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Magnesium and Carbonate in Enamel and Synthetic Apatites

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Abstract—This study aimed to: determine the Mg and CO_3 distribution in the outer (surface), middle, and inner (closest to the enamel-dentin junction, EDJ) layers of human enamel; and determine the factors affecting the incorporation of Mg into synthetic apatites and the consequence of such incorporation on the properties of the apatites. Results demonstrated that the concentrations of Mg, CO_3, and organic components increased from the surface to the inner layers close to the EDJ and a difference in crystallinity from the outer to the inner layers. Initial results indicated that the extent of dissolution of the inner layer enamel is greater than that in the outer or surface enamel. Results on synthetic apatites showed the following: (1) Limited Mg incorporation into apatite was dependent on solution [Mg/Ca] molar ratio, temperature, pH, and the presence of CO_3 or fluoride (F); (2) incorporation of Mg causes reduction in crystallinity and an increase in the extent of dissolution of the apatite: (3) the negative effect of Mg on the properties of apatites is synergistic to that of CO_3 and antagonistic to that of F; and (4) exposure to acid of Mg-containing apatites causes the dissolution of Mg-rich apatite and precipitation of Mg-poor apatite. The observed decrease in the [Mg/Ca] of enamel and synthetic apatites after acid exposure may explain the observed 'preferential loss' of Mg and CO_3 in the initial stages of caries.

Key words: Magnesium, carbonate, enamel, apatite.

The mineral phases of enamel, dentin, and bone are apatites differing in crystallinity, with the enamel mineral showing considerably higher crystallinity than either dentin or bone, as shown by their x-ray diffraction patterns (Fig. 1) and transmission electron micrographs. Magnesium (Mg) and carbonate (CO_3) are two minor but important elements associated with biological apatites. The average concentrations of both Mg and CO_3 are much lower in human enamel (0.26 wt% Mg; 3.8 wt% CO_3) compared with those in either dentin (0.8 wt% Mg; 6 wt% CO_3) or bone (0.8 wt% Mg; 7.5 wt% CO_3) (LeGeros, 1981, 1984). In human enamel, it was observed that Mg and CO_3 concentrations increased from the surface toward the enamel-dentin junction, EDJ (Weatherell *et al.*, 1968; Robinson *et al.*, 1981, 1984; Sakae, 1988). In addition, Mg and CO_3 were implicated in dental caries in that they are 'preferentially lost' during the early stages of caries (Hallsworth *et al.*, 1972, 1973).

Based on convincing structural and chemical evidence from studies on biological and synthetic apatites (LeGeros, 1967; LeGeros *et al.*, 1971), it is now generally accepted that biological apatites should be described as carbonate hydroxyapatite and not as pure hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$ (Elliott, 1973; LeGeros, 1981). CO_3 is a substituent in the structure or lattice of biological and synthetic apatites obtained from aqueous systems by precipitation of hydrolysis methods in the presence of either CO_3^{2-} or HCO_3^- ions (Zapanta-LeGeros, 1965; LeGeros, 1967, 1981; LeGeros *et al.*, 1971; Okazaki *et al.*, 1981; Featherstone *et al.*, 1983; LeGeros and Tung, 1983); the substitution is principally CO_3-for-PO_4 coupled with Na-for-Ca (LeGeros, 1967, 1981; LeGeros *et al.*, 1971). The incorporation of CO_3 into the apatite causes changes in lattice parameters: contraction in the *a*- and expansion in the *c*-axis dimensions, as a consequence of the CO_3-for-PO_4 substitution (Zapanta-LeGeros, 1965; LeGeros, 1967, 1981; LeGeros *et al.*, 1971); reduction in crystal size and changes in crystal shape (LeGeros, 1967, 1981; LeGeros *et al.*, 1971); decrease in phosphate content while still giving an apatitic structure (Zapanta-LeGeros, 1965; LeGeros, 1967, 1981; LeGeros *et al.*, 1971); and increase in solubility (Okazaki *et al.*, 1981; LeGeros and Tung, 1983).

The nature of the association of Mg is less clear. Hypotheses regarding Mg in calcified tissues include: (1) Mg is mainly associated with the organic phase (Robinson *et al.*, 1981, 1984); (2) Mg is associated with a non-apatitic phase, possibly dolomite, $(Ca,Mg)(CO_3)_2$ (Driessens and Verbeeck, 1985; Terpstra and Driessens, 1986); (3) Mg is mainly surface-bound (Neuman and Mulryan, 1971; Featherstone *et al.*, 1983); or (4) Mg is, to a limited extent, a substituent (for Ca) in the apatite (LeGeros, 1984; Aoba *et al.*, 1992).

