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Effects Of Fluoride, Bicarbonate, And Magnesium Ions On The Acid Resistance Of Recrystallized And Remineralized Surfaces Of Enamel.

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EFFECTS OF FLUORIDE, BICARBONATE, AND MAGNESIUM IONS ON THE ACID RESISTANCE OF RECRYSTALLIZED AND REMINERALIZED SURFACES OF ENAMEL.

THE UNIVERSITY OF ALABAMA IN BIRMINGHAM,
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EFFECTS OF FLUORIDE, BICARBONATE, AND MAGNESIUM IONS ON THE ACID RESISTANCE OF RECRYSTALLIZED AND REMINERALIZED SURFACES OF ENAMEL

by

SUKHUM THIRADILOK

A DISSERTATION

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the degree of Doctor of Philosophy in the Department
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1977

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LIST OF ABBREVIATIONS

^{45}Ca = Radioactive calcium as calcium chloride.

Ca = The total calcium and/or ionic calcium added to or measured in solutions.

Mg = The total magnesium added in solutions.

PO_4 = The total inorganic phosphate measured or added in solutions, which included H_3PO_4, $H_2PO_4^-$, HPO_4^{2-}, and PO_4^{3-}.

F = The total and/or ionic fluoride added to or measured in solutions

HCO_3 = The total carbonate added in solutions, which included H_2CO_3, HCO_3^-, CO_3^{2-}.

OH = The total NaOH added in the reaction solutions.

Ci = Curie.

mCi = Millicurie.

DPM = Disintegration per minute.

Rc = Recrystallized.

Rm = Remineralized.

EDTA = Ethylenediamine Tetracetate.

INTRODUCTION

Dental caries is a multifactorial microbial disease characterized by early loss of surface and subsurface minerals and later loss of the tooth. It is the most prevalent chronic disease of the child and young adult (101). Although the exact mechanisms of the disease processes are incompletely known, caries may be arrested, and even reversed to some extent, by meticulous control of dental plaque and diet and by optional utilization of fluoridation (25). Fluoridation greatly reduces the incidence and severity of caries in widespread, diverse populations somewhat independent of hygiene and diet. Applications of fluoride ions directly on tooth surfaces also inhibit carious processes, but less predictably than fluoridation (20). Fluoride ions seem to be even more effective in caries prevention for high risk than low risk populations (63).

Several possible mechanisms of the fluoride ion action of caries prevention are known. The contribution of each mechanism probably varies at different points in the carious process. In broad concept fluoride ions inhibit acid production of plaque bacteria, enhance reprecipitation of minerals in the tooth, increase the acid resistance of the minerals, and diminish the adsorption of organic substances on the surface minerals of the tooth (44). The source of fluoride ions whether from tooth minerals, oral fluids, or exogenous substances seems unimportant. Rather, the concentrations and frequencies of fluoride ion presence at the tooth surface seem to control its effectiveness (101).

Clinical and laboratory evidence indicates that early carious lesions frequently undergo arrest and reversal by a phenomenon described as remineralization (90). Remineralization is remarkably stimulated by the low fluoride ion concentrations of fluoridation and the consequent levels of fluoride found in dental plaque (27, 41). The remineralizing effect of fluoride and the subsequent acid resistance of the tooth minerals provide a most logical mechanism of action of caries prevention. However, the processes of remineralization depend to varying degrees on the total environment of tooth surfaces. Therefore it is difficult to obtain knowledge of the remineralization potential of a particular tooth or surface site for any conditions. The basic components require study of the individual reactants under the simplest possible conditions before attempting an understanding of the complex in situ phenomena of remineralization.

The principal minerals of teeth and bones are calcium apatites, variously described as a family of minerals similar to hydroxyapatite (103). Deviations in structure and composition of biological apatites are often attributed to substitution of "foreign ions" in the crystal lattice of hydroxyapatite and excessive accumulation of extraneous ions in layers of surface hydration (70). Such ionic substitutions and adsorptions occur during mineralization and later by exchange and recrystallization. The calcium apatite family is also described as a continuum in which the initial minerals deposit as acidic calcium phosphates which then hydrolyze to hydroxyapatites (16, 78). The early mineral deposits appear structureless or relatively noncrystalline whereas the aged minerals are more crystalline (94). Compared to bone the principal minerals of tooth enamel are almost pure crystalline hydroxyapatite (14).

Early x-ray analysis showed that biological apatites were similar in lattice structure to hydroxyapatite. These early studies were based on the x-ray diffraction of almost pure fluorapatite (67, 79). Later studies of electron diffraction (3), neutron diffraction (105), and infrared spectroscopy (50) confirmed the basic structure of biological apatites. Precise determination of the detailed structure of biological apatites and similar synthetic precipitates has been almost impossible because of their poor crystallinity (14, 97). In addition, the minerals of interest undergo varying degrees of change during preparation of samples for study by physical methods.

The methods of chemical analysis, x-ray diffraction, and electron microscopy confirmed that newly calcified tissues contain poorly crystallized apatites that slowly change to well crystallized minerals in the mature animal (83, 94). Synthetic apatites prepared from solutions partially mimic the process of bone mineral crystallization (16, 21, 78). These studies led to the assumption that the initial minerals in bone are relatively nonstructured calcium phosphates that on maturation recrystallized to hydroxyapatites. It has been assumed that enamel also develops by the growth of precursor minerals to form much better crystallized apatites than found in bone (14).

Besides being a poorly crystallized mineral of colloidal dimensions, biological apatites exhibit wide variations of chemical composition. Calcium and phosphate make up the major inorganic content of bone and tooth minerals, nearly all of which is thought to be in the apatite structure. Enamel and the other biological minerals contain slightly variable amounts of magnesium, sodium, pyrophosphate, chloride, fluoride, citrate, and carbonate (103). Several additional trace elements have also been found in these minerals (22). It has not been possible to distinguish which fractions of the trace elements, potassium, and some organic substances, are exclusively in the matrix or in the mineral phase of bones and teeth (98).

In vitro studies of solution precipitates have led to much understanding of the nature of bone mineral (70). Little success has been attained in studies of enamel chemistry by solution precipitates because they do not reproduce the relatively large crystal size of enamel apatite (68). However, crystals formed in remineralized enamel from solutions that contained optimal levels of fluoride resembled natural crystals of enamel (26, 28). Thus studies of remineralization hold promise of providing needed information of the surface reactions of enamel.

The composition of biological fluids accounts for many of the physical and chemical variations of bone and tooth minerals (68, 91). The nonstoichiometry of biological apatites relative to the calcium and phosphate composition of hydroxyapatite, i.e., Ca/P ratio of 1.67, has been attributed to one or more of the following factors: (a) The adsorption of acidic phosphate ions on apatite surfaces (14, 37); (b) Ionic substitutions for calcium or statistically vacant calcium positions in the crystal lattice (70, 77); (c) The inclusion of dicalcium phosphate (19) and/or octacalcium phosphate (6), on growing crystal facets; (d) The presence of amorphous calcium phosphates with accompanying foreign ion inclusions in water of hydration (70); (e) Substitutions of bicarbonate and carbonate ions for phosphate in lattice sites (51, 72), or as an admixed phase of calcium carbonates (80); (f) Substitutions of bicarbonate or carbonate for hydroxyl sites in the crystal structure (24). The assumptions (a) to (d) would cause ratios of Ca/P below the ratio of hydroxyapatite, whereas (e) to (f) would cause higher ratios than hydroxyapatite.

Neuman and Neuman concluded that the status of bone mineral in terms of nucleation, growth, maturation, and dissolution involved primarily the dynamic surface chemistry of hydroxyapatite (70). The small crystalline minerals interact with the surrounding fluids first by ionic interchange in surface layers of hydration, then at the crystal surface, and much more slowly by intracrystalline exchange. Thus, the reactive interface may be thought of as a gel of increasing density from the solution into the anterior of the apatite crystal. The basic ionic reactions of the interface have been described as adsorption, exchange, recrystallization, and precipitation.

Adsorption is a poorly known process. It is a rather nonspecific uptake of a substance in the hydration shell or on crystal surfaces involving weak electrostatic forces. Adsorption of a substance to hydroxyapatite is generally 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 calcium for calcium in apatite. Heteroionic exchange is the process of replacement of one ion by a different ion, such as the replacement of hydroxyl ions by fluoride ions in apatite. Heteroionic exchange causes either minor or major alterations of some characteristics of apatites.

Recrystallization implies the dynamic processes of crystal dissolution and redeposition. Neuman and Neuman suggested that intracrystalline exchange represented a type of recrystallization of apatites (70). The term recrystallization will be used herein to mean surface exchange and intracrystalline exchange relative to reactions between solutions not saturated with apatite and minerals in the surfaces of enamel. Recrystallization by our definition means that the minerals may show a net gain or loss of calcium, or phosphate, or fluoride, but not a combination of calcium and phosphate. Net gains of both calcium and phosphate from supersaturated solutions by the surface minerals in enamel are considered as remineralization (90). Conversely net losses of both calcium and phosphate are defined as demineralization. Adsorption, exchange, recrystallization, demineralization, and remineralization may occur simultaneously within enamel. Recrystallization in the classic sense (70) has the same meaning as remineralization except when the redeposited minerals are noncrystalline (46).

The fluoride ion in appropriate concentrations in enamel and plaque fluids has a unique ability to prevent dental caries (61). Fluoride inhibits the bacterial production of harmful substances (40) and by incorporation as fluorapatites increases the resistance of enamel to decay (99). Relatively high levels of fluoride interfere with the adherence of pellicle-like substances on enamel minerals, but whether this action relates to caries prevention is unknown (25).

The anti-metabolic (antibacterial) theory of fluoride action in caries prevention assumes an effective level of fluoride ions in plaque most of the time. This is feasible since in vitro studies showed that bacterial production of CO_2 from glucose was inhibited by as little as 5 ppm or 0.25 mM fluoride in the media (84). Plaque recovered from tooth surfaces of residents of fluoridated and low-fluoride communities contained considerably higher levels of total fluoride than required to reduce CO_2 production (40). However, the percentage of the total fluoride as the ionic form in plaque is not known. One of the possible harmful effects of plaque is that it provides a sink for the inactivation of fluoride ions, thus preventing free diffusion to the minerals of the tooth (45).

The reactions between fluoride ions and tooth minerals and the subsequent resistance of the minerals to caries have been the subject of intensive investigation since the work of Volker in 1939 (99). Largely as a result of this first laboratory investigation the anti-caries action of fluoride is considered to be caused primarily by the relative insolubility of fluorapatites (100). Although the differences in thermodynamic solubility of hydroxyapatite and fluorapatite are small (7) their rates of dissolution are vastly different in all but the strongest acids (13).

The following reactions between fluoride ions and tooth minerals have been described by Brudevold (9):

(a) Adsorption of fluoride ions on surface minerals and in hydrated mineral phases from solutions of relative low concentrations, followed by fluoride exchange for the hydroxyl ions of hydroxyapatite and recrystallization of acidic calcium phosphates to fluorapatite;

(b) Decomposition of surface minerals and precipitation of CaF_2 on surfaces from solutions of relatively high fluoride ion concentrations followed by dissolution of CaF_2 and incorporation of fluoride ions in fluorapatite by the exchange, recrystallization, and remineralization;

(c) Remineralization of slightly demineralized surfaces by direct precipitation of fluorapatites within the tooth from supersaturated solutions of calcium, phosphate, and fluoride ions.

The effect of fluoride ions on remineralization is not distinct from the increased acid resistance of fluoride containing tooth minerals. The concept embodies the law of mass action and must include the dynamic reactions of recrystallization, demineralization, and remineralization across the tooth mineral plaque fluid or saliva interface (46). However, the direct reformation of hydroxy- and fluorapatites can occur only by recrystallization since apatites do not directly precipitate from biological or like solutions (92). The initial products of remineralization theoretically must undergo phase transformation (recrystallization) to apatites (95). The presence of fluoride ions in the solution phase markedly increases the rates of recrystallization of the initial precipitates to both hydroxy- and fluorapatites (27). Enamel remineralized in the presence of fluoride has been shown to resist acid dissolution to a greater extent than non-fluoride exposed enamel (27, 52). Remineralization has been demonstrated by chemical means (27), hardness (47), microradiography (88), electron microscopy (32), and polarized light microscopy (89).

The levels of bicarbonate and carbonate ions found in plasma inhibited the remineralization of enamel as determined by rehardening (30). Carbonates in enamel reduce its resistance to caries (17, 104). Clearly, minerals that contain relatively high levels of carbonate dissolve in acid more readily than does the apatite of enamel (54). Although carbonates of saliva buffer acids, the CO_2 produced by fermentation of carbohydrates is a source of plaque acid that dissolves tooth minerals. Rats fed a high carbonate diet exhibited more extensive caries than controls, both groups on sugar diets (91). However, both fluoride and bicarbonate in the diet reduced the incidence of caries in the rat more than fluoride alone (56). The apparent contradictions may be resolved by consideration of whether the carbonates were incorporated and increased the reactivity of the tooth minerals or served as buffers of plaque acids.

The nature of carbonates in geological and biological apatites has been debated without clear resolution. The arguments consider whether carbonates are present as a separate phase (80) or at a deformed site in the apatite lattice (51, 72). Evidence has indicated exchange of carbonate for phosphate and hydroxyl ions in synthetic apatites (23, 72), or phosphate ions alone (48). Still other studies indicated bicarbonate restriction to layers of hydration on surfaces of apatites of bone and teeth (72).

The carbonates exist in progressively higher concentrations from the surface to the deeper layers of enamel in erupted teeth (8). The crudely determined gradients of carbonates in enamel have generally been presented as evidence of post-eruptive maturation and remineralization of surface enamel (54). However, a similar carbonate profile has been reported for the enamel of unerupted teeth (102). The possibility exists that the reported carbonate gradients in enamel result from storage of extracted teeth in water or even a moist atmosphere.

Carbonates in enamel theoretically increase caries susceptibility by preferential dissolution in weak acids (53, 55). The presence of carbonates in solutions inhibits crystallization of apatites and stabilizes amorphous precipitates (49). This effect of carbonates probably intervenes in the natural processes of recrystallization and remineralization of enamel. If so, then carbonates of plaque and salivary origins may be considered potentially cariogenic in the absence of fluoride.

The distribution of fluoride ions in enamel has been found to be the reverse of the carbonate gradient (11). Generally the older the post-eruptive age of the tooth the higher the fluoride content of the surface enamel. However, most determinations of fluoride in enamel are relatively insensitive and provide only rough estimates of contents in surface enamel. Recent estimates of surface fluoride in enamel show only small differences for teeth from fluoridated localities and low fluoride areas (1). It is possible that fluoride ions and bicarbonate or carbonate ions compete for sites in enamel minerals (73), although most evidence indicates competition between carbonates and phosphate ions in apatites (71). Bicarbonate-carbonate exchange for phosphate in enamel might interfere with the fluoride ion effect of caries prevention.

Despite the widespread importance of magnesium metabolism, its role in calcification is relatively unknown. Exchange studies indicated that the apatite structure strongly rejects magnesium ions. The small amounts of exchanged magnesium were confined to the hydration shell or surfaces of apatite crystals (69). This study suggested that bone minerals provide a reservoir of magnesium which is rapidly mobilized on magnesium deficient diets.

The effects of magnesium ions on enamel reactivity and carious processes have not been studied sufficiently. Magnesium in enamel dissolves in acids at different rates than calcium. This led to the suggestion that most of the magnesium in enamel exists as an organic complex (10). Plasma levels of magnesium in mineralizing solutions greatly reduce the rates of enamel rehardening (30). Similar levels of magnesium in supersaturated solutions slow the rates of calcium phosphate precipitations and stabilize amorphous precipitates (2, 69, 96). Magnesium ions in saliva and plaque may promote caries by interfering with remineralization of enamel.

Evidence of the effects of magnesium on caries is unclear. McClure observed increased caries in rats by addition of magnesium in the drinking water (60). Parma and associates, according to Harris, found a lower incidence of caries in rats given fluoride and magnesium than fluoride alone in drinking water (36). This effect could be attributed to increase of tooth mineral activities toward fluoride. Additions of magnesium in the dry diet of rats did not affect caries (62). Ingestion of magnesium phosphate has been reported to increase caries resistance of human teeth (82). However, these studies did not differentiate between the effects of phosphate supplementation and those of magnesium on caries. Recent studies indicated that the preferential loss of a magnesium-rich mineral was associated with the advancing front of demineralization in the early carious lesion of enamel (35).

The relationships between fluoride ions and magnesium ions in mineralization of normally calcified tissues are incompletely known, but obviously are of paramount importance. Appropriate levels of fluoride ions seemingly enhance mineralization of bones and teeth and prevent calcification of soft tissues. Whereas excessive magnesium ions tend to prevent mineralization, magnesium deficiency seems to promote ectopic calcifications of soft tissues (81). Relatively large intake of fluoride also increased the magnesium content of dentin and enamel of the rat (59). The effects of fluoride on magnesium uptake and the effects of magnesium on fluoride uptake by recrystallized or remineralized enamel have not been investigated.

The basic structure of enamel consists of rods or prisms radiating from the dentinoenamel junction toward the outer surfaces of the tooth crown. The prisms are described as keyhole-shaped in sections cut approximately parallel to the surface of the crown, i.e., perpendicular to the long axis of the prism. The prisms have rounded heads and narrow tails and contain many hydroxyapatite crystallites. The crystallites lie mostly parallel to the long axis of the prisms in the central portions of the head region. The crystallites are inclined away from the long axis in the peripheral and tail regions of the prisms (86, 93).

Scanning and transmission electron micrographs show preferential dissolution of minerals in the central regions of enamel prisms in weak acids (5, 74), whereas the calcium chelators at basic pH preferentially dissolve the sheath or peripheral regions of the prisms (43, 76). Selected micrographs of early carious enamel indicated acid-type dissolution of enamel (65). The preferential dissolution of crystallites by acids has been ascribed to crystal orientation, differences in mineralization of individual crystals, and the relationship between organic components and the crystallites (75, 87).

The size and shape of a few crystals found in carious regions of enamel indicated that remineralization had occurred in the lesion (42). Studies of remineralization of slightly demineralized surfaces of enamel in solutions of 0.15 mM NaF showed the filling of prisms with well crystallized apatites (31, 32). However, artificial subsurface lesions contained minerals described as amorphous calcium phosphates after remineralization in similar solutions to those referred to above (66). Gwinnett and associates demonstrated the formation of globular minerals on the surfaces of demineralized enamel (34). Apparently the micrographic appearance of remineralized enamel depends on the conditions of demineralization, the composition of mineralizing solutions, and the duration of exposure in supersaturated solutions.

Objectives of this study were to:

(a) Study the demineralization of natural surfaces of enamel in weak acids;

(b) Determine the effects of fluoride, bicarbonate, and magnesium ions on ionic exchange, recrystallization, and remineralization of surface enamel;

(c) Distinguish the uptake of fluoride ions in surface enamel by recrystallization and remineralization;

(d) Determine whether surfaces remineralized in the presence of fluoride ions contained crystalline or amorphous minerals;

(e) Test the acid resistance of recrystallized and remineralized surfaces of enamel.

Exposure of tooth and bone minerals and synthetic preparations of apatites to fluoride ions almost invariably increases the acid resistance of the minerals, even if the initial exposure to fluoride occurred in the acid (39). The acid resistance of tooth minerals treated with fluoride ions has consistently been used as an indication of potential caries prevention in terms of the relative insolubility of fluorapatites. The relationship between acid and caries resistance of fluoride treated enamel has been questioned based on faulty premises (12). This erroneous opinion arose from the concept that any agent which reduced the rates of calcium and phosphate dissolution from enamel must also prevent caries when directly applied on tooth surfaces. However, coating of surface minerals of enamel with heavy metals such as lead, tin, titanium, silver, etc., prevents the formation of fluorapatites. The subsequent acid testing of such coated minerals reflects the retarded rates of ionic diffusion through the surface coatings.

The purpose of this work was to determine the acid resistance, and the potential caries resistance, of natural surfaces of enamel and demineralized, recrystallized, and remineralized surfaces of enamel after exposures in solutions of 0.4 mM NaF at pH 7.0 and in acid that contained fluoride at pH 4.5.

METHODS AND PROCEDURES OF EXPERIMENTATION

1. Preparation of Enamel Surfaces

Completely erupted bovine incisor teeth were extracted at the time of slaughter and stored in deionized water at 4°. The water contained thymol crystals for bacteriostasis. Teeth with developmental and traumatic defects or mineralized surface deposits were discarded. Each incisor was sectioned as previously described to provide 1 cm^2 surface of intact labial enamel (29). The labial surface was lightly abraded and pumiced by the usual clinical procedure to remove extraneous materials. The surfaces of the sections, except the 1 cm^2 of unaltered surface enamel, were coated with fingernail varnish. Each section of enamel was attached with dental sticky wax on a plastic plate as shown in figure 1.

The surface enamel was partially demineralized in 10 mM acetic acid of pH 5.5. Six 1-cm^2 surfaces were attached with sticky wax on the inside wall of a 600 ml beaker. The surfaces were immersed 24 hours in 500 ml of the acid with constant magnetic stirring at approximately 100 rpm. The six surfaces, while attached in the beaker, were rinsed in several changes of deionized water and then soaked in 500 ml of the water for 24 hours, again with constant stirring. The calcium and phosphate dissolved in the acid

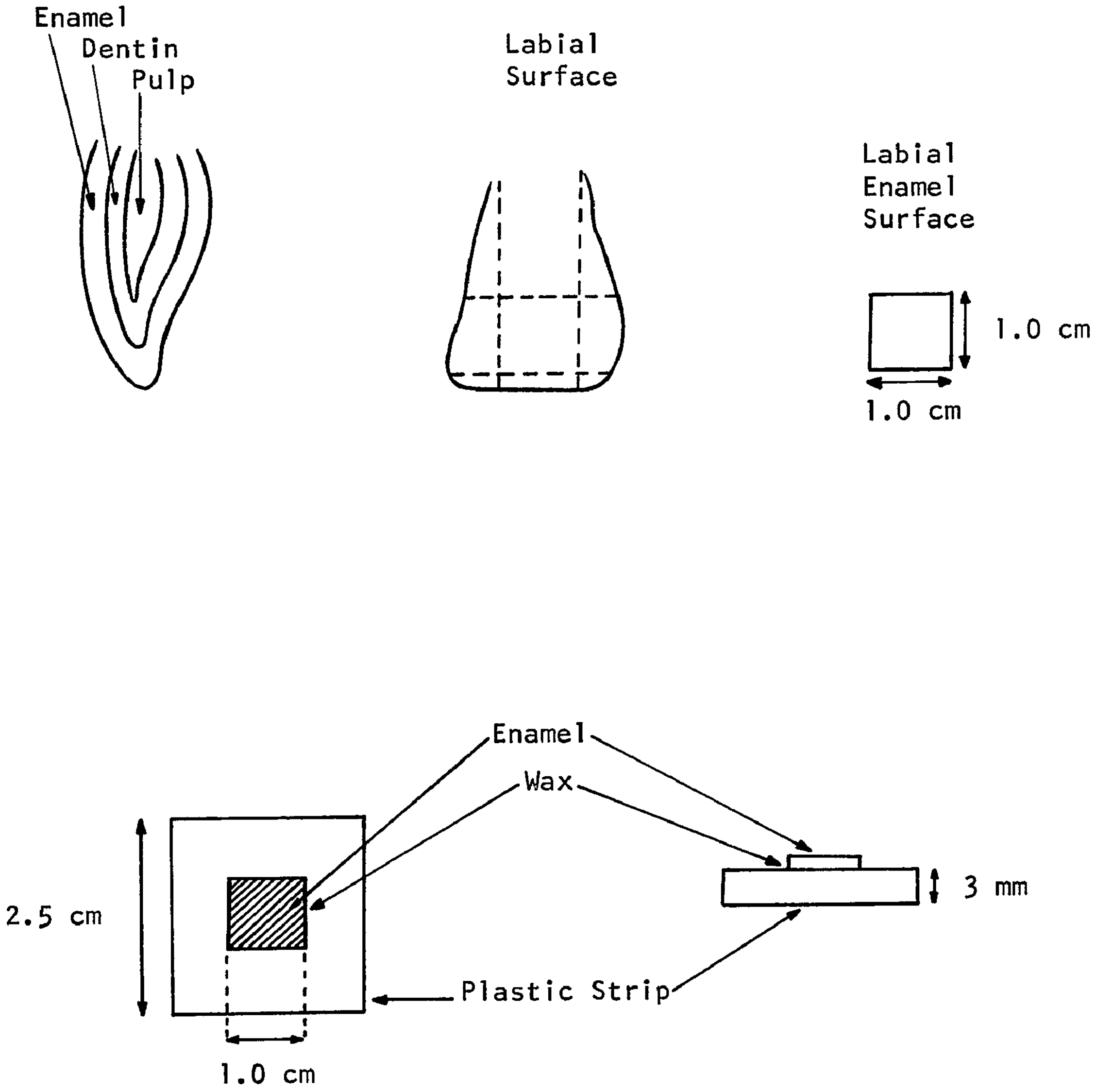


FIGURE 1 Preparation of Enamel Surfaces

One cm^2 surfaces were cut from bovine incisors and attached on plastic blocks with sticky wax to leave exposed only the labial enamel as illustrated.

and in the wash water were determined at the 24 hour times of treatments.

2. Preparation of Solutions

Stock solutions were prepared by dissolution of quantitated amounts of reagent grade chemicals in distilled water to provide known concentrations of reactants. The following stock solutions were prepared: 1.0 M NaCl, 0.1 M NaOH, 0.1 M HCl, 0.5 M acetic acid, 0.05 M $CaCl_2$, 0.05 M NaH_2PO_4, 0.10 M $NaHCO_3$, 0.05 M $MgCl_2$ and 0.025 M NaF. Radioactive calcium, obtained as 17 mCi ^{45}Ca per mg $CaCl_2$ was diluted with distilled water to provide a stock solution of 1.0 μCi per ml. The solution of NaOH was gassed with N_2 and tightly sealed to prevent CO_2 contamination.

Experimental solutions were prepared by dilution of stock solutions in distilled water to provide known concentrations of reactants. Solutions prepared to contain calcium and phosphate in concentrations above saturation levels were carefully mixed in the following procedure to prevent precipitation: NaCl was added to a relatively large volume of distilled water to obtain 150 mM final concentration; then respective stock solutions were added slowly with stirring to attain 1.5 mM $CaCl_2$, 1.0 mM NaH_2PO_4, and 0.4 mM NaF; the solutions were then slowly titrated to pH 7.0 with NaOH and diluted to final volume with distilled water. The same procedures were followed to attain concentrations of 5, 15, or 25 mM $NaHCO_3$ except that the solutions were bubbled with appropriate mixtures of N_2 and CO_2 from the beginning and throughout the use of the solutions. Each experimental solution contained approximately 0.01 μCi/ml ^{45}Ca.

Solutions were prepared at a final pH of 7.0 to contain 1.5 mM calcium and/or 1.0 mM phosphate, each in the absence and presence of 0.4 mM NaF. Solutions were also prepared to contain 5, 15, or 25 mM bicarbonate or 1.0 mM magnesium in the presence and absence of the various combinations of calcium, phosphate, or fluoride.

Solutions designated as mineralizing solutions contained 1.5 mM $CaCl_2$ and 1.0 mM NaH_2PO_4. Solutions described as recrystallizing solutions contained 1.5 mM $CaCl_2$ or 1.0 mM NaH_2PO_4, or neither $CaCl_2$ nor NaH_2PO_4.

The solution of acetic acid was prepared by dilution of 20 ml stock acetic acid in about 950 ml water, followed by titration with NaOH to pH 4.5 or 5.5 before dilution to 1 liter. Then 500 ml of the 10 mM acetic acid of pH 5.5 was used to partially demineralize six 1- cm^2 surfaces of enamel for 24 hours. The 10 mM acetic acid at pH 4.5 was used in continuous laminar flow to dissolve minerals in the surfaces of enamel for collection in separation 10 minute aliquots. In some experiments the 10 mM acetic acid solution was prepared to contain 0.4 mM NaF before titration to pH 4.5.

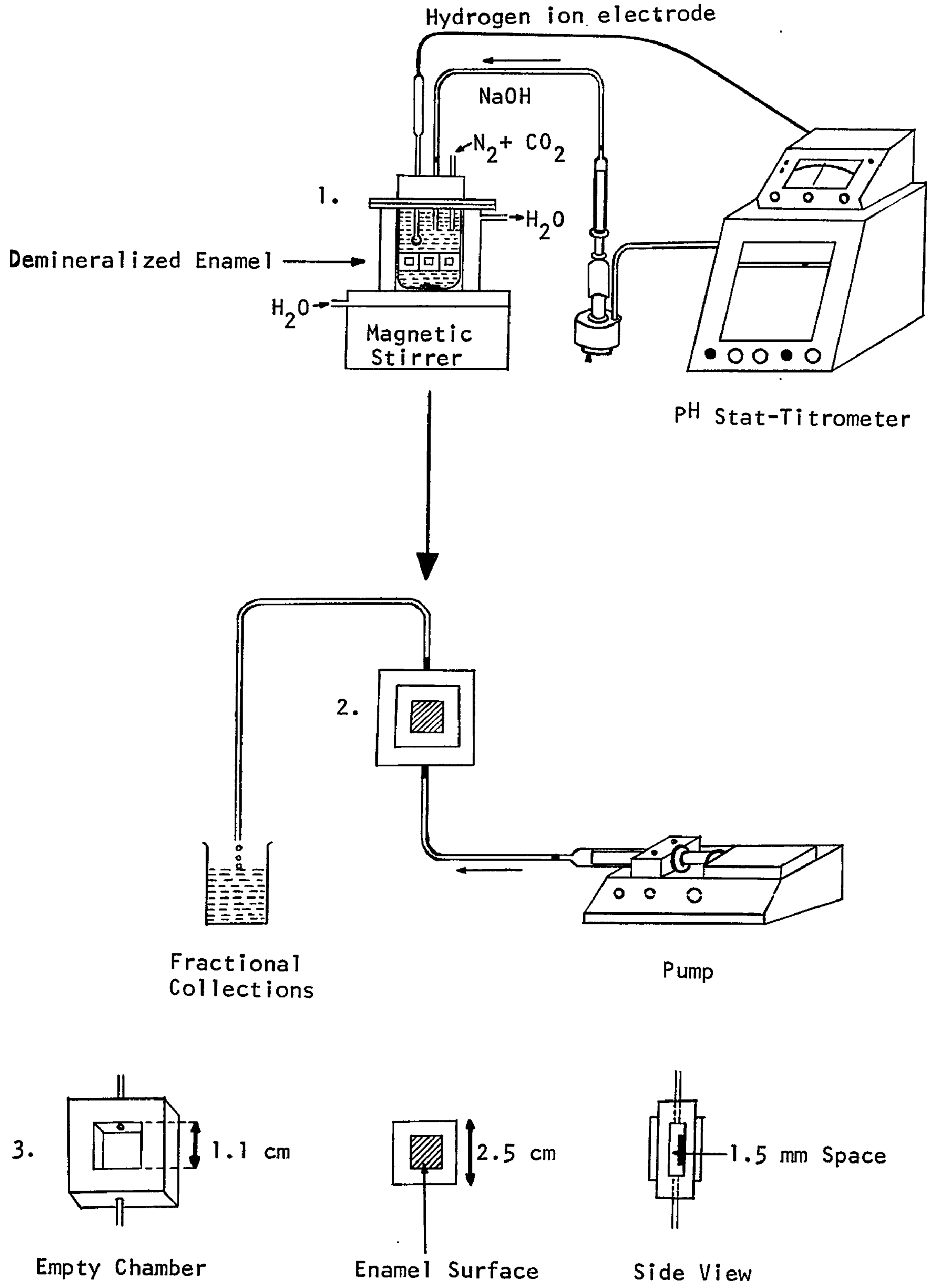
3. Procedures of Remineralization and Recrystallization of Partially Demineralized Surfaces of Enamel

Six of the enamel surfaces, after partial demineralization and the water wash, were attached with sticky wax on the side wall of a 60 ml beaker. The beaker was placed in a water-jacketed constant temperature chamber at 25°. Then 50 ml of solution were poured into the beaker to immerse the enamel surfaces. The constant temperature chamber was placed on a magnetic stirrer to mix the solution at a constant rate. A combination pH electrode was inserted through the top wall of the chamber. The chamber contained ports for titrant addition and tubing for gassing the solutions. An automatic potentiometer-titrator (pH stat) was used to maintain constant pH 7.0 of the solution in the chamber. The assembled apparatus is illustrated in Figure 2.

