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Crystal Growth of Bone Mineral

By

Walter E. Brown, Ph.D.*

* Research Associate of the American Dental Association Research Division, National Bureau of Standards, Washington, D. C. 20234.

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NATIONAL BUREAU OF STANDARDS

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Abstract

Formation of hydroxyapatite *in vitro* occurs either (1) through direct precipitation, or (2) by the initial formation of octacalcium phosphate followed by hydrolysis *in situ* to hydroxyapatite. The two processes lead to crystals with distinctive morphologies. A third process for the formation of hydroxyapatite (alternate precipitation of octacalcium phosphate and hydrolysis to hydroxyapatite on the unit-cell level) could lead to crystals with either morphology. Octacalcium phosphate plays a vital, albeit transitory, role in two of these processes and allows the interpretation of properties of apatitic materials without assumptions regarding the existence of arbitrary and ill-defined crystalline species. The effects of fluoride ions in particular, on the properties of apatitic materials become understandable in terms of octacalcium phosphate as an intermediary in the crystallization of hydroxyapatite.

The role of octacalcium phosphate in the formation of tooth and bone is likely to be equally as great as that *in vitro*. Some of these possibilities are discussed for the first time.

It is now generally accepted that hydroxyapatite (HA), $Ca_{10}(OH)_2(PO_4)_6$, is the structural prototype for the principal inorganic crystalline constituent of tooth and bone. There appears to be no reason to question this view even though the crystallites are so small and poorly formed that definite proof is lacking. Chemical analyses reveal that tooth and bone, as well as many synthetic HA precipitates deviate considerably from the composition given by the above formula; the crystal structure, therefore, must also deviate from the ideal HA arrangement that has been computed from x-ray and neutron diffraction data (39). With most crystalline substances, one may be assured that evidence in the form of an x-ray powder diffraction pattern suffices to identify the material. Bone, however, is so finely divided that its diffraction patterns are not very distinctive, especially since there are at least two other crystalline compounds, octacalcium phosphate (OCP), $Ca_8H_2(PO_4)_6 \cdot 5H_2O$, and tetracalcium phosphate, $Ca_4O(PO_4)_2$, with x-ray powder diffraction patterns somewhat similar to that of HA. Even with fairly sharp HA diffraction patterns, it is not possible to be certain that the other two salts are entirely absent, and, of course, in a diffuse pattern it is even more difficult.

The point of view presented in this paper is that OCP plays an important transitory role even though it is not the major crystalline calcium phosphate in tooth or bone except perhaps in the very early stages of mineralization. The role relates to the mechanisms by which the crystals grow. Thus its importance would go far beyond its actual concentration at any instant because many of the dynamic and static properties of bone relate closely to events that occur during growth of the crystallites. Tetracalcium phosphate has not been shown to form from aqueous systems and for this reason not much attention is given to it here.

In recent years considerable evidence has accumulated to indicate that OCP participates in the formation of tooth and bone as the initial precipitate which then hydrolyzes to HA. The precipitation and hydrolysis reactions may be written,

$$8Ca^{++} + 2H^{+} + 6PO_4^{---} + 5H_2O \rightleftharpoons Ca_8H_2(PO_4)_6 \cdot 5H_2O \qquad (1)$$

$$Ca_8H_2(PO_4)_6 \cdot 5H_2O + 2Ca^{++} = Ca_{10}(OH)_2(PO_4)_6 + 4H^{+} + 3H_2O \qquad (2)$$

If OCP participates in this way in the formation of tooth and bone mineral, then obviously these two reactions would be of great physiological importance, ranking among the most important inorganic reactions in the vertebrates. Some of the reasons for believing that OCP participates in bone formation are summarized later along with a discussion of the crystal-growth mechanisms through which this could take place; but before these points can be taken up, attention is given briefly to the problem of nonstoichiometric apatitic materials, and the relationship between the crystal structures of OCP and HA is examined.

The term "hydroxyapatite" is used here to denote the more or less ideal crystalline compound, $Ca_{10}(OH)_2(PO_4)_6$; the terms "apatite" and "apatitic" cover a much larger class of materials that deviate from the ideal composition either in calcium or

phosphate content or by having extraneous materials such as fluoride, carbonate, or water tightly held within the crystals. These are practical terms used here to describe materials that give x-ray powder diffraction patterns similar to, but often not as sharp as, that of pure well-crystallized HA. Because mixtures of OCP and HA give HA-like powder patterns they, too, are apatitic in this sense. Materials deviating from ideal HA composition, particularly when the Ca/P ratio is not 10/6, are referred to frequently as nonstoichiometric apatites.

Difficulties with stoichiometry of apatitic materials began when the calcium phosphates were being investigated by J. J. Berzelius (4) in the first half of the 19th century, and they remain with us today almost unabated. Some idea of the number of investigations and the ingenuity in proposing explanations to account for what, at first sight, appears to be a simple problem can be seen from the listing in Table 1. A detailed discussion of each of these proposals would be lengthy and is not attempted here. Implicit in much of the following discussion, however, is the view that proposal 8a in Table 1, "Intracrystalline mixtures of HA and OCP," is a major cause of stoichiometric variability in apatites. This view is taken, first, because it appears to have the most relevance to our major consideration, crystal-growth mechanisms, and, second, because it is the only proposal among those listed in Table 1 for which direct proof is available (12). This proof came from single-crystal x-ray studies which revealed that individual "crystals" with optical properties intermediate to HA and OCP were indeed intracrystalline mixtures of HA and OCP of the type that had been proposed (7).

The omission of the other proposals from this discussion does not mean that we ascribe no part to them in nonstoichiometric apatitic materials. Of the proposals listed in Table 1, perhaps the most widely accepted are those based on some form of calcium deficiency or substitution in the HA lattice; many papers have contained attempts to establish the reasonableness of one modification of this proposal over another. Undoubtedly lattice defects of various kinds are present; this is amply demonstrated by the way many apatitic precipitates retain extraneous water or pick up carbon dioxide (3,20,49). It is common knowledge that defects are present in nearly all crystals. In spite of their ubiquity, however, defects usually occur in extremely low concentrations compared to those that would have to be present in apatites to account for the observed variations in the Ca/P ratio of apatitic precipitates. The experimental evidence that has been put forward to prove proposals listed under the first item in Table 1 fails to establish their validity, largely because it has related mostly to colligative properties rather than specific structural features.

Terms such as "solid solution," "isomorphous series," and "alpha-tricalcium phosphate hydrate" have been applied to nonstoichiometric apatites without precise structural models and as such tend to be labels rather than descriptions of the phenomenon. In bone, surface phenomena undoubtedly are of great importance. The other proposals listed ir Table 1 are either of minor importance to this problem or in need of further experimental verification. Proposal 8a is the only one for which we see an obvious casual relationship between crystal growth and the nonstoichiometric character of apatites. It is obvious, however, that as long as there is uncertainty as to the causes of this phenomenon, progress in working out the details of the HA crystal growth mechanism, as well as many other physical chemical properties of apatites, will be retarded. This fact alone suffices to remove the problem from the category into which it is frequently placed -- an academic problem of interest only to crystallographers.