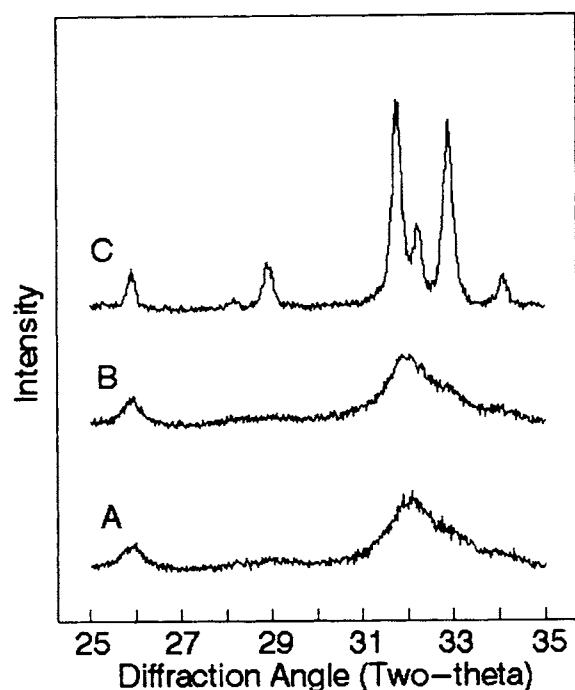It was reported that diet-induced incorporation of

Fig. 1—X-ray diffraction patterns of the mineral phases of human enamel (A), dentin (B), and bone (C) indicating the much higher crystallinity (i.e., greater crystal size) of enamel apatite crystals compared with those of either dentin (B) or bone (C). The average concentrations of Mg and CO_3 are: (A) 0.26 wt% Mg, 3.8 wt% CO_3; (B) 0.8 wt% Mg, 6.2 wt% CO_3; and (C) 0.8 wt% Mg, 7.5 wt% CO_3.

magnesium into rat dental enamel caused the formation of enamel apatite of poorer crystallinity than that without Mg (Spencer *et al.*, 1989). The nature of Mg incorporation into this rat enamel was not clear. Synthetic apatites prepared by precipitation or hydrolysis of anhydrous dicalcium phosphate, $CaHPO_4$, in the presence of Mg^{2+} ions in solution incorporate a limited amount of Mg into the apatite structure, as evidenced by concomitant small but significant changes in lattice parameters (LeGeros, 1984). Some Mg^{2+} ions may also be located on the surface (Featherstone *et al.*, 1983; Aoba *et al.*, 1992). Studies on synthetic apatite demonstrated that the formation of Mg-containing apatites is critically dependent on solution [Mg/Ca] molar ratio, $[Mg/Ca]_S$: Up to 0.03 promotes the formation of only apatite; at higher levels, Mg-substituted tricalcium phosphate $(Ca,Mg)_3(PO_4)_2$, ß-TCMP, forms; and at still higher levels, Mg-containing amorphous calcium phosphate, ACP-Mg, forms (LeGeros, 1967, 1984; LeGeros *et al.*, 1989), as shown in Fig. 2. The incorporation of Mg causes the formation of apatite of poorer crystallinity compared with Mg-free apatites (LeGeros, 1967; LeGeros *et al.*, 1989, 1995) and higher extent of dissolution in acid buffer (LeGeros, 1984; Okazaki *et al.*, 1986; Okazaki and LeGeros, 1992, 1996; LeGeros *et al.*, 1995), enhancing the observed effects of carbonate (LeGeros, 1967, 1981; LeGeros *et al.*, 1971; Okazaki *et al.*, 1981; LeGeros and Tung, 1983). When present simultaneously in solution, Mg
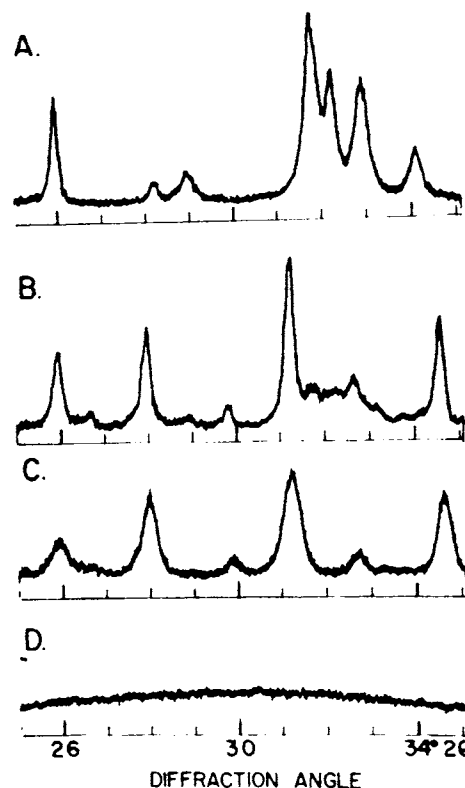