In at least 3 replicate experiments the six surfaces were immersed in a given solution for 24 hours under conditions of constant pH 7.0, temperature of 25°, and constant rate of stirring. Duplicate 0.2 ml samples were taken of the solutions at intervals of 0, 1, 2, 3, 6, and 24 hours for ^{45}Ca analysis. Samples taken at the initial time and the 24 hour time of enamel-solution contact were used to determine the calcium, phosphate, and fluoride concentrations of the solutions. The surfaces were rinsed

Figure 2. Schematic Procedures for Exposure of Surface Enamel in Solutions of Constant pH.

1) Exposure of 6-1 cm^2 surfaces in 50 ml. solution of constant pH, temperature, rate of stirring, and atmosphere.
2) Exposure of 1 cm^2 surface in constant rate laminar flow of acid at constant pH for sequential analysis of dissolved minerals.
3) Construction of laminar flow chamber.


Hydrogen ion electrode
NaOH
N_2+ CO_2
1.
$\rightarrow H_2O$
Demineralized Enamel
H_2O
Magnetic Stirrer
pH Stat-Titrometer
2.
Fractional Collections
Pump
3.
1.1 cm
2.5 cm
1.5 mm Space
Empty Chamber
Enamel Surface
Side View

in 4 changes of deionized water before removal from the 60 ml beaker.

4. Procedure of Determining the Acid Resistance of Natural and Altered Surface Minerals in Enamel

Natural, untreated surfaces of enamel and partially demineralized surfaces, before and after 24 hour exposure in the solutions of neutral pH, were exposed in a laminar flow of acid. Each surface was separately inserted in a small chamber of 0.5 ml capacity as shown in figure 2. Ten mM acetic acid at pH 4.5 was pumped through the chamber at a rate of 1.2 ml per minute. The off-flow was collected in 6 sequential 10 minute (12 ml) aliquots. The calcium, calcium-45, phosphate and fluoride dissolved from the surfaces were determined in each of the acid collections. Four of the 6 surfaces exposed in each of the solutions of neutral pH were subjected to the acid resistance test, providing data of at least 12 surfaces for each of the studied variables.

5. Methods of Chemical Analyses

Calcium levels in experimental solutions were determined by the spectrophotometric measurement of O-cresolphthalein complexation of Ca^{+2} (85). The method measures Ca^{+2} in the range of 0.1 to 10 μg.

The levels of phosphate in the solutions were determined by the molybdate reduction method described by

Chen, Toribara, and Warner (15). This method measures inorganic orthophosphate in the 0.1 to 8 μg range.

Fluoride ion levels in the solutions were determined using the specific ion electrode method of McCann (58). Acid solutions were buffered to pH 7.0 with EDTA (ethylenediamine tetracetate) before determination of fluoride ion levels. The EDTA complexed cations that might have interfered with fluoride ion determinations. The lower limit of accurate determination of fluoride was equivalent to 0.0005 mM NaF.

Isotopic ^{45}Ca was determined by liquid scintillation counting in commercial solvent (Aquasol, New England Nuclear). Duplicate 0.2 ml or 0.5 ml samples at each interval of sampling, were each mixed in a separate 15 ml volume of scintillation fluid for radioactive counting. The samples were counted for 10 minutes, recorded as counts per minute and later calculated as disintegrations per minute.

Total carbonates were measured in some of the solutions and in samples of surface minerals in some experiments by the method of Curtress (18). The levels of magnesium in specified solutions were estimated using the spectrophotometric method described by Mann and Yoe (57).

6. Preparation of Enamel Surfaces for Scanning Electron Microscopy

Selected surfaces of natural, pumiced enamel and demineralized surfaces before and after exposure in the solutions of neutral pH, and after testing of acid resistance, were prepared for scanning in the electron microscope. The surfaces were dried in room atmosphere for at least 1 week then coated with an alloy of 60 percent gold and 40 percent palladium in a vacuum evaporator. The prepared surfaces were scanned and photographed at magnifications of 60 to 12,000 in the electron microscope operated at 20 KV (Stereoscan Mark II, Cambridge).

7. Methods of Statistical Analysis

Throughout the investigations differences were tested statistically using student's "t" test at the 5 percent level of significance, $\alpha = 0.05$ (106). In addition the data were subject to analysis of variance and tested by General Linear Models Procedure in computer programmed analyses (4). These procedures confirmed the previously completed "t" tests.

RESULTS

1. Calcium and Phosphate Dissolved from Demineralized Surfaces of Enamel in 24 Hour Acid Exposure

Table 1 shows the calcium and phosphate dissolved in 24 hour exposure of six 1-cm^2 surfaces of unaltered enamel in a 500 ml volume of 10 mM acetic acid of pH 5.5. Means of 21.8 μMol calcium and 13.5 μMol phosphate were dissolved from each of the surfaces in calcium to phosphate ratio (Ca/P) of 1.63. The dissolution of the enamel increased the pH of the acid from 5.5 to 6.1 in 24 hours.

Table 1 also shows the calcium and phosphate dissolved from the acid demineralized enamel during 24 hour exposure of the six 1-cm^2 surfaces in a 500 ml volume of deionized water. Approximately 1 μMol calcium and 1 μMol phosphate dissolved from each surface during the 24 hour water wash at pH 7.0. (The demineralized, water washed surfaces were hereafter simply termed demineralized surfaces).

2. Reactions of Demineralized Surfaces of Enamel in Solutions at pH 7.0

Table 2 shows the calcium, ^{45}Ca, phosphate, bicarbonate, and fluoride compositions of solutions at the initial and at the 24 hour time of enamel-solution interaction. In 3 experiments each, six 1-cm^2 surfaces of

TABLE 1

Calcium and Phosphate Dissolved in 24 Hour Exposure of Unaltered Surfaces of Enamel in Acid and in the Subsequent 24 Hour Exposure in Deionized Water*

	μMol/cm^2 (S.D.)**			24 Hour
24 Hour Exposure in:	Ca	PO_4	Ca/P	pH
10 mM acetic acid, pH 5.5	21.8(1.5)	13.5(1.6)	1.6(0.1)	6.1(0.2)
Deionized Water, pH 7.0	1.0(0.1)	1.0(0.2)	1.0(0.2)	7.0(0.1)

* Six-1 cm^2 surfaces of enamel were immersed 24 hours in 500 ml of 10 mM acetic acid of pH 5.5. The surfaces were rinsed and washed in 500 ml. deionized water for 24 hours.

** The table shows the means and standard deviations for 6 experiments.

TABLE 2

Reactions of Demineralized Surfaces of Enamel in Solutions at pH 7.0: Composition of Solutions at the Initial and 24 Hour Time of Enamel-Solution Interaction. Each table value represents the mean and standard deviation for 3 experiments.

Initial Solution Compositions					Composition of Solutions at the 24 Hour Time of Enamel Solutions			
mM				DPM x 10^2/ml (S.D.)		mM (S.D.)x10^{-2}		
$CaCl_2$	NaH_2PO_4	NaF	$NaHCO_3$	Ca-45	Ca-45	Ca	PO_4	F
-	-	-	-	229(6)	111(10)	11(0)	8(1)	-
-	-	0.4	-	207(8)	52(6)	6(0)	5(0)	29(2)
-	1.0	-	-	248(7)	75(14)	-	81(8)	-
-	1.0	0.4	-	266(2)	23(4)	2(0)	95(2)	-
-	-	-	15.0	254(4)	139(5)	8(0)	15(1)	-
-	-	0.4	15.0	250(4)	72(10)	6(1)	9(0)	31(2)
-	1.0	-	15.0	219(1)	103(4)	2(0)	2(1)	-
-	1.0	0.4	15.0	215(1)	42(6)	1(0)	-	31(2)
1.5	-	-	-	279(18)	254(11)	147(5)	-	-
1.5	-	0.4	-	279(4)	235(6)	145(0)	-	23(1)
1.5	-	-	15.0	304(17)	279(9)	145(5)	-	-
1.5	-	0.4	15.0	310(6)	279(16)	136(6)	-	34(3)
1.5	1.0	-	-	225(5)	168(11)	109(5)	61(7)	-
1.5	1.0	0.4	-	219(3)	58(1)	38(3)	38(3)	10(0)
1.5	1.0	-	5.0	213(1)	170(10)	131(2)	80(1)	-
1.5	1.0	0.4	5.0	205(2)	48(6)	39(2)	30(3)	10(1)
1.5	1.0	-	15.0	203(1)	173(7)	139(5)	90(5)	-
1.5	1.0	0.4	15.0	205(2)	64(14)	44(11)	49(6)	15(2)
1.5	1.0	-	25.0	217(4)	176(2)	130(4)	86(2)	-
1.5	1.0	0.4	25.0	182(5)	61(3)	53(2)	42(2)	15(0)

demineralized enamel were exposed in 50 ml of each of the designated solutions at constant pH 7.0 for 24 hours. The ^{45}Ca activity in solutions at the initial time of enamel-solution contact varied because of isotopic decay between times of experimentation. The small concentrations of calcium and phosphate found at the 24 hour time of enamel-solution interaction in solutions that did not initially contain calcium and/or phosphate were accurately measurable and statistically valid. No attempt was made to measure changes of bicarbonate in the solutions because of continuous gassing with appropriate mixtures of N_2 and CO_2.

Table 3 (Fig. 3) shows the uptake of ^{45}Ca in the surfaces of enamel at the 1, 2, 3, 6, and 24 hour times of reaction in the solutions of pH 7.0. The isotopic uptake was calculated as DPM x $10^3/cm^2$ from the differences of ^{45}Ca in solutions at the initial time of enamel-solution contact and at the indicated sampling intervals. Since the solutions contained slightly different levels of the isotope at the initial time of reaction, the uptake was normalized for purposes of comparison by recalculation as the percentage of the ^{45}Ca in solutions.

The level of 15 mM bicarbonate in solutions of the isotope alone slightly reduced ^{45}Ca uptake in the enamel throughout the 24 hour time of reaction. Addition of 1.0 mM phosphate greatly increased the uptake of ^{45}Ca beginning in the second hour of reaction, in the presence or absence of bicarbonate in the solutions. The presence of

TABLE 3

Reactions of Demineralized Surfaces of Enamel in Solutions at pH 7.0: The Rates of Exchange-Recrystallization and Remineralization of ^{45}Ca in Surfaces of Enamel. Each table value represents the mean and standard deviation for 3 experiments.

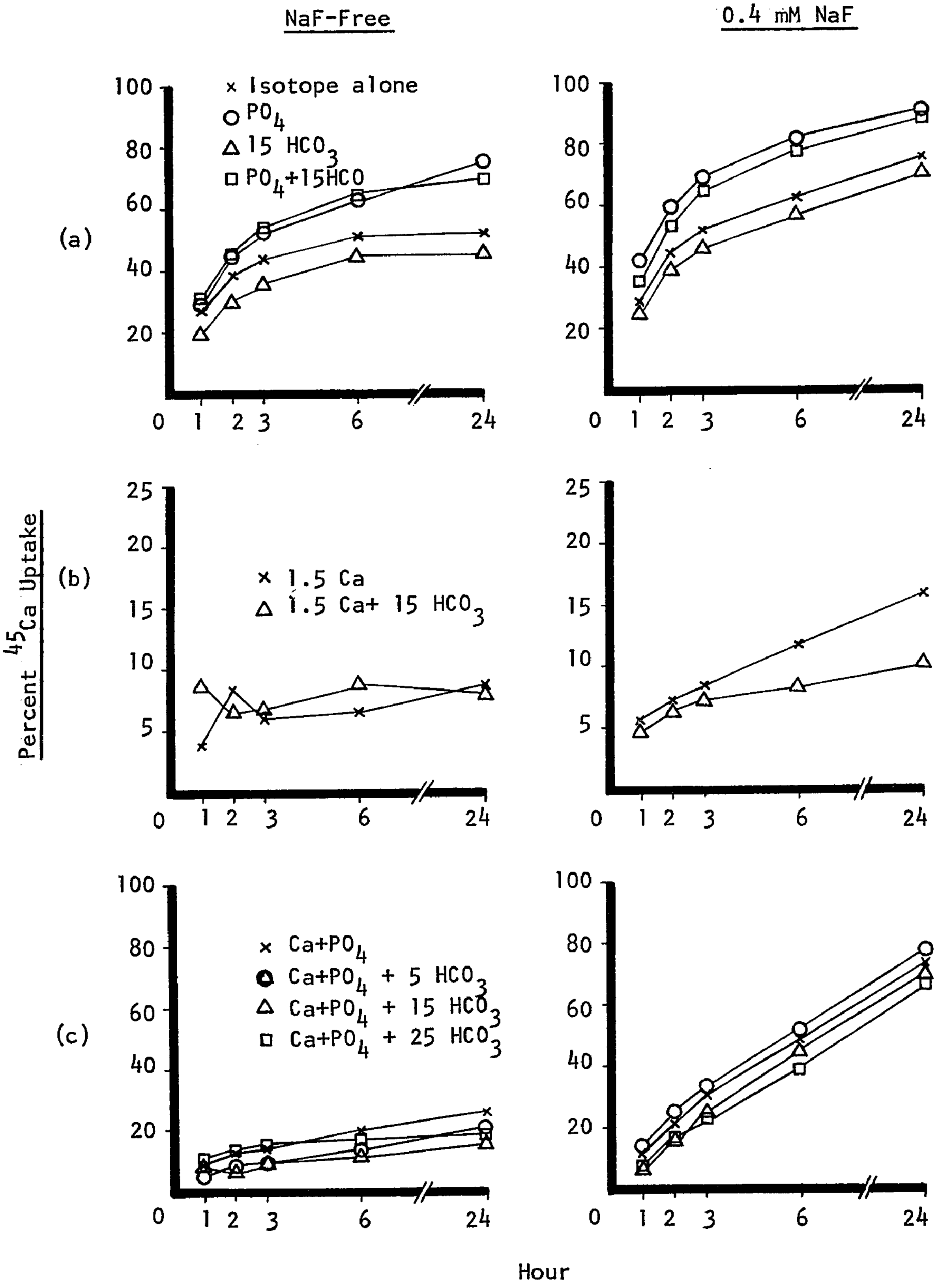
Solutions (mM)				Percent ^{45}Ca (S.D.) Uptake by Enamel Surfaces at Hr:				
$CaCl_2$	NaH_2PO_4	NaF	$NaHCO_3$	1	2	3	6	24
-	-	-	-	27(4)	39(4)	44(3)	51(1)	52(3)
-	-	0.4	-	29(4)	44(4)	52(3)	62(3)	75(4)
-	1.0	-	-	27(7)	43(7)	51(6)	63(5)	70(5)
-	1.0	0.4	-	42(11)	59(11)	69(10)	81(6)	92(2)
-	-	-	15.0	20(4)	30(3)	36(2)	45(5)	45(1)
-	-	0.4	15.0	25(4)	39(4)	46(4)	57(3)	71(4)
-	1.0	-	15.0	31(3)	45(2)	53(1)	64(1)	69(2)
-	1.0	0.4	15.0	35(7)	53(6)	65(7)	77(5)	89(3)
1.5	-	-	-	4(1)	8(6)	5(2)	7(3)	9(4)
1.5	-	0.4	-	6(2)	7(2)	8(2)	12(2)	16(1)
1.5	-	-	15.0	9(3)	7(5)	7(4)	9(4)	8(4)
1.5	-	0.4	15.0	5(3)	6(2)	7(3)	8(4)	10(5)
1.5	1.0	-	-	9(2)	13(3)	14(3)	20(4)	25(7)
1.5	1.0	0.4	-	12(2)	22(3)	31(3)	48(3)	73(1)
1.5	1.0	-	5.0	6(1)	8(2)	9(2)	13(1)	20(4)
1.5	1.0	0.4	5.0	13(1)	24(1)	34(1)	52(2)	77(3)
1.5	1.0	-	15.0	6(1)	8(1)	10(1)	11(1)	16(4)
-	1.0	0.4	15.0	8(2)	16(4)	25(6)	45(7)	69(8)
1.5	1.0	-	25.0	11(1)	13(1)	15(1)	16(1)	19(2)
1.5	1.0	0.4	25.0	8(3)	17(4)	24(4)	38(3)	67(1)

Figure 3. The Uptake of ^{45}Ca by Demineralized Surfaces of Enamel in 24 Hour Exposures in Solutions at pH 7.0. The uptake of ^{45}Ca was calculated as the percentage of the total amount of isotope in solution which was taken up by the enamel. Note the effects of 0.4 mM NaF in solutions illustrated in the figure.

a) ^{45}Ca uptake by enamel exposed to solutions of the isotope in the presence and absence of 1.0 mM NaH_2PO_4 and 15 mM $NaHCO_3$.

b) ^{45}Ca uptake by enamel exposed to solutions of the isotope and 1.5 mM $CaCl_2$ in the absence and presence of 15 mM $NaHCO_3$.

c) ^{45}Ca uptake by enamel exposed to solutions of 1.5 mM $CaCl_2$, 1.0 mM NaH_2PO_4, and 0, 5, 15, and 25 mM $NaHCO_3$.


NaF-Free
0.4 mM NaF
(a)
(b)
(c)
× Isotope alone
○ PO4
△ 15 HCO3
□ PO4+15HCO
× 1.5 Ca
△ 1.5 Ca+ 15 HCO3
× Ca+PO4
● Ca+PO4 + 5 HCO3
△ Ca+PO4 + 15 HCO3
□ Ca+PO4 + 25 HCO3
Percent 45Ca Uptake
100
80
60
40
20
25
20
15
10
5
0
1
2
3
6
24
Hour

0.4 mM NaF in these solutions (absence of 1.5 mM calcium) markedly increased the uptake of ^{45}Ca, particularly between the first and sixth hour of enamel-solution interaction.

The presence of 1.5 mM calcium in the absence of added phosphate in the solutions tremendously reduced the uptake of ^{45}Ca in the enamel throughout the 24 hour reactions. Additions of 0.4 mM NaF in these solutions marginally increased the uptake of ^{45}Ca, and then only in the absence of carbonate in the solutions.

The uptake of ^{45}Ca in the surfaces of enamel from solutions of combined calcium and phosphate (absence of NaF) was practically the same in the first 3 hours as from solutions of 1.5 mM calcium alone. The isotopic uptake increased slightly to about 20 percent from these solutions of calcium and phosphate in 24 hours. Additions of 0.4 mM NaF in the solutions of calcium plus phosphate greatly increased the rates of ^{45}Ca uptake to attain the same 24 hour uptake as from solutions of NaF alone and solutions of phosphate alone. However, the rates of ^{45}Ca uptake were higher between the initial time of enamel-solution contact and the sixth hour of reaction from solutions of NaF alone than from solutions of mixed calcium and phosphate. The 5 mM bicarbonate level tended to increase, whereas 25 mM bicarbonate tended to decrease the uptake of ^{45}Ca from solutions of calcium phosphate.

Table 4 shows the estimated gains and losses by the enamel surfaces of calcium, phosphate, and fluoride during 24 hour interactions in the solutions at pH 7.0. The table also shows the 24 hour uptake of ^{45}Ca in the demineralized surfaces as the percentage of the total isotope in solution at the initial time of enamel-solution contact. Note that the ^{45}Ca uptake of 52 percent from solutions of isotope alone increased to 75 percent from solutions of NaF alone, and further increased to 92 percent from solutions of NaF and phosphate in the absence of added calcium. Additions of 1.5 mM calcium to solutions of the isotope alone reduced the uptake of ^{45}Ca from 52 to 9 percent. Calcium and/or bicarbonate reduced, whereas fluoride increased the uptake of ^{45}Ca in the enamel.

Table 4 also shows the individual and combined effects of 1.0 mM phosphate, 0.4 mM NaF and 15 mM bicarbonate in absence of added calcium on the enamel-solution reactions. Compared to the 52 percent ^{45}Ca uptake by enamel from solutions of isotope alone, NaF and phosphate, with or without bicarbonate, increased ^{45}Ca uptake to levels of 89 to 92 percent NaF alone or phosphate alone, NaF and bicarbonate and phosphate and bicarbonate increased ^{45}Ca uptake to levels of 69 to 75 percent whereas bicarbonate alone slightly reduced the uptake of ^{45}Ca.

Except in solutions of phosphate alone, and solutions of phosphate plus NaF, small amounts of both calcium and

TABLE 4

Reactions of Demineralized Surfaces of Enamel in Solutions at pH 7.0: Estimated Uptake of ^{45}Ca and Gain or Loss of Surface Calcium, Phosphate and Fluoride by Enamel in 24 Hour Interaction with Solutions of pH 7.0. Each table value represents the mean and standard deviation for 3 experiments.

Initial Solution				24 Hour Uptake by Enamel			
Concentrations, mM				% (S.D.)	$\mu Mol/cm^2$ (S.D.) x 10^{-2}		
$CaCl_2$	NaH_2PO_4	NaF	$NaHCO_3$	^{45}Ca	Ca	PO_4	F
-	-	-	-	52(3)	-88(2)*	-70(8)*	-
-	-	0.4	-	75(4)	-49(2)	-40(3)	111(13)
-	1.0	-	-	70(5)	0	227(73)	-
-	1.0	0.4	-	92(2)	-12(2)	47(18)	110(12)
-	-	-	15.0	45(1)	-64(3)	-124(11)	-
-	-	0.4	15.0	71(4)	-47(8)	-77(2)	93(13)
-	1.0	-	15.0	69(2)	-19(2)	-17(8)	-
-	1.0	0.4	15.0	89(3)	-6(1)	0	92(15)
1.5	-	-	-	9(4)	27(14)	0	-
1.5	-	0.4	-	16(1)	43(3)	0	138(10)
1.5	-	-	15.0	8(4)	46(40)	0	-
1.5	-	0.4	15.0	10(5)	161(50)	0	77(25)

TABLE 4 (Continued)

1.5	1.0	-	-	25(7)	344(43)	328(56)	-
1.5	1.0	0.4	-	73(1)	934(27)	519(26)	246(2)
1.5	1.0	-	5.0	20(4)	162(20)	170(8)	-
1.5	1.0	0.4	5.0	77(3)	930(20)	583(26)	251(10)
1.5	1.0	-	15.0	16(4)	104(27)	90(21)	-
1.5	1.0	0.4	15.0	69(8)	892(88)	434(51)	210(16)
1.5	1.0	-	25.0	19(2)	169(29)	119(16)	-
1.5	1.0	0.4	25.0	67(1)	810(18)	449(12)	213(3)

* The negative sign (-) indicated calcium or phosphate dissolved.

phosphate dissolved from the enamel in the absence of added calcium in the solutions. Calcium and phosphate dissolved in almost the same amounts in the solution of isotope alone and in the 24 hour wash in deionized water. Addition of NaF in the solution of isotope alone reduced the amounts of minerals dissolved in the neutral pH solutions about 44 percent. Additions of phosphate reduced the amounts of calcium dissolved to the lower limits of accurate measurement, but apparently caused uptake of phosphate in the enamel. Addition of 15 mM bicarbonate increased the amounts of phosphate that dissolved from the enamel unless the solutions contained phosphate and NaF. However, bicarbonate alone and with NaF reduced the amounts of calcium dissolved from the enamel.

In the absence of added calcium the fluoride uptake of 1.1 μMol/cm^2 was the same in the presence as in the absence of phosphate. The presence of 15 mM bicarbonate reduced the uptake of fluoride, but not significantly ($0.2>P>0.1$).

Table 4 also shows the effects of 1.5 mM calcium, 0.4 mM NaF and 15 mM bicarbonate each in the <u>absence of added phosphate</u>, on the reactions of enamel in the solutions of neutral pH. Compared to ^{45}Ca uptake of 52 percent from solutions of the isotope alone, only 9 percent of the ^{45}Ca was taken up by enamel exposed 24 hours in solutions that contained 1.5 mM calcium alone. Calcium and bicarbonate together in the solutions did not significantly alter the uptake of ^{45}Ca. Calcium plus NaF increased uptake of the isotope, but to levels of only 16 percent. Calcium, NaF and bicarbonate combined slightly reduced the ^{45}Ca uptake compared to the absence of bicarbonate. An apparent, small net uptake of calcium was found for enamel exposed in the solutions of 1.5 mM calcium, which was statistically meaningful only in the presence of NaF and bicarbonate. The extremely small amounts of phosphate that dissolved in the solutions from the enamel were below the levels of accurate measurement.

Almost one-half of the 0.4 mM NaF in the solutions of 1.5 mM calcium was taken up as 1.4 μMol fluoride in each of the enamel surfaces. This uptake of fluoride was significantly greater in the presence than in the absence of the added 1.5 mM calcium concentrations. However, 15 mM bicarbonate in solutions of 1.5 mM calcium significantly reduced the uptake of fluoride nearly one-half.

Table 4 shows the effects of 1.5 mM calcium plus 1.0 mM phosphate without and with 0.4 mM NaF, and without and with 5, 15, or 25 mM bicarbonate on interactions between the demineralized surfaces of enamel and the solutions at pH 7.0. The uptake of ^{45}Ca by the enamel of 25 percent in solutions of combined 1.5 mM calcium and 1.0 mM phosphate was one-half its uptake from solutions of the isotope alone. Additions of 5 and 15 mM bicarbonate to solutions of calcium and phosphate in the absence of NaF slightly reduced the calcium-45 uptake to about 20 and 16 percent, respectively.

The level of 25 mM bicarbonate had no more effect on ^{45}Ca uptaken than 5 mM bicarbonate. The isotopic uptake increased in the range of 67 to 77 percent when 0.4 mM NaF was added to these solutions regardless of the levels of bicarbonate. However, the fluoride uptake of about 2.5 μMol/cm^2 (75 percent of the 0.4 mM NaF in solutions) from solutions, without bicarbonate and with 5 mM bicarbonate, was reduced to 2.1 μMol/cm^2 from solutions of 15 and 25 mM bicarbonate. Note that 25 mM bicarbonate had no more effect on the uptake of fluoride than 15 mM bicarbonate.

The estimated uptake of 3.4 μMol calcium and 3.3 μMol phosphate in each of the surfaces of enamel from solutions of 1.5 mM calcium and 1.0 mM phosphate was reduced at least one-half in the presence of either 5, 15, or 25 mM bicarbonate (absence of NaF). The apparent ratios of Ca/P for the minerals deposited in the enamel were approximately 1, except for the mean ratio of 1.4 from solutions of 25 mM bicarbonate.

Additions of 0.4 mM NaF in solutions of 1.5 mM calcium and 1.0 mM phosphate, without and with 5 mM bicarbonate, increased calcium uptake a factor of 2.7 and phosphate uptake a factor of about 1.7. The presence of 15 mM bicarbonate in these solutions had no effect on calcium uptake ($P = 0.50$), but reduced slightly the uptake of phosphate. Additions of 25 mM bicarbonate in the presence of NaF reduced the uptake of calcium and phosphate, but only by small amounts. The presence of NaF in these solutions increased the ratios of Ca/P for the minerals precipitated in the enamel from about 1 in the absence of NaF to about 1.8. The ratios of Ca/F for the precipitated minerals ranged between 3.7 and 4.2.

The minerals precipitated in the enamel surfaces from bicarbonate-free solutions of calcium plus phosphate and NaF replaced almost one-half of the amounts of calcium and phosphate dissolved in the 24 hour demineralization in the acid. In contrast, minerals deposited in the surfaces from the same solutions, but in the absence of NaF, replaced only 16 percent of the calcium and 24 percent of the phosphate removed by demineralization.

3. The Effects of 24 Hour Reactions of Demineralized Surfaces of Enamel in Solutions of pH 7.0 on the Dissolution of Surface Minerals in Laminar Flow of Acid

Table 5 (Fig. 4) shows the recovery of ^{45}Ca from minerals of the surface enamel in each of 6 sequential, 10 minute (12 ml) collections of 10 mM acetic acid at pH 4.5. The recovery of ^{45}Ca was recalculated as the percentage of the amounts taken up in the enamel at the 24 hour time of reaction because of the different levels of uptake from the various solutions.

TABLE 5

Effects of 24 Hour Reactions of Demineralized Surfaces of Enamel in Solutions at pH 7.0 on the Rates of ^{45}Ca Recovery in Laminar Flow of 10 mM Acetic Acid at pH 4.5. Each table value represents the mean and standard deviation for 12 surfaces of enamel.