The first report of the existence of OCP was made by Berselius (4) over a century ago. Yet, less than a decade ago (10) the existence of a discrete crystalline compound with OCP composition was so much in question that reactions (1) and (2) could not have been written with much assurance that they were meaningful. The apparent continuous variability in compositions of apatitic precipitates made it difficult to select a unique crystalline entity with a lower Ca/P ratio. This was particularly true since so many of these materials yielded nearly the same x-ray pattern. The presence of certain characteristic lines in the x-ray patters of materials with Ca/P ratios near 8/6 (5) and the reproducibility of their optical properties (12) pointed to the existence of a discrete compound with the composition of OCP. Final proof of its existence, at least for this reviewer, came when crystals large enough for single-crystal x-ray study were grown. This x-ray study (10) gave a set of unit-cell dimensions which, along with the density, yielded rational unit-cell contents consistent with the crystal symmetry and a Ca/P ratio of 8/6. The structure of OCP, when calculated from single-crystal x-ray data (7), proved to be completely in accord with these views and substantiated the proposal (10) that it had a layer-type structural relationship to HA.

The existence of the salt OCP with its unique relationship to HA has introduced a new factor that makes it possible to understand many hitherto enigmatic problems in the chemistry of apatitic precipitates in vitro; it has not achieved the same indispensable role in the chemistry of tooth and bone mineral, we believe, because the smallness of their crystallites precludes study by more rigorous methods. In spite of this, enough evidence for the presence of OCP in vivo has accumulated to warrant careful consideration of its physiological and pathological implications.

The physical chemist can study the factors that influence the formation of OCP and its hydrolysis, but it remains for those in physiological and medical research to learn how these relate to growth and diseases of mineralized tissues. Fluoride ion is believed to play a particularly active role in connection with its ability to accelerate reaction (2). This review may be particularly appropriate at this time because of the increased interest in the use of fluorides for the treatment of osteoporosis, and because it may lead to a better understanding of some of the reactions between fluoride ions and bone crystallites.

A number of reviews (15,21,55,56) on bone and tooth mineral have appeared in recent years, including four (22,49,53,64) in the last decade in this journal. A rereading of the latter four reviews is recommended for additional background and for insight into changes in knowledge and attitudes with respect to the role of OCP in mineralization. Three of these (22,49,64) make no mention of OCP; the fourth (53) suggests that OCP may be a contributing factor to the nonstoichiometric character of apatitic materials, but devotes most of its discussion on this subject to surface adsorption and defects in the HA lattice. Another review on a related subject that has appeared recently in this journal (71) discusses factors that influence the nucleation process. No mention is made of OCP, and it is assumed that the first aggregate in calcification (i.e., the nucleus) is a dicalcium phosphate. Our view is that the initial nucleus is more likely to be related structurally to HA and OCP. The present review differs from the others also, in that it places much greater emphasis on the role of OCP, and it is more concerned with growth -- in contrast to nucleation -- processes. A third distinction relates to the much greater emphasis that is placed on the ideal crystal structures of HA and OCP as models in our attempts to interpret the chemical properties of tooth and bone. Thus, the properties of bone and tooth crystallites are examined in terms of the properties of the two end members Ha and OCP. This is in contrast to the more prevalent view that nonstoichiometric crystals are more or less homogeneous and that their chemical properties vary continuously with composition from those of OCP, at one extreme, to those of HA, at the other.

Crystal Structure of HA

The structure of HA (39) is shown in Figure 1 projected on the c face (i.e., the basal plane of the hexagonal prism). Six unit cells are shown. The hexagonal arrangement of the phosphates and some of the calcium atoms is indicated by the two hexagons. The hexagonal arrangement of the remaining four calciums (the "column" calciums in the acute corners) would be apparent if a few more of the adjacent unit cells were drawn in. The HA structure has been described many times (39,53,64), but usually the origin of the unit cell is chosen to be at the center of the cell shown in Figure 1. The arrangement as shown in Figure 1 is especially easy to visualize since it is necessary to fix one's attention on only four groups of ions (i.e., the hexagons of calciums and phosphates, the hydroxyl ions at their common center, and the column calciums). It also makes it easy to relate the structure of HA to that of OCP which is described later.

Three of the calciums at the corners of the inner hexagon lie on a mirror of symmetry 1/4 of a unit-cell length up from the basal plane, and the alternate three are on a plane 3/4 of the way up. Hence, the six calciums form a puckered hexagon. The remaining four calciums occur as superimposed pairs of column calciums in the acute corners of the cell. This gives a total of 10 calciums in the unit cell. The phosphorus atoms also form a puckered hexagon so that a phosphorus on a mirror at $z = 3/4$ occupies a site very close to the calcium on the mirror at $z = 1/4$, and v.v. Since the phosphorus lies on a mirror, two of the oxygens of the PO_4 unit also lie on the mirror and the other two oxygens lie in symmetrical positions above and below the mirror. The latter oxygens are quite strongly coordinated to the calcium in the adjacent corner of the inner hexagon as well as to the corresponding calcium in the vertically adjacent unit cell. This results in a chain of atoms, --Ca-O O-Ca-O O--, parallel to the c axis which probably contributes substantially to the stability of the HA crystal. Each of the remaining two oxygens of a PO_4 unit (the two that lie on the mirror) is bonded to a pair of column calciums. This coordination serves to tie the HA structure together laterially (i.e., parallel to the plane of the diagram). The two hydroxyl ions in the center of the hexagons are replaced by fluoride ions in

fluorapatite.

Crystal Structure of OCP

The corresponding projection of three unit cells of OCP (7) is shown in Figure 2. The unit-cell dimensions of OCP are compared with those of HA in Table 2. The c dimensions, normal to the plane of the paper, and the b dimensions, parallel to the horizontal in Figures 1 and 2, are nearly the same for the two salts, but the a dimension of OCP (the full length of the side in Figure 2) is more than twice that of HA.

What is left of a puckered hexagon of calcium ions, somewhat distorted and with only four of the corners filled, can be seen in Figure 2; a similar fragment of a puckered hexagon of PO_4 ions is also present. The two column calciums in the acute corner near the section A-A are present in OCP, but they are slightly shifted so that they are no longer exactly superimposed. In place of the two hydroxyl ions of HA are a water molecule and phosphate oxygen.

The atoms in the immediate vicinity of the section A-A of OCP are only slightly shifted from the sites of the corresponding atoms near section A-A of HA shown in Figure 1. The differences between the two salts increase with distance from sections A-A until, in the region of unshaded atoms, there is no formal resemblance. The closeness of fit between the two salts is illustrated in Figure 3 which shows a half unit cell of OCP joined to a full unit cell of HA at the plane A-A (i.e., the lower half of Figure 1 and the upper half of Figure 2 are transposed to Figure 3). There should be very little strain in two unit cells joined in this way, and this is the only way the two salts can be joined and have a strain-free juncture of two lattices. Another identical juncture could take place at the lower edge of the HA cell in Figure 3, but another half unit cell of OCP would have to be added to the top before the OCP part of the structure would return to the plane of compatibility with HA.