Fig. 2—X-ray diffraction patterns of precipitates obtained from solutions with various $[Mg/Ca]_S$, initial pH 9, precipitation temperature, from 95 to 100°C. (A) Apatite from solutions without Mg. From solutions with $[Mg/Ca]_S$ of up to 0.03, only apatite with decreasing crystallinity was obtained. Mg-substituted tricalcium phosphate, ß-TCMP, formed at the expense of apatite from [Mg/Ca], = 0.1 (B) and 0.2 (C). Mg-containing amorphous calcium phosphate (C) formed when $[Mg/Ca]_S = 0.5$.

and CO_3 become incorporated into the forming apatite, causing synergistic effects on its crystal and dissolution properties (LeGeros *et al.*, 1995; Okazaki *et al.*, 1996).

The aims of this study were to: (1) determine the Mg and CO3 distribution from the outer (surface), middle, and inner (close to EDJ) layers of human enamel; (2) determine the factors affecting the incorporation of Mg into apatite; (3) determine the effect of Mg on properties of apatite with and without the simultaneous incorporation of CO_3 or F.

MATERIALS AND METHODS

Preparation of enamel samples

Enamel samples were obtained from 10 third molar adult human teeth, caries-free, extracted for orthodontic purposes. A Buehler diamond disc was used to obtain from 5 to 7 thin sections from each tooth. Starting from the enamel surface, about 20 μ were sectioned for each layer: outer or surface (O), middle (M), and inner (I), closest to the enamel-dentin junction, EDJ, but away from dentin.
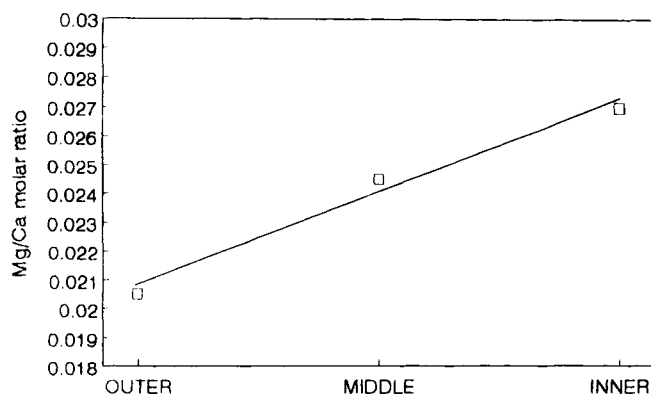
Fig. 3—[Mg/Ca] in different layers of human enamel showing increasing Mg concentrations from outer (surface) to inner (close to the EDJ) layers of human enamel.
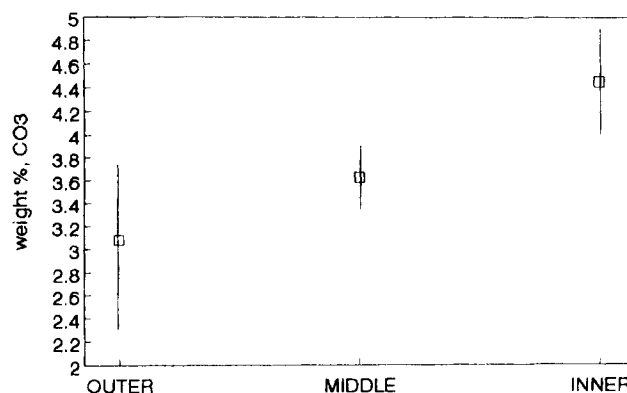

Fig. 4—Carbonate, CO_3, content increasing from outer to inner layers of human enamel. Average CO_3 content for 5 sets: outer, 3.1; middle, 3.6; and inner, 4.4 wt% CO_3.

