47. Koulourides, T., Feagin, F., and Pigman, W.: Remineralization of dental enamel by saliva in vitro. Ann. N. Y. Acad. Sci. 131:771-775, 1965.

48. Manly, R. S. and Harrington, D. P.: Solution rate of tooth enamel in an acetate buffer. J. Dent. Res. 38(5):910-919, 1959.

49. Maron, S. H. and Prutton, C. F.: "Surface Phenomena" In Principles of Physical Chemistry. Chapter 7, pp. 214-231, The Macmillan Co., New York, 1964.

50. McCann, H. G.: Determination of fluoride in mineralized tissues using the fluoride ion electrode. Arch. Oral Biol. 13:475-477, 1968.

51. McClure, F. J.: Water Fluoridation: The Search and the Victory. Nat. Inst. Health, Dept. Health, Education and Welfare, Washington, D. C., 1970.

52. McKay, F. S. and Black, G. V.: Investigation of mottled teeth. Dental Cosmos 58:477, 627, 781, 894, 1916.

53. Meckel, A. H.: The keyhole concept of enamel microstructure. In Symposium: Chemistry and Physiology of Enamel, pp. 25-42, The University of Michigan, Sept. 1971.

54. Meckel, A. H., Griebstein, W. J., and Neal, R.-J.: Structure of mature human dental enamel as observed by electron microscopy. Arch. Oral Biol. 10:775-783, 1965.

55. Muhlemann, H., Lenz, H., and Rossenky, K.: Electron microscopic appearance of rehardened enamel. Helv. Odont. Acta 8:108-111, 1964.

56. Nancollas, G. H. and Mohan, M. S.: The growth of hydroxyapatite crystals. Archs. Oral Biol. 15:731-745, 1970.

57. Neuman, W. F.: Bone as a problem in surface chemistry metabolic interrelations. Trans. Sec. Conf. Josiah Macy, Jr. Foundation, 1950.

58. Neuman, W. F. and Neuman, M. W.: The Chemical Dynamics of the Bone Mineral, University of Chicago Press, Chicago, 1958.

59. Orban, B. J.: Orban's Oral Histology and Embryology (ed. Bhaskar, S. N., 8th ed.), C. V. Mosby Co., St. Louis, 1976.

60. Orion, R.: Instruction Manual, Calcium Ion Electrode Model 93-20 Orion Research, Cambridge, Massachusetts, 1975.

61. Pickerill, H. P.: The Prevention of Dental Caries and Oral Sepsis, pp. 90-96, Tendal and Cox, London, Bailliere, 1912.

62. Poole, D. F. G. and Brooks, A. W.: The arrangement of crystallites in enamel prisms. Archs. Oral Biol. 5:14-26, 1961.

63. Poole, D. F. G. and Johnson, N. W.: The effects of different demineralization agents on the human enamel surfaces studied by scanning electron microscopy. Archs. Oral Biol. 12:1621-1634, 1967.

64. Pruitt, K. M.: Macromolecular components of oral fluids at tooth surface. Swedish Dent. J. 1:225-240, 1977.

65. Pruitt, K. M. and Turner, M. E.: "A Kinetic Theory for Analysis of Complex Systems." In Cellular Function and Molecular Structure A Symposium on Biophysical Approaches to Biological Problems, Academic Press, in press.

66. Rasmussen, H., and Bordier, P.: The Physiological and Cellular Basis of Metabolic Bone Diseases, 1st ed., Williams and Wilkins Co.. Baltimore, 1974.

67. Ruthrauff, W. M.: Landmarks in Dental Research Leading to the Establishment of the Calcification Theory of Immunity to Dental Caries, pp. 96-97, Ruthrauff Co., Philadelphia, Pa., 1923.

68. Sarkar, B. C. R. and Chauhan, U. P. S.: A new method for determining microquantities of calcium in biological materials. Analyt. Biochem. 20:155-166, 1967.

69. Scott, D. B., Simmelink, J. W., and Nygaard, V.: Mineralization of dental enamel. In Symposium: Chemistry and Physiology of Enamel, pp. 6-24, The University of Michigan, Sept., 1971.