Interlayered Crystals

The presence of a layer common to both salts is the structural basis for saying that the two form interlayered mixtures (10). It was noted earlier that a "single-crystal" x-ray study (12) fully substantiates the predictions based on the above structural considerations. In this study it was apparent from the sharpness of the x-ray reflections that the individual layers of HA and OCP were at least several hundred angstroms thick. In some powder diffraction studies, the intensities of reflections arising from planes parallel to the common structural layer were abnormally weak and diffuse (12), indicating that the individual layers were much thinner. Depending on the conditions under which the crystals form, the intracrystalline layers probably vary widely in their relative number and thickness; thus yielding compositions ranging from that of OCP to that of HA and allowing wide variations in chemical behavior. There are two routes which may lead to interlayered crystals. These are discussed under the sections relating to Processes II and III.

The result that HA and OCP may be present in the same crystal is accepted even by those who advocate other explanations listed in Table 1, and whose present view appears to be that it is not permissible to extrapolate these results as far as to say that interlayering occurs in the very much smaller crystallites of bone and tooth. There appears to be no reason that precludes such an extrapolation, and, in fact, there is chemical and physical evidence summarized next that supports the idea that OCP is present in bone and tooth crystallites during at least part of their existence.

Evidence for OCP in Bone and Tooth

Several types of circumstantial evidence indicate that OCP has at least a transient existence in tooth and bone. These are described briefly in the following listing.

1. A platy or blade-like shape is characteristic of OCP. HA is usually found as hexagonal needles or rods, occasionally as hexagonal plates. It is becoming more generally accepted that bone and dentin contain at least some platy crystallites (38,61,78). The initial precipitate in early enamel is a ribbon (60). Therefore, crystallite morphology in both bone and tooth favors the view that OCP plays an important part in formation of the mineral.

2. A Ca/P ratio less than 10/6 is in accord with the presence of OCP. Low Ca/P ratios have been found in the early stages of mineralization of tissues (23).

3. When OCP is heated above about 200°C, it is partially converted to a pyrophosphate because it is an acid calcium phosphate. Mineralized tissues also form pyrophosphate when heated (34). The amount of pyrophosphate is greatest in tissues in early stages of mineralization (26) and decreases with age and with time after formation of the mineral (68). These findings are in accord with the presence of OCP in these materials, with its participation in mineralization according to Process II or III, and with its disappearance according to reaction (2).

4. Solubility data indicate that calf and child bone contain a solid phase with a Ca/P ratio of about 8/6; the indicated solid in human adult bone has a ratio close to 10/6 (44). The presence of greater amounts of OCP in growing tissues such as child and calf bone, as compared to adult bone, is to be expected if OCP is an intermediate in the formation of HA.

5. Three diffuse lines in the x-ray pattern of fetal enamel were attributed (35) to a protein fraction. Later, they were found to correspond to lines of OCP (11). They were missing in demineralized samples, and, therefore, they should be ascribed to OCP. This is perhaps the most direct evidence for the presence of OCP in mineralized tissue.

6. The greater ease of formation of OCP over that of HA, and its probable lower surface energy are both important in the early stages of formation of crystals and favor OCP as the initial precipitate (11).

Individually none of the evidence listed above proves that OCP participates in the formation of tooth and bone crystallites, but their cumulative effect is convincing. Those that hold that lattice defects are the major causes for nonstoichiometric behavior in apatites would naturally take issue with the interpretation given to the second and third of the above listed points, but the other four points are less amenable to alternative explanations.

Crystal Growth Mechanisms

Most crystal growth processes are thought to take place in two stages: a nucleation stage (41,54) (formation of the incipient crystal) and a three dimensional growth stage. The nucleation step is believed to require supersaturation to overcome an "activation" energy; it is difficult to study because the nuclei are extremely small and because impurities facilitate the nucleation step. A number of investigators (29,71,72) have studied this part of the process with particular emphasis on the role of collagen and other proteins as catalysts for nucleation. It is held (55,71) that differences in nucleating ability among the various proteins in the body may account for the fact that some tissues calcify and others do not. The crucial question here is whether there is a critical supersaturation which must be exceeded before nucleation can occur and whether its value is characteristic for each type of heterogeneity in the system. If the answer to both parts of this question is no, that nucleation will occur at any degree of supersaturation at any nucleation site given enough time, then much of the work that has been done on nucleation of apatites is on a shaky theoretical basis. This question has not been answered completely, but the models that are usually used to describe the critical nucleus do not lead to the result that there is a critical supersaturation in the precise sense. In view of this situation, it would appear that more serious consideration be given to other mechanisms which might differentiate between mineralizing and soft tissues. The reverse of catalysis, poisoning, has been suggested, and the pyrophosphate ion has been implicated (25). The metabolic products and the transport functions of osteoblasts, odontoblasts, and ameloblasts could be dominant factors and should be examined for the roles they may have in mineralization. Because of the structural similarity between HA and OCP, supposed epitaxial relations between certain proteins and HA (28,56,71) would hold equally well between these proteins and OCP. Thus, the controversy as to whether or not such epitaxy is important in vivo (62) does not relate directly to the mechanisms discussed here.

Direct observation of the detailed mechanics of the three dimensional stage of growth from solution is, of course, impossible; yet, considerable insight into these processes has been gained from theoretical and experimental investigations (18,76). At first thought one might think that growth on a crystal facet takes place by deposition of ions more or less randomly over the full area of the facet, but this is not the generally accepted mechanism. Instead growth is thought to occur by the addition of ions to the leading face (i.e., riser) of a "step" or a terrace on the facet. The height of the step is taken to be a rational multiple of the unit-cell thickness so that the flat surfaces above and below the step are similar. The character of the face of the riser is such that it captures ions from the solution more effectively than the flats above and below it. In some instances, probably as the result of

bunching of smaller steps, the steps are high enough to be seen with an optical microscope.

As ions deposit on the riser, the growth layer spreads until it has covered the entire facet. Further growth on that facet must then await a nucleation process, the formation of a "pill box" somewhere on the facet. Once formed, the pill box becomes a growth layer that again spreads over the facet by "two dimensional growth." The surface nucleation step (referred to as two dimensional nucleation), just like formation of the original nucleus of the crystal, is believed to require supersaturation and catalytic help from heterogeneities, and very often nucleation is the rate controlling step.

In the presence of certain types of dislocations in the lattice, the surface nucleation process is unnecessary because growth does not eliminate the step from the facet of the crystal. The spiral or screw dislocation growth mechanism (27) is a common example of this; since it does not require a periodic nucleation process, it may lead to particularly rapid growth. Shortly after the discovery of the screw dislocation growth mechanism, Amelinckx reported two instances which indicate that this mechanism occurs in apatitic minerals. One of the minerals (1) was thought to be an apatite produced from a melt and is of limited interest here; the other (2) was thought to have grown from solution. The significance of his observations, if any, to biological apatites is not obvious, but they should be kept in mind.