Preparation of synthetic apatites

Synthetic apatites containing Mg with and without fluoride (F) or CO_3 were prepared by precipitation as previously described (LeGeros, 1967, 1984; LeGeros et al., 1989, 1995). Variables investigated included temperature (37, 60, and 100°C); pH (5 and 7.5); $[Mg/Ca]_S$; presence of CO_3 ($[C/P]_S$, 1 to 3) or F ($[F/P]_S$, 0.05 to 0.2). Earlier studies showed that when the $[Mg/Ca]_S$ is higher than 0.03, ß-TCMP (Mg-substituted ß-TCP) forms at the expense of apatite (LeGeros, 1967, 1981, 1984; LeGeros et al., 1989). Therefore, for the preparations in this study, the maximum $[Mg/Ca]_S$ of 0.03 was used.

Analyses of enamel and synthetic apatites

(1) Elemental analyses:

Some thin sections were carbon-coated and analyzed by energy-dispersive analyses EDS) for Mg, Ca, Na, K, and chloride (Cl) of the outer, middle, and inner layers of enamel in different areas of the tooth (Fig. 4). Scans were made of four points per region (O, M, I) per section, with 3 sections used per tooth. Powdered pooled samples from each region from several thin sections were analyzed for calcium and magnesium by atomic absorption spectrophotometry (Perkin-Elmer 3030B); phosphate was analyzed by the colorimetric method. Carbonate content was determined by means of an infrared (IR) method based on the ratios of intensities of the CO_3 and PO_4 absorption bands, and using as standards carbonate apatite analyzed by the Conway diffusion method (LeGeros, 1967; LeGeros and LeGeros, 1983). Organic content was deduced from the weight loss between 200 and 400°C as determined by TGA analyses (Sakae et al., 1984; Sakae, 1988).

(2) X-ray diffraction (XRD), infrared (IR), and thermogravimetric (TGA) analyses:

Some thin sections separated into layers were pooled for each tooth, powdered for XRD, IR, and TGA analyses. XRD analyses were made on a Philips APD3520, with KCl as internal standard for the lattice parameter measurements. IR analyses were made on KBr pellets

(about 1 mg sample/300 mg KBr) with the Perkin-Elmer 983G KBr pellet. TGA analyses were made by means of the Rigaku TGA-DTA simultaneous analyzer, which provided weight loss as a function of ignition temperature.

(3) Dissolution experiments:

Dissolution properties of powdered (to pass 200-mesh) enamel and synthetic apatite samples were determined in acidic buffer (0.1 M KAc, pH 6, 37°C, 60 min) with solid/solution ratio of 25 mg/50 mL and stirring rate of 500 rpm. The extent of dissolution was determined from the amount of Ca^{2+} ions released to the buffer with time and was monitored by a calcium-ion-specific electrode. The [Mg/Ca] of the enamel and synthetic apatites was determined before and after exposure to the acid buffer.

RESULTS

Enamel

The concentrations of Mg, CO_3, Na, and the organic contents of human enamel increased from outer (surface) to inner (close to the EDJ) layers of enamel, in the order: inner > middle > outer layers (Figs. 3-5). EDAX analyses showed uneven distribution of Ca, Mg, P, Cl, and Na in different areas of the enamel (Fig. 3). The Cl concentration was higher at the outer than the inner layers. The line broadening, $ß_{1/2}$, of the XRD peaks was greater in the patterns from the inner compared with those from the outer enamel layers (Fig. 6), indicating that the apatite crystals present in the outer layer are larger (lower $ß_{1/2}$ values = higher crystallinity = larger crystals) than those present in the inner layers. Larger a-axis dimensions were observed for apatite from the outer layers compared with those from the inner layers. Initial results on two sets of pooled samples from the outer, middle, and inner enamel layers showed that the extent of dissolution is much greater for the inner compared with the outer and middle layers (Fig. 7).

Synthetic apatites

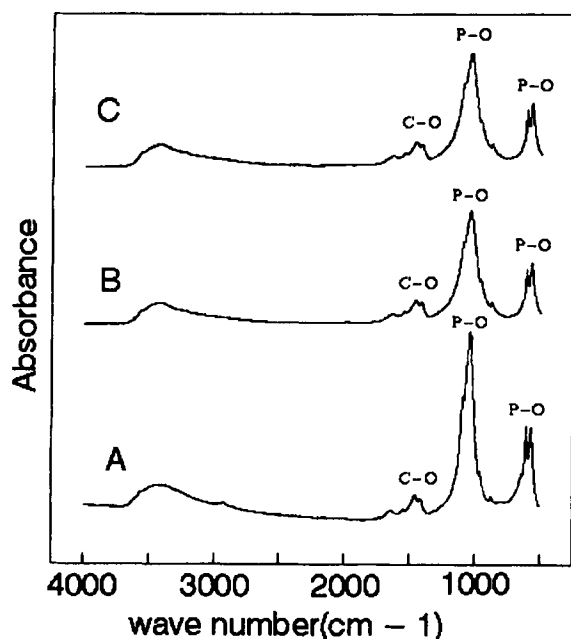The extent of Mg incorporation into synthetic apatites