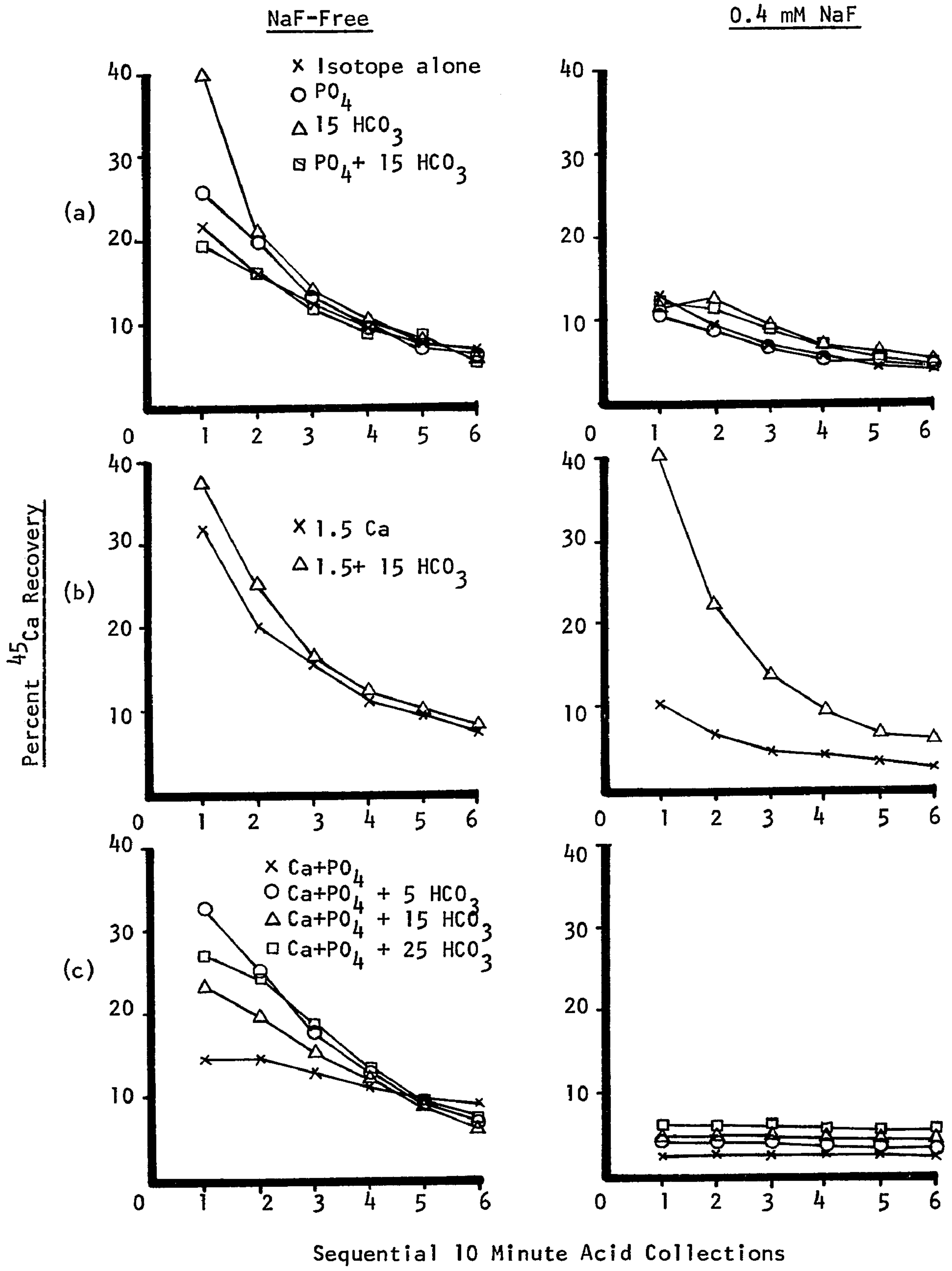
Solutions (mM)				Percent ^{45}Ca (S.D.) Recovery in Sequential 10 Minute Collections of Acid					
$CaCl_2$	NaH_2PO_4	NaF	$NaHCO_3$	1	2	3	4	5	6
-	-	-	-	22(4)	16(4)	12(3)	10(2)	8(1)	7(1)
-	-	0.4	-	13(2)	10(1)	7(1)	6(1)	5(1)	4(1)
-	1.0	-	-	26(7)	20(4)	13(2)	10(2)	7(2)	6(1)
-	1.0	0.4	-	11(1)	9(1)	7(1)	5(1)	5(1)	4(1)
-	-	-	15.0	40(5)	21(2)	14(1)	10(1)	8(1)	6(1)
-	-	0.4	15.0	12(2)	13(2)	9(2)	7(2)	6(2)	5(1)
-	1.0	-	15.0	19(4)	16(1)	12(1)	9(1)	8(1)	6(1)
-	1.0	0.4	15.0	12(4)	11(1)	9(1)	7(4)	6(1)	5(1)
1.5	-	-	-	32(2)	20(1)	16(2)	11(1)	9(1)	7(2)
1.5	-	0.4	-	10(1)	7(1)	5(1)	4(1)	4(1)	3(0)
1.5	-	-	15.0	37(1)	25(4)	16(4)	12(4)	10(3)	8(2)
1.5	-	0.4	15.0	40(6)	22(3)	14(2)	9(2)	7(1)	6(3)
1.5	1.0	-	-	15(1)	15(1)	13(1)	11(1)	10(0)	9(1)
1.5	1.0	0.4	-	3(0)	3(0)	3(0)	2(0)	3(0)	2(0)
1.5	1.0	-	5.0	23(3)	20(3)	15(4)	12(6)	9(3)	6(3)
1.5	1.0	0.4	5.0	4(1)	4(0)	4(0)	4(0)	3(0)	3(1)
1.5	1.0	-	15.0	33(6)	25(5)	18(6)	12(6)	9(5)	7(4)
1.5	1.0	0.4	15.0	5(1)	5(1)	5(1)	5(1)	4(0)	4(1)
1.5	1.0	-	25.0	27(1)	25(1)	18(2)	13(2)	10(2)	7(2)
1.5	1.0	0.4	25.0	6(1)	6(1)	6(1)	6(0)	6(1)	6(0)

Figure 4. Effects of 24 Hour Exposures of Enamel Surfaces in Solutions that Contained 0.4 mM NaF at pH 7.0 on the Recovery of ^{45}Ca from the Surfaces in Laminar Flow of 10 mM Acetic Acid at pH 4.5. The acid flow from each surface was collected in separate 10 minute (12 ml) aliquotes for 1 hour. The recovery of ^{45}Ca was calculated as the percentage of the isotopic uptake from solutions of pH 7.0.

a) ^{45}Ca recoveries from surfaces exposed 24 hours to solutions of the isotope in the presence and absence of 1.0 mM NaH_2PO_4 and 15 mM $NaHCO_3$.

b) ^{45}Ca recoveries from surfaces exposed 24 hours to solutions of 1.5 mM $CaCl_2$ in the absence and presence of 15 mM $NaHCO_3$.

c) ^{45}Ca recoveries from surfaces exposed 24 hours to solutions of 1.5 mM $CaCl_2$, 1.0 mM NaH_2PO_4, and 0, 5, 15 and 25 mM $NaHCO_3$.


NaF-Free
0.4 mM NaF
× Isotope alone
○ PO_4
△ 15 HCO_3
⊠ PO_4+ 15 HCO_3
(a)
(b)
(c)
× 1.5 Ca
△ 1.5+ 15 HCO_3
× Ca+PO_4
○ Ca+PO_4 + 5 HCO_3
△ Ca+PO_4 + 15 HCO_3
□ Ca+PO_4 + 25 HCO_3
Percent ^{45}Ca Recovery
0
10
20
30
40
1
2
3
4
5
6
Sequential 10 Minute Acid Collections

The ^{45}Ca recovery from surfaces exposed in solutions of the isotope alone decreased smoothly from 22 to 7 percent between the first and sixth collections of the acid to provide total recovery of 74 percent. ^{45}Ca recovery from surfaces exposed in solutions of phosphate plus bicarbonate or phosphate alone was the same as from surfaces exposed in isotope alone, i.e., a total of 70 percent was recovered from surfaces exposed in phosphate plus bicarbonate and 81 percent was recovered from surfaces exposed in phosphate alone. ^{45}Ca recovery from surfaces exposed in 15 mM bicarbonate alone was greater in the initial 10 minute collection of acid than in any subsequent collection. Additions of 0.4 mM NaF in each of these pretreatment solutions (without added calcium) reduced the recovery of ^{45}Ca almost by one-half. The total isotopic recoveries for the NaF exposed surfaces ranged between 40 and 51 percent and averaged 46 percent. The recovery of ^{45}Ca in the sequential acid collections after NaF treatments could realistically be considered as describing one line.

^{45}Ca recoveries from surfaces exposed in solutions of 1.5 mM calcium without and with 15 mM bicarbonate (absence of added phosphate) were rapid in the first and second collections of the acid. The ^{45}Ca recoveries from surfaces exposed in solution of calcium, bicarbonate, and NaF combined were the same as in the absence of NaF in the solution. Calcium and NaF in the absence of bicarbonate greatly depressed the recovery of ^{45}Ca. The total recovery of ^{45}Ca was complete (about 100 percent) for surfaces exposed in 1.5 mM calcium alone and calcium plus bicarbonate, in the presence or absence of NaF. But a total of only 32 percent of the ^{45}Ca was recovered from surfaces exposed in calcium plus NaF in the absence of bicarbonate.

The recovery of ^{45}Ca from surfaces exposed in NaF-free solutions that contained calcium and phosphate together was about the same as recovery from surfaces exposed in solutions of the isotope alone. The level of 5 mM bicarbonate in these solutions increased the recovery of ^{45}Ca from the surfaces in the first 2 or 3 collections of the acid. Both 15 and 25 mM bicarbonate additionally increased the recovery of ^{45}Ca in the first and second acid collections. Additions of NaF in the solutions of combined calcium and phosphate greatly reduced the recoveries of ^{45}Ca, particularly in the initial collections of the acid. The rates of isotopic recovery were about 3 percent in each of the consecutive collections of acid from surfaces exposed in solutions of combined NaF, calcium, and phosphate. The rates of recovery were also practically constant in collections of acid from surfaces exposed in these solutions in the presence of bicarbonate. Each level of bicarbonate slightly increased the rates of ^{45}Ca recovery. Note the total recoveries of 15 percent in the absence of bicarbonate, 22 percent for 5 mM bicarbonate, 28 percent for 15 mM bicarbonate, and 35 percent for 25 mM bicarbonate.

Table 6 (Fig. 5) shows the amounts of calcium dissolved from the surfaces of enamel in each of the 6 sequential collections of acid. Calcium dissolved in slightly increasing amounts in each successive collection of acid from surfaces exposed in solutions of the isotope alone. EXcept for the first collections of acid, calcium dissolved at practically constant rates from the surfaces within an experimental group. Significantly less calcium dissolved from surfaces exposed in phosphate plus bicarbonate (absence of added calcium or NaF) than surfaces exposed in solutions of isotope alone. Larger amounts of calcium dissolved in the acid from surfaces exposed in either phosphate or bicarbonate than from the surfaces exposed in solutions of the isotope alone. Additions of NaF in these solutions (no added calcium) reduced the amounts of calcium dissolved in the acid 17 to 20 percent for surfaces exposed in solutions of isotope alone or solutions of both phosphate and bicarbonate. Bicarbonate and NaF reduced the calcium dissolved about 30 percent, whereas phosphate and NaF reduced the calcium dissolved a total of 42 percent. However, exposure of the enamel in NaF-free solutions of combined phosphate and bicarbonate, compared to either alone, reduced the amounts of calcium dissolved about

TABLE 6

Effects of 24 Hour Reactions of Demineralized Surfaces of Enamel in Solutions at pH 7.0 on the Rates of Calcium Dissolution in Laminar Flow of 10 mM Acetic Acid at pH 4.5. Each table value represents the mean and standard deviation for 12 surfaces of enamel.

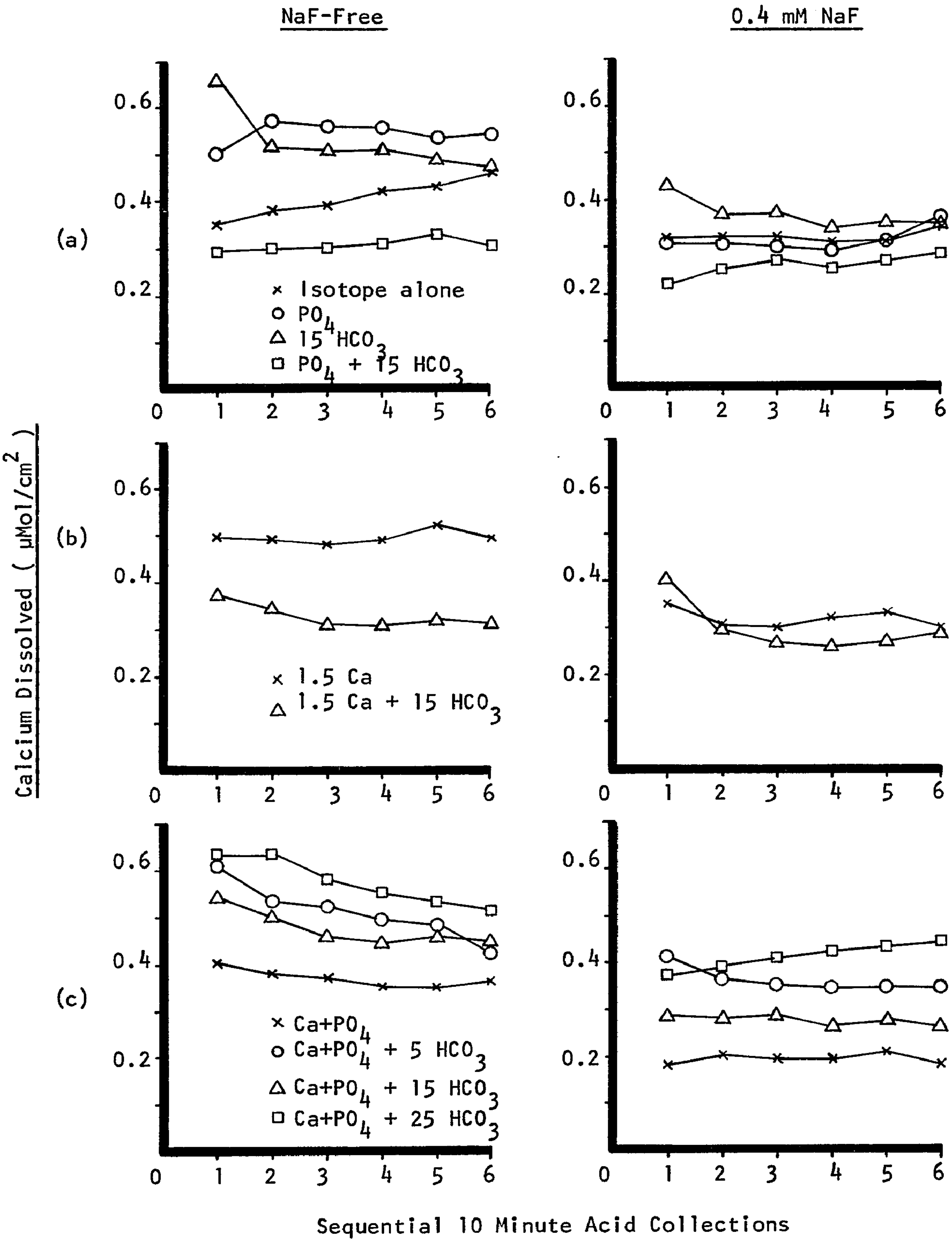
Solutions (mM)				Calcium Dissolved in Sequential 10 Minute Collections of Acid μMol/cm^2 (S.D.) x 10^{-2}					
$CaCl_2$	NaH_2PO_4	NaF	$NaHCO_3$	1	2	3	4	5	6
-	-	-	-	35(2)	38(5)	39(1)	42(4)	43(5)	46(7)
-	-	0.4	-	32(4)	32(4)	32(5)	31(3)	31(1)	34(1)
-	1.0	-	-	50(3)	57(11)	56(12)	56(12)	53(16)	54(10)
-	1.0	0.4	-	31(6)	31(1)	30(1)	29(3)	31(8)	36(8)
-	-	-	15.0	66(9)	52(7)	51(8)	51(6)	49(7)	47(4)
-	-	0.4	15.0	43(7)	37(8)	37(8)	34(7)	35(4)	35(5)
-	1.0	-	15.0	29(4)	30(1)	30(1)	31(1)	33(2)	30(3)
-	1.0	0.4	15.0	22(3)	25(1)	27(2)	25(1)	27(3)	28(3)
1.5	-	-	-	50(8)	49(10)	48(12)	49(9)	52(10)	49(10)
1.5	-	0.4	-	35(1)	31(2)	30(1)	32(2)	33(2)	30(3)
1.5	-	-	15.0	37(3)	34(3)	31(3)	31(3)	32(5)	31(2)
1.5	-	0.4	15.0	40(8)	30(1)	27(2)	26(4)	27(6)	29(1)
1.5	1.0	-	-	40(4)	38(4)	37(4)	35(4)	35(4)	36(6)
1.5	1.0	0.4	-	18(2)	20(3)	19(2)	19(3)	21(2)	18(2)
1.5	1.0	-	5.0	61(11)	53(2)	52(1)	49(4)	48(4)	42(2)
1.5	1.0	0.4	5.0	41(7)	36(7)	35(6)	34(4)	34(4)	34(1)
-	1.0	-	15.0	54(6)	50(4)	46(3)	44(2)	46(2)	44(5)
1.5	1.0	0.4	15.0	28(5)	28(3)	28(3)	26(3)	27(2)	26(3)
1.5	1.0	-	25.0	63(4)	63(1)	58(2)	55(2)	53(3)	51(3)
1.5	1.0	0.4	25.0	37(3)	39(6)	41(5)	42(6)	43(7)	44(10)

Figure 5. The Effects of 24 Hour Exposures of Enamel Surfaces in Solutions of 0.4 mM NaF at pH 7.0 on the Rates of Calcium Dissolution in Laminar Flow of 10 mM Acetic Acid at pH 4.5. The acid flow from each surface was collected in 6 sequential 10 minute (12 ml) aliquotes for 1 hour.

a) Calcium dissolved from surface exposed 24 hours to solutions of the isotope in the absence and presence of 1.0 mM NaH_2PO_4 and 15 mM $NaHCO_3$.

b) Calcium dissolved from surfaces exposed 24 hours to solutions of 1.5 mM $CaCl_2$ in the absence and presence of 15 mM $NaHCO_3$.

c) Calcium dissolved from surface exposed 24 hours to solutions of 1.5 mM $CaCl_2$, 1.0 mM NaH_2PO_4, and 0, 5, 15, and 25 mM $NaHCO_3$.


NaF-Free
0.4 mM NaF
(a)
(b)
(c)
Calcium Dissolved (μMol/cm^2)
0.6
0.4
0.2
0
1
2
3
4
5
6
× Isotope alone
○ PO$_4$
△ 15 HCO$_3$
□ PO$_4$ + 15 HCO$_3$
× 1.5 Ca
△ 1.5 Ca + 15 HCO$_3$
× Ca+PO$_4$
○ Ca+PO$_4$ + 5 HCO$_3$
△ Ca+PO$_4$ + 15 HCO$_3$
□ Ca+PO$_4$ + 25 HCO$_3$
Sequential 10 Minute Acid Collections

40 percent.

Pretreatment of the enamel surfaces in solutions of 1.5 mM calcium alone caused the dissolution of approximately the same amounts of calcium in the acid as exposure in solution of either 15 mM bicarbonate or 1.0 mM phosphate. Compared to 1.5 mM calcium alone, exposure of the surfaces in solutions of combined calcium and bicarbonate reduced the amounts of calcium dissolved about 34 percent. Additions of NaF in solutions of calcium alone or with bicarbonate had no more effect on calcium dissolution than NaF-free solutions of calcium and bicarbonate combined.

Exposure of the enamel surfaces in solutions of calcium and phosphate together reduced the amounts of calcium dissolved from 0.40 to 0.35 $\mu Mol/cm^2$ in the first to fourth collections of the acid. This slight trend of decreased calcium dissolution was opposite the trend of increased calcium dissolution from surfaces exposed in solutions of the isotope alone. The levels of bicarbonate in the NaF-free solutions of calcium phosphate significantly increased the amounts of calcium dissolved in the acid collections, particularly for solutions of 25 mM bicarbonate. However, differences between the amounts of calcium dissolved for surfaces exposed in the 3 levels of bicarbonate were insignificant for practical purposes, even though slightly less calcium dissolved in each successive collection of the acid.

Additions of 0.4 mM NaF to the bicarbonate-free solutions of calcium phosphate reduced the amounts of calcium dissolved in the collections of the acid about 50 percent. The NaF in the solutions with bicarbonate, compared to NaF-free solutions of bicarbonate, reduced the amounts of calcium dissolved between 30 and 40 percent, with 15 mM bicarbonate apparently causing the greatest reductions. However, the amounts of calcium dissolved were nearly the same for surfaces exposed in NaF-free solutions of calcium phosphate and surfaces exposed in solutions of calcium, phosphate, NaF and 5 and 25 mM bicarbonate.

Table 7 (Fig. 6) shows the amounts of phosphate dissolved in the sequential collections of acid that correspond to the amounts of calcium dissolved shown in Table 6 (Fig. 5). In general smaller amounts of phosphate dissolved in the first than in the subsequent collections of the acid, but with exceptions. Phosphate dissolved in increasing amounts and almost the same in successive collections of acid from surfaces exposed in solutions of the isotope alone and in solutions of 1.5 mM calcium alone. Exposure of the enamel in solutions of either phosphate or bicarbonate increased the phosphate dissolved in each collection of the acid compared to exposure in solutions of phosphate plus bicarbonate. Additions of NaF in these solutions almost equally reduced the acid dissolution of phosphate and calcium from the enamel except in the initial collections of the acid.

TABLE 7

Effects of 24 Hour Reactions of Demineralized Surfaces of Enamel in Solutions at pH 7.0 on the Rates of Phosphate Dissolution in Laminar Flow of 10 mM Acetic Acid at pH 4.5. Each table value represents the mean and standard deviation for 12 surfaces of enamel.

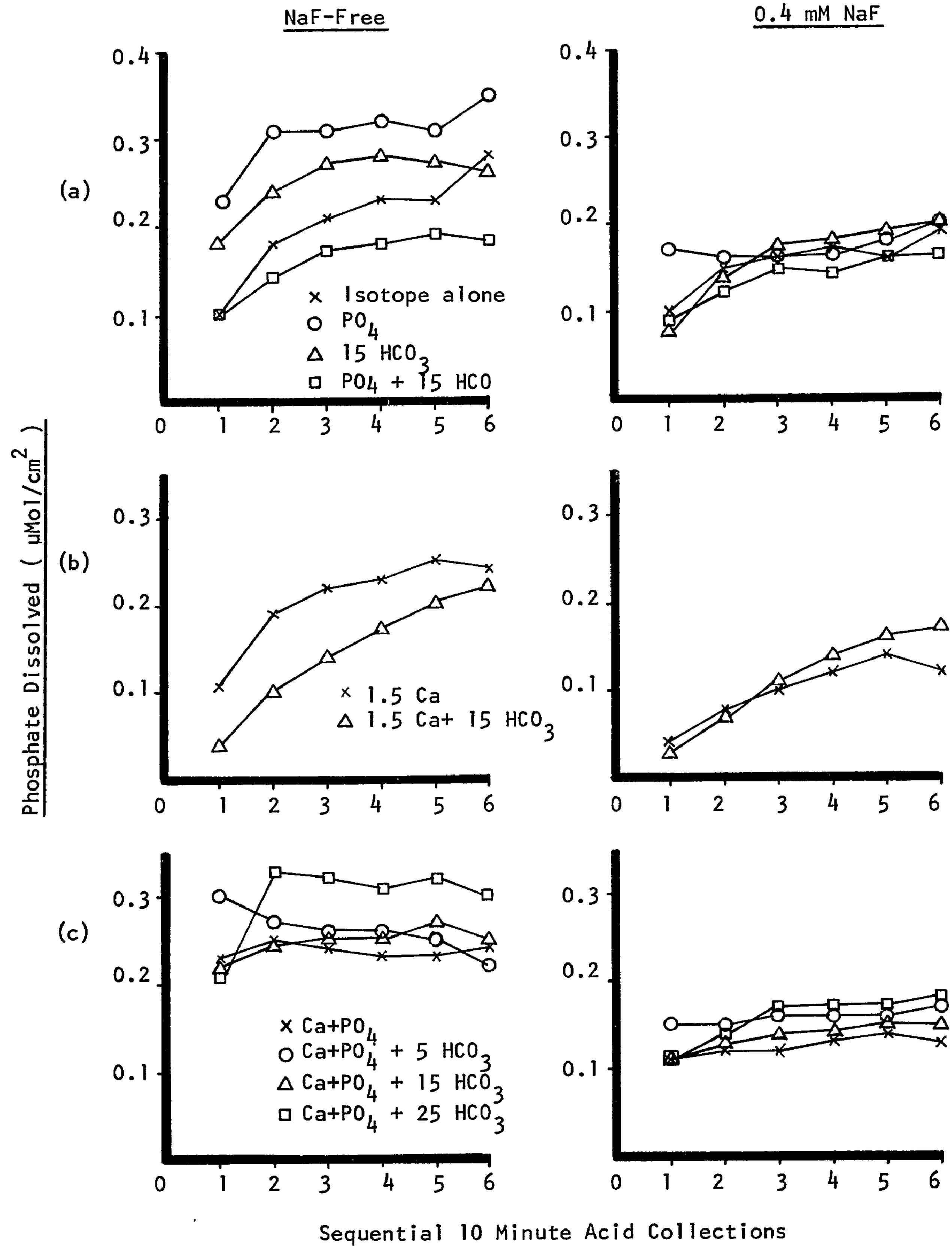
Solutions (mM)				Phosphate Dissolved in Sequential 10 Minute Collections of Acid $\mu Mol/cm^2$ (S.D.) x 10^{-2}					
$CaCl_2$	NaH_2PO_4	NaF	$NaHCO_3$	1	2	3	4	5	6
-	-	-	-	10(0)	18(1)	21(1)	23(1)	23(1)	28(4)
-	-	0.4	-	10(1)	15(3)	16(2)	17(1)	16(0)	19(0)
-	1.0	-	-	23(2)	31(8)	31(10)	32(11)	31(13)	35(7)
-	1.0	0.4	-	17(6)	16(0)	16(0)	16(2)	18(6)	20(7)
-	-	-	15.0	18(4)	24(3)	27(4)	28(2)	27(4)	26(2)
-	-	0.4	15.0	8(3)	14(4)	17(4)	18(4)	19(3)	20(4)
-	1.0	-	15.0	10(2)	14(2)	17(1)	18(1)	19(1)	18(2)
-	1.0	0.4	15.0	9(1)	12(1)	15(1)	14(0)	16(2)	16(2)
1.5	-	-	-	11(3)	19(4)	22(4)	23(3)	25(5)	24(5)
1.5	-	0.4	-	4(0)	8(1)	10(1)	12(2)	14(1)	12(1)
1.5	-	-	15.0	4(1)	10(1)	14(1)	17(1)	20(4)	22(3)
1.5	-	0.4	15.0	3(2)	7(2)	11(3)	14(3)	16(3)	17(1)
1.5	1.0	-	-	23(2)	25(2)	24(2)	23(2)	23(2)	24(4)
1.5	1.0	0.4	-	11(0)	12(1)	12(0)	13(1)	14(0)	13(1)
1.5	1.0	-	5.0	30(8)	27(1)	26(1)	26(2)	25(4)	22(2)
1.5	1.0	0.4	5.0	15(3)	15(2)	16(2)	16(2)	16(2)	17(3)
1.5	1.0	-	15.0	22(2)	25(2)	25(2)	25(1)	27(3)	25(2)
1.5	1.0	0.4	15.0	11(1)	13(1)	14(1)	14(1)	15(1)	15(1)
1.5	1.0	-	25.0	21(2)	33(3)	32(2)	31(2)	32(4)	30(5)
1.5	1.0	0.4	25.0	11(3)	14(1)	17(0)	17(2)	17(1)	18(1)

Figure 6. The Effects of 24 Hour Exposures of Enamel Surfaces in Solutions of 0.4 mM NaF at pH 7.0 on the Rates of Phosphate Dissolution in Laminar Flow of 10 mM Acetic Acid at pH 4.5. The acid flow from each surface was collected in separate 10 minute (12 ml) fractions for 1 hour.

a) Phosphate dissolved from surfaces exposed 24 hours to solutions of the isotope in the absence and presence of 1.0 mM NaH_2PO_4 and 15 mM $NaHCO_3$.

b) Phosphate dissolved from surfaces exposed 24 hours to solutions of 1.5 mM $CaCl_2$ in the absence and presence of 15 mM $NaHCO_3$.

c) Phosphate dissolved from surface exposed 24 hours to solutions of 1.5 mM $CaCl_2$, 1.0 mM NaH_2PO_4, and 0, 5, 15, and 25 mM $NaHCO_3$.


NaF-Free
0.4 mM NaF
(a)
(b)
(c)
Phosphate Dissolved (μMol/cm2)
0.4
0.3
0.2
0.1
0
1
2
3
4
5
6
× Isotope alone
○ PO4
△ 15 HCO3
□ PO4 + 15 HCO
× 1.5 Ca
△ 1.5 Ca+ 15 HCO3
× Ca+PO4
○ Ca+PO4 + 5 HCO3
△ Ca+PO4 + 15 HCO3
□ Ca+PO4 + 25 HCO3
Sequential 10 Minute Acid Collections

Pretreatment of the enamel surfaces in NaF-free solutions of 1.5 mM calcium plus 15 mM bicarbonate significantly reduced phosphate dissolution except for the last collection of the acid, compared to exposure in solutions of calcium alone. NaF in the solutions of calcium and calcium plus bicarbonate reduced phosphate dissolution to the same levels, which were almost one-half the amounts dissolved from surfaces exposed in NaF-free solutions of calcium alone.

Except for phosphate dissolved in the first collection of the acid, and for each of the acid collections from surfaces exposed in 25 mM bicarbonate, similar amounts of phosphate dissolved from surfaces exposed in NaF-free solutions of combined calcium, phosphate, and 5 and 15 mM bicarbonate. NaF in these solutions of calcium phosphate in the absence of bicarbonate reduced phosphate dissolution about 50 percent. The 3 levels of bicarbonate in these solutions with NaF tended to increase slightly the amounts of phosphate dissolved in the acid.

Table 8 (Fig. 7) shows the molecular ratios of calcium to phosphate for the minerals dissolved from the surfaces of enamel in the sequential collections of the acid. The ratios in the first 10 minute collections of the acid were above 2 except for some of the surfaces exposed in solutions that contained 1.0 mM phosphate. Ratios for minerals dissolved from surfaces exposed in solutions of phosphate alone

TABLE 8

Molecular Ratios of Calcium to Phosphate of Minerals Dissolved from Surfaces of Enamel in Laminar Flow of Acid. Each table value represents the mean and standard deviation for 12 surfaces of enamel.

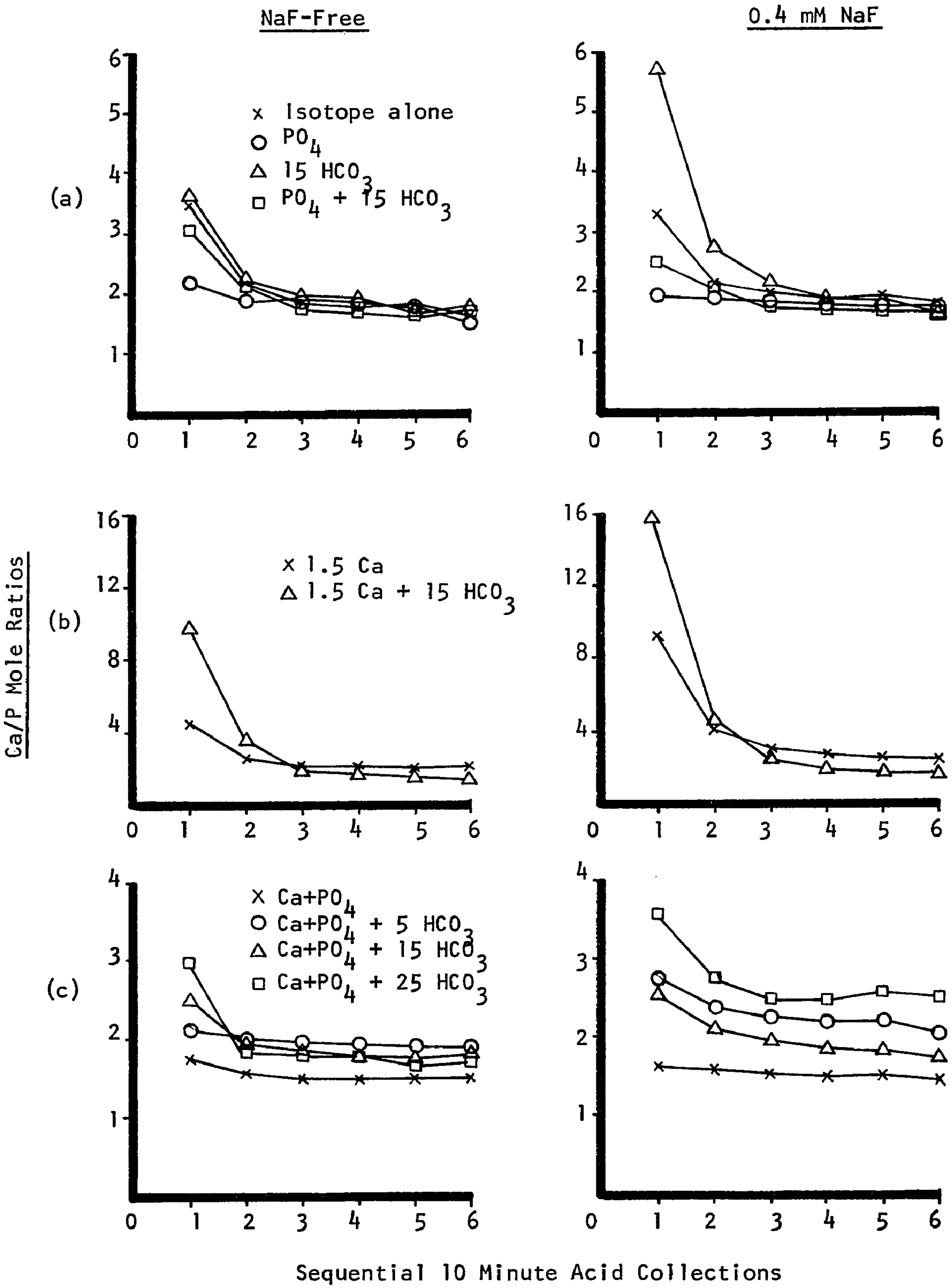
Solutions (mM)				Ca/P Mole Ratios of Minerals Dissolved in Sequential 10 Minute Collections of Acid					
$CaCl_2$	NaH_2PO_4	NaF	$NaHCO_3$	1	2	3	4	5	6
-	-	-	-	3.5(0.1)	2.2(0.2)	1.8(0.0)	1.8(0.1)	1.8(0.2)	1.6(0.2)
-	-	0.4	-	3.3(0.1)	2.2(0.1)	2.0(0.1)	1.9(0.1)	1.9(0.1)	1.8(0.1)
-	1.0	-	-	2.2(0.2)	1.9(0.2)	1.8(0.3)	1.8(0.3)	1.8(0.3)	1.5(0.2)
-	1.0	0.4	-	1.9(0.3)	1.9(0.1)	1.9(0.1)	1.8(0.1)	1.8(0.1)	1.8(0.2)
-	-	-	15.0	3.67(0.4)	2.2(0.0)	1.9(0.1)	1.8(0.1)	1.8(0.1)	1.8(0.1)
-	-	0.4	15.0	5.7(2.1)	2.7(0.5)	2.2(0.3)	1.9(0.2)	1.8(0.1)	1.7(0.1)
-	1.0	-	15.0	3.0(0.3)	2.1(0.4)	1.8(0.2)	1.7(0.2)	1.7(0.1)	1.7(0.1)
-	1.0	0.4	15.0	2.5(0.4)	2.1(0.1)	1.8(0.0)	1.8(0.0)	1.7(0.0)	1.7(0.1)
1.5	-	-	-	4.6(1.0)	2.6(0.4)	2.2(0.3)	2.2(0.2)	2.0(0.2)	2.1(0.2)
1.5	-	0.4	-	4.3(0.5)	4.0(0.4)	3.0(0.3)	2.7(0.3)	2.5(0.1)	2.4(0.1)
1.5	-	-	15.0	9.7(1.0)	3.5(0.5)	2.2(0.1)	1.7(0.1)	1.6(0.1)	1.4(0.1)
1.5	-	0.4	15.0	15.9(6.0)	4.6(1.7)	2.4(1.0)	1.9(0.2)	1.7(0.2)	1.7(0.1)
1.5	1.0	-	-	1.8(0.1)	1.6(0.0)	1.5(0.0)	1.5(0.1)	1.5(0.0)	1.5(0.0)
1.5	1.0	0.4	-	1.6(0.1)	1.6(0.1)	1.5(0.1)	1.5(0.2)	1.5(0.2)	1.5(0.1)
1.5	1.0	-	5.0	2.1(0.2)	2.0(0.0)	2.0(0.1)	1.9(0.0)	1.9(0.2)	1.9(0.2)
1.5	1.0	0.4	5.0	2.7(0.2)	2.4(0.3)	2.2(0.3)	2.2(0.3)	2.2(0.3)	2.0(0.3)
1.5	1.0	-	15.0	2.5(0.2)	2.0(0.1)	1.9(0.0)	1.8(0.0)	1.7(0.2)	1.8(0.1)
1.5	1.0	0.4	15.0	2.6(0.4)	2.1(0.2)	2.0(0.1)	1.9(0.1)	1.8(0.1)	1.8(0.1)
1.5	1.0	-	25.0	3.0(0.4)	1.9(0.1)	1.8(0.2)	1.8(0.1)	1.7(0.2)	1.7(0.2)
1.5	1.0	0.4	25.0	3.6(0.3)	2.8(0.3)	2.5(0.2)	2.5(0.4)	2.6(0.4)	2.5(0.5)

Figure 7. The Molecular Ratios of Calcium to Phosphate (Ca/P) of the Minerals Dissolved from Surfaces of Enamel in Laminar Flow of 10 mM Acetic Acid at pH 4.5 after 24 Hour Exposures in Solutions at pH 7.0. The ratios of Ca/P was calculated from data in tables 6 and 7 for each 10 minute collection of acid.

a) Ca/P ratios of minerals dissolved from surfaces exposed 24 hours to solutions of the isotope in the absence and presence of 1.0 mM NaH_2PO_4 and 15 mM $NaHCO_3$.

b) Ca/P ratios of minerals dissolved from surfaces exposed 24 hours to solutions of 1.5 mM $CaCl_2$ in the presence and absence of 15 mM $NaHCO_3$.

c) Ca/P ratios of minerals dissolved from surfaces exposed 24 hours to solutions of 1.5 mM $CaCl_2$, 1.0 mM NaH_2PO_4, and 0, 5, 15 and 25 mM $NaHCO_3$.