It is apparent from the foregoing, that growth on a given face may take place by more than one mechanism (e.g., those requiring periodic nucleation versus various types of dislocation mechanisms). It should be apparent, also, that the details of the mechanism will differ for each type of face on a crystal. Thus for HA, the dominant growth mechanism for the six prism faces may differ drastically from that for the two basal faces. In this instance because of the structural similarity between HA and OCP, there is the added complication that OCP may participate by precipitating on the prism faces of HA.

Because HA can be formed either directly or through OCP as an intermediary, it becomes necessary here to distinguish between the "growth mechanism" and the "chemical process" by which HA is formed. "Mechanism" is used here to mean details of how ions from solution become attached to the lattice of the crystal and involves concepts such as surface nucleation, growth at dislocations and transport of ions; "process" relates here to the sequence of crystalline entities that are formed after the ions leave the solution. We know very little about the growth mechanisms of HA; we know more about the processes. First, we know that pure, well crystallized OCP can be converted to HA in situ. Also, crystals of HA that form in vitro very often have the blade-like habit of OCP. This was taken (12) to indicate that they precipitated as OCP and hydrolyzed subsequently to HA. At other times, HA crystals appear as well-formed hexagonal needles. It was proposed (12), therefore, that HA can be produced through either of two totally different processes (direct formation of HA crystals, or precipitation of OCP crystals followed by hydrolysis to HA). Subsequent considerations concerning the complications that could arise from the similarities between the structures of OCP and HA led to a proposal for a third process (8) (alternation of reactions (1) and (2) at the unit-cell level) which could also lead to crystals with hexagonal morphology.

Process I: It is highly unlikely that intermediate formation of OCP could take place in crystallization of HA in a hydrothermal bomb because the stability of OCP decreases rapidly as the temperature is raised. This is probably true also for the precipitation of HA from highly basic solutions or from solutions containing relatively high concentrations of fluoride. It is necessary to assume, therefore, that HA can form by direct precipitation of ions from solution. This process would naturally lead to HA crystals with good hexagonal morphology because a crystal of lower symmetry does not intervene in the growth process. It should be noted that the evidence for this mechanism is of a negative nature -- it is inconceivable that OCP could be an intermediate under some of the conditions for formation of HA. It would, of course, be unrealistic to assume that any salt cannot form directly.

The observation by Amelinckx (2) that mineral apatite crystals, presumed to have grown from solutions, revealed growth spirals on the prism faces can be interpreted as indicating a mechanism leading directly to HA. These crystals, however, had the hexagonal tabular habit in contrast to that of tooth and bone crystallites which always show an elongation along the c axis. Furthermore, OCP could participate in a spiral growth mechanism. At this stage, it is precarious to speculate as to what importance, if any, Process I has relative to apatites formed in vivo, and what the details of this growth mechanism should be.

Process II: It is especially significant that the hydrolysis of OCP usually occurs in situ. It is a simple matter, by boiling OCP in water or alkaline solution, to obtain products anywhere in the range of compositions between OCP and nearly pure HA. Hydrolysis in situ of OCP is to be clearly distinguished from a process in which OCP dissolves and then HA precipitates as a separate phase. In the first place, the HA crystallites produced by hydroylsis of OCP, because of the difficulties usually associated with solid-state reactions, very probably have properties that differ from those of HA formed without going through an OCP intermediate. Secondly, the product crystals will be pseudomorphs after the original OCP. Thirdly, the hydrolysis may not be complete so that the product may be an intracrystalline mixture of HA and OCP. Since OCP invariably is platy or blade-like, its hydrolysis products will not have the morphology and the hexagonal external symmetry normally attributed to HA. This is a matter of significance relative to the surface properties of the crystallites. More importantly, the occurrence of platy crystallites in bone (38,61,78) suggests that Process II may be operative in their formation. It is clear, on the other hand, from the hexagonal symmetry of enamel crystallites (60) that they could not be formed by Process II.

That this mechanism has received much less attention than it deserves is illustrated by the fact that it is very difficult in vitro to produce HA in a nonplaty form (except at very high pH or in a hydrothermal bomb) and in a form that does not yield at least some pyrophosphate when heated. Presumably, the mechanism for growth of the initial OCP crystals that form in Process II is the same as that described below for Process III except for omission of the hydrolysis step in the third stage.

Process III: In a sense this process is a compromise between the first two. It is assumed that the product that precipitates first is a single unit-cell thickness of OCP, thus taking advantage of the favorable rate of reaction (1), but it also assumes that the rate of the hydrolysis, reaction (2), is sufficient to more or less keep up with the precipitation so that even during growth the major body of the crystal is HA. It may yield crystals with hexagonal symmetry like Process I.

As noted earlier, most growth mechanisms may be divided into two stages, nucleation and three-dimensional growth. The mechanism proposed for Process III (8) is unique in that it includes three stages of growth; the second stage is unusual; and the third stage is divided into two steps.

First stage. Formation of the nucleus (a minimum grouping of ions that is capable of subsequent growth into a crystal). This stage could be the same for processes I, II and III.

Second stage. Two-dimensional growth of nucleus. This produces a two-dimensional lattice, presumably a single unit-cell thickness of OCP (11).

Third stage. Three-dimensional growth.

1. Precipitation step: A unit-cell thickness of OCP is deposited on a prism face per reaction (1) (see Fig. 4a-4b).

2. Hydrolysis step: A unit-cell thickness of OCP hydrolyzes to a layer of HA two unit cells in thickness per reaction (2) (see Fig. 4b-4c).

If a second precipitation step follows immediately after the preceding one (i.e., before the hydrolysis step can occur) the first layer of OCP becomes buried and its hydrolysis may be prevented. Thus, if the precipitation step is much faster than the hydrolysis step, the product would be pure OCP; if the two steps are about equally fast, the product would be an interlayered crystal; and if hydrolysis is much the more rapid of the two steps, the product would be pure HA. Herein lies an important aspect of this growth mechanism because it suggests the possibility of regulating the nature of the precipitate by controlling the relative rates of the two steps. The relative rate of the hydrolysis step, for example, should be enhanced by high calcium concentration, high pH, or by the presence of fluoride ions. Fluoride ions are believed to facilitate hydrolysis because they would initiate this step by tending to form fluorapatite. Once the hydrolysis step is initiated in the OCP layer, at any point on the face, it should spread spontaneously over the remainder of the layer for reasons given later.

A two-dimensional growth stage is not common to most crystal-growth mechanisms. It was proposed (11) to account for the ribbon-like morphology that was observed in

the electron micrographs of tooth enamel in early stages of mineralization (60). Thus the importance of this stage would be in its great influence on the final morphology of the crystallites. The extremely long, fibrous habit of enamel crystals and the platy habit of bone and dentin crystallites are believed to relate primarily to the events that control the second stage in each case. Bone crystallites are so thin (circa 50A.) that it would be extremely difficult to distinguish experimentally whether they grow by Process II or III. For the average bone crystallite the distinction does not appear to be important because it amounts to a difference between (1) a single hydrolysis step involving a two unit-cell thickness of OCP (Process II) and, (2) two hydrolysis steps, each involving a single unit-cell thickness of OCP (Process III). Enamel crystallites are much thicker (about 10 times that of bone crystallites) and a distinction between Processes II and III would be revealed by differences in the cross-sectional shapes of the crystallites and in the way they react with fluoride.