Fig. 5—Infrared (IR) absorption spectra of apatites from the outer (A), middle (B), and inner (C) layers of human enamel showing increasing CO_3 as calculated from the ratio of intensities of the CO_3 (C-O) and PO_4 (P-O) absorption bands (LeGeros, 1967; LeGeros and LeGeros, 1983).
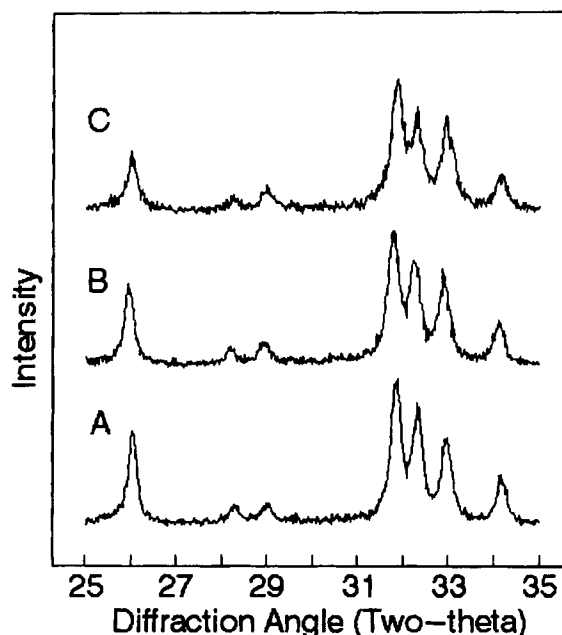

Fig. 6—X-ray diffraction patterns of apatite from outer (A), middle (B), and inner (C) layers of human enamel showing increasing line broadening ($\beta_{1/2}$) of the diffraction peaks. Corresponding average line-broadening values in mm: (A) 4.3, (B) 5.0, and (C) 6.1. The increasing $\beta^{1/2}$ values indicate that the crystals located in the outer layer (A) are larger than those from the inner (C) enamel layer. The average a-axis dimensions were: (A) 0.9449, (B) 0.9445, and (C) 0.9440 ± 0.0005 nm.

increased with the increase in the following variables: $[Mg/Ca]_s$, temperature of reaction, initial pH, and simultaneous presence of carbonate or fluoride (Figs. 8-10). At lower pH, the appearance of ß-TCMP with apatite occurred at $[Mg/Ca]_s < 0.03$. However, in the presence of F, at the same pH, the appearance of ß-TCMP was delayed to higher $[Mg/Ca]_s$ and allowed for the incorporation of higher levels of Mg (Fig. 9). The crystallinity of apatite became poorer with increasing Mg content (Fig. 2) and with the combined incorporation of Mg and CO_3, while crystallinity improved with the simultaneous incorporation of F (Fig. 11). Dissolution experiments demonstrated that increase in extent of dissolution increased with Mg content with and without the simultaneous incorporation of CO_3 or F (Fig. 12). The effect of Mg on the dissolution of apatite was synergistic to that of CO_3, confirming earlier observations (LeGeros, 1984; Okazaki and LeGeros, 1992). The negative effect of Mg on the extent of dissolution of synthetic apatites was minimized when F was simultaneously incorporated into the apatite (Fig. 12). Analyses of [Mg/Ca] of some of the apatite preparations before and after exposure to acid showed a decrease in [Mg/Ca] after acid exposure similar to earlier observations (LeGeros, 1984; Okazaki and LeGeros, 1992).

DISCUSSION

The incorporation of Mg into synthetic apatites was shown to increase with increasing temperature, pH, and in the presence of CO_3 or F ions. The simultaneous incorporation of Mg caused synergistic effects with CO_3, antagonistic effects with

F, on the properties of apatite. For example, simultaneous incorporation of Mg + CO_3 caused synergistic effects on reducing the crystallinity and increasing the extent of dissolution of the (Mg,CO_3)-apatite (Fig. 12). On the other hand, the simultaneous incorporation of Mg + F into (Mg,F)-apatites caused antagonistic effects on these properties: F promoting crystal growth and comparative resistance to dissolution, and Mg inhibiting crystal growth and promoting greater extent of dissolution.