NaF-Free
0.4 mM NaF
Ca/P Mole Ratios
(a)
x Isotope alone
○ PO4
△ 15 HCO3
□ PO4 + 15 HCO3
(b)
× 1.5 Ca
△ 1.5 Ca + 15 HCO3
(c)
× Ca+PO4
○ Ca+PO4 + 5 HCO3
△ Ca+PO4 + 15 HCO3
□ Ca+PO4 + 25 HCO3
0 1 2 3 4 5 6
1 2 3 4 5 6
4 8 12 16
1 2 3 4
Sequential 10 Minute Acid Collections

decreased from 2.2 in the first to about 1.7-1.8 in the later collections of the acid. NaF in the solutions of phosphate alone caused dissolution of minerals in ratios of 1.9 in the first to 1.8 in the last collections of the acid. The ratios for minerals dissolved from surfaces exposed in solutions of either the isotope alone, bicarbonate alone, or phosphate plus bicarbonate, each in the presence or absence of NaF, decreased from ranges of 2.5 to 5.7 in the first to about 1.6 to 1.8 in the final collections of acid.

The ratios of calcium to phosphate for minerals dissolved from surfaces exposed in solutions of 1.5 mM calcium alone were exceptionally high (4.6 to 9) in the early and late (2 to 2.5) collections of the acid. The ratios for surfaces exposed in calcium plus bicarbonate were even higher in the first collections of acid than for surfaces exposed in calcium alone, but these ratios sharply decreased to ranges of 1.4 to 1.7 in the last collections of the acid. NaF in these solutions of 1.5 mM calcium (absence of added phosphate) additionally increased the ratios of calcium to phosphate of the dissolved minerals.

The ratios of calcium to phosphate of the minerals dissolved from surfaces exposed in solutions of calcium phosphate with or without NaF, decreased from 1.6 to 1.7 in the initial to about 1.5 in subsequent collections of the acid. Additions of bicarbonate in these solutions increased the ratios for minerals dissolved from the surfaces of enamel, particularly in the first collections of the acid. The ratios decreased to a range of 1.7 to 1.9 in the second or subsequent collections of acid from surfaces exposed in the NaF-free solutions of bicarbonate, but the ratios remained relatively high with NaF in the solutions of bicarbonate, calcium, and phosphate.

Table 9 (Fig. 8) shows the fluoride dissolved in the sequential collections of acid from the surfaces of enamel which were exposed 24 hours at pH 7.0 in the solutions that contained 0.4 mM NaF. Fluoride dissolved in relatively constant, extremely small amounts in each of the successive collections of acid from surfaces exposed in solutions without added calcium. Totals of only 2.5 to 3.8 percent of the fluoride uptake of the 24 hour exposure in the solutions (absence of added calcium) were recovered in the acid.

The amounts of fluoride dissolved from surfaces exposed 24 hours in solutions of 1.5 mM calcium, in the absence of added phosphate, decreased slightly in each sequential collection of the acid. Approximately one-half the amounts of fluoride dissolved in each of the acid collections from surfaces exposed in solutions of calcium plus bicarbonate than from surfaces exposed in calcium without added bicarbonate. A total of only 7.0 percent of the fluoride taken up from solutions of calcium was recovered in the acid. Almost 10 percent of the fluoride uptake was recovered from surfaces exposed in solutions of calcium plus bicarbonate. Much larger amounts of fluoride dissolved from

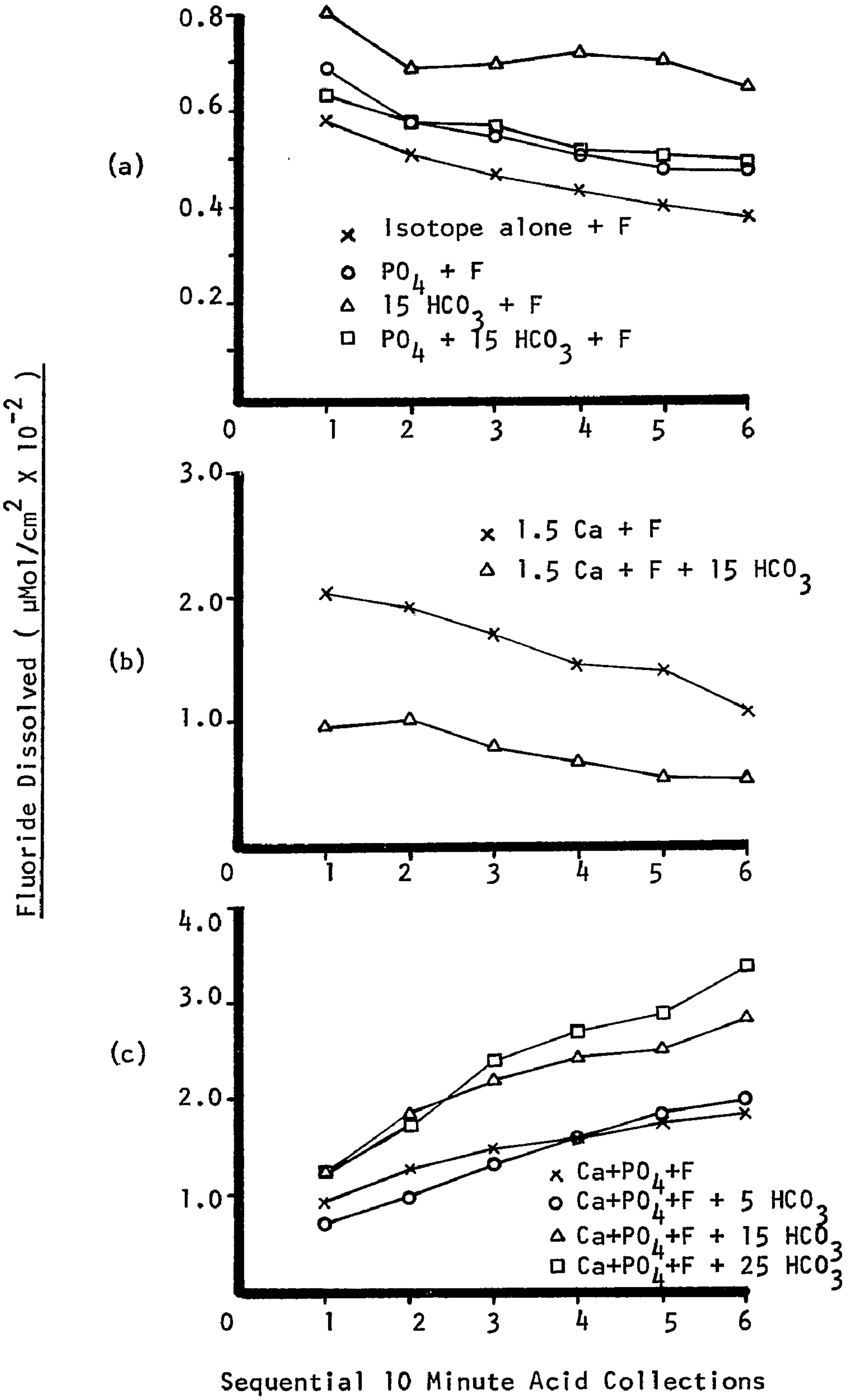
TABLE 9

Effects of 24 Hour Reactions of Demineralized Surfaces of Enamel in Solutions at pH 7.0 on the Rates of Fluoride Recovery in Laminar Flow of 10 mM Acetic Acid at pH 4.5. Each table value represents the mean and standard deviation for 12 surfaces of enamel.

Initial Solution Compositions (mM)				Fluoride Dissolved in Sequential 10 Minute Collections of Acid $\mu Mol/cm^2$ (S.D.) x 10^{-4}					
$CaCl_2$	NaH_2PO_4	NaF	$NaHCO_3$	1	2	3	4	5	6
-	-	0.4	-	58(9)	51(8)	47(7)	43(6)	40(4)	38(4)
-	1.0	0.4	-	69(9)	58(3)	55(5)	51(1)	48(5)	49(7)
-	-	0.4	15.0	81(17)	69(9)	70(2)	72(20)	71(20)	65(8)
-	1.0	0.4	15.0	64(9)	58(8)	56(7)	52(6)	51(4)	49(4)
1.5	-	0.4	-	203(32)	193(12)	169(11)	145(8)	140(17)	107(9)
1.5	-	0.4	15.0	96(27)	102(17)	80(13)	67(7)	55(1)	55(10)
1.5	1.0	0.4	-	91(18)	127(18)	146(5)	159(7)	175(5)	183(25)
1.5	1.0	0.4	5.0	71(27)	99(23)	134(31)	160(29)	182(26)	200(30)
1.5	1.0	0.4	15.0	124(4)	183(14)	221(17)	244(18)	250(23)	285(40)
1.5	1.0	0.4	25.0	124(26)	178(31)	237(26)	271(18)	291(30)	338(19)

Figure 8. The Rates of F^- Dissolution from Enamel Surfaces in Laminar Flow of 10 mM Acetic Acid at pH 4.5 after 24 Hour Exposures in Solutions of 0.4 mM NaF at pH 7.0. The acid flow from each surface was collected in 10 minute (12 ml) fractions for 1 hour. The figure shows the F^- dissolved from surfaces exposed 24 hours to solutions that contained:

a) The isotope in the absence and presence of 1.0 mM NaH_2PO_4 and 15 mM $NaHCO_3$;
b) 1.5 mM $CaCl_2$ in the presence and absence of 15 mM $NaHCO_3$; and
c) 1.5 mM $CaCl_2$, 1.0 mM $NaHPO_4$, and 0, 5, 15 and 25 mM $NaHCO_3$.


Fluoride Dissolved (µMol/cm^2 X 10^{-2})
(a)
0.8
0.6
0.4
0.2
0
1
2
3
4
5
6
× Isotope alone + F
○ PO$_4$ + F
△ 15 HCO$_3$ + F
□ PO$_4$ + 15 HCO$_3$ + F
(b)
3.0
2.0
1.0
0
1
2
3
4
5
6
× 1.5 Ca + F
△ 1.5 Ca + F + 15 HCO$_3$
(c)
4.0
3.0
2.0
1.0
0
1
2
3
4
5
6
× Ca+PO$_4$+F
○ Ca+PO$_4$+F + 5 HCO$_3$
△ Ca+PO$_4$+F + 15 HCO$_3$
□ Ca+PO$_4$+F + 25 HCO$_3$
Sequential 10 Minute Acid Collections

surfaces exposed in solutions of 1.5 mM calcium than from surfaces exposed in solutions of 1.0 mM phosphate in the absence of added calcium.

Fluoride dissolved in slightly increasing amounts in sequential collections of acid from surfaces exposed in solutions of calcium phosphate. The same amounts of fluoride dissolved from surfaces exposed in the presence and absence of 5 mM bicarbonate, to yield a total recovery of 3.5 percent of the fluoride uptake in these surfaces. Greater amounts of fluoride dissolved in the acid from surfaces exposed to solutions of 15 and 25 mM bicarbonate than from surfaces pretreated in the solutions of calcium phosphate without added bicarbonate. However, total fluoride recovery from these surfaces was still less than 7 percent of the fluoride taken up from the pretreatment solutions.

Table 10 (Fig. 9) shows the molecular ratios of Ca/F for minerals dissolved in the collections of acid from surfaces exposed 24 hours in the solutions of 0.4 mM NaF at pH 7.0. The ratios progressively increased from as low as 30 to as high as 90 in sequential collections of acid from surfaces exposed in solutions that did not contain added calcium, except from surfaces exposed in NaF plus bicarbonate which varied between 45 to 55 in the collections of acid. The mean ratios of Ca/F for the total minerals dissolved in the acid from these surfaces varied between 47 and 70.

The ratios of calcium to fluoride of the minerals dissolved from surfaces exposed in 1.5 mM calcium (absence

TABLE 10

Effects of 24 Hour Reactions of Demineralized Surfaces of Enamel in Solutions at pH 7.0 on the Mineral Dissolution in 1 Hour Laminar Flow of Acid

Solutions				Total Minerals Dissolved in 1 Hour Acid Collections						
mM				%(S.D.)	μMol/cm^2(S.D.)x10^{-2}			Mole Ratio		Percent Recovery Ratio
$CaCl_2$	NaH_2PO_4	NaF	$NaHCO_3$	^{45}Ca	Ca	PO_4	F	Ca/P	Ca/F	^{45}Ca/F
-	-	-	-	74(13)	242(14)	123(08)	-	2.0(0.1)	-	-
-	-	0.4	-	44(4)	192(16)	93(07)	2.8(0.4)	2.1(0.1)	70(4)	18
-	1.0	-	-	81(13)	326(61)	183(47)	-	1.8(0.2)	-	-
-	1.0	0.4	-	40(2)	188(20)	103(19)	3.3(0.2)	1.8(0.2)	57(5)	14
-	-	-	15.0	98(7)	316(40)	150(17)	-	2.1(0.1)	-	-
-	-	0.4	15.0	51(9)	221(33)	96(20)	3.5(1.9)	2.3(0.3)	52(1)	14
-	1.0	-	15.0	71(2)	184(06)	97(07)	-	1.9(0.2)	-	-
-	1.0	0.4	15.0	49(3)	153(13)	81(06)	3.3(0.3)	1.9(0.0)	47(8)	14
1.5	-	-	-	95(5)	297(56)	124(24)	-	2.4(0.3)	-	-
1.5	-	0.4	-	32(1)	190(08)	59(07)	9.6(0.3)	3.2(0.2)	20(1)	5
1.5	-	-	15.0	109(17)	196(17)	88(08)	-	2.2(0.1)	-	-
1.5	-	0.4	15.0	98(5)	178(20)	68(12)	4.6(0.5)	2.7(0.2)	39(1)	17
1.5	1.0	-	-	72(4)	220(26)	141(14)	-	1.6(0.0)	-	-
1.5	1.0	0.4	-	15(0)	115(13)	75(02)	8.8(0.3)	1.5(0.1)	13(1)	4
1.5	1.0	-	5.0	85(18)	305(13)	156(09)	-	2.0(0.1)	-	-
1.5	1.0	0.4	5.0	22(1)	214(26)	94(09)	8.5(1.5)	2.3(0.3)	26(3)	6
1.5	1.0	-	15.0	103(32)	284(19)	148(10)	-	1.9(0.1)	-	-
1.5	1.0	0.4	15.0	28(3)	163(20)	82(06)	13.1(0.9)	2.0(0.1)	12(1)	4
1.5	1.0	-	25.0	99(7)	342(86)	181(18)	-	1.9(0.2)	-	-
1.5	1.0	0.4	25.0	35(3)	246(31)	93(03)	14.4(1.4)	2.7(0.4)	17(4)	5

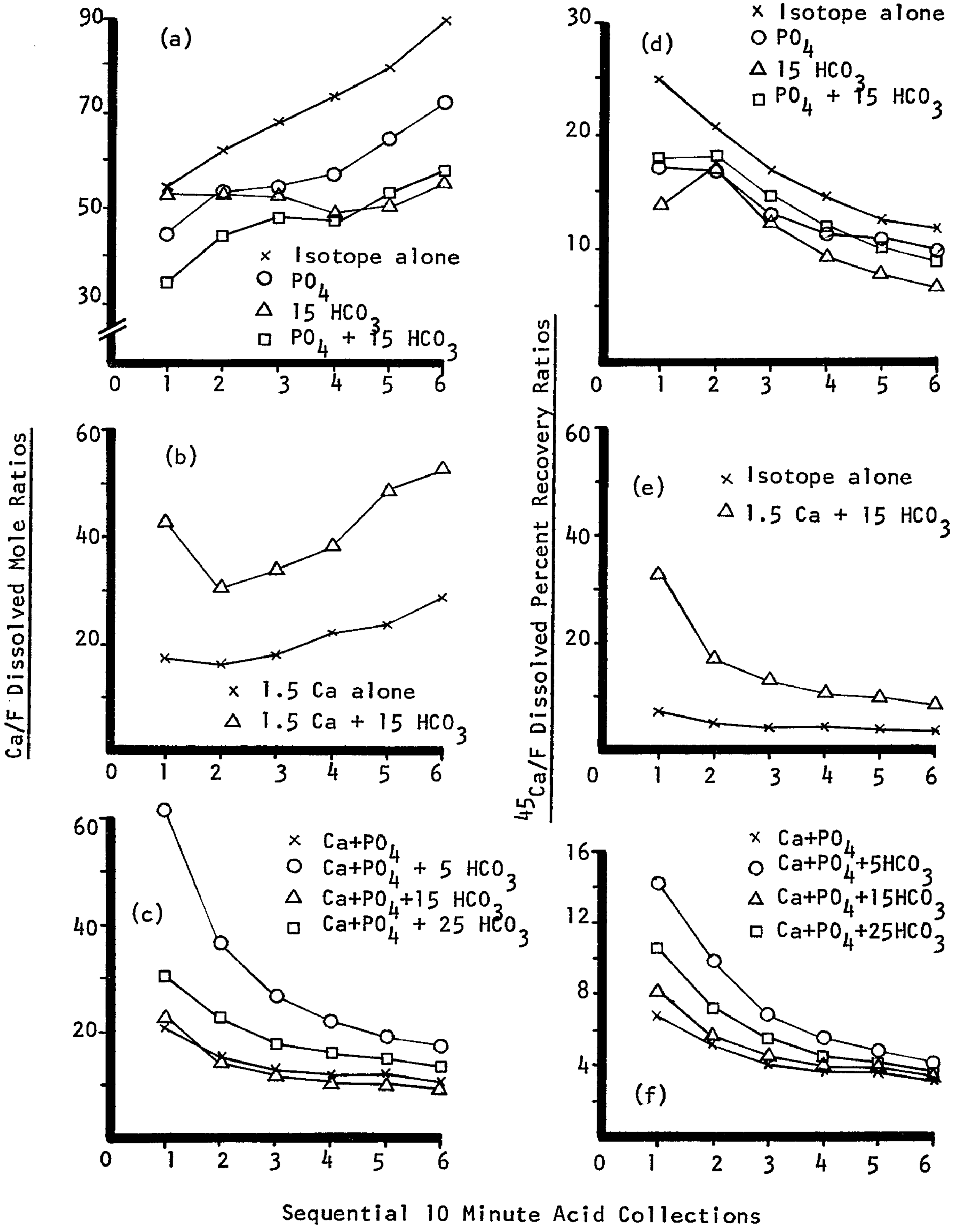
* Each table value represents the mean and standard deviation for 12 surfaces of enamel.

Figure 9. The Molecular Ratios of Ca/F and the Corresponding Ratios of the Percentage Recovery of ^{45}Ca to the Percentage Recovery of F^- in the Laminar Flow of Acid from Surfaces of Enamel Exposed in Solutions of 0.4 mM NaF at pH 7.0. The surfaces were exposed 24 hours to solutions that contained:

a) Isotope in the presence and absence of 1.0 mM NaH_2PO_4 and 15 mM $NaHCO_3$;

b) 1.5 mM $CaCl_2$ in the presence and absence of 15 mM $NaHCO_3$; and

c) 1.5 mM $CaCl_2$, 1.0 mM NaH_2PO_4, and 0, 5, 15 and 25 mM $NaHCO_3$.


(a)
90
70
50
30
× Isotope alone
○ PO_4
△ 15 HCO_3
□ PO_4 + 15 HCO_3
(b)
60
40
20
× 1.5 Ca alone
△ 1.5 Ca + 15 HCO_3
(c)
× Ca+PO_4
○ Ca+PO_4 + 5 HCO_3
△ Ca+PO_4+15 HCO_3
□ Ca+PO_4 + 25 HCO_3
(d)
30
20
10
× Isotope alone
○ PO_4
△ 15 HCO_3
□ PO_4 + 15 HCO_3
(e)
× Isotope alone
△ 1.5 Ca + 15 HCO_3
(f)
16
12
8
4
× Ca+PO_4
○ Ca+PO_4+5HCO_3
△ Ca+PO_4+15HCO_3
□ Ca+PO_4+25HCO_3
0 1 2 3 4 5 6
Ca/F Dissolved Mole Ratios
^{45}Ca/F Dissolved Percent Recovery Ratios
Sequential 10 Minute Acid Collections

of added phosphate) increased from about 16 in the first and second to 28 in the sixth collection of the acid (mean Ca/F of 20). The ratios decreased from 42 to 30 between the first and second, then increased in subsequent collections of the acid to 53 from surfaces exposed in bicarbonate plus calcium (average of about 40).

The ratios of calcium to fluoride decreased almost the same from about 20 to 10 in the sequential collections of acid from surfaces exposed in solutions of calcium phosphate without and with 15 mM bicarbonate. Levels of 5 and 25 mM bicarbonate in these solutions increased the ratios of Ca/F for minerals dissolved in the acid, but these ratios also decreased in the sequential collections to attain ratios of 13 to 17. The means of the Ca/F ratios for the total minerals dissolved in the acid were 12 to 13 in the absence of bicarbonate and in the presence of 5 mM bicarbonate, and 26 and 17 for solutions of 15 and 25 mM bicarbonate.

Table 10 also shows the total amounts of calcium, ^{45}Ca, phosphate, and fluoride dissolved from the surfaces of enamel in the collections of acid. NaF in solutions without added calcium reduced the recovery of ^{45}Ca, 41, 50, 48, and 30 percent respectively, for solutions of either isotope alone, phosphate alone, bicarbonate alone, or phosphate plus bicarbonate. NaF in solutions of 1.5 mM calcium (absence of added phosphate) reduced ^{45}Ca recovery in the acid 67 percent, but reduced the isotope recovery only 10 percent with bicarbonate in the pretreatment solution. NaF in solutions of calcium phosphate, in the presence or absence of 5 and 15 mM bicarbonate, reduced the recovery of ^{45}Ca from the enamel in the acid 73 to 79 percent.

The NaF in solutions of phosphate plus 15 mM bicarbonate reduced calcium dissolution 42 to 48 percent. NaF in solutions of 15 mM bicarbonate alone, calcium alone, and calcium phosphate plus 5 or 25 mM bicarbonate reduced the total amounts of calcium dissolved 28 to 36 percent. The additions of NaF to the solutions reduced the total amounts of phosphate dissolved in acid almost the same percentages as for calcium, except for solutions of calcium in the absence of added phosphate and solutions of calcium phosphate with 25 mM bicarbonate. In these solutions NaF reduced phosphate dissolution much more than calcium dissolution.

Exposure of enamel surfaces in NaF-free solutions of phosphate alone significantly increased the amounts of calcium and phosphate dissolved in the acid, compared to exposure in solutions of isotope ($0.05<P<0.10$). Exposure of enamel in solutions of bicarbonate alone also increased calcium and phosphate dissolution but not as much as exposure in solutions of phosphate alone. However, phosphate plus bicarbonate in NaF-free solutions reduced the amounts of calcium and phosphate dissolved in the acid to the same levels as NaF in solutions of the isotope alone. Additions of NaF in the solutions of phosphate plus bicarbonate further reduced the amounts of minerals dissolved in the acid.

Exposure of the enamel surfaces in solutions of only calcium tended to increase the amounts of calcium, but not phosphate, dissolved in the acid compared to solutions of the isotope alone. Exposure in NaF-free solutions of calcium plus bicarbonate significantly reduced the amounts of calcium and phosphate dissolved in the acid compared to either solutions of calcium alone or isotope alone. This effect of calcium plus bicarbonate accounts for the relatively small reduction of mineral dissolution caused by the addition of NaF to the solution.

The presence of 5 and 25 mM bicarbonate in NaF-free solutions of calcium plus phosphate greatly increased the amounts of calcium and phosphate dissolved in the acid compared to the absence of bicarbonate in the solutions. The calcium dissolved in the acid was slightly decreased, but phosphate dissolved was slightly increased from surfaces exposed in calcium plus phosphate compared to surfaces exposed in solutions of the isotope alone.

The total amounts of minerals dissolved in the acid from surfaces exposed 24 hours in solutions of calcium phosphate were less than the estimated mineral uptake except for the surfaces exposed in solutions that also contained bicarbonate in the absence of NaF. The total amounts of fluoride dissolved in the acid were less than 10 percent of the estimated uptake of fluoride during reactions of the surfaces in the solutions of neutral pH.

The averages of the Ca/P ratios for the total minerals dissolved in the acid from the pretreated surfaces were 1.8 or higher except for surfaces exposed in solutions of calcium phosphate without added bicarbonate. Combinations of NaF and bicarbonate in the solutions of calcium phosphate appeared to cause higher Ca/P ratios of the dissolved minerals than either NaF or bicarbonate. Phosphate in the pretreatment solutions generally lowered the ratios of the dissolved minerals, whereas calcium in the absence of phosphate caused higher ratios.

4. The Scanning Electron Micrographic Appearance of Untreated, Demineralized, and Remineralized Surfaces of Enamel

Figure 10 shows representative examples of labial surfaces of bovine enamel after (a,b) mild pumicing, (c,d) after 24 hour demineralization in 10 mM acetic acid at pH 4.5. Figure 11 shows representative examples of labial surfaces of human enamel after (a) mild-pumicing, (b) after 24 hour demineralization in 10 mM acetic acid at pH 4.5, (c) after 24 hour exposure of demineralized surfaces in NaF-free mineralizing solution, and (d) after 1 hour exposure of NaF-free remineralized surfaces in a laminar flow of the

Figure 10. Scanning Electron Micrographs of Natural and Altered Surfaces of Enamel (Bovine).

a,b) Pumiced, untreated surface enamel.

c,d) Surface demineralized 24 hours in 10 mM acetic acid of pH 5.5, then washed 24 hours in deionized water.