The rationale behind the precipitation and hydrolysis steps can be summarized as follows. Since HA and OCP have a common structural layer, the transition to the aqueous phase from this plane in the outermost unit-cell should be the same whether the crystal is OCP or HA. In the "crystal" depicted in Figure 4a the transition layer has been taken to be a half unit cell of OCP, for the reason, described elsewhere (8, 11), that this would probably reduce the surface energy. If this is so, then the surface exposed to the solution will be the "hydrated" layer of OCP (Fig. 2) in which interatomic bonding is relatively weak, and, therefore, attachment of ions to this surface for growth of the lattice will be quite inefficient. Once a "pill box" of OCP a unit-cell in thickness forms on this surface, its edges expose the "apatitic" layer of OCP (Fig.2) to the solution. Here the bonding is ionic and relatively strong, and efficiency of attachment of ions should be relatively high; lateral growth (i.e., spreading of the pill box over the surface) would be rapid. Clearly, this type of mechanism is in accord with the generally held views on crystal growth.

The hydrolysis step is represented by the transition from Figure 4b to Figure 4c. It should be noted first that this transition requires a considerable shifting of the two phosphate groups in the hydrated layer, the two protons and five water molecules must depart, and two calcium ions must enter the unit cell. Along with these changes there must be a contraction in the lattice from 18.68 A. (d_{100} of OCP) to 16.32 A. ($2d_{100}$ of HA) and a lateral shift as well. It is because of this contraction and shift that we believe the hydrolysis step, once initiated anywhere in the layer, will spread spontaneously over the remainder of the layer.

Another proposed nucleation mechanism has received considerable attention (56). In this the nucleus is believed to be a dicalcium phosphate and not an HA-OCP type of nucleus as proposed here. In support of this view it is stated that serum appears to be supersaturated with respect to HA but undersaturated with respect to dicalcium phosphate dihydrate. Thus, under normal conditions dicalcium phosphate dihydrate would not precipitate for thermodynamic reasons while HA would not for kinetic reasons, and nucleation would occur only when serum becomes supersaturated with respect to dicalcium phosphate. This it would almost certainly do if the serum became sufficiently supersaturated because, under many conditions, dicalcium phosphate dihydrate will precipitate more rapidly than the other calcium phosphates; OCP shares this property and under some conditions it precipitates even more rapidly than dicalcium phosphate dihydrate. With regard to the degree of saturation of serum, the solubility of OCP in solutions in the pH range of serum is intermediate to that of HA and the dicalcium phosphates (6,52). Hence, serum saturated with respect to OCP would be supersaturated with respect to HA and undersaturated with respect to dicalcium phosphate dihydrate; the presence of OCP would thus account for the degrees of saturation of serum with respect to calcium phosphates without the need to assume that a structurally unrelated salt, such as dicalcium phosphate dihydrate, participates in the nucleation of HA. The dicalcium phosphate nucleation hypothesis was put forth before the structural relationship between HA and OCP was known. In the absence of direct substantiative experimental evidence, there appears to be no reason at this time to hold the view that a dicalcium phosphate initiates precipitation in bone. It is exceedingly unlikely that the ribbon-like crystallites that form in early mineralization of enamel (60) could have a structural relationship to a dicalcium phosphate.

Reactions with Fluoride

Although it might appear that reaction of fluoride ion with HA is a relatively simple process, several reactions with significantly different thermodynamic consequences are conceivable. Depending on the model chosen to describe the reaction, one can visualize a range of situations in which the fluoride concentration has no

influence on the calcium and phosphate concentrations in serum to others in which they are strongly dependent on the fluoride concentration. Experimental studies of whether fluoride reduces the solubility of dental enamel have yielded ambiguous results (36). One should, therefore, examine other possible models that may explain how very small amounts of fluoride prevent dental caries. One such model relates to the reaction between fluoride and OCP, as discussed below. In view of the possible importance of fluoride on a host of other physiological reactions as well as those involving calcium phosphates, additional studies of the solubility of apatite in the presence of fluoride are certainly warranted even though such studies are likely to prove difficult.

The reaction of fluoride with OCP also has received relatively little experimental study. In macrocrystalline form, OCP is less stable than HA under physiological conditions (6,52) and OCP will hydrolyze spontaneously to HA if the kinetics of the reaction are favorable. An important role of fluoride appears to be the acceleration of the hydrolysis reaction (12,58). A particularly significant aspect of this reaction is that fluoride concentrations far too low to produce stoichiometric fluorapatite should suffice to initiate the reaction to form apatite ; once initiated, the reaction would proceed spontaneously to produce an apatite with a low fluoride content. This is a possible explanation for the well known fact (47) that the presence of remarkably small amounts of fluoride during the formation of the mineral serves to protect teeth against caries. According to the view presented here, the effect of a small amount of fluoride is to eliminate a relatively unstable salt, OCP, by converting it to a more stable salt, HA. Fluoride owes its ability to accelerate hydrolysis of OCP to the fact that a fluoride ion concentration greater than 10^{-7} to 10^{-8} molar (0.0002 to 0.002 p.p.m.) would make fluorapatite less soluble than HA, thus increasing the thermodynamic driving force for the conversion of OCP to an apatite.

The dramatic effect of fluoride on the precipitation of apatitic materials was clearly illustrated by an experiment reported by Newesely (58). In his process for "homogeneous" precipitation of OCP, appropriate calcium and phosphate solutions are mixed together at room temperature, warmed to about 40°C., and allowed to stand until a precipitate is produced. Newesely found that, in the absence of fluoride, well crystallized OCP precipitated; when the solution was made about 10^{-6} molar in fluoride (0.02 p.p.m.), the product was apatite. Thus a fluoride concentration about one-fiftieth that recommended for drinking water sufficed to completely alter the nature of the precipitate. The solubility of HA was exceeded in all his solutions, but only in the presence of fluoride was the formation of OCP crystals prevented. Newesely concluded that fluoride ions induced formation of fluorapatite nuclei on which, in turn, growth of HA occurred by epitaxy. In other words, he assumed that the HA crystallized by Process I. The results can be interpreted also on the basis that the fluoride induced hydrolysis of OCP formed by Process II or III. Concentrations of fluoride somewhat less than 10^{-6} did not prevent formation of OCP. He attributed this to a lack of supersaturation with respect to fluorapatite so that its nuclei could not form. The compositions of these solutions would indicate, however, that they, too, were supersaturated with respect to fluorapatite. A more likely explanation appears to be that lower fluoride ion concentrations were less effective in accelerating the hydrolysis reaction so that the rate of reaction (2) remained slow relative to that of reaction (1). These were *in vitro* studies but there appears to be no reason to believe that the effects of fluoride *in vivo* will be any less subtle and far reaching.