Results from the present study where different analytical techniques (EDAX, IR, TGA) were used on the outer, middle, and inner layers (i.e., from the surface enamel region to near EDJ) confirmed earlier observations of increasing levels of Mg and CO_3 toward the EDJ (Robinson et al., 1981, 1984; Sakae, 1988; Sakae and LeGeros, 1990). In addition, this study showed a decrease in crystallinity of apatite from these layers, paralleling the increase in Mg and CO_3 levels. Since Mg and CO_3, individually and in combination, were shown to cause a reduction in the crystallinity (i.e., crystal size) of synthetic apatite (LeGeros, 1967, 1981, 1984; LeGeros et al., 1971, 1995; Okazaki et al., 1981, 1986; Okazaki and LeGeros, 1992, 1996), the observed decrease in crystallinity from surface enamel to inner (close to the EDJ) layers is consistent with the concomitant changes in composition (specifically, increases in Mg and CO_3
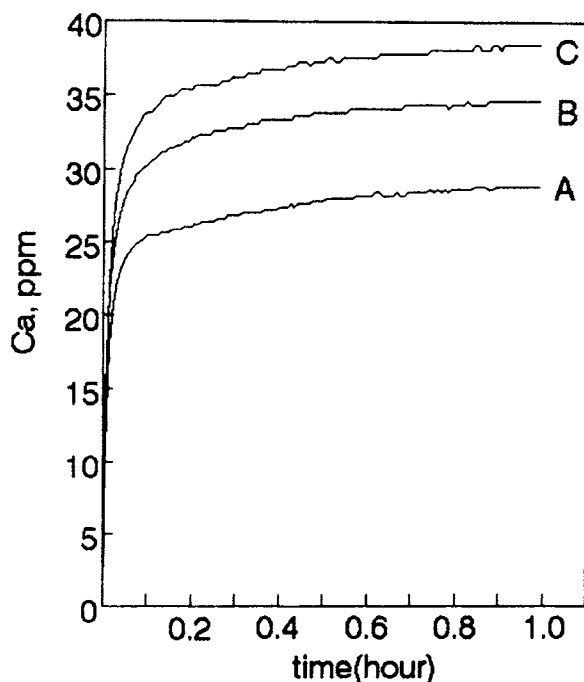
Fig. 7—*Comparative extent of dissolution (expressed as ppm Ca released with time) in acid buffer (0.1 M KAc, pH 6, 37°C) of outer (A), middle (B), and inner (C) enamel layers.*

Fig. 8—*Effect of initial pH of precipitation on the incorporation of Mg into synthetic apatites. (A) pH 5, (B) pH 7.5. Mg incorporation increases with pH. At pH 5, β-TCMP appears, with apatite from [Mg/Ca]$_S$ = 0.02.*

consistent with the higher concentrations of Mg and CO_3 in these regions and the reported effects of these ions on the solubility of synthetic apatites (Okazaki *et al.*, 1981; LeGeros and Tung, 1983; LeGeros, 1984; Okazaki and LeGeros, 1992, 1996; LeGeros *et al.*, 1995).

SUMMARY AND CONCLUSIONS

The observed increase in Mg and CO_3 concentrations from surface to inner (close to the EDJ) layers of enamel was also reflected in the decrease in crystallinity: The outer layer, with lowest Mg and CO_3, had higher crystallinity than the inner layer, with higher Mg and CO_3 concentrations. This difference in crystallinity suggests that the apatite crystals located at the enamel surface are larger than those located in the inner layers closer to the EDJ. Future ultrastructural studies by transmission electron microscopy should be able to confirm this.