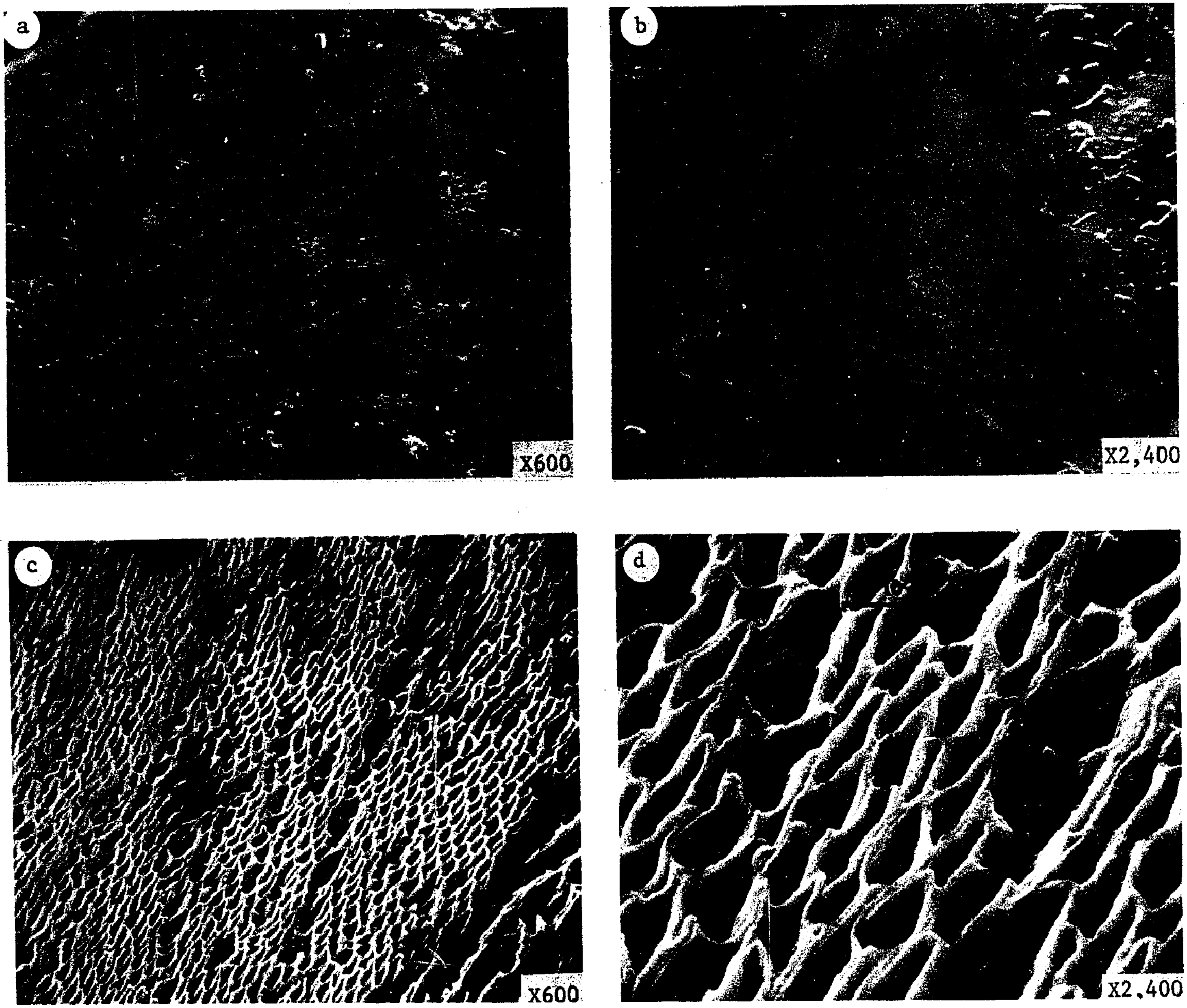

a
X600
b
X2,400
c
X600
d
X2,400

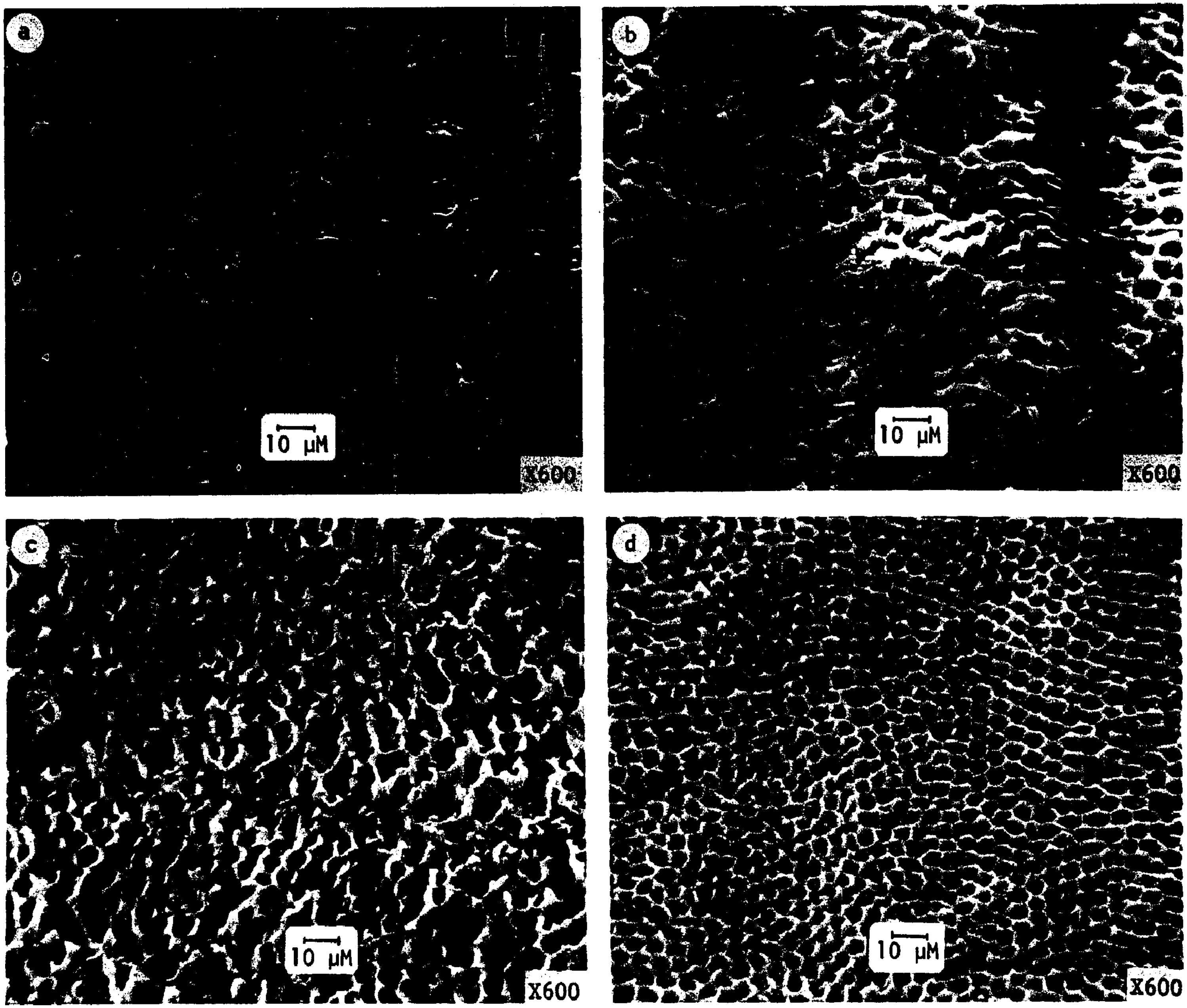
Figure 11. Scanning Electron Micrographs of Natural and Altered Surfaces of Enamel (Human).

a) Pumiced untreated surface enamel.

b) Surface demineralized 24 hours in 10 mM acetic acid, pH 4.5.

c) Demineralized surface after 24 hour exposure in 1.5 mM $CaCl_2$ and 1.0 mM NaH_2PO_4 at pH 7.0.

d) Surface treated as in c) after 1 hour laminar flow of acid at pH 4.5.


a
10 µM
X600
b
10 µM
X600
c
10 µM
X600
d
10 µM
X600

acid. The features and soundness of the pumiced surfaces were apparent. The 24 hour exposure in the acid primarily dissolved the minerals of the prism core leaving the sheath more or less intact. It appeared as though the sheath regions remineralized to a small extent in the NaF-free solutions. Subsequent 1 hour exposure of the remineralized surfaces in the acid flow caused further demineralization of the sheath regions of the enamel prisms.

Figure 12 shows examples of one area of a demineralized surface of human enamel after 24 hour exposure in mineralizing solution that contained 0.4 mM NaF. The remineralized surface appeared in low magnification to be coated with porous minerals. The higher magnifications showed the crystalline minerals were formed on the previously demineralized sheath regions of the enamel prisms. The degree of remineralization was insufficient to completely coalesce and fill the enamel rods.

Figure 13 shows an area of NaF-remineralized surface after 1 hour exposure in the acid flow. The 1 hour acid exposure apparently dissolved and redistributed the remineralized component.

Figure 12. Scanning Electron Micrographs of Demineralized Surfaces of Enamel After 24 Hour Exposure in Solution at pH 7.0 that Contained 1.5 mM $CaCl_2$, 1.0 mM NaH_2PO_4, and 0.4 mM NaF (Remineralized surfaces).

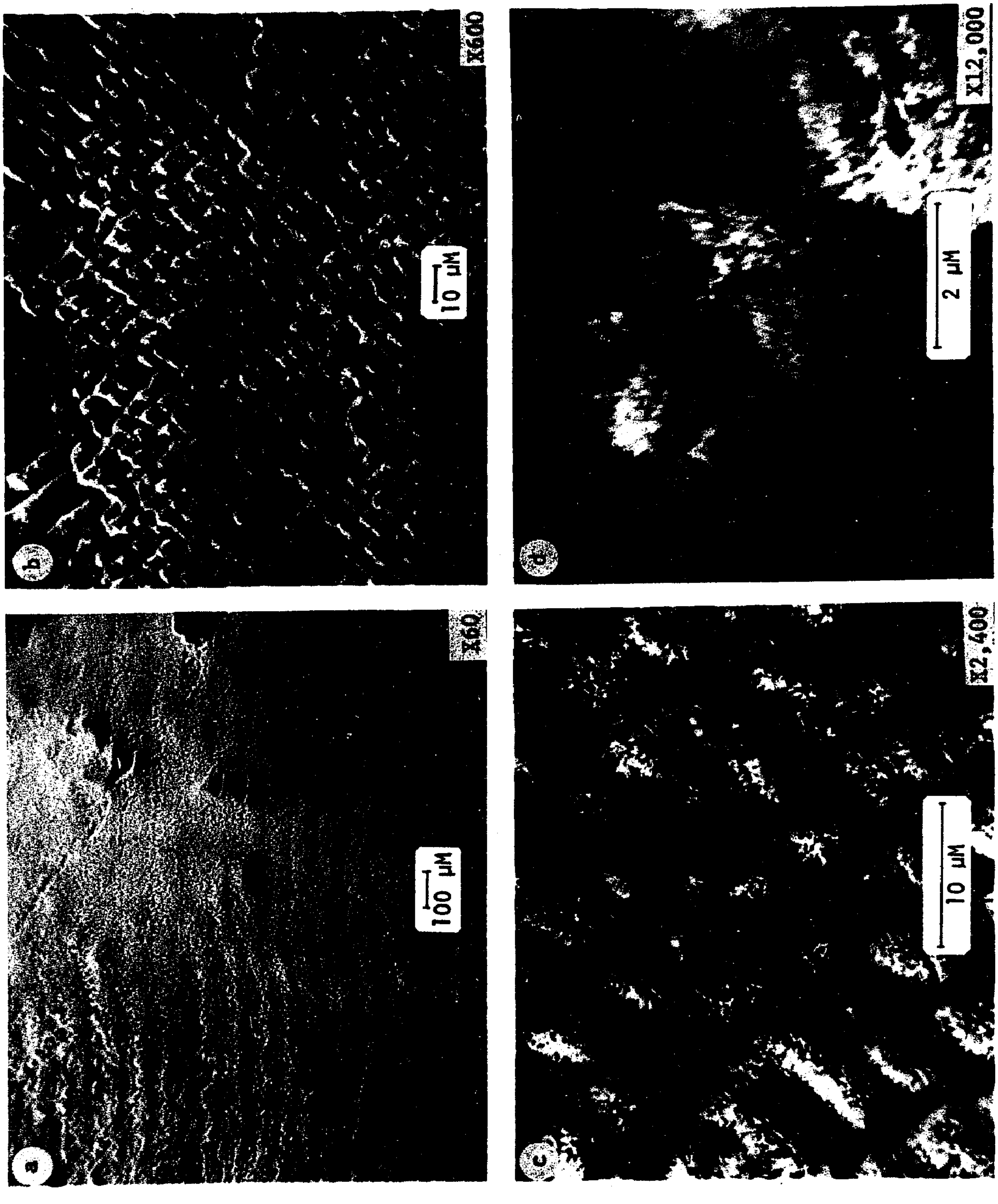
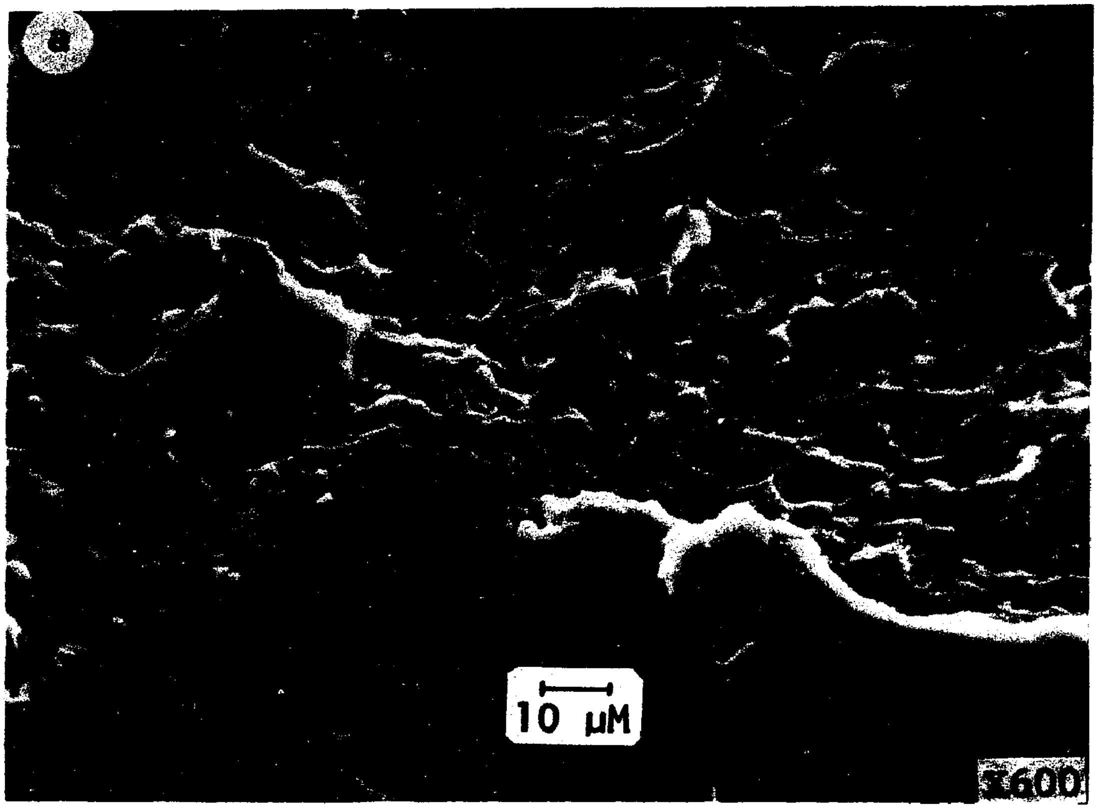
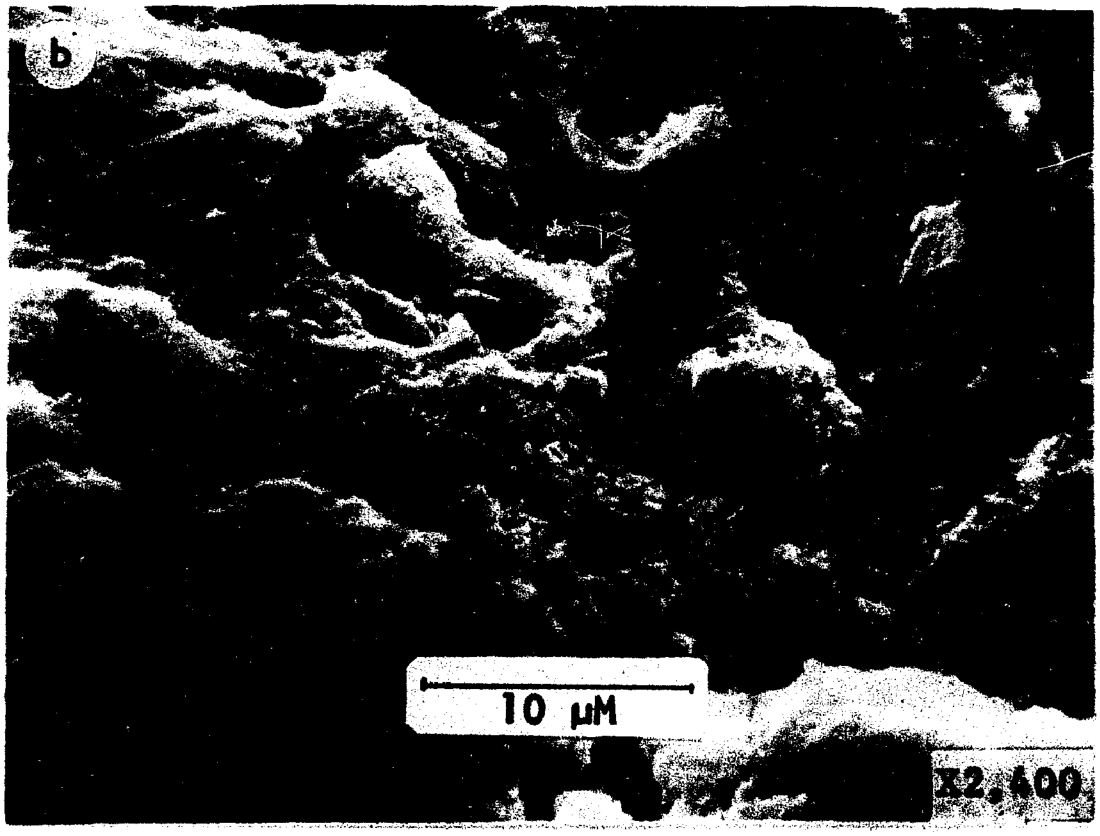

a
100 μM
X60
b
10 μM
X600
c
10 μM
X2,400
d
2 μM
X12,000

Figure 13 (a and b). Scanning Electron Micrographs of Surfaces Remineralized in the Presence of 0.4 mM NaF After 1 Hour Exposure to Laminar Flow of 10 mM Acetic Acid at pH 4.5.


a
10 μM
X600


b
10 μM
X2,400

5. Comparison of the Acid Resistance of Enamel Remineralized in the Presence of 0.4 mM NaF at pH 7.0 and the Presence of 0.4 and 0.05 mM NaF in the Laminar Flow of Acid at pH 4.5

The demineralized surfaces of enamel were remineralized 24 hours in solutions that contained 1.5 mM calcium, 1.0 mM phosphate, and either no fluoride or 0.4 mM fluoride, at constant pH 7.0.

Groups of untreated surfaces, demineralized surfaces and remineralized surfaces were exposed individually in a laminar flow of 10 mM acetic acid at pH 4.5. The acid was prepared free of NaF or with 0.4 mM NaF at pH 4.5. The acid was collected in six successive 10 minute (12 ml) aliquots from each surface. Tables 11 (Fig. 14), 12 (Fig. 15) and 13 (Fig. 15) show the ^{45}Ca recovery, and the calcium and phosphate dissolved, respectively, in the collections of acid from these surfaces.

The recovery of ^{45}Ca decreased from about 15 percent in the first, smoothly to about 9 percent in the last collection of NaF-free acid from surfaces remineralized in the absence of fluoride. The ^{45}Ca recoveries from these surfaces were reduced approximately one-half in each of the corresponding collections of acid NaF. A total of 72 percent of the isotope was recovered from surfaces remineralized in the absence of fluoride in NaF-free acid,

TABLE 11

Effects of NaF in 10 mM Acetic Acid at pH 4.5 on the Recovery of Calcium-45 from Remineralized Surfaces of Enamel.

Surfaces	Acid Conditions	Percent Ca^{45} Recovery in Sequential 10 Minute Collections of Acid					
		1	2	3	4	5	6
Remin, NoF	1) NaF-Free	14.6(1.3)	14.6(1.2)	13.0(0.7)	11.1(0.3)	9.9(0.2)	9.2(0.7)
	2) 0.4 mM NaF	7.9(1.8)	7.6(0.8)	6.8(0.7)	5.4(0.3)	5.5(1.0)	4.0(0.8)
	3) 0.05 mM NaF	15.5(1.8)	14.9(1.9)	13.0(1.7)	9.2(1.1)	7.3(0.3)	6.5(3.8)
Remin + F	1) NaF-Free	2.5(0.1)	2.7(0.1)	2.5(0.1)	2.4(0.1)	2.6(0.1)	2.3(0.1)
	2) 0.4 mM NaF	2.5(0.7)	2.5(0.6)	2.3(0.7)	2.2(0.6)	2.1(0.6)	2.2(0.5)

* Each table value represents the mean and standard deviation for 6 surfaces of enamel.

Figure 14. The Recovery of ^{45}Ca in Laminar Flow of Acid, in the Absence and Presence of 0.05 or 0.4 mM NaF at pH 4.5, from Surfaces of Enamel Remineralized 24 Hours in the Presence and Absence of 0.4 mM NaF at pH 7.0.

a) ^{45}Ca recovery from surfaces remineralized in the presence and absence of NaF in 10 minute collections of acid that did not contain added NaF.

b) ^{45}Ca recovery from surfaces remineralized in the presence and absence of NaF in 10 minute collections of acid that contained 0.05 or 0.4 mM NaF at pH 4.5.

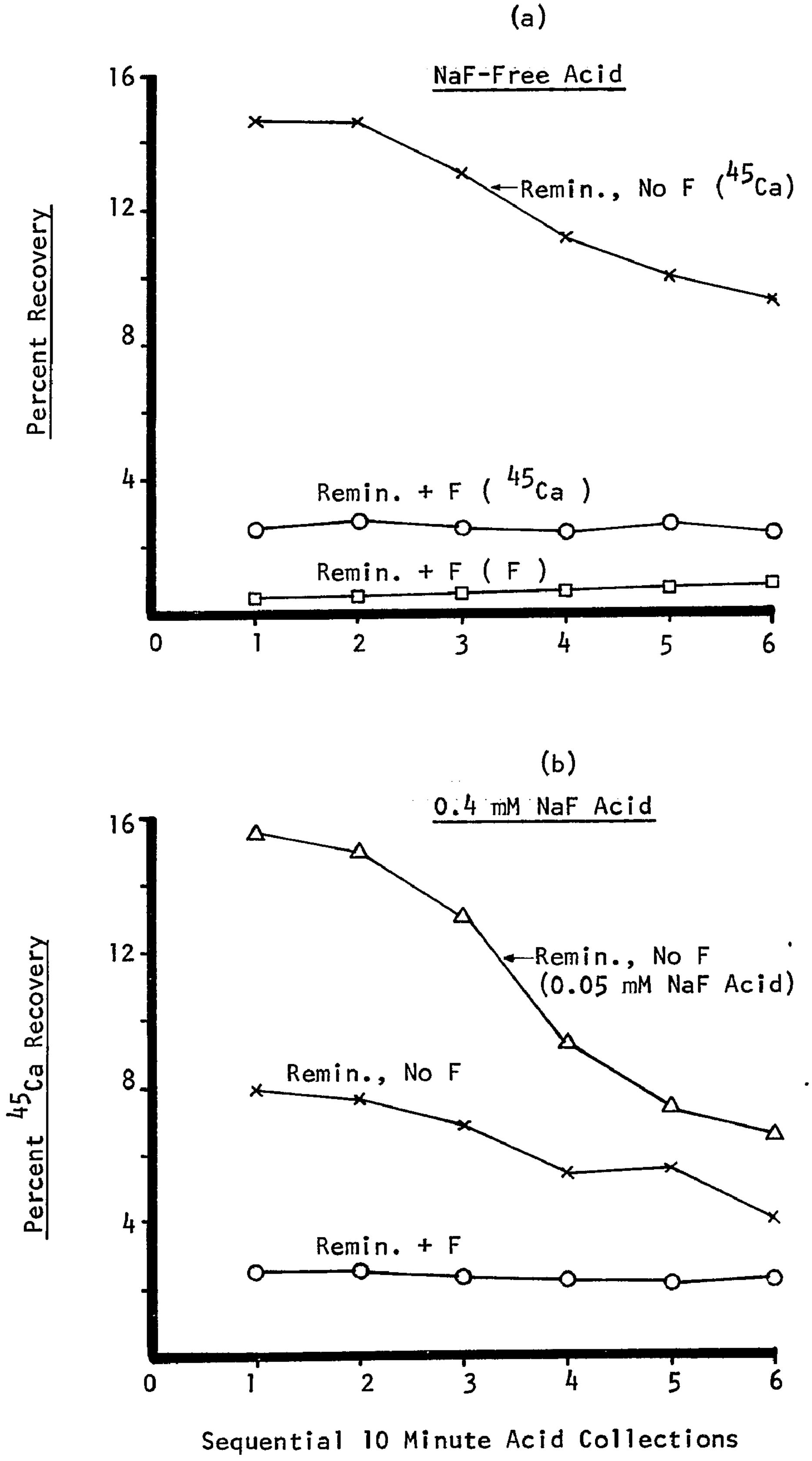

(a)
NaF-Free Acid
Percent Recovery
16
12
8
4
0
1
2
3
4
5
6
Remin., No F (^{45}Ca)
Remin. + F (^{45}Ca)
Remin. + F (F)
(b)
0.4 mM NaF Acid
Percent ^{45}Ca Recovery
16
12
8
4
0
1
2
3
4
5
6
Remin., No F
(0.05 mM NaF Acid)
Remin., No F
Remin. + F
Sequential 10 Minute Acid Collections

TABLE 12

Effects of NaF in 10 mM Acetic Acid at pH 4.5 on the Rates of Calcium Dissolution from Surfaces of Enamel.

Surfaces	Acid Conditions	Calcium Dissolved in Sequential 10 Minute Collection of Acid $\mu Mol/cm^2$ (S.D.) x 10^{-2}					
		1	2	3	4	5	6
Natural	1) NaF-Free	56(20)	48(7)	49(5)	49(6)	51(6)	47(6)
	2) 0.4 mM NaF	40(2)	31(2)	29(2)	29(2)	30(5)	28(3)
Demin.	1) NaF-Free	41(6)	39(6)	40(6)	39(5)	40(5)	44(3)
	2) 0.4 mM NaF	34(3)	25(1)	24(1)	23(1)	21(3)	22(2)
Remin, NoF	1) NaF-Free	40(4)	38(4)	37(4)	35(4)	35(4)	36(6)
	2) 0.4 mM NaF	31(5)	29(2)	26(2)	23(1)	24(2)	19(2)
	3) 0.05 mM NaF	42(2)	47(3)	44(3)	42(3)	44(4)	38(1)
Remin. + F	1) NaF-Free	18(6)	20(4)	19(4)	19(4)	21(4)	17(6)
	2) 0.4 mM NaF	27(4)	27(5)	27(4)	26(4)	26(4)	29(5)

* Each table value represents the mean and standard deviation for 6 surfaces of enamel.

TABLE 13

Effects of NaF in 10 mM Acetic Acid at pH 4.5 on the Rates of Phosphate Dissolution from Surfaces of Enamel.

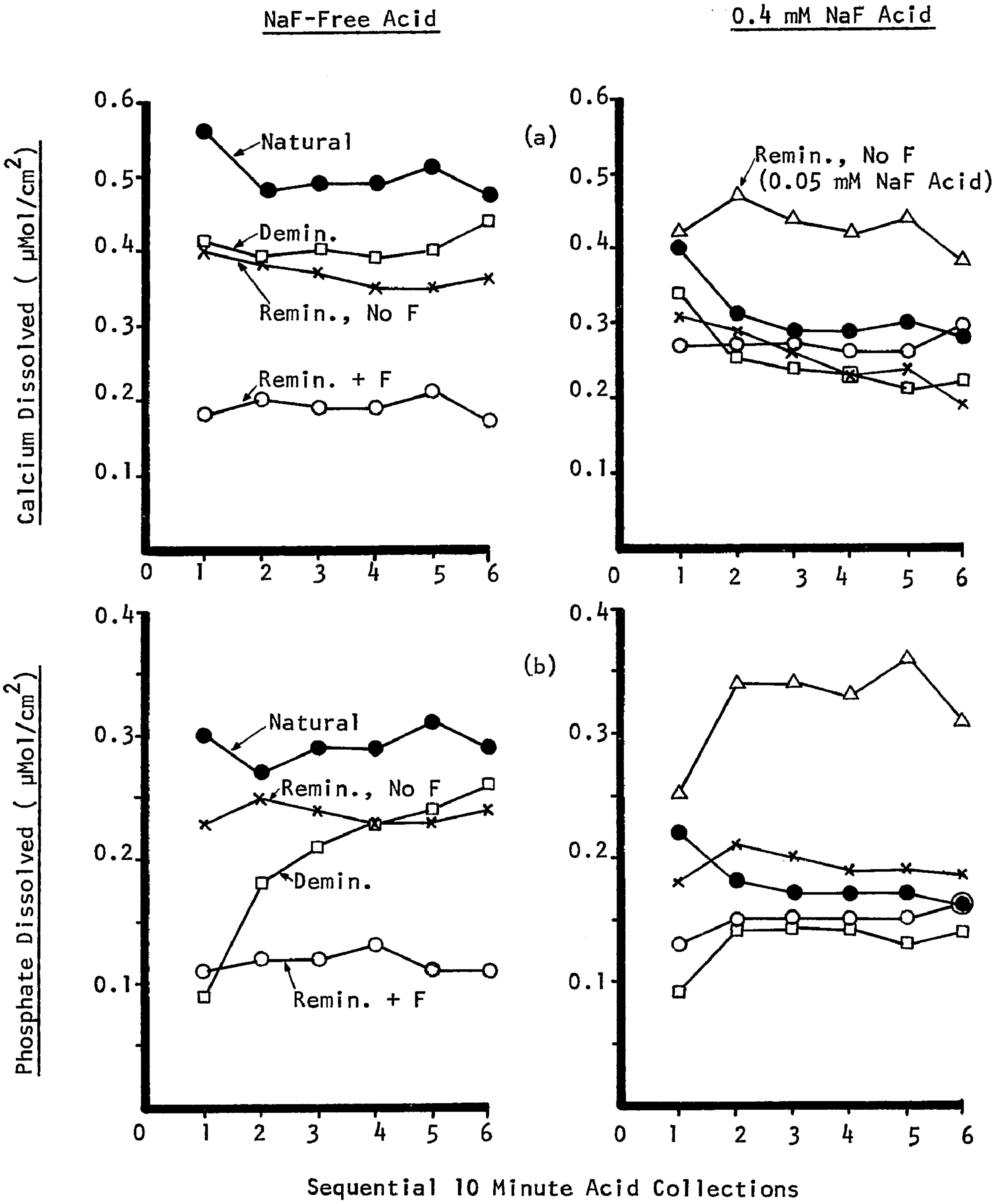
Surfaces	Acid Conditions	Phosphate Dissolved in Sequential 10 Minute Collections of Acid $\mu Mol/cm^2$ (S.D.) x 10^{-2}					
		1	2	3	4	5	6
Natural	1) NaF-Free	30(12)	27(4)	29(4)	29(5)	31(5)	29(5)
	2) 0.4 mM NaF	22(2)	18(1)	17(0)	17(1)	17(1)	16(3)
Demin.	1) NaF-Free	9(1)	18(3)	21(3)	23(3)	24(3)	26(1)
	2) 0.4 mM NaF	9(0)	14(1)	14(1)	14(0)	13(1)	14(1)
Remin, NoF	1) NaF-Free	23(2)	25(2)	24(2)	23(2)	23(2)	24(4)
	2) 0.4 mM NaF	18(3)	21(3)	20(2)	19(3)	19(2)	16(0)
	3) 0.05 mM NaF	25(2)	34(1)	34(2)	33(2)	36(3)	31(2)
Remin. + F	1) NaF-Free	11(3)	12(2)	12(2)	13(2)	11(5)	11(4)
	2) 0.4 mM NaF	13(2)	15(4)	15(3)	15(3)	15(3)	16(4)

* Each table value represents the mean and standard deviation for 6 surfaces of enamel.

Figure 15. The Rates of Calcium and Phosphate Dissolutions from Untreated, Demineralized, and Remineralized (Absence and Presence of 0.4 mM NaF) Surfaces of Enamel in Laminar Flow of 10 mM Acetic Acid in the Presence and Absence of Added NaF at pH 4.5.

a) Calcium dissolved from enamel surfaces in 10 minute collections of acid in the absence and presence of added NaF at pH 4.5.

b) Phosphate dissolved from enamel surfaces in 10 minute collections of acid in the absence and presence of added NaF at pH 4.5.


NaF-Free Acid
0.4 mM NaF Acid
Calcium Dissolved (µMol/cm^2)
Phosphate Dissolved (µMol/cm^2)
(a)
(b)
Natural
Demin.
Remin., No F
Remin. + F
Remin., No F (0.05 mM NaF Acid)
0.6
0.5
0.4
0.3
0.2
0.1
0
1
2
3
4
5
6
Sequential 10 Minute Acid Collections

whereas, only 37 percent was recovered in acid NaF. The recovery of ^{45}Ca was constant at a rate of about 2.5 percent in each of the sequential collections of NaF-free acid and acid NaF from surfaces remineralized in the presence of fluoride. The recovery of fluoride was also essentially constant at a rate of about 1 percent of the amounts taken up in the remineralized enamel in each of the collections of NaF-free acid (Table 11, Fig. 14).