70. Scott, D. B., Simmelink, J. W., and Nygaard, V.: Structural Aspects of Dental Caries. J. Dent. Res. Suppl. to No. 2, 53:165-178, 1974.

71. Sharpe, A. N.: Influence of the crystal orientation in human enamel on its reactivity to acid as shown by high resolution microradiography. Archs. Oral Biol. 12:583-591, 1967.

72. Silverstone, L. M.: Remineralization phenomena. Caries Res. 11:59-84, 1977.

73. Smith, H. V. and Smith, M. C.: Mottled enamel in Arizona and its correlation with concentration of fluorides in water supplies. Univ. Arizona Agr. Expt. Sta. Tech. Bull. 43:395-398, 1932.

74. Sobel, A. E.: Interrelationship of tooth composition, body fluids, diet and caries susceptibility. Ann. N. Y. Acad. Sic. 85:96-109, 1960.

75. Termine, J. D. and Posner, A. S.: Infra-red analysis of rat bone: Age dependency of amorphous and crystalline mineral fractions. Science N. Y. 153: 1523-1525, 1966.

76. Termine, J. D., Wuthier, R. E., and Posner, A. S.: Amorphous crystalline mineral changes during endochondral and periosteal bone formation. Proc. Soc. Exptl. Biol. Med. 125:4, 1967.

77. Thiradilok, S.: Effect of Fluoride, Bicarbonate and Magnesium Ions on the Acid Resistance of Recrystallized and Remineralized Surfaces of Enamel, Ph.D. dissertation, University of Alabama in Birmingham, 1977.

78. Thomas, J. P. and Feagin, F. F.: The effects of 0.05 to 5.0 mM NaF on calcium phosphate mineral accretion and subsequent acid dissolution at enamel surfaces. Ala. J. Med. Sci. 10(1):52-59, 1973.

79. Vaughan, J. M.: The Physiology of Bone, 2nd ed., Clarendon Press, Oxford, 1975.

80. Volker, J. F.: Effect of fluorine on solubility of enamel and dentin. J. Proc. Soc. Exp. Biol. 42:725-727, 1939.

81. Volker, J. F.: Solubility of fluorosed enamel and dentin. J. Proc. Soc. Exp. Biol. 43:643-645, 1940.

82. Volker, J. F., Hodge, H., Wilson, H. J. and Van Voorhis, S. N.: The adsorption of fluoride by enamel, dentin, bone and hydroxyapatite as shown by the radioactive isotope. J. Biol. Chem. 134:543-548, 1940.

83. Volker, J. F. and Russell, D. L.: "The Epidemiology of Dental Caries," In Clinical Pedodontics (ed. S. B. Finn) 4th ed., pp. 454-561, W. B. Saunders Co., Philadelphia, London and Toronto, 1973.

84. Volker, J. F. and Russell, D. L.: "The Prevention of Dental Caries with Fluoride," In Clinical Pedodontics (ed. S. B. Finn) 4th ed., pp. 498-517, W. B. Saunders Co., Philadelphia, London and Toronto, 1973.

85. Weikel, J. H., Neuman, W. F., and Feldman, I.: The surface chemistry of Bone. VIII. On the mechanism of ionic exchange. J. Am. Chem. Soc. 76:5202-5207, 1954.

86. Zar, J. H.: Biostatistical Analysis (eds. McElroy, W. D., Swanson, C. P.), Prentice Hall Inc., Englewood Cliffs, N. J., 1974.

GRADUATE SCHOOL
UNIVERSITY OF ALABAMA IN BIRMINGHAM
DISSERTATION APPROVAL FORM

Name of Candidate Luis Antonio Aponte-Merced

Major Subject Physiology and Biophysics

Title of Dissertation Effects of Fluoride on Mineral Uptake and Acid Resistance of Enamel

Dissertation Committee:

, Chairman

Edwin Bradley Jr.

Director of Graduate Program

Dean, UAB Graduate School S B Barker

Date 6 May 1978