The observed decrease in the [Mg/Ca] in biological and

concentrations) in the same regions. The observed higher *a*-axis dimensions of enamel apatite from the surface compared with those in the inner layers are also consistent with the lower CO_3 content in the surface enamel apatite (Zapanta-LeGeros, 1965; LeGeros, 1967; LeGeros *et al.*, 1971). The higher Cl concentration in the surface enamel apatite can also contribute to the difference in the *a*-axis dimension (LeGeros, 1974; LeGeros *et al.*, 1978). Initial observations on the greater extent of dissolution of the inner compared with the outer (surface) and middle layers of enamel are also
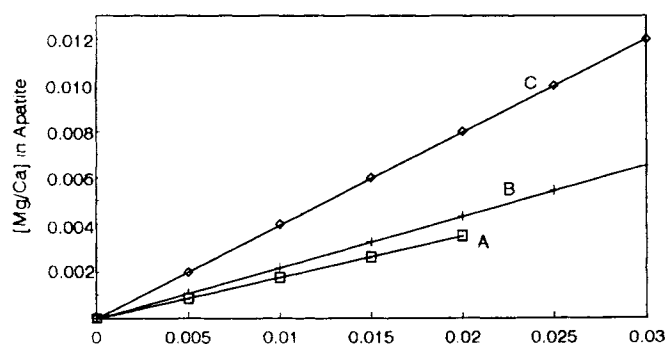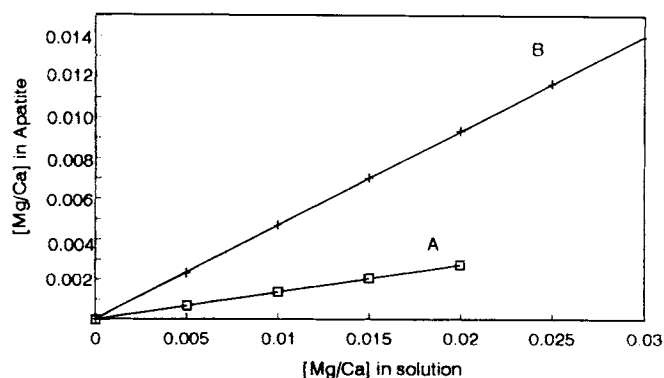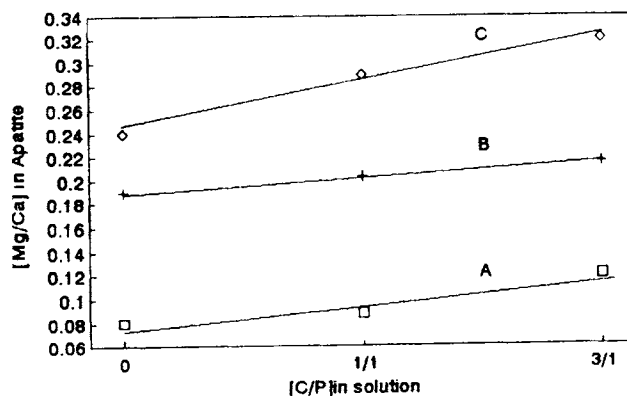

Fig. 9—*Fluoride (F) effect on the incorporation of Mg at pH 5 (A) without F in solution; (B) with 0.5 mM F: (C) with 2 mM F. Mg incorporation increased with increasing F in solution.*

Fig. 10—*Carbonate (CO_3) effect on the incorporation of Mg. Solution [Mg/Ca], [Mg/Ca]S: (A) 0.01, (B) 0.02, and (C) 0.03.*
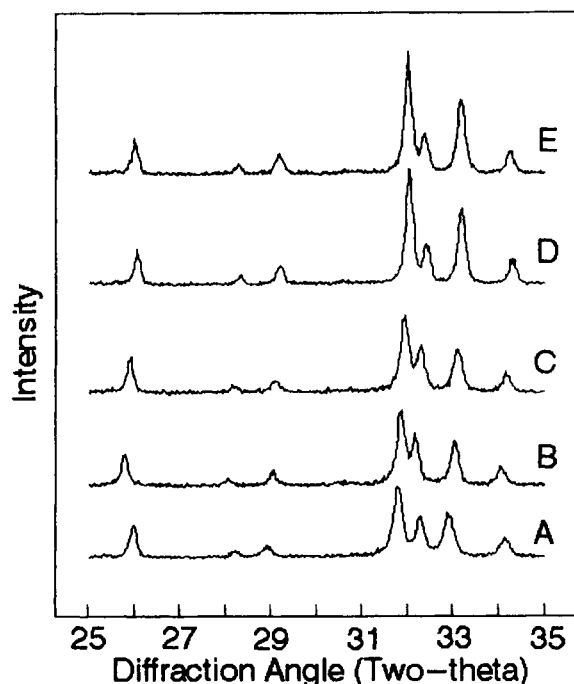
Fig. 11—X-ray diffraction patterns of synthetic apatites from calcium and phosphate solutions containing: (A) Mg, (B) CO_3, (C) Mg + CO_3, (D) Mg + F; and (E) F.