The tables (12 and 13) show that slightly greater amounts of calcium dissolved in each of the acid collections from natural surfaces than from demineralized surfaces in both NaF-free acid and acid NaF. Almost the same amounts of calcium dissolved in the acid from surfaces remineralized in the absence of fluoride and from demineralized surfaces of enamel. Compared to surfaces remineralized in the absence of fluoride, one-half the amounts of calcium and phosphate dissolved from the surfaces remineralized in solutions of fluoride in the NaF-free acid. The relationships between the amounts of phosphate dissolved in the NaF-free acid were essentially the same as for calcium dissolved from natural surfaces and both groups of remineralized surfaces. However, phosphate dissolved from demineralized surfaces in small amounts in the first collection of acid and progressively in higher amounts in subsequent collections of the NaF-free acid.

Except for greater variability in the first and last collections of acid NaF, calcium dissolved in almost the same amounts from natural, demineralized, and remineralized surfaces of enamel. Phosphate also dissolved in a relatively close range from these surfaces, except in the first collections of acid NaF. The amounts of calcium and phosphate dissolved in each of the collections of acid NaF were significantly less than the amounts dissolved in the NaF-free acid from the respective groups of surfaces, except the surfaces remineralized in the presence of fluoride and the first collections of acid from demineralized surfaces.

The calcium to phosphate ratios of minerals dissolved from the natural surfaces of enamel decreased smoothly from about 1.90 to 1.66 in the successive collections of NaF-free acid; and from 1.85 to 1.66 in the first four collections, then to 1.78 in the final two collections of acid NaF (Table 14, Fig. 16). The ratios for minerals dissolved from demineralized surfaces were 4.6 in the first collections of NaF-free acid and about 4.0 in the first collections of acid NaF. These ratios decreased slowly to the range of 1.68 to 1.70 in NaF-free acid and to the range of 1.56 to 1.59 in acid NaF.

The calcium to phosphate ratios of minerals dissolved from surfaces remineralized in the absence of fluoride decreased from 1.75 in the first to a range of 1.55 to 1.50 in subsequent collections of NaF-free acid, but decreased sharply to 1.4 in the second to 1.25 in the last collections

TABLE 14

The Effects of NaF in Acid at pH 4.5 on the Ratios of Calcium to Phosphate Dissolved from Enamel Surfaces.

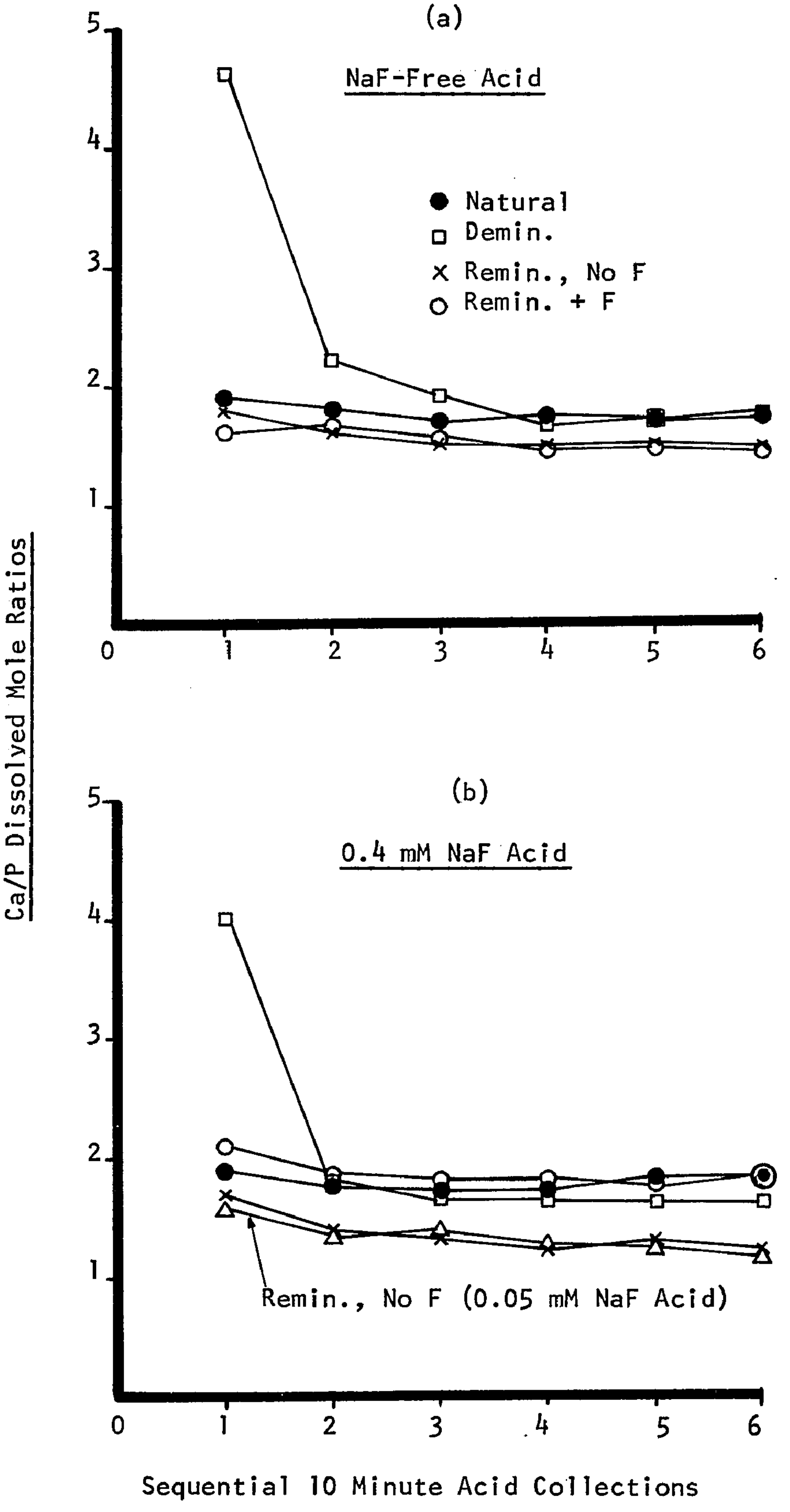
Surfaces	Acid Conditions	Ca/P (S.D.) Ratio in Sequential 10 Minute Collections of Acid					
		1	2	3	4	5	6
Natural	1) NaF-Free	1.9(0.1)	1.8(0.0)	1.7(0.1)	1.7(0.1)	1.7(0.1)	1.7(0.2)
	2) 0.4 mM NaF	1.9(0.1)	1.8(0.2)	1.7(0.1)	1.7(0.1)	1.8(0.2)	1.8(0.3)
Demin	1) NaF-Free	4.6(0.7)	2.2(0.3)	1.9(0.1)	1.7(0.1)	1.7(0.0)	1.7(0.1)
	2) 0.4 mM NaF	4.0(0.3)	1.8(0.0)	1.7(0.1)	1.6(0.1)	1.6(0.1)	1.6(0.1)
Remin; NoF	1) NaF-Free	1.8(0.1)	1.6(0.0)	1.5(0.0)	1.5(0.1)	1.5(0.0)	1.5(0.0)
	2) 0.4 mM NaF	1.7(0.1)	1.4(0.1)	1.3(0.1)	1.2(0.2)	1.3(0.2)	1.2(0.2)
	3) 0.05 mM NaF	1.7(0.1)	1.4(0.1)	1.3(0.1)	1.3(0.1)	1.2(0.1)	1.2(0.1)
Remin; + F	1) NaF-Free	1.6(0.2)	1.6(0.2)	1.5(0.2)	1.5(0.2)	1.5(0.2)	1.5(0.1)
	2) 0.4 mM NaF	2.1(0.2)	1.8(0.1)	1.8(0.1)	1.8(0.1)	1.8(0.1)	1.8(0.1)

* Each table value represents the mean and standard deviation for 6 surfaces of enamel.

Figure 16. The Molecular Ratios of Ca/P of Minerals Dissolved from Untreated, Demineralized, and Remineralized Surfaces of Enamel in the Laminar Flow of Acid in the Absence and Presence of NaF at pH 4.5. The acid flow was collected from each surface in 10 minute (12 ml) fractions.

a) Acid without added NaF.

b) Acid with added NaF.


(a)
NaF-Free Acid
● Natural
□ Demin.
× Remin., No F
○ Remin. + F
5
4
3
2
1
0
1
2
3
4
5
6
Ca/P Dissolved Mole Ratios
(b)
0.4 mM NaF Acid
5
4
3
2
1
Remin., No F (0.05 mM NaF Acid)
0
1
2
3
4
5
6
Sequential 10 Minute Acid Collections

of acid NaF. The ratios decreased from 1.63 smoothly to 1.50 for surfaces remineralized in the presence of fluoride in the successive collections of NaF-free acid. However, the ratios for minerals dissolved from these surfaces decreased from about 2.10 to 1.76 in the collections of acid NaF.

Calcium, and particularly phosphate, dissolved in greater amounts in the first 2 or 3 collections of acid that contained 0.05 mM NaF than in NaF-free acid from these surfaces. However, there was a definite trend of decreased dissolution of calcium and phosphate in the third to sixth collections of the 0.05 mM NaF acid.

6. The Effects of Magnesium on the Recrystallization of Demineralized Surfaces of Enamel at pH 7.0 and on the Subsequent Dissolution of Surface Minerals in Acid

Table 15 shows the effects of magnesium ions on the uptake of ^{45}Ca and fluoride ions in demineralized surfaces of enamel from solutions at pH 7.0. The solutions did not contain added calcium or phosphate. However, small amounts of calcium and phosphate dissolved from the surfaces in the solutions. The 1.0 mM level of magnesium particularly suppressed calcium dissolution. Fluoride slightly reduced the dissolution of calcium and phosphate in the solutions at pH 7.0. Fluoride increased the uptake of ^{45}Ca about 30 percent in the absence of magnesium. Magnesium

TABLE 15

Effects of Magnesium Ions on the Uptake of Fluoride and ^{45}Ca in Demineralized Surfaces of Enamel from Solutions of pH 7.0 Without Added Calcium or Phosphate

Initial Concentrations			24 Hour Concentrations			
(mM)		DPMx10^{4}/ml(S.D.)	DPMx10^{4}/ml(S.D.)	mM (S.D.)		
NaF	MgCl$_2$	^{45}Ca	^{45}Ca	Ca	PO$_4$	F
-	-	2.29(0.06)	1.11(0.10)	0.11(0.00)	0.08(0.01)	-
-	1.0	2.28(0.10)	1.40(0.05)	0.02(0.00)	0.06(0.00)	-
0.4	-	2.07(0.08)	0.52(0.06)	0.06(0.00)	0.05(0.00)	0.29(0.02)
0.4	1.0	2.44(0.06)	1.14(0.05)	0.03(0.00)	0.04(0.00)	0.30(0.00)

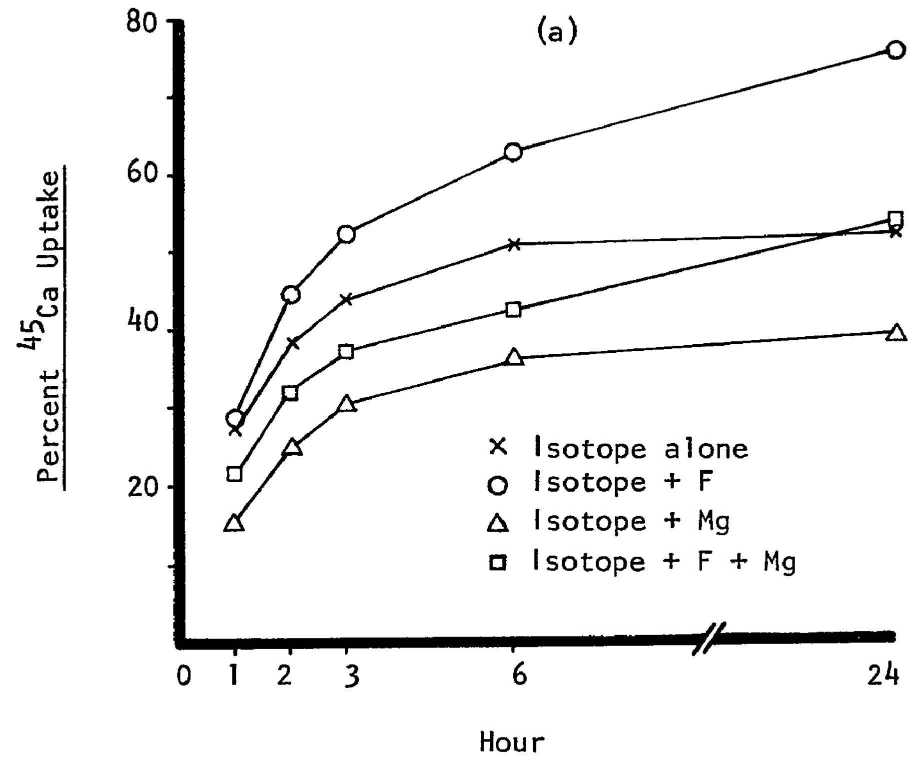
a) The percentages uptake of ^{45}Ca in the order listed were: (1) 52(±3), (2) 40(±), (3) 75(±4) and (4) 53(±).

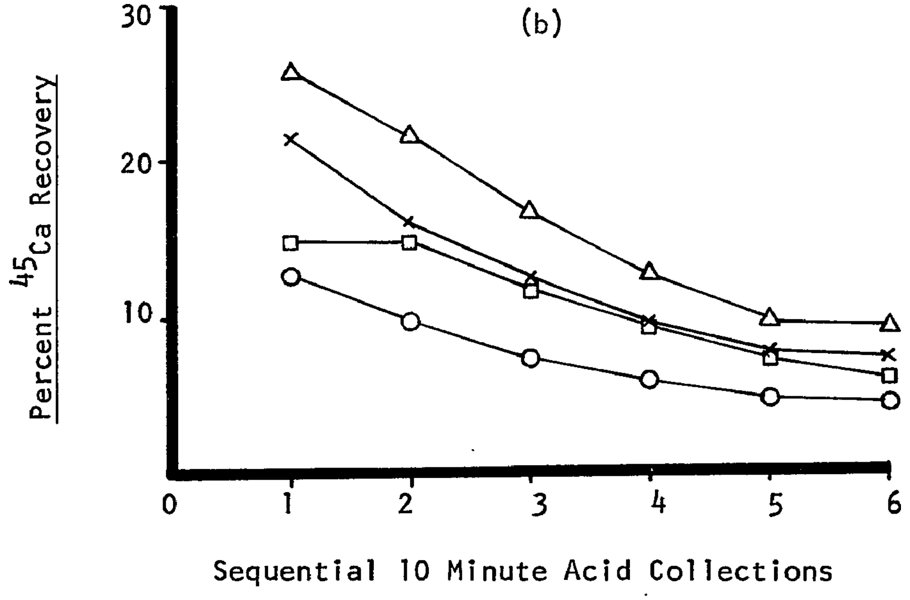
b) The uptake of fluoride per surface (μMol/cm^{2} was 1.11 (±0.13) for solutions of fluoride alone and 1.03 (±0.02) for solutions of fluoride plus magnesium.

Figure 17. The Effects of 0.4 mM NaF and 1.0 mM $MgCl_2$ on Recrystallization and Subsequent Acid Resistance of Minerals in Enamel Surfaces.

a) ^{45}Ca uptake in demineralized enamel exposed 24 hours in solutions at pH 7.0 that contained only the isotope or 0.4 mM NaF alone and combinations of 1.0 mM $MgCl_2$ and the NaF.

b) Recovery of ^{45}Ca from the surfaces in sequential 10 minute (12 ml) collections of 10 mM acetic acid at pH 4.5.


(a)
Percent ^{45}Ca Uptake
80
60
40
20
0
1
2
3
6
24
Hour
× Isotope alone
○ Isotope + F
△ Isotope + Mg
□ Isotope + F + Mg


(b)
Percent ^{45}Ca Recovery
30
20
10
0
1
2
3
4
5
6
Sequential 10 Minute Acid Collections

in the absence of fluoride reduced the 24 hour uptake of ^{45}Ca about 26 percent. Fluoride in the presence of magnesium restored the uptake of ^{45}Ca to the same levels as in the absence of fluoride or magnesium in solutions. Figure 17(a) showed that these effects of fluoride and magnesium on the uptake of ^{45}Ca in the enamel followed the same trends throughout the 24 hour reactions as indicated by the net uptake at the 24 hour time of determinations. The uptake of fluoride in the enamel was the same in the presence as in the absence of magnesium in the solutions.

Figure 17(b) shows the percentage recoveries of ^{45}Ca from the enamel surfaces in the sequential, 12 ml (10 minute) collections of acid. The ^{45}Ca was rapidly recovered in the collections of acid from surfaces exposed in solutions of magnesium in the absence of fluoride. The ^{45}Ca was least recoverable in the acid collections from surfaces exposed in solutions of fluoride in the absence of magnesium, whereas, the recoveries of ^{45}Ca were similar for surfaces exposed in solutions of magnesium alone and solutions that did not contain fluoride or magnesium. Magnesium in the absence of fluoride increased the total ^{45}Ca recovery about 23 percent. Fluoride in the absence of magnesium decreased the total ^{45}Ca recovery 41 percent, but fluoride and magnesium together in solutions increased the recovery of ^{45}Ca 31 percent compared to the effect of fluoride alone.

Table 16 (Fig. 18) shows the effects of pretreatment of enamel in the solutions of magnesium and fluoride on the subsequent rates of calcium, phosphate, and fluoride dissolution in the collections of the acid. Calcium dissolved in slightly increasing amounts in successive collections of acid from surfaces exposed in magnesium alone and solutions without fluoride or magnesium. Greater amounts of calcium and phosphate dissolved in each collection of acid from surfaces exposed in solutions of magnesium alone than surfaces exposed in the control solutions. Calcium dissolved at constant rates from surfaces exposed in solutions of fluoride alone. The amounts of calcium dissolved were the same for surfaces exposed in fluoride alone and solutions of fluoride plus magnesium.

The amounts of phosphate dissolved in the initial collections of the acid were less than those dissolved in subsequent collections. Closely similar amounts of phosphate dissolved in the acid from surfaces exposed in solutions without fluoride or magnesium, fluoride alone, and fluoride plus magnesium. The calcium to phosphate ratios of the dissolved minerals were exceptionally high for the surfaces exposed in solutions that did not contain magnesium. Exposure in solutions of magnesium reduced these ratios from about 2.2 in the initial to 1.68 in the subsequent collections of acid.

The amounts of fluoride dissolved from the surfaces pretreated in solutions of fluoride plus magnesium were

TABLE 16

Effects of 24 Hour Exposure of Demineralized Surfaces of Enamel in Solutions of 1.0 mM Magnesium and 0.4 mM Fluoride at pH 7.0 (Absence of Added Calcium or Phosphate) on Subsequent Rates of Mineral Dissolution in Laminar Flow of Acid (10 mM Acetic Acid, pH 4.5, Flow Rate of 1.2 ml/min.).

A) Calcium Dissolved

Solutions mM		Sequential 10 Minute Collections of Acid (μMol/cm^2(S.D.) x 10^{-2}						
NaF	$MgCl_2$	1	2	3	4	5	6	Total
-	-	35(2)	38(5)	39(1)	42(4)	43(5)	46(7)	242(14)
-	1.0	43(3)	48(6)	50(8)	52(5)	50(3)	58(4)	301(20)
0.4	-	32(4)	32(4)	32(5)	31(3)	31(1)	34(1)	192(16)
0.4	1.0	31(6)	33(9)	34(9)	36(6)	35(5)	35(3)	205(35)

B) Phosphate Dissolved

Solutions mM		Sequential 10 Minute Collections of Acid (μMol/cm^2(S.D.) x 10^{-2}						
NaF	$MgCl_2$	1	2	3	4	5	6	Total
-	-	10(0)	18(1)	21(1)	23(1)	23(1)	28(4)	123(8)
-	1.0	21(3)	27(4)	30(4)	31(3)	30(2)	35(3)	174(11)
0.4	-	10(1)	15(3)	16(2)	17(1)	16(0)	19(0)	93(7)
0.4	1.0	14(3)	18(5)	20(5)	21(3)	21(2)	21(1)	115(17)

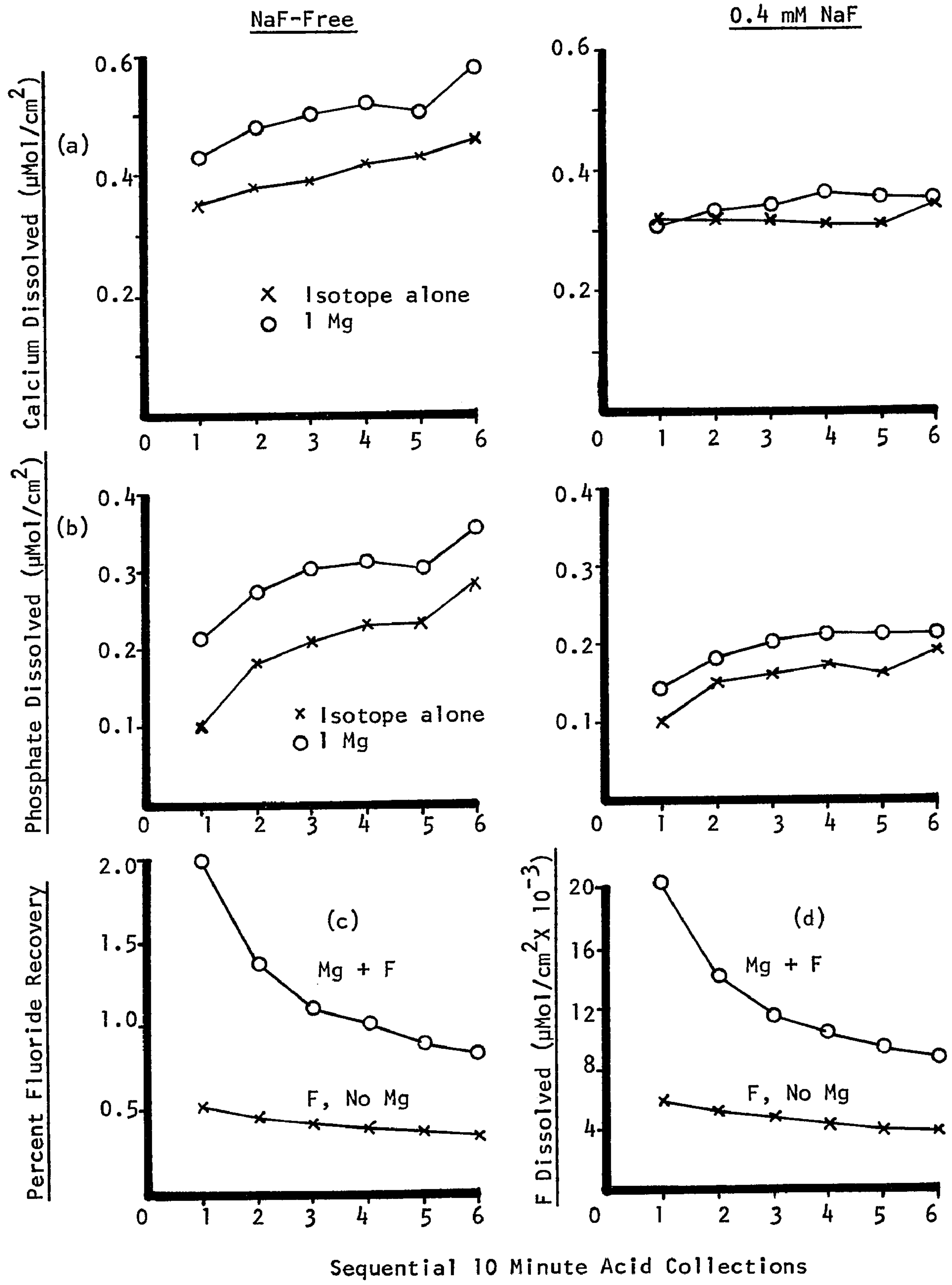
TABLE 16 (continued)

C) Fluoride Dissolved

Solutions mM		Sequential 10 Minute Collections of Acid (μMol/cm^2(S.D.) x 10^{-5}						
NaF	$MgCl_2$	1	2	3	4	5	6	Total
0.4	-	579(92)	511(79)	470(71)	428(57)	398(44)	383(41)	2769(373)
0.4	1.0	2040(361)	1411(194)	1135(56)	1036(64)	920(117)	857(73)	7399(918)

* Each table value represents the mean and standard deviation for 12 surfaces of enamel.

Figure 18. The Effects of 24 Hour Exposure of Enamel Surfaces in Solutions at pH 7.0 that Contained 0.4 mM NaF and 1.0 mM $MgCl_2$ on Subsequent Rates of a) Calcium, b) Phosphate, and c) F^- Dissolution in Laminar Flow of 10 mM Acetic Acid at pH 4.5. The acid flow from each surface was collected in sequential 10 minute (12 ml) fractions for 1 hour. The illustrations in the right side of the figure represent surfaces exposed 24 hours in solutions that contained 0.4 mM NaF.


NaF-Free
0.4 mM NaF
(a)
Calcium Dissolved (μMol/cm²)
0.6
0.4
0.2
0
1
2
3
4
5
6
× Isotope alone
○ 1 Mg
(b)
Phosphate Dissolved (μMol/cm²)
0.4
0.3
0.2
0.1
× Isotope alone
○ 1 Mg
(c)
Percent Fluoride Recovery
2.0
1.5
1.0
0.5
Mg + F
F, No Mg
(d)
F Dissolved (μMol/cm² X 10⁻³)
20
16
12
8
4
Mg + F
F, No Mg
Sequential 10 Minute Acid Collections

greater, by a factor of 4 in the initial to a factor of 2 in the final collections of acid, than from surfaces exposed in fluoride alone. However, the total recovery of fluoride was only 7 percent of the uptake of fluoride for surfaces exposed in solutions of fluoride plus magnesium, and only 2.5 percent for surfaces exposed in solutions of fluoride alone.

7. Effects of Magnesium Ions on the Remineralization and Subsequent Acid Resistance of Enamel Surfaces

The mineralizing solutions were prepared to contain 1.5 mM calcium, 1.0 mM phosphate, either no added fluoride or 0.4 mM fluoride, and either 1.0 mM or no magnesium, each as the sodium or chloride salt. Table 17 shows the fluoride, magnesium, and ^{45}Ca in solutions at the initial time of contact with the demineralized surfaces of enamel. The table also shows the concentrations of ^{45}Ca, calcium, phosphate, and fluoride in solution at the 24 hour time of enamel-solution interaction. The B part of Table 17 shows the estimated uptake of minerals in the remineralized enamel and the amounts of base titrant added to maintain constant pH 7.0 of the solutions. The pH, calcium, ^{45}Ca, phosphate and fluoride levels in the solutions were stable until contacted with the enamel.

Small amounts of calcium and phosphate were taken up in a ratio of one in surfaces exposed in solutions without magnesium or fluoride. Magnesium in the absence of fluoride

TABLE 17

Effects of Magnesium Ions on the Remineralization of Enamel in Solutions of 1.5 mM $CaCl_2$, 1.0 mM NaH_2PO_4 and 0.4 mM Fluoride at pH 7.0.

A) Composition of Solutions,

Initial Solutions			After 24 Hour Reaction with Enamel			
mM		DPMx10^4/ml(S.D.)	DPMx10^4/ml(S.D.)	mM(S.D.)		
NaF	$MgCl_2$	^{45}Ca	^{45}Ca	Ca	PO_4	F
-	-	2.88(0.07)	2.15(0.14)	1.13(0.05)	0.63(0.07)	-
0.4	-	2.80(0.04)	0.75(0.02)	0.39(0.03)	0.39(0.03)	0.10(0.00)
-	1.0	2.15(0.08)	1.64(0.06)	1.37(0.03)	0.76(0.06)	-
0.4	1.0	2.14(0.12)	0.83(0.12)	1.02(0.01)	0.36(0.01)	0.12(0.02)

TABLE 17 (Continued)

B) Calculated Mineral Uptake in Remineralized Mineral,

Solutions		24 Hour Mineral Uptake				
mM		μMol/cm^2(S.D.)				
NaF	$MgCl_2$	OH	Ca	PO_4	F	%^{45}Ca
-	-	2.98(0.53)	3.44(0.43)	3.28(0.56)	-	25.23(6.81)
0.4	-	6.85(0.33)	9.34(0.27)	5.19(0.26)	2.46(0.02)	73.30(0.66)
-	1.0	2.82(0.66)	1.09(0.25)	2.02(0.49)	-	24.04(0.60)
0.4	1.0	6.04(0.26)	3.98(0.12)	5.35(0.11)	2.33(0.13)	61.26(3.76)

a) Estimated magnesium uptake of 0.5 μMol/cm^2 from solutions without fluoride and 1.0 μMol/cm^2 from solutions with fluoride.

prevented calcium uptake, but slightly reduced phosphate uptake in the enamel compared to solutions without magnesium or fluoride. The amounts of base titrant added and the uptake of ^{45}Ca correlated with surfaces exposed in the two just described solutions. The ^{45}Ca, calcium, and phosphate uptake in enamel was greatly increased in the presence of fluoride and the absence of magnesium. Magnesium in the presence of fluoride markedly reduced the uptake of calcium, but it had no effect on phosphate uptake in the enamel. The uptake of fluoride in the enamel was not affected by the presence of magnesium in the solutions.

Figure 19(a,b) shows the uptake of ^{45}Ca in the enamel and the base titrant added to the solutions at the 1, 2, 3, 6, and 24 hour times of reaction with the enamel. Magnesium in the absence of fluoride had little effect on titrant additions for maintenance of pH 7.0 or on ^{45}Ca uptake in the enamel. Fluoride in the absence of magnesium increased ^{45}Ca uptake and titrant additions throughout the 24 hour reactions. Magnesium in the presence of fluoride, compared to fluoride alone, decreased titrant additions from the beginning, but reduced the uptake of ^{45}Ca only after the first hour of reaction. The total effects of magnesium on these two parameters were relatively small but consistent.

Table 18 (Fig. 19c) shows the recoveries of ^{45}Ca in the laminar flow of acid from the surfaces of enamel after 24

Figure 19. The Effects of 0.4 mM NaF and 1.0 mM $MgCl_2$ on the Remineralization of Enamel Surfaces. Demineralized surfaces were exposed 24 hours to solutions at pH 7.0 that contained 1.5 mM $CaCl_2$ and 1.0 mM NaF in the absence and presence of the NaF and $MgCl_2$.

a) Base additions to solutions that were required to maintain pH 7.0 during 24 hour exposure of enamel surfaces.

b) ^{45}Ca uptake of the surface enamel corresponding to the base additions shown in illustration a).

c) ^{45}Ca recovery from the remineralized surfaces in laminar flow of 10 mM acetic acid at pH 4.5 in sequential 10 minute (12 ml) collections.