It was suggested (8) that too high a concentration of fluoride ion may so accelerate the hydrolysis step that it impedes precipitation of OCP either in the second stage or in the precipitation step of the third stage. Premature alternation of OCP to an apatite in the second stage could limit the length and breadth of the crystal; similarly, if the rate of the hydrolysis step is too fast, the unit-cell layer of OCP (Fig. 4b) could be destroyed before it could spread over the entire area of that face of the crystal. These are the reasons that were given (11) as a possible explanation for mottling of teeth in areas with excessively high fluoride in the drinking water. By largely eliminating growth by Processes II and III, high fluoride concentrations could actually reduce the rate of crystal growth. This may account for the hypomerali-zed zone in dentin produced by the administration of fluoride (51,63).

Physiological Considerations

In the following we speculate on some possible physiological and pathological consequences of the presence of OCP in hard tissues. Elevated Ca x P products in child serum, as compared to adult, appear to be directly related to a greater presence of OCP in child bone (44). It is easy to conclude that high calcium phosphate activities in child serum result simply from the greater presence of this more soluble

constituent in bone. On the other hand, calcium homeostasis and phosphate metabolism should affect the rate of reaction (1) and, therefore, the amount of OCP in the tissue. This reciprocal relationship makes it difficult to define a clear causal relationship between OCP and elevated Ca x P products of the serum. In any case, the greater solubility of OCP should have important effects on parathyroid activity, on phosphate metabolism, and on the localized change such as those that normally occur as the result of osteolytic and osteoblastic activities, and the role of OCP in these relationships deserves greater physiological attention.

Obviously, any mechanism that would reduce the rate of reaction (2) would also have the effect of increasing the OCP content of bone. If the fluoride ions normally present in serum influence the rate of reaction (2), then metabolic activity could enter the picture in the following way. It is known that fluoride ions react with ATP and ADP to form the fluorophosphate ion, PO_3F^{--}, which has a modest life time in an aqueous medium (37). Removal of fluoride ions by this mechanism could, therefore, slow the rate of reaction (2). Thus a higher metabolic rate in the child could account, in part, for the greater concentration of OCP believed to be present in child bone (44). Generally, fluoride is considered to be a poison of metabolic reactions involving ATP and ADP, but the possibility should be left open that actually these reactions with fluoride are essential for control of the properties of bone crystallites.

Reference has been made to the views that serum is supersaturated with respect to HA (55,46) and that child bone in vitro behaves as though OCP is the principal solid phase that saturates the solution (44). Both of these effects would result if bone contained both HA and OCP and if the rate of dissolution of OCP were considerably greater than the formation of HA by Process I. Under these circumstances, crystal growth would effectively stop whenever the ion-activity product of OCP in the serum drops below its saturation value even though the serum remains supersaturated with respect to HA. Dissolution of HA could not begin until the ion-activity product of HA in the serum drops below its saturation value. Between these extremes, HA could neither precipitate (through Process II or III) nor dissolve, giving rise to a "solubility gap" in which reaction between serum and the lattice of the bone crystallites would be static. Such a situation would allow some latitude or lag in the regulatory mechanisms controlling dissolution and crystallization. The gap represents a factor of about 10 in the calcium and phosporus concentrations represented by the solubilities at serum pH of well crystallized HA and OCP (8); the gap may be much less, however, for finely divided crystallites such as those in bone. A similar, but much larger, gap is implicit to the mechanism which postulates dicalcium phosphate as the nucleating substance, but the gap would disappear once the dicalcium phosphate nuclei were converted to apatitic crystallites. The reason this would be true is that the dicalcium phosphate is thought to participate only in the nucleation stage and not in the three-dimensional growth stage as is suggested here for the precipitation of OCP in Processes II and III. The idea of an HA solubility gap was not created to account for the apparent supersaturation of serum with respect to HA, but instead it is a natural consequence of the growth mechanism associated with Processes II and III. The evidence for supersaturation of serum with respect to HA is not conclusive, but if it exists, it indicates that Process I is not very effective in the growth of bone crystallites.

This reviewer is not qualified to discuss the many other physiological and medical implications that would derive from the presence of OCP in mineralized tissues and its participation in growth of apatite crystals. It would be desirable for those more closely associated with these subjects to enlarge on the implications. As a specific example, it should be asked if any diseases of bone relate to an improper functioning (i.e., excessively slow or rapid rates) of reactions (1) and (2).

It is hoped that the examples that were given above adequately illustrate the clarity and detail of the concepts that derive from the use of two crystalline entities, HA and OCP, to describe reactions that occur under physiological conditions. This is in contrast to other mechanisms to account for nonstoichiometric apatites listed in Table 1 which generally do not lead to precision in the physical chemical descriptions of such processes and are less likely to suggest definitive experiments.

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Table 1

Proposals to Account for Nonstoichiometric Apatites

		Reference
1.	Calcium deficiencies in lattice	
	a. H_2O in place of OH^- ions	33,77
	b. Interstitial protons	65,67,73
	c. H_2O or H_3O^+ in Ca^{++} sites	5,56
	d. Interstitial protons and missing OH^- ions	3,40
2.	Solid solutions	14,19,31,32
3.	Isomorphous series	17,48
4.	Hydrated tricalcium phosphate	17,31,79
5.	Lattice substitutions	
	a. CO_3^{--} for PO_4^{---}	48
	b. Na^+ or K^+ for Ca^{++}	5
	c. $(OH)_4^{----}$ for PO_4^{---}	50
6.	Excess Ca or PO_4 on surface of HA	
	a. Adsorbed Ca^{++} or PO_4^{---}	15,43,75
	b. Heterionic exchange	55
	c. Substitution in surface unit cells	5
	d. Sorption on "inner surfaces"	5,31
	e. Coating of OCP 1/2 unit cell in thickness	8
	f. Surface complex of $Ca_2(HPO_4)(OH)_2$	69
	g. Surface complex of $CaHPO_4$ or $Ca(H_2PO_4)_2$	5
7.	Occlusion of phosphate	66

Table 1 (continued)

		References
8.	Intracrystalline mixtures	
	a. OCP with HA	12
	b. Tetracalcium phosphate with HA	9
9.	Subunit-cellular twinning of OCP (paracrystallinity)	59
10.	Mixtures of HA with other compounds	15,70
11.	Coprecipitation	74
12.	$Ca(OH)_2$ adsorbed on tricalcium phosphate hydrate	16

Table 2

Comparison of Hydroxyapatite and Octacalcium Phosphate Unit Cell Dimensions

	Octacalcium Phosphate	Hydroxyapatite
a	19.87 Å	9.42 Å
b	9.63	9.42
c	6.87	6.89
α	89°17'	90°
β	92°13'	90°
γ	108°57'	120°