Fig. 12—Comparative dissolution of synthetic apatites containing: (A) F; (B) Mg + F; (C) without Mg, F, or CO_3; (D) Mg; (E) CO_3; and (F) Mg + CO_3. The presence of Mg increased the extent of dissolution (D vs. C; B vs. A; F vs. E). Mg and CO_3 have synergistic effects (F compared with E or D). Mg and F have antagonistic effects (B compared with A or D).

synthetic apatites after exposure to acid may explain the reported 'preferential loss' of Mg and CO_3 during the early stages of caries (Hallsworth et al., 1972, 1973) and the hypothesis involving the dissolution of Mg-rich, CO_3-rich, and F-poor apatites and the precipitation of the Mg-poor, CO_3-poor, and F-rich apatites after caries challenge (LeGeros, 1990).

The synergistic effects of Mg and CO_3 in reducing the crystallinity of synthetic apatites may partially explain the considerable difference in crystallinity (crystal size) between enamel and dentin or bone (Fig. 1), a difference which parallels their difference in Mg and CO_3 levels.

ACKNOWLEDGMENTS

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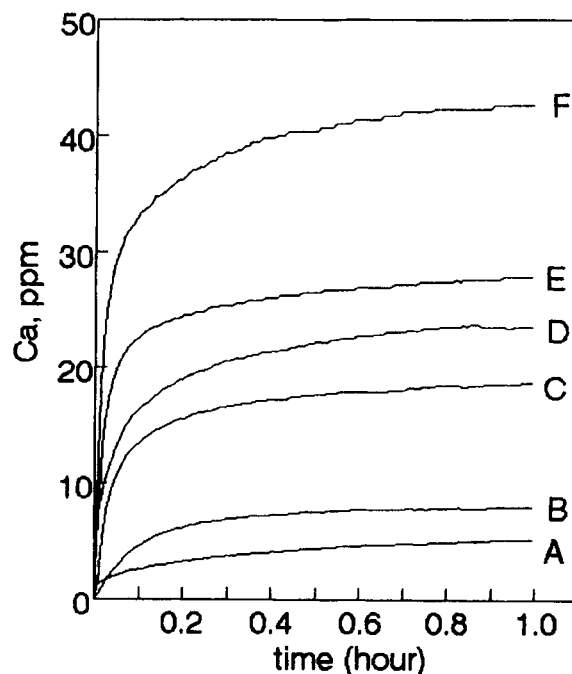Driessens FCM, Verbeeck RMH (1985). Dolomite as a possible magnesium-containing phase in human tooth enamel. Calcif Tissue Int 37:376-380.

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DISCUSSION

Deutsch: Do you know where the magnesium is in the structure of the apatite? Is it with carbonate or phosphate? *LeGeros*: We have shown that carbonate is in the lattice structure, and we believe that the magnesium is, too. They affect the a- and c-axes. The amount of magnesium in the structure is very small, unless there is high carbonate or fluoride. I imagine that magnesium substitutes for calcium. It seems the only place it could be. *Deutsch*: Can you distinguish between the magnesium on the surface and in the lattice structure? *LeGeros*: I believe that Dr. Aoba has done that with absorption studies. *Crenshaw*: With the high levels of fluoride and magnesium you have used, are you forming magnesium-fluor-phosphate on the surfaces of the crystals? *LeGeros*: If we are, we cannot detect it with x-ray diffraction. *Simmer*: With the cyclic oscillations in pH, the crystals may be cleansing themselves of impurities. The carbonate- and magnesium-contaminated crystals would be most soluble. Robinson's slides show that uptake of calcium is greatest when the pH in the enamel is lowest. So, the crystals continue to grow during this time of low pH. *LeGeros*: Depending on the calcium/phosphate content, by removing those impurities, the crystals may get bigger, because the impurities make the crystals smaller. *Margolis*: The primary factor controlling the rate of crystal growth or dissolution is the calcium/phosphorus saturation, regardless of the pH. We found the same gradients for carbonate and magnesium in enamel. The solubility is greatest nearest the DEJ, where the carbonate is higher (similar to LeGeros' findings). *Bawden*: Is there more magnesium in fluorosed enamel? *Robinson*: Yes. Magnesium is quite a lot higher. The removal of matrix proteins may be driven by reactions at the crystal surfaces. If fluoride improves the crystal structure, why does it allow the magnesium to fit in better? *LeGeros*: One speculation is that F accelerates the formation of the apatite and the process becomes less discriminating. *Wright*: Since magnesium goes up with elevated fluoride, does chloride increase? *LeGeros*: I don't know. *Crenshaw*: This is the third reference to the paper on zone refinement just published. *Fincham*: Do you think that increased solubility near the DEJ may be due to crystal size and orientation? *LeGeros*: It seems to be primarily related to composition, because carbonate and magnesium content is higher there.