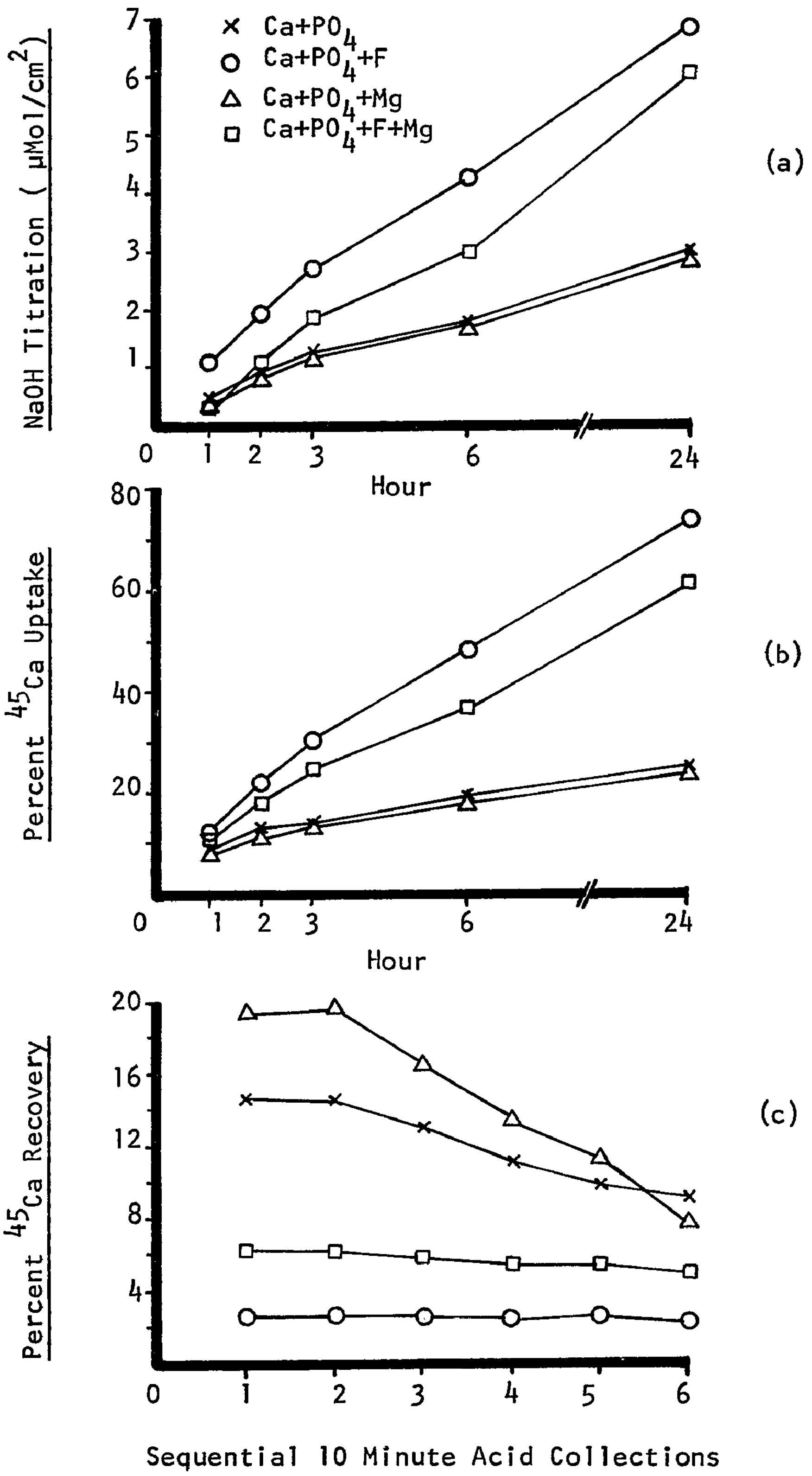

× Ca+PO$_4$
○ Ca+PO$_4$+F
△ Ca+PO$_4$+Mg
□ Ca+PO$_4$+F+Mg
(a)
NaOH Titration (μMol/cm^2)
7
6
5
4
3
2
1
0
1
2
3
6
24
Hour
(b)
Percent ^{45}Ca Uptake
80
60
40
20
0
1
2
3
6
24
Hour
(c)
Percent ^{45}Ca Recovery
20
16
12
8
4
0
1
2
3
4
5
6
Sequential 10 Minute Acid Collections

TABLE 18

Effects of Fluoride and Magnesium Ions on Recovery of ^{45}Ca from Remineralized Enamel in Laminar Flow of Acid

Solutions mM		Percent ^{45}Ca (S.D.) Recovery in Sequential 10 Minute Collections of Acid						
NaF	$MgCl_2$	1	2	3	4	5	6	Total %
-	-	14.6(2.6)	14.6(1.9)	13.0(1.0)	11.1(0.7)	9.9(1.0)	9.2(1.2)	72.4
0.4	-	2.5(0.6)	2.7(0.2)	2.5(0.3)	2.4(0.3)	2.6(0.4)	2.3(0.3)	15.0
-	1.0	18.4(4.4)	18.5(4.7)	15.8(3.5)	12.7(3.1)	10.9(2.8)	7.9(2.2)	84.2
0.4	1.0	5.6(2.0)	5.5(1.8)	5.3(1.6)	5.1(1.2)	5.2(0.8)	4.6(1.1)	36.5

* Each table value shows the mean and standard deviation for 12 surfaces. Each surface was separately exposed in laminar flow of 10 mM acetic acid at pH 4.5.

hour exposure in the mineralizing solutions of magnesium and/or fluoride. Slightly greater percentages of ^{45}Ca were recovered in the successive collections of acid from surfaces exposed in solutions of magnesium alone than solutions without magnesium or fluoride. ^{45}Ca was recovered from surfaces exposed in solutions of fluoride at almost constant rates in the collections of acid. Twice the percentages of ^{45}Ca were recovered from surfaces remineralized in solutions of magnesium plus fluoride than solutions of fluoride alone. The recoveries of ^{45}Ca from surfaces remineralized in the absence of fluoride were more than twice the recoveries from surfaces remineralized in solutions of magnesium plus fluoride.

Table 19 (Fig. 20a) shows the amounts of calcium dissolved in the successive collections of acid from the remineralized enamel. Greater amounts of calcium dissolved from surfaces exposed in solutions of magnesium without fluoride than from surfaces remineralized in the absence of magnesium and fluoride, except for the first collections of acid. However, the amounts of calcium dissolved in the successive collections of acid were generally the same for surfaces remineralized in the presence of fluoride plus magnesium, magnesium alone, and the absence of both magnesium and fluoride. Calcium dissolved from surfaces remineralized in the presence of fluoride without magnesium was about one-half the amounts dissolved from surfaces remineralized in the absence of fluoride.

TABLE 19

Effects of Fluoride and Magnesium Ions in Remineralized Enamel on Calcium Dissolution in 10 mM Acetic Acid at pH 4.5.

Solutions mM		Calcium Dissolved in Sequential 10 Minute Collections of Acid μMol/cm^2 (S.D.)					
NaF	$MgCl_2$	1	2	3	4	5	6
-	-	0.40(0.08)	0.36(0.11)	0.37(0.05)	0.33(0.10)	0.35(0.05)	0.36(0.06)
0.4	-	0.18(0.06)	0.20(0.04)	0.19(0.04)	0.19(0.04)	0.21(0.04)	0.17(0.06)
-	1.0	0.44(0.06)	0.47(0.06)	0.46(0.03)	0.45(0.03)	0.46(0.05)	0.44(0.05)
0.4	1.0	0.48(0.15)	0.39(0.08)	0.40(0.07)	0.41(0.09)	0.42(0.08)	0.40(0.09)

* Each table value represents the mean and standard deviation for 12 surfaces.

Table 20 (Fig. 20b) shows the phosphate dissolved from the remineralized surfaces in the collections of acid. Phosphate dissolved almost the same from surfaces remineralized in solutions of fluoride plus magnesium and solutions without fluoride or magnesium, which was slightly less than from surfaces remineralized in solutions of magnesium alone. Almost exactly one-half the amounts of phosphate dissolved from surfaces remineralized in solutions of fluoride alone than from surfaces remineralized in the absence of fluoride or magnesium. The calcium to phosphate ratios of the dissolved minerals varied between 1.63 to 1.75 in the first collections of acid, except that the ratio was 1.85 for surfaces remineralized in magnesium alone. The ratios decreased between 1.6 to 1.7 in the second collections of the acid from all surfaces. After the second collections of acid the ratios smoothly decreased between 1.5 and 1.6, except the ratios increased to 1.8 for minerals dissolved from surfaces remineralized in solutions of fluoride plus magnesium.

Table 21 (Fig. 20c) shows the fluoride dissolved in the collections of acid from surfaces remineralized in solutions of fluoride. Small amounts of fluoride were recovered from the surfaces in slightly increasing amounts in successive collections of the acid. The total amounts of fluoride recovered were only 3.9 to 4.5 percent of the fluoride uptake in the remineralized enamel. The fluoride recoveries were slightly greater in each collection of acid from surfaces

TABLE 20

Effects of Fluoride and Magnesium Ions in Remineralized Enamel on Phosphate Dissolution in 10 mM Acetic Acid at pH 4.5.

Solutions mM		Phosphate Dissolved in Sequential 10 Minute Collections of Acid μMol/cm^2(S.D.)					
NaF	$MgCl_2$	1	2	3	4	5	6
-	-	0.23(0.04)	0.25(0.03)	0.24(0.03)	0.23(0.00)	0.20(0.07)	0.22(0.08)
0.4	-	0.11(0.03)	0.12(0.02)	0.12(0.02)	0.13(0.02)	0.11(0.05)	0.11(0.04)
-	1.0	0.24(0.05)	0.31(0.05)	0.30(0.03)	0.29(0.03)	0.30(0.05)	0.28(0.03)
0.4	1.0	0.28(0.10)	0.24(0.05)	0.24(0.04)	0.23(0.04)	0.24(0.03)	0.22(0.03)

* Each table value represents the mean and standard deviation for 12 surfaces.

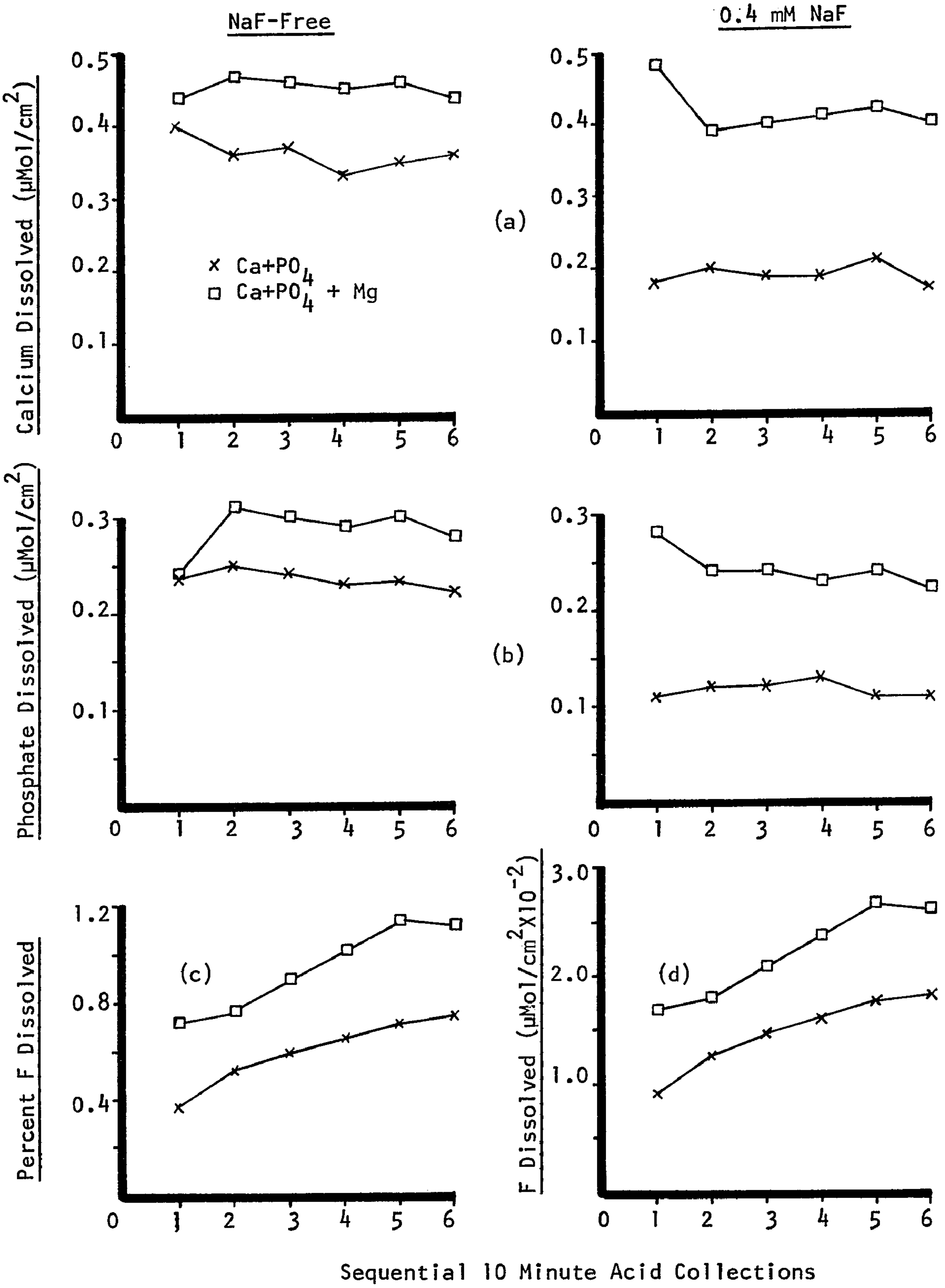
TABLE 21

Effects of Magnesium Ion in Remineralized Enamel on Fluoride Recovery in 10 mM Acetic Acid at pH 4.5.

Solutions mM		Fluoride Dissolved in Sequential 10 Minute Collections of Acid μMol/cm^2 (S.D.) x 10^{-2}					
NaF	$MgCl_2$	1	2	3	4	5	6
0.4	-	0.91(0.17)	1.27(0.18)	1.46(0.05)	1.60(0.07)	1.75(0.05)	1.83(0.25)
0.4	1.0	1.68(0.52)	1.78(0.44)	2.09(0.45)	2.37(0.49)	2.66(0.27)	2.61(0.38)

* Each table value represents the mean and standard deviation for 12 surfaces.

Figure 20. The Effects of 24 Hour Exposure (Remineralization) in Solutions of 0.4 mM NaF and 1.0 mM $MgCl_2$ on the Rates of a) Calcium, b) Phosphate, and c) F^- Dissolution from Enamel Surfaces in Laminar Flow of 10 mM Acetic Acid at pH 4.5. The acid flow from each surface was collected in sequential 10 minute (12 ml) fractions for 1 hour.


NaF-Free
0.4 mM NaF
Calcium Dissolved (µMol/cm^2)
Phosphate Dissolved (µMol/cm^2)
Percent F Dissolved
F Dissolved (µMol/cm^{2}X10^{-2})
(a)
(b)
(c)
(d)
× Ca+PO$_4$
□ Ca+PO$_4$ + Mg
0.5
0.4
0.3
0.2
0.1
1.2
0.8
0.4
3.0
2.0
1.0
0
1
2
3
4
5
6
Sequential 10 Minute Acid Collections

remineralized in the presence of magnesium than in its absence.

The calcium to fluoride ratios of the dissolved minerals decreased from 28 to 15 in the collections of acid from surfaces remineralized in solutions of magnesium plus fluoride. Similarly, the ratios decreased from 20 smoothly to 10 in collections of acid from surfaces remineralized in fluoride alone. Ratios of the recovery of ^{45}Ca to the recovery of fluoride, both as percentages of uptake, decreased smoothly from 10 to 3 in the successive collections of the acid.

DISCUSSION

1. Demineralization of Surface Enamel in Acid for 24 Hours

Calcium and phosphate dissolved in ratio of 1.63 during the 24 hour demineralization in the weak acid. This was essentially the same as the ratio reported for mixed samples of human enamel (103). The 1.63 ratio of calcium to phosphate for the dissolved minerals indicated that they dissolved as enamel apatites. This suggested that the residual surface minerals in the enamel after the acid demineralization were also apatite. However, since the minerals dissolved in the acid in ratio of 1.63 rather than the 1.67 ratio of hydroxyapatite the residual surface phase contained a slight excess of phosphate (about 0.3 μMol per surface of enamel relative to hydroxyapatite). Previous investigations showed that small excess amounts of phosphate in acid demineralized surfaces of enamel were mostly HPO_4^{-2} (27).

Small amounts of calcium and phosphate dissolved from the acid demineralized surfaces of enamel in the wash water. Since calcium and phosphate dissolved in ratio of about 1 in the water, phosphate dissolved in excessive amounts relative to calcium if the surface phase undergoing dissolution was apatite. Appropriate calculations indicated that each of the washed, demineralized surfaces contained excess calcium of 0.6 μMol relative to the composition of hydroxyapatite. However, the water dissolution of calcium and phosphate in ratio of 1 could have indicated that the surface phase undergoing dissolution was dicalcium phosphate residual in the acid demineralized enamel (33). However, later experimentation clearly showed that the demineralized, water washed surfaces contained excessive amounts of calcium relative to the composition of hydroxyapatite. The excess calcium was either retained in the hydrated layer of the mineral surfaces or was bound to the organic matrix of the demineralized enamel.

2. Reactions of Demineralized Surfaces of Enamel in Solutions at pH 7.0

Groups of the demineralized, washed surfaces were exposed 24 hours at pH 7.0 in solutions that contained either 150 mM NaCl (isotope alone), 1.0 mM phosphate, 15 mM bicarbonate, or the phosphate and bicarbonate combined, each in the absence and presence of 0.4 mM NaF. These solutions were unsaturated relative to the solubility of calcium phosphates, calcium carbonates, and calcium fluoride. The net uptake of ^{45}Ca, phosphate, and fluoride in the enamel surfaces occurred by ionic exchange and recrystallization.

Exchange-recrystallization reactions between the demineralized surfaces of enamel and the NaF-free solutions of the isotope alone removed about one-half of the ^{45}Ca

in solution. The curves for ^{45}Ca uptake in the enamel simulated the classical description of Neuman for ionic exchange and recrystallization of hydroxyapatite (70). Calcium and phosphate dissolved from the enamel in the solutions of the isotope alone in almost the same amounts, and ratio, as in the water wash after demineralization. Thus during exchange-recrystallization reactions in solutions of isotope alone excess calcium continually accumulated in the surface minerals. The fraction collection method of surface exposure to the laminar flow of acid confirmed that the surface minerals contained excessive amounts of calcium relative to phosphate. Calcium dissolved in almost constant amounts, phosphate dissolved in initially small but progressively increasing amounts, and ^{45}Ca dissolved in decreasing amounts in successive collections of acid from these surfaces. The characteristics of acid dissolution showed that the surface minerals were deficient in phosphate and that the exchanged ^{45}Ca was mostly confined to the mineral surfaces.

The demineralized surfaces of enamel removed 75 percent of the ^{45}Ca from solutions that contained 0.4 mM NaF only. The NaF increased the rates of ^{45}Ca uptake throughout the 24 hour exposure of the enamel in the solution, and equally reduced the small amounts of calcium and phosphate dissolved in the solution. This indicated that fluoride enhanced the recrystallization of surface minerals, but that the surfaces still retained the small amounts of excess calcium. The estimated fluoride uptake per surface of 1.1 μMol possibly reacted with the excess calcium in the surfaces to form CaF_2 or CaF^+. However, the equal effect of the NaF on calcium and phosphate dissolution at neutral pH indicated fluoride uptake by recrystallization of surface minerals to fluorapatites. The extremely small recoveries of ^{45}Ca and fluoride in the collections of acid from these surfaces confirmed that most of the ^{45}Ca and fluoride were taken up in the enamel as fluorapatite. The calcium to phosphate ratios of the minerals dissolved from these surfaces in the laminar flow of acid showed that the surfaces contained a readily soluble phase of excess calcium. If the fluoride was taken up as CaF^+ in the surfaces, then it dissolved in the acid and rapidly recrystallized to fluorapatite. The minerals dissolved in the laminar flow of acid obviously represented the more soluble surface phases leaving fluorapatites undissolved.

Small amounts of calcium and phosphate dissolved from the enamel surfaces in each of the solutions without added calcium, except the solutions that contained phosphate in the absence of bicarbonate. Phosphate alone in the solutions stimulated recrystallization of the surface minerals almost as effectively as NaF alone. This was shown by the increased uptake of ^{45}Ca, absence of calcium dissolution, and net uptake of phosphate by the enamel. It appeared that the phosphate was taken up in reaction with the excess calcium in the demineralized surfaces. However, the calcium to phosphate ratio of the minerals dissolved in the first collections of acid from these surfaces indicated that the surfaces still contained excess calcium. Thus only a small fraction of the phosphate uptake must have reacted with the excess calcium in the surfaces. This possibly meant that the excess calcium in the demineralized, washed surfaces was bound to acid soluble components of the organic matrix of the demineralized enamel. If so, then the excess calcium may be considered as a site of nucleation for new mineral formation rather than simply calcium adsorbed in the hydrated layers of crystal surfaces.

The minerals formed in the surfaces exposed in the NaF-free solutions of phosphate alone dissolved in greater amounts in the laminar flow of acid than the minerals recrystallized in solutions of the isotope alone. However, the calcium to phosphate ratios and recoveries of ^{45}Ca in the acid from these two groups of surfaces were closely alike. This indicated that the minerals formed in the surfaces from the two solutions, whether amorphous or crystalline calcium phosphates, differed only in amount.

Inclusion of NaF in solutions of phosphate alone reduced the apparent uptake of phosphate, but greatly increased the uptake of ^{45}Ca so that 92 percent of the isotope in the solution was taken up in the mineral. This additive effect of fluoride and phosphate on the uptake of ^{45}Ca occurred mostly in the first hour of enamel-solution interaction. Surprisingly, the uptake of fluoride was the same from solutions of isotope alone and solutions of phosphate plus NaF. If CaF_2 had been a principal reaction production in the surface minerals, then the 1.0 mM level of phosphate probably would have prevented fluoride uptake as CaF_2 since the solutions did not contain added calcium. The 43 percent decrease of the minerals dissolved, and the calcium to phosphate ratios of the minerals dissolved, in the laminar flow of acid indicated that a relatively large fraction of the surface minerals were converted to fluorapatites. The small recoveries of fluoride and ^{45}Ca in the acid also showed that the surfaces contained recrystallized fluorapatites.

The additions of 15 mM bicarbonate in solutions of isotope alone slightly reduced the uptake of ^{45}Ca and reduced the amounts of calcium dissolved, but doubled the amounts of phosphate dissolved from the enamel in the solutions of neutral pH. Bicarbonate displaced phosphate (72) and thereby apparently doubled the amounts of excess calcium in the surface minerals. This was obvious in the calcium to phosphate ratios and the rapid recovery of ^{45}Ca from these surfaces in the laminar flow of acid. The high calcium to phosphate ratios of the minerals dissolved in the first collections of the acid indicated the presence of calcium carbonates in the surfaces.

Bicarbonate in solutions of NaF alone slightly reduced the uptake of ^{45}Ca and fluoride in the enamel and increased the amounts of phosphate dissolved in the solutions. The uptake of fluoride increased the uptake of ^{45}Ca in the presence and absence of bicarbonate. The extremely high ratios of calcium to phosphate of the minerals dissolved from the surfaces in the first collections of acid indicated that both bicarbonate and fluoride displaced surface phosphate. However, the small recoveries of ^{45}Ca and fluoride in the acid indicated that the undissolved surface minerals contained fluorapatites. The minerals dissolved from these surfaces in the laminar flow of acid were not the minerals which contained the major fractions of the ^{45}Ca and fluoride.

Bicarbonate in solutions of phosphate without and with NaF has no effects on the uptake of ^{45}Ca and fluoride in the enamel. Bicarbonate plus phosphate reduced the dissolution of calcium and phosphate from the enamel in the solutions to negligible levels. In these solutions of phosphate neither bicarbonate nor fluoride displaced surface phosphate. Therefore the surface minerals contained the same amounts of excess calcium after exposure in these solutions as after the water wash. The presence of the excess calcium, or the deficiency of surface phosphate, was again obvious in the initial ratios of calcium to phosphate of the minerals dissolved in the acid.

The small recoveries of ^{45}Ca and fluoride and the effect of the fluoride in reducing calcium and phosphate dissolutions in the acid were not affected by bicarbonate and phosphate in the pretreatment solutions. The presence of both phosphate and bicarbonate, in the presence and absence of NaF, in the solutions reduced the amounts of minerals dissolved from the enamel in the acid. Thus the combined buffering action of bicarbonate and phosphate in the minerals partially masked the effects of fluoride on the acid resistance of the surfaces. Bicarbonate and phosphate apparently reacted with surface calcium and adsorbed as ionic complexes in the hydrated mineral surfaces in amounts sufficient to partially reduce the strength of the acid.

3. Reactions of Demineralized Surfaces of Enamel in Solutions of 1.5 mM Calcium in the Absence of Added Phosphate at pH 7.0

The uptake of ^{45}Ca in enamel from solutions of 1.5 mM calcium, in the absence of phosphate, was relatively small. The large amounts of ^{40}Ca in the solutions competitively overwhelmed ^{45}Ca in the exchange reactions with the surface minerals. Inclusion of NaF in the solutions of calcium slightly increased the uptake of ^{45}Ca but not in the presence of bicarbonate. Compared to solutions of phosphate, the 1.5 mM calcium in the absence of bicarbonate slightly increased fluoride uptake in the enamel. The fluoride was taken up by recrystallization or by ionic association with the excess calcium in the surface minerals. The solutions were not saturated in relation to the solubility of CaF_2 or calcium carbonate. However, an apparent small uptake of calcium occurred in the enamel from solutions of calcium, bicarbonate, and fluoride combined. This small uptake of calcium possibly resulted from adsorption of association complexes of calcium and bicarbonate ions in the surface minerals. Whatever the reactions of calcium and bicarbonate, they reduced the uptake of fluoride one-half compared to the fluoride uptake of the surfaces in solutions without the bicarbonate.

The calcium to phosphate ratios were unbelievably high for minerals dissolved from the surfaces after reactions in the solutions of 1.5 mM calcium. These high ratios were not caused by carry-over of the 1.5 mM calcium of the pretreatment solutions as shown by the small recovery of ^{45}Ca and fluoride from the surfaces exposed in solutions of calcium and NaF. Additional evidence that the high ratios were not caused by solution carry-over was the finding that less calcium dissolved from these surfaces than dissolved in the collections of acid from surfaces exposed in solutions of phosphate in the absence of added calcium.

The extremely high calcium to phosphate ratios of the minerals dissolved in the initial 1 or 2 collections of the acid were caused by saturation of the surfaces of the enamel minerals with hydrated calcium ions and complexes of calcium-fluoride or calcium-bicarbonate. These ions and ionic complexes probably occluded the surface minerals and thereby almost eliminated phosphate dissolution in the first collections of acid from the surfaces. The failure to find phosphate in the solutions of calcium after the 24 hour exposure of the enamel surfaces indicated that the ionic complexes occluded rather than displaced the surface phosphate.

Pretreatment of the enamel surfaces in solutions of calcium plus bicarbonate reduced the amounts of calcium dissolved in the acid as effectively as pretreatments in solutions of calcium plus NaF with or without bicarbonate. However, NaF in solutions of calcium in the presence and absence of bicarbonate reduced the already depressed dissolution of phosphate. This indicated that the calcium-bicarbonate and calcium-fluoride complexes in the mineral surfaces effectively reduced the strength of the acid in the enamel.

The recoveries of ^{45}Ca in the acid from the surfaces exposed in solutions of 1.5 mM calcium alone, and solutions of calcium plus bicarbonate in the absence and presence of NaF, were rapid and essentially complete. This showed that the ^{45}Ca uptake occurred almost exclusively in the mineral surfaces as relatively soluble phases. However, very small recoveries of ^{45}Ca were found in the collections of acid from surfaces exposed in solutions of calcium plus NaF in the absence of bicarbonate. The uptake of fluoride in the enamel greatly increased the acid resistance of the fractions of the surface minerals that contained ^{45}Ca. Bicarbonate in the solutions of calcium plus NaF greatly reduced the uptake of fluoride in the enamel and completely eliminated the fluoride effect of increased acid resistance of the minerals that contained ^{45}Ca.

The amounts of fluoride taken up in the enamel from solutions of 1.5 mM calcium were greater by a factor of almost 3 in the absence of bicarbonate than in the presence of bicarbonate. Twice the amounts of fluoride dissolved in the acid from the former than from the latter groups of surfaces, but the percentage recoveries were the same for both groups of surfaces. This indicated that fluoride was taken up in, and dissolved from the same mineral phase regardless of the presence or absence of bicarbonate. Bicarbonate probably reduced the uptake of fluoride and inhibited recrystallization of calcium phosphate to fluorapatites by competition for and occlusion of surface binding sites. The bicarbonate effect was not caused by a significant reduction of calcium activity in the bulk solution. This was shown by the failure of bicarbonate to inhibit fluoride uptake from solutions that did not contain added calcium. We assumed that a calcium-bicarbonate complex formed in the solution which was then adsorbed in the enamel or that the complex formed in the enamel.

4. The Effects of Bicarbonate and Fluoride Ions on the Remineralization of Enamel Surfaces

The solutions of 1.5 mM calcium plus 1.0 mM phosphate at pH 7.0 (mineralizing solutions) were supersaturated relative to the solubility of the apatite family of calcium phosphate (64). The 0.4 mM NaF in the solutions increased the supersaturation respective to fluorapatites, but the solutions were not saturated with CaF_2 (7). Bicarbonate ions reduced the activity of calcium and increased the stability of the solutions. The mineralizing solutions were stable for several months when stored at 25° in the absence of apatites or other seed materials.

Contact between demineralized enamel and the mineralizing solutions caused decreases of the calcium and phosphate concentrations and release of hydrogen ions in the solutions. These changes in the solutions ceased at the moment of removal of the enamel even though the solutions were still supersaturated relative to the solubility of apatites. Minerals precipitated in the enamel, but not in the mineralizing solutions. This phenomenon has been defined as remineralization (90). The mechanisms of enamel remineralization have not been determined. Whether the organic matrix is involved or if the precipitation in the enamel represents growth only on existing minerals remains unsolved. Most descriptions of remineralization or like phenomena have derived from studies in which solutions were supersaturated beyond the levels of spontaneous precipitation of dicalcium phosphate. The solutions used in this study were unsaturated relative to the solubility of dicalcium phosphate. Therefore formation of dicalcium phosphate in the surfaces of enamel was not possible by mechanisms of precipitation, at least not in the context of the classical definition of precipitation (38).

The mineralizing solutions contained 12.0 μMol calcium, 8.33 μMol phosphate, and 3.33 μMol fluoride, in the presence of 0.4 mM NaF, available for uptake by each of the surfaces of enamel. Surfaces remineralized in the absence of bicarbonate and fluoride removed 28 percent of the calcium and 39 percent of the phosphate from the solutions. Calcium and phosphate were taken up in the same ratio as dicalcium phosphate. However, precipitation of dicalcium phosphate was not possible.

The demineralized surfaces each contained excess calcium, relative to the composition of apatites, before exposure in the mineralizing solutions. Uptake of phosphate in the surfaces from the NaF-free mineralizing solutions was only one-third greater than the uptake of phosphate from solutions of phosphate alone, whereas the uptake of calcium was negligible from solutions of calcium alone. Phosphate alone in the solutions remarkably increased the exchange-recrystallization uptake of ^{45}Ca in the enamel. Calcium alone in the solutions competitively inhibited ^{45}Ca uptake in the enamel. This effect of calcium was evident in the mineralizing solutions, but phosphate in the presence of calcium (absence of NaF) increased the uptake of ^{45}Ca by a factor of 3. This indicated that the uptake of phosphate from the mineralizing solution tripled the number of sites for calcium binding in the enamel. The uptake of calcium increased the binding sites for phosphate about one-third. The 1.67 ratio of new sites for remineralization, above the concurrent uptake of calcium and phosphate by recrystallization, was the ratio required for crystal growth of hydroxyapatite.

It was not possible to differentiate the fractions of net mineral or isotope uptake as ionic exchange, recrystallization, or remineralization since these reactions occurred simultaneously in the enamel. However, preferential dissolution of the surface minerals in the laminar flow of acid showed that dicalcium phosphate was not present in the enamel. Calcium and phosphate dissolved from these surfaces in the initial collections of acid as apatites and later as apatites and octacalcium phosphate. The existence of dicalcium phosphate would have been detected in the acid. The amounts of calcium and phosphate dissolved in each collection of the acid were much smaller than the amounts taken up in the enamel from the NaF-free mineralizing solution.

The presence of 5, 15, and 25 mM bicarbonate in the NaF-free mineralizing solutions practically prevented remineralization of the enamel. The 5 and 25 mM levels of bicarbonate reduced the uptake of ^{45}Ca about 20 percent, but 15 mM bicarbonate inhibited uptake of the isotope 40 percent. The extent of bicarbonate inhibition of ^{45}Ca uptake indicated the percentage of the available reactive sites which were occluded by calcium-bicarbonate interaction on the surface minerals. The 25 mM bicarbonate level was less effective than 15 mM bicarbonate because of the greater reduction of calcium activity in the solution rather than in the surface minerals. It was apparent that small amounts of calcium bicarbonate formed in the enamel. Greater amounts of calcium and ^{45}Ca dissolved in the laminar flow of acid from the surfaces remineralized in the presence of bicarbonate than in its absence. In addition, the ratios of calcium to phosphate for minerals dissolved in the initial collections of acid from these surfaces indicated the dissolution of mixed calcium phosphates and bicarbonates.