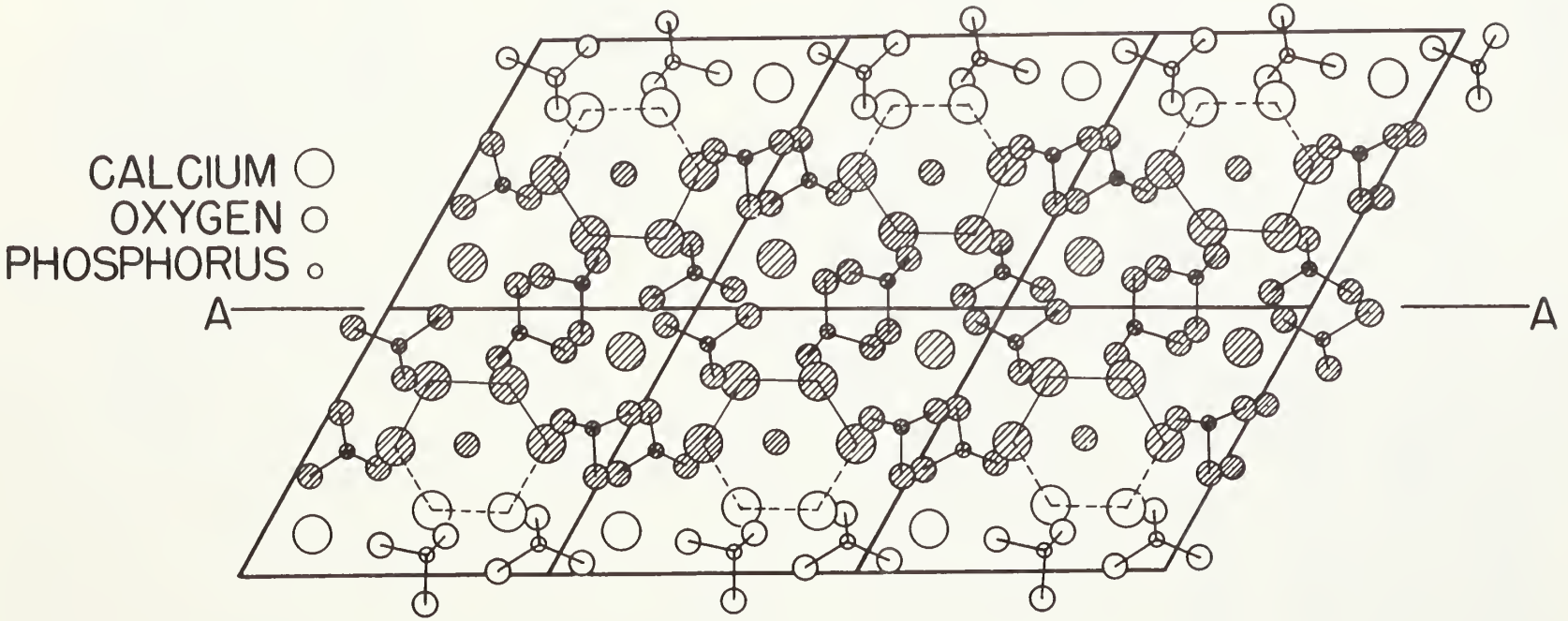

Figure 1. Six unit cells of hydroxyapatite projected on the c face.

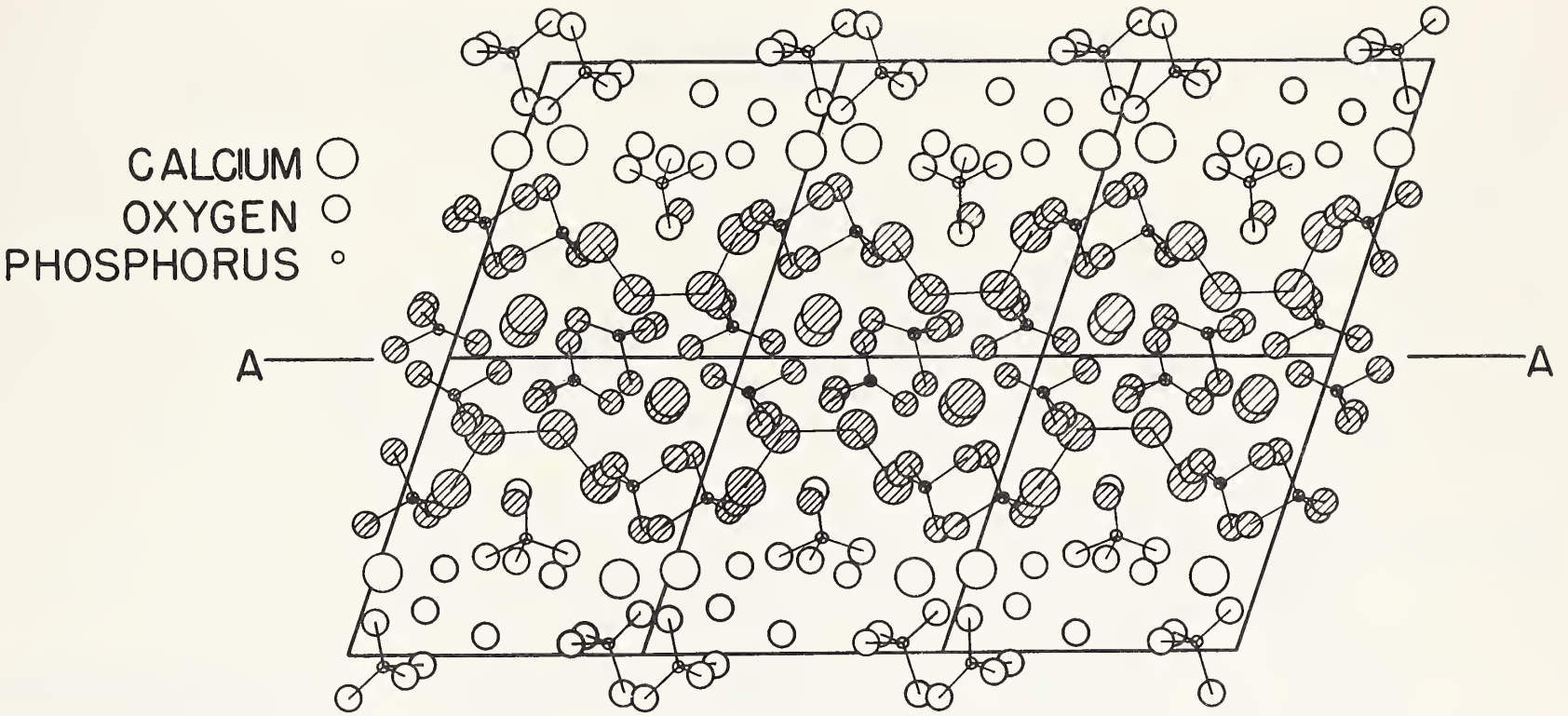

Figure 2. Three unit cells of octacalcium phosphate projected on the c face.