The presence of fluoride in the mineralizing solution increased the uptake of ^{45}Ca, calcium, and phosphate 63 to 66 percent. The net uptake of calcium and phosphate occurred in ratio of 1.8, which was slightly too high to have indicated the formation of apatite alone, whereas the Ca/F ratio of net mineral uptake in the remineralized enamel of 3.8 was too low to have indicated the precipitation of fluorapatite. However, about 50 percent of the fluoride was taken up in the enamel independent of the remineralization process, i.e., from solutions that did not contain calcium or solutions that contained calcium in the absence of phosphate. The fractions of the fluoride uptake attributable to remineralization possibly resulted in the formation of fluorhydroxyapatite in the enamel. CaF_2 clearly was not a product of the fluoride uptake in the remineralized enamel. If fluoride was taken up by association on mineral surfaces as CaF^+, then the remineralized fraction of the surface minerals contained calcium and phosphate in the exact ratio of hydroxyapatite. Minerals dissolved from these surfaces in the initial collections of the laminar flow of acid in the calcium to phosphate ratio of hydroxyapatite.

The fluoride ion effect of increased rates of exchange-recrystallization and remineralization of the demineralized surfaces of enamel was as evident in the presence as in the absence of bicarbonate. The 15 and 25 mM levels of bicarbonate slightly reduced the uptake of ^{45}Ca, calcium, phosphate, and fluoride in the remineralized enamel. Obviously the uptake of fluoride in the surface minerals prevented bicarbonate inhibition of remineralization. This indicated that fluoride was taken up by exchange-recrystallization of surface minerals and remineralized hydroxyapatite to fluorapatites even in the presence of bicarbonate. The initial uptake of fluoride possibly occurred at discrete sites of excess calcium in the surfaces. Fluoride bound at these sites of crystal growth possibly prevented the binding of bicarbonate and thereby eliminated bicarbonate inhibition of recrystallization and remineralization of apatites.

The extremely small recoveries of ^{45}Ca and fluoride in the laminar flow of acid from the surfaces remineralized in the presence of NaF showed that these ions were taken up in relatively insoluble minerals. Fluoride increased the acid resistance of the surface minerals as shown by almost 50 percent reductions of calcium and phosphate dissolution in the acid. Bicarbonate in the mineralizing solution increased the amounts of calcium and phosphate dissolved from the surfaces. The ratios of calcium to phosphate of the minerals dissolved in the acid indicated that the surfaces remineralized in the presence of bicarbonate and fluoride contained small amounts of calcium bicarbonate. However, as discussed above, the bicarbonate in the surfaces had only a small effect on remineralization in solutions of fluoride. Therefore the small amounts of bicarbonate on the surfaces remineralized in the presence of fluoride were probably in mineral sites that were not critical for the growth or recrystallization of fluorapatites.

The calcium to fluoride ratios of the minerals dissolved from the surfaces remineralized in the presence of fluoride decreased to about 10 in the successive collections of acid. The calcium to phosphate ratios in the same collections of acid indicated apatite dissolution initially and then recrystallization of octacalcium phosphate to apatite in the surfaces. The ratios of the percentage recoveries of ^{45}Ca to the recoveries of fluoride from these surfaces decreased from 7 to 3 in the successive collections of acid. These ratios would have been one if the ^{45}Ca and fluoride had been taken up in and dissolved from the same minerals, even if the minerals were a random mixture of fluorapatites and calcium fluoride. The decreases of the calcium to phosphate ratios and calcium to fluoride ratios in the successive collections of the acid demonstrated recrystallization of relatively soluble surface minerals to fluorapatites. The fluorapatites formed in the surfaces during remineralization were practically insoluble in the weak acid.

The scanning electron micrographic images of labial surfaces of bovine and human enamel were essentially identical. The features of surface enamel after exposure in weak acids were the same as those described previously (76). The weak acid dissolved principally the surface minerals of the core region of each enamel rod. Transmission electron micrographs showed that the crystals in the sheath of the rods were almost or entirely unaffected by the weak acid (31).

The small mineral uptake of the demineralized surfaces from NaF-free solutions was not clearly evident. Mineral uptake of the enamel from NaF-free solutions was less than 20 percent of the amounts of apatite dissolved in the 24 hour acid exposure. The 1 hour exposure of the NaF-free remineralized surfaces in the acid flow obviously dissolved minerals of the prism sheaths. The amounts of minerals dissolved in the 1 hour acid exposure were almost the same as the amounts deposited in NaF-free remineralized surfaces.

The demineralized surfaces exhibited relatively massive growths of crystalline minerals after remineralization in the presence of NaF. The new crystals projected from the sheath regions of the enamel prisms as if the sheath contained sites of nucleation for crystal growth. The crystals coalesced in some areas of the surface to almost completely fill the enamel prisms. The mineral growth replaced more than 50 percent of calcium phosphates dissolved during demineralization. Subsequent exposure of these surfaces for 1 hour in the acid flow dissolved only small amounts of calcium and phosphate and even smaller amounts of the ^{45}Ca and fluoride taken up during remineralization. The chemically determined dissolution characteristic of the NaF-remineralized surfaces and the electron micrographs indicated recrystallization and redistribution of the surface minerals in the enamel.

5. The Effects of Fluoride at pH 4.5 on the Acid Resistance of Surface Minerals in Enamel

Relatively small amounts of calcium and phosphate, in ratios of 1, were taken up in demineralized surfaces from the mineralizing solutions that did not contain fluoride. Previous results indicated that the calcium and phosphate were taken up by recrystallization of altered surface minerals and by remineralization of apatites (octacalcium phosphate and hydroxyapatite). The calcium to phosphate ratios of the minerals dissolved from these surfaces in the collections of NaF-free acid indicated the dissolution of slightly calcium rich apatites initially and then the dissolution of a mixture of octacalcium phosphate and hydroxyapatite. Minerals dissolved from these surfaces in the acid NaF also as calcium-rich apatites in the initial collection of the acid, but the decreasing ratios of calcium to phosphate in subsequent collections of the acid indicated the recrystallization of octacalcium phosphate and hydroxyapatite to fluorapatite in the surfaces.

The amounts of calcium and phosphate dissolved from the remineralized surfaces (absence of fluoride) in the NaF-free acid equaled the concentrations that would have dissolved in the acid NaF if recrystallization to fluorapatites had not occurred. The maximum concentrations attained in the NaF-free acid were 3 x 10^{-5} M calcium, 2 x 10^{-5} M phosphate, and 3 x 10^{-5} M hydrogen ions. The addition of 1 x 10^{-4} M NaF at pH 4.5 possibly exceeded the solubility of fluorapatite, but not CaF_2. The conversion of octacalcium phosphate and hydroxyapatite to fluorapatites reduced calcium dissolution about 30 percent, phosphate dissolution about 20 percent, and ^{45}Ca recovery about 50 percent in the laminar flow of acid from surfaces remineralized in the absence of fluoride.

Surfaces of enamel remineralized in the presence of fluoride exhibited the well known Volker effect of acid resistance (99). Calcium and phosphate dissolution from these surfaces were reduced about 50 percent in NaF-free acid compared to surfaces remineralized in the absence of fluoride. The calcium to phosphate ratios of the minerals dissolved in the laminar flow of acid from the surfaces remineralized in the presence of fluoride indicated recrystallization of octacalcium phosphate to fluorapatite. We suspected that fluorapatites were formed in the surfaces during remineralization and also later in the acid by release of fluoride and its recrystallization with octacalcium phosphate to fluorapatites. However, the fluoride released in the acid attained maximum concentrations equivalent to only 2 x 10^{-6} M NaF. Addition of 0.4 mM NaF in the acid at pH 4.5 actually increased the amounts of calcium and phosphate dissolved from the surfaces remineralized in the presence of fluoride, but did not affect the small recovery of ^{45}Ca from these surfaces. This indicated that the acid resistance of the surface minerals was caused by the fluorapatites formed in the remineralized enamel rather than the release of fluoride in the acid and its recrystallization to fluorapatites. Apparently the laminar flow of acid dissolved the more soluble surface minerals. The very small recoveries of ^{45}Ca and fluoride in the acid collections showed the relative insolubility of the remineralized fluorapatites in the enamel. The acid NaF did not affect the fluorapatites formed by recrystallization and remineralization as shown by the same ^{45}Ca recoveries from the remineralized surfaces in NaF-free acid and acid NaF.

Addition of NaF in the acid reduced the amounts of calcium and phosphate dissolved from untreated surfaces of enamel progressively from 30 percent in the first 10 minute collection to about 45 percent in the sixth collection of the acid. This effect of the acid NaF was obviously caused by exchange-recrystallization of surface minerals to fluorapatites. The natural surfaces were almost as insoluble after 30 minute exposure in acid NaF as surfaces remineralized 24 hours in the presence of fluoride. Theoretically, then, as little as 8 ppm fluoride (0.4 mM NaF) in dental plaque should effectively increase the acid resistance of the surface minerals of enamel and thereby prevent or reduce the severity of caries.

Natural, untreated surfaces of enamel dissolved as calcium rich apatites in the first two collections of acid and as almost pure hydroxyapatite in the subsequent collections of acid. The dissolution of small amounts of calcium carbonates in the surface minerals in the initial collections of acid accounted for the slightly high calcium to phosphate ratios of the natural surfaces. Slightly smaller amounts of calcium and phosphate dissolved from demineralized surfaces in the collections of acid than from untreated surfaces. This indicated that the acid demineralization and water wash removed the more soluble and more accessible surface minerals of the enamel. The minerals in surfaces remineralized in the absence of fluoride were also slightly less dissolved in the collections of acid than untreated surfaces.

Exposure of demineralized surfaces of enamel in acid NaF progressively reduced calcium dissolved from 15 percent in the first collection to about 60 percent in the sixth collection of acid. Whereas the phosphate dissolved from these surfaces was not affected in the first 10 minute exposure in the acid NaF, the acid NaF reduced the amounts of phosphate dissolved from 20 percent to 45 percent between the second and sixth collections of the acid. This showed the phosphate deficiency of the washed, demineralized surfaces before exposure in the acid. The excess calcium in the washed surface minerals simply dissolved in the acid without reacting with the acid NaF to any significant extent. The small differences of the fluoride effect on reduced calcium and phosphate dissolutions in the acid indicated recrystallization of surface minerals, acidic calcium phosphates, to fluorapatites. Scanning electron micrographs of the demineralized surfaces, and the rapid dissolution of surface calcium in the collections of acid, indicated that the excess calcium was bound to acid soluble components of the organic matrix. The apparent dissolution of apatites from these surfaces after exposure in mineralizing solutions suggested that the excess surface calcium participated in reactions of remineralization of surface minerals.

The level of 0.05 mM NaF (1 ppm fluoride) in the acid slightly increased calcium and phosphate dissolution and recovery of ^{45}Ca in the first to fourth collections of acid from surfaces remineralized in the absence of fluoride. This conclusively showed that fluoride released in the acid from surfaces remineralized in the presence of fluoride was not significantly involved in acid resistance of these surfaces. However, there was a trend of reduced calcium and phosphate dissolution in the fifth and sixth collections of acid that contained 0.05 mM NaF. This level of acid NaF effectively converted surface minerals to fluorapatites and increased the acid resistance of enamel when the surfaces were exposed in the laminar flow of acid for at least one-half hour.

The studies herein showed that levels of fluoride ions readily attainable at tooth surfaces in the mouth were highly effective in the formation of fluorapatites in surface minerals of enamel. Surface minerals recrystallized to fluorapatites and exhibited markedly increased acid resistance within the first 10 minutes of exposure in 0.4 mM NaF at pH 4.5. Remineralized and recrystallized fluorapatites formed in surfaces of enamel at neutral pH were highly resistance to dissolution at pH 4.5 in acid that contained less than 2 x 10^{-6} M NaF. The combinations of recrystallization to fluorapatites in acid and recrystallization-remineralization to fluorapatites of enamel minerals at neutral pH undoubtedly account for most of the caries preventing effect of fluoridation.

6. The Effects of Magnesium Ions on Recrystallization of Surface Minerals in Enamel

The solutions used in this part of the study for pretreatment of demineralized surfaces of enamel did not contain calcium or phosphate before the time of contact with the enamel. Thus the net uptake of ^{45}Ca and fluoride occurred by ionic exchange and recrystallization of surface minerals. Fluoride ions in the absence of magnesium increased the net uptake of ^{45}Ca in the enamel. Magnesium ions decreased the uptake of ^{45}Ca in the surfaces. Apparently this effect of magnesium was due to competition with ^{45}Ca for sites of surface exchange. The prevention of calcium dissolution in the solutions of neutral pH by magnesium indicated that calcium and magnesium reacted at the same sites in the surface minerals. The uptake of fluoride ions in the enamel was not affected by magnesium. Therefore, fluoride exchanged with different sites than ^{45}Ca. We assumed that fluoride ion uptake mostly represented exchange with hydroxyl ions in crystal surfaces of hydroxyapatite.

Magnesium alone in the pretreatment solutions almost equally increased the total amounts of calcium, ^{45}Ca, and phosphate recovered from the surface minerals in acid. Fluoride alone decreased the amounts of surface minerals dissolved in the acid collections, but fluoride decreased the recovery of ^{45}Ca twice as much as calcium and phosphate. Fluoride plus magnesium had no effect on the acid dissolution of phosphates or the recovery of ^{45}Ca in the acid. However, fluoride plus magnesium reduced the acid dissolution of calcium as effectively as fluoride alone. Even though almost 3 multiples of fluoride were recovered from surfaces exposed in solutions of magnesium, the total recoveries were a small fraction of the fluoride uptake.

These studies indicated that the uptake of fluoride ions in the surface minerals of enamel occurred by recrystallization to fluorapatites at pH 7.0 and pH 4.5. The small fractions of the fluoride uptake which were recovered in the collections of acid probably indicated the adsorbed rather than the exchanged fluoride. Magnesium ions tripled the amount of adsorbed fluoride, which was only 7 percent of the total fluoride uptake. Magnesium increased the acid dissolution of calcium and phosphate and by competition with calcium prevented recrystallization to less soluble apatites.

The high calcium to phosphate ratios of the minerals dissolved from the surfaces in the initial collections of acid were caused by a relative deficiency of phosphate in the surface minerals before experimentation. Magnesium reduced these ratios by slightly increasing the dissolution of phosphate, more so than calcium, as if a mixture of calcium-magnesium phosphates were undergoing dissolution. This suggested that magnesium reacted with phosphate in the surface minerals to form the relative soluble, amorphous calcium-magnesium phosphate salts recently reported by Tomazic et al. (96).

7. Effects of Magnesium and Fluoride Ions on the Remineralization and Subsequent Acid Resistance of Surface Minerals in Enamel

Fluoride ions exerted their well known effects on the remineralization and acid resistance of surface minerals in enamel. The fluoride of the mineralizing solutions enhanced the recrystallization of calcium phosphates to fluorapatites and at the same time formed fluorapatites by remineralization. The scanning electron micrographs showed that remineralized constituents from solutions with fluoride crystallized in the sheath regions of demineralized enamel rods to more or less fill the rods.

Magnesium almost completely inhibited the uptake of calcium in the remineralized enamel. However, magnesium had little effect on the uptake of ^{45}Ca, fluoride, the release of acid in the surface minerals, or the uptake of phosphate in the presence of fluoride. These findings indicated magnesium and calcium competition for the same reactive sites in the remineralized enamel, and effective substitution of magnesium for calcium. Magnesium possibly substituted for calcium in either sites of recrystallization or remineralization.

The presence of magnesium in the remineralized enamel prevented the fluoride ion effect of increased acid resistance of the surface minerals. However, the small recoveries of ^{45}Ca and fluoride from these surfaces indicated that a large fraction of the minerals existed as fluorapatites despite the presence of magnesium. The increase of the calcium to phosphate ratios of the minerals dissolved in the successive collections of acid from these surfaces, and the slightly greater recoveries of ^{45}Ca and fluoride from these surfaces than from surfaces remineralized in the absence of magnesium, indicated that fluoride released in the acid did not recrystallize as fluorapatites in the presence of magnesium. A larger fraction of the surface minerals was relatively acid soluble in enamel remineralized in the presence than in the absence of magnesium. Apparently magnesium stabilized acidic calcium phosphates in the surface minerals (96) which was most noticed in the presence of fluoride.

These studies suggested that magnesium substituted for calcium in surface limited acid calcium phosphates. The substituted magnesium prevented the hydrolysis of acid phosphate radicals and thereby inhibited recrystallization of apatites. The reduced levels of recrystallized apatites limited the number of sites available for epitaxic growth by remineralization. Fluoride in the solutions overwhelmed the magnesium inhibition by initiation of new sites for mineral formation, possibly at sites of matrix bound calcium in the demineralized surfaces.

Speculatively, the results of this study indicated that magnesium in enamel and in oral fluids may be considered potentially cariogenic. If remineralization is a primary mechanism of the fluoride action of caries prevention, then prevention of the magnesium effects may be one of its actions.

CONCLUSIONS

1. The Effects of Recrystallization and Remineralization in NaF-free Solutions on the Acid Resistance of Surface Minerals in Enamel

Table 22 summarizes the effects of recrystallization and remineralization in the various NaF-free solutions on the total minerals dissolved from enamel surfaces in 1 hour exposure in the laminar flow of acid. Increased recovery of ^{45}Ca indicated that a fraction of the isotope was taken up in more accessible surface sites or in relatively soluble minerals. Surfaces recrystallized in the presence of phosphate showed no increase of ^{45}Ca recovery. We assumed that ^{45}Ca recrystallized in the same minerals from solutions of isotope alone and solutions of phosphate even through a much greater uptake was found for solutions of phosphate. Bicarbonate, calcium, or magnesium in recrystallizing solutions and bicarbonate or magnesium in remineralizing solutions increased the subsequent acid recovery of ^{45}Ca.

Bicarbonate or phosphate in recrystallizing solutions increased the dissolution of calcium and phosphate in the acid. However, bicarbonate plus phosphate decreased mineral dissolution from the recrystallized surfaces. These ions reduced the recrystallization of apatites in the surface

TABLE 22

The Effects of Recrystallization and Remineralization in NaF-Free Solutions of Bicarbonate, Phosphate, Calcium, and/or Magnesium on the Acid Resistance of Enamel Surfaces.

Percentage increase (+) or decrease (-) of minerals dissolved in 1 hour laminar flow of acid.

		Percentage Effects [+,-]		
Method*	Variables**	Calcium	Phosphate	^{45}Ca
Rc	15 HCO_3	+31	+22	+34
Rc	1 PO_4	+35	+49	0
Rc	1 PO_4 + 15 HCO_3	-24	-21	0
Rc	1.5 Ca	+23	0	+29
Rc	1.5 Ca + 15 HCO_3	-19	-29	+48
Rc	1 Mg	+24	+18	+30
Rc	Rm	0	0	0
Rm	5 HCO_3	+39	0	0
Rm	15 HCO_3	+29	0	+43
Rm	25 HCO_3	+56	+28	+37
Rm	1 Mg	+24	+22	+22

* Rc = Recrystallized.
Rm = Remineralized.

** Numeral = mM concentration.

minerals and probably favored the formation of more soluble amorphous minerals. The combined adsorption of calcium bicarbonate and calcium phosphates in the hydrated mineral surfaces apparently buffered the acid and reduced the dissolution of surface minerals.

Calcium alone in the solutions increased the dissolution of surface calcium but not phosphate. The increased calcium dissolution represented adsorbed calcium, whereas the surfaces were deficient in phosphate before recrystallization reactions. Calcium plus bicarbonate in the solutions reduced the amounts of calcium and phosphate dissolved in the acid. The apparent greater effect on phosphate than calcium was again because the demineralized water washed surfaces were deficient in phosphate. Also bicarbonate displaced surface phosphate during recrystallization and then slightly reduced the strength of the acid at the mineral surfaces. Magnesium ions in the recrystallizing solutions increased the acid dissolution of calcium more than phosphate. We assumed that magnesium partially substituted for calcium in the formation of amorphous surface minerals and thereby increased the relative solubility of the minerals.

Minerals dissolved almost exactly the same from surfaces remineralized and recrystallized under the respective control conditions, i.e., with no additive in the solutions except calcium and phosphate in mineralizing solutions. This indicated close similarity of the composition of recrystallized and remineralized surface minerals. The levels of 5 and 15 mM bicarbonate in the mineralizing solutions increased calcium, but had no effect on phosphate dissolved in the acid. The level of 25 mM bicarbonate further increased calcium dissolution, above the amounts due to 15 mM bicarbonate, and caused significant dissolution of phosphate. The 5 and 15 mM carbonate levels interfered with recrystallization, and displaced surface phosphate, leaving calcium bicarbonates to dissolve along with the same amounts of amorphous calcium phosphate in surfaces remineralized in the absence of bicarbonate. However, the 25 mM bicarbonate so overwhelmed remineralization that it actually caused the formation of additional amounts of readily soluble amorphous salts in the enamel surfaces.

Magnesium in the mineralizing solutions had the same effects as in the recrystallizing solutions. However, magnesium almost completely inhibited remineralization. These results indicated that magnesium substituted for calcium in sites required for nucleation of crystal growth. Literature reports showed that magnesium inclusion in precipitated calcium phosphates stabilized amorphous, non-crystalline minerals (96).

2. Effects of Recrystallization and Remineralization in Solutions of 0.4 mM NaF at pH 7.0 on the Acid Resistance of Enamel Surfaces

Table 23 shows the percentage decreases of minerals dissolved in 1 hour flow of acid from surfaces recrystallized or remineralized in solutions of 0.4 mM NaF, compared to the respective NaF-free solutions. Recrystallization in solutions of the isotope and fluoride alone decreased the amounts of calcium and phosphate dissolved in the acid 21 to 24 percent, but decreased the recovery of ^{45}Ca about 41 percent. The fluoride effect on decreased recovery of ^{45}Ca indicated the percentages of the isotope which were taken up by recrystallization of surface minerals to fluorapatites. Whereas, the decreases of calcium and phosphate dissolved in the acid indicated the percentages of the acidic calcium phosphates (octacalcium phosphate) which were recrystallized to fluorapatites.

Phosphate increased the recrystallization of surface minerals to fluorapatites compared to solutions of fluoride alone. Bicarbonate reduced the recrystallization of surface minerals to fluorapatite compared to solutions of phosphate. Bicarbonate plus phosphate almost completely inhibited the recrystallization of surface minerals to fluorapatite, as did solutions of calcium plus bicarbonate. Apparently bicarbonate competed with phosphate for calcium binding

TABLE 23

The Effects of Recrystallization and Remineralization in Solutions of 0.4 mM NaF at pH 7.0 on the Acid Resistance of Enamel Surfaces.

Percentage reductions of fluoride treated minerals dissolved in 1 hour laminar flow of acid, each compared to the respective control surfaces.

		Percentage Decreases			Average
Method*	Variables**	Calcium	Phosphate	^{45}Ca	Ca/F
Rc	-	21	24	41	70
Rc	1 PO_4	42	44	50	57
Rc	15 HCO_3	30	36	48	52
Rc	1 PO_4 + 15 HCO_3	17	17	30	47
Rc	1.5 Ca	36	52	67	20
Rc	1.5 Ca + 15 HCO_3	9	23	10	39
Rc	1 Mg	32	33	33	28
Rm	-	48	47	79	13
Rm	5 HCO_3	30	40	74	26
Rm	15 HCO_3	43	45	73	12
Rm	25 HCO_3	28	49	65	17
Rm	1 Mg	8	16	57	19

* Rc = Recrystallized.
Rm = Remineralized.

** Numeral = mM concentration.

in the surface minerals. Calcium in the absence of bicarbonate increased the recrystallization of total surface minerals to fluorapatites. The calcium to fluoride ratios of the minerals dissolved in the acid from these surfaces indicated that as much as 25 percent of the minerals were fluorapatite.

Magnesium inhibited recrystallization and remineralization in the absence of fluoride. However, recrystallization of apatites appeared uninhibited in the presence of fluoride and magnesium in the absence of phosphate in the solutions. Magnesium in mineralizing solutions inhibited remineralization of apatites even in the presence of fluoride. We assumed that relatively soluble calcium magnesium phosphates precipitated in the surfaces. Apparently the calcium in the mineralizing solutions enhanced the recrystallization of fluorapatites even in the presence of magnesium. This was indicated by similar acid resistance of the ^{45}Ca in these surfaces and surfaces recrystallized in solutions that contained calcium and only fluoride.

Surfaces remineralized in solutions of fluoride were twice as acid resistant as surfaces remineralized in the absence of fluoride. The minerals dissolved from the fluoride remineralized surfaces contained slightly less fluoride than fluorhydroxyapatite. Almost 80 percent of the total ^{45}Ca uptake in surfaces remineralized in the presence of fluoride was found in the fluorapatites. Bicarbonate in the mineralizing solutions only slightly reduced the fluoride effect on acid resistance of the recrystallized-remineralized surface minerals.

3. The Effects of Fluoride in the Acid at pH 4.5 on Dissolution of Surface Enamel in 1 Hour Exposure to Laminar Flow of the Acid

Table 24 shows the effects of fluoride in the acid at pH 4.5 on the dissolution of surface enamel compared to the mineral dissolution in the acid without added fluoride. The level of 0.4 mM NaF in the acid increased the acid resistance of natural untreated surfaces and demineralized surfaces 36 to 39 percent. This showed that the acid fluoride recrystallized with surface minerals to form fluorapatites since the levels of dissolved calcium and acid fluoride were below the solubility of calcium fluoride.

The acid fluoride significantly increased the acid resistance of enamel remineralized in the absence of fluoride. However, the effect was greater for calcium than phosphate which showed that the acid fluoride caused the recrystallization of octacalcium phosphate and possibly other acidic calcium phosphates to fluorapatites. The level of 0.05 mM NaF (1 ppm F) in the acid failed to increase the acid resistance of the surface minerals in 1 hour. This relatively small level of fluoride in the acid slightly increased the dissolution of phosphate, but not calcium, which showed that the NaF-free remineralized surfaces

TABLE 24

The Effects of 0.4 mM NaF in Acid at pH 4.5 on the Dissolution of Surface Minerals of Enamel.

Percentage reductions of mineral dissolution in acid NaF compared to NaF-free acid in 1 hour.

	Percentage Decrease (-), Increase (+)		
Surfaces*	Calcium	Phosphate	^{45}Ca
Natural	-38	-39	-
Demineralized	-39	-36	-
Rm., No F, pH 7.0	-31	-21	-49
Rm. + F, pH 7.0	+40	0	0
Rm., No F acid contained 0.05 mM NaF at pH 4.5	+17	+37	-8
Rm. + F, NaF-free acid	-48	-47	-79

* Rm. = Remineralized

contained acidic calcium phosphates.

Surfaces remineralized in the presence of fluoride were much less dissolved (about 50 percent in calcium and phosphate and 90 percent less in ^{45}Ca) than surfaces remineralized in the absence of fluoride, both in the acid without added NaF. However, the acid fluoride slightly increased calcium and phosphate, but not ^{45}Ca, dissolution from the surfaces remineralized in the presence of fluoride, compared to their dissolution from identically remineralized surfaces in acid without added NaF. The important fact for consideration was that acid fluoride did not additionally increase the acid resistance of remineralized enamel that contained fluoride, but markedly increased the acid resistance of enamel (sound, demineralized, and remineralized) that did not contain fluoride. The effect of acid fluoride on natural, untreated enamel in the acid environment was most important to understanding the mechanism of action of fluoridation in prevention of dental caries. As little as 0.4 mM fluoride (8 ppm F) at tooth surfaces should greatly slow acid decay processes. The remineralization and recrystallization effects of fluoride would then replace the dissolved minerals with acid resistant fluorapatites as acid production ceased and the environment returned to neutral pH.

SUMMARY

Labial surfaces of bovine incisor enamel were pumiced, demineralized 24 hours in 10 mM acetic acid of pH 5.5, then washed 24 hours in deionized water (83 ml/cm^2). The minerals of the surfaces were recrystallized 24 hours at pH 7.0 in solutions of either 1.5 mM $CaCl_2$, 1.0 mM NaH_2PO_4, or neither the calcium nor the phosphate. Demineralized surfaces were also remineralized 24 hours at pH 7.0 in solutions of both 1.5 mM calcium and 1.0 mM phosphate. The solutions were prepared to contain either 0.00 or 0.4 mM NaF, and/or 5, 15, 25 mM $NaHCO_3$, and/or 1.0 mM $MgCl_2$. The recrystallization and remineralization of surface minerals were estimated from changes of ^{45}Ca, calcium, phosphate, and fluoride ion concentrations in the solutions during the time of contact with the enamel.

The effects of fluoride, bicarbonate, and magnesium on the acid resistance of the surface minerals in the enamel were determined in a laminar flow of 10 mM acetic acid at pH 4.5. The amounts of ^{45}Ca, calcium, phosphate, and fluoride dissolved from each of the surfaces were measured in 6 sequential 10 minute (12 ml) collections of the acid. The effects of fluoride on the acid resistance of untreated and remineralized surfaces were also determined by direct addition of 0.4 mM NaF in the acid at pH 4.5.

The central regions of the enamel rods dissolved to variable depths in the demineralized surfaces of bovine and human incisors. The surface minerals dissolved as apatites in acid demineralization, but in the subsequent water wash phosphate dissolved in preference to calcium. The residual excess of surface calcium was evident in high calcium to phosphate ratios of minerals later dissolved in the laminar flow of acid from most of the recrystallized and remineralized surfaces of enamel.

Phosphate alone in solutions increased the uptake of ^{45}Ca in recrystallized surfaces. Fluoride had an additive effect with phosphate to markedly stimulate ^{45}Ca uptake in the enamel. Bicarbonate or magnesium alone slightly depressed the uptake of ^{45}Ca, but fluoride in these solutions largely offset the bicarbonate and magnesium effects. Calcium alone and in the presence of fluoride almost eliminated the uptake of ^{45}Ca by direct competition for sites of recrystallization in the enamel.

Bicarbonate or magnesium in solutions almost completely inhibited remineralization in the absence, but not in the presence of fluoride. The fluoride uptake by recrystallization alone was approximately one-half the fluoride uptake of simultaneous recrystallization and remineralization of the enamel surfaces. Bicarbonate and magnesium did not meaningfully interfere with the uptake of fluoride in the surface minerals. Well crystallized minerals were observed in the sheath regions of the enamel prisms in remineralized surfaces after exposure in solutions of fluoride.

Surfaces recrystallized in NaF-free solutions of either phosphate, bicarbonate, calcium, or magnesium generally exhibited greater mineral dissolution in the acid than control surfaces. Surfaces recrystallized in solutions of bicarbonate and calcium or bicarbonate and phosphate were less dissolved in the acid than controls. Surfaces remineralized in NaF-free solutions of 15 and 25 mM bicarbonate also exhibited greater mineral dissolution than their respective controls.

Fluoride in the solutions increased the acid resistance of recrystallized and remineralized surfaces. This effect of fluoride was most notable on the acid resistance of the fractions of the recrystallized and remineralized surface minerals that contained ^{45}Ca and fluoride. The results indicated that most of the ^{45}Ca and fluoride existed in the surfaces as fluorapatites.

The addition of 0.4 mM NaF directly in the acid at pH 4.5 significantly increased the acid resistance of untreated, demineralized, and NaF-free remineralized surfaces of enamel. The acid NaF effect occurred within the first 10 minute exposure of the surfaces in the acid flow. Addition of 0.05 mM NaF in the acid was ineffective in one hour, but appeared to increase the acid resistance in more lengthy exposures. The acid NaF did not further increase the acid resistance of surfaces remineralized in the presence of fluoride. These results may partially explain the reasons why water fluoridation more effectively prevents dental caries than the direct applications of concentrated solutions of fluoride on tooth surfaces.

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