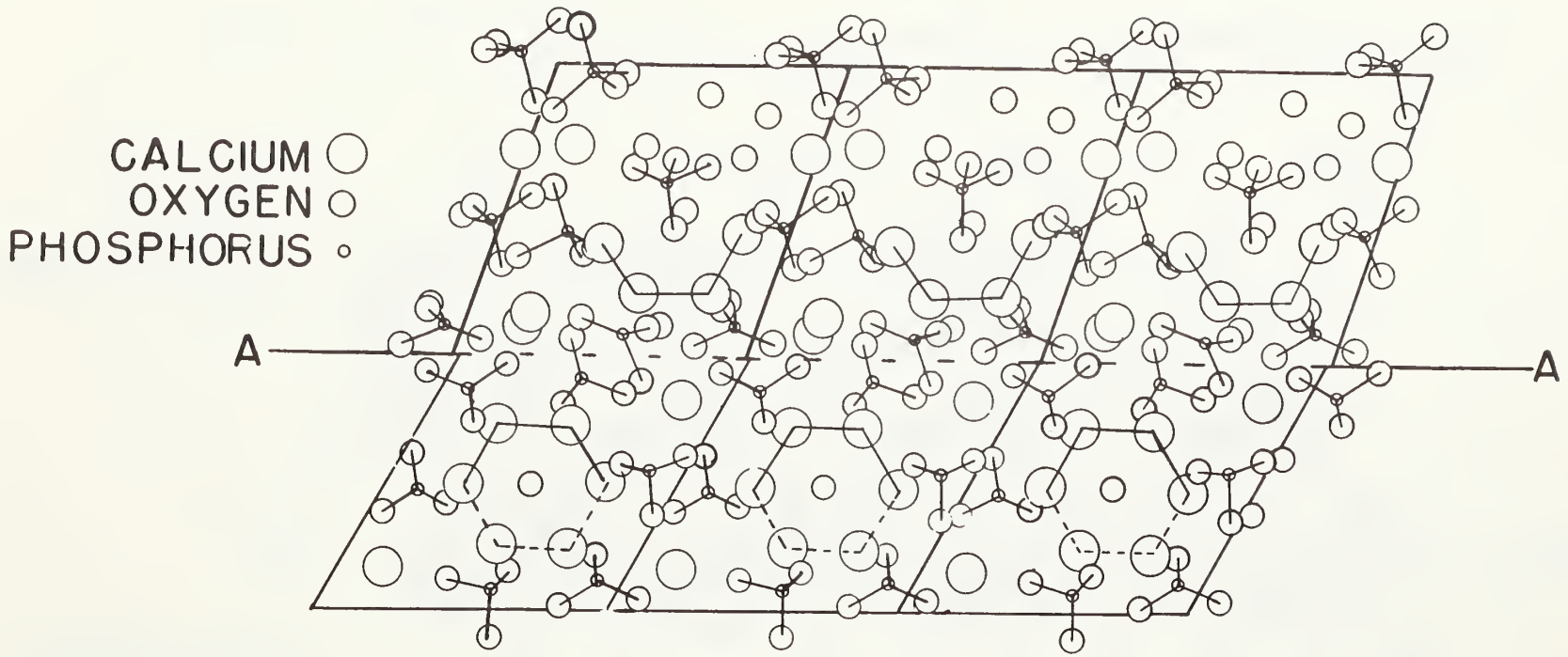

Figure 3. Three unit cells of hydroxyapatite and one and one-half unit cells of octacalcium phosphate joined on a plane parallel to the a face.

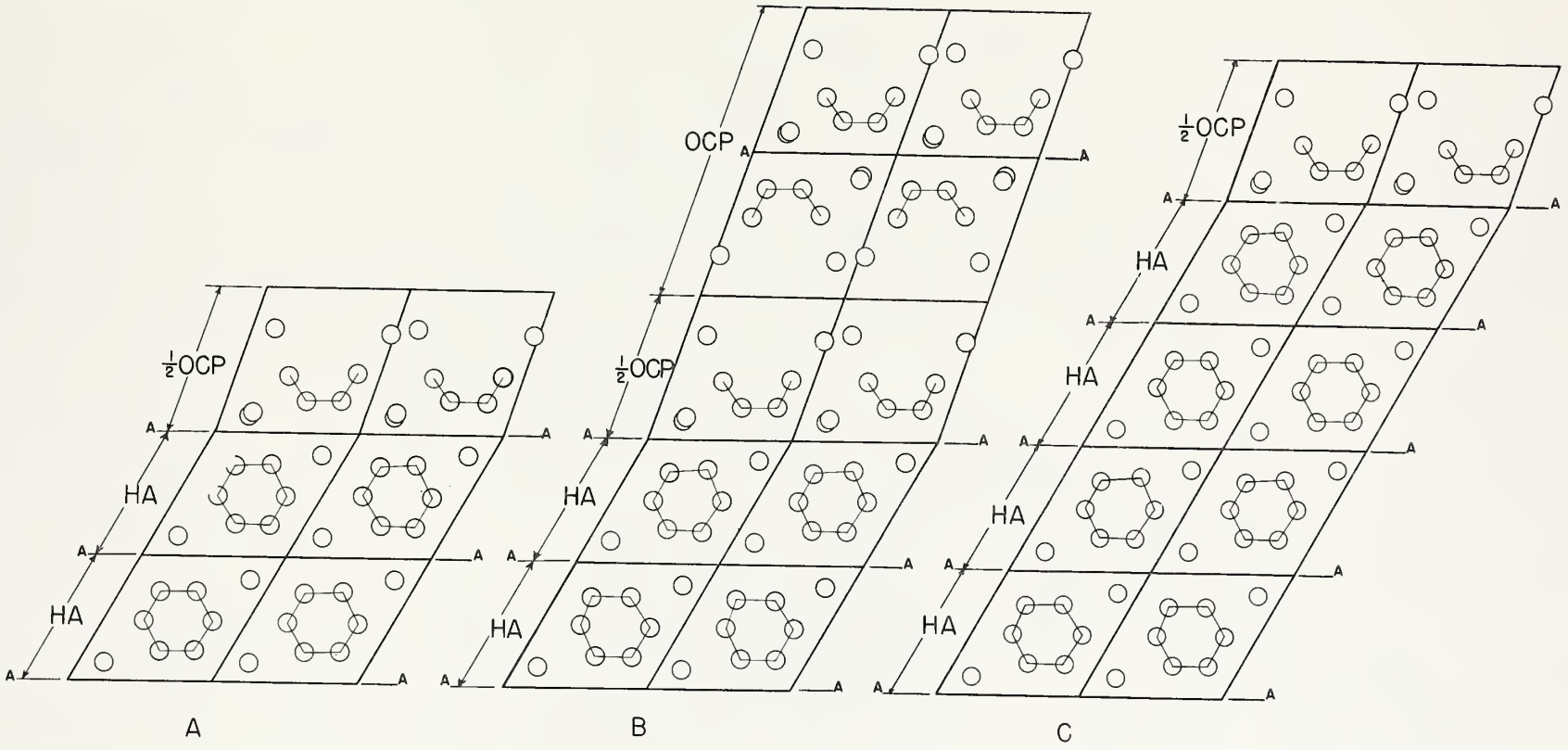

Figure 4. Growth of hydroxyapatite by Process III. Only the calcium atoms are depicted.