Structural features of biologically involved phosphates

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A review of the physico-chemical characteristics of the biologically involved phosphates was presented. Schematic drawings of the inorganic salts of dental and physiologic interest were included and discussed.

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The crystallography and the crystal chemistry of phosphates are becoming increasingly important for the dental researcher. Excellent models of organic molecules are readily available commercially, and good descriptions are present in biochemical textbooks. Corresponding representations of inorganic compounds are more difficult to come by, especially as regards phosphates.

The following schematic drawings of unit cells are thought to be helpful — especially for teaching purposes — in bringing out some of the salient characteristics of inorganic salts of dental and physiologic interest.

The unit cell

The unit cell is a parallellopiped which is conceived of as being repeated throughout a crystal structure. The unit cell contains all the characteristics — the symmetry and chemical elements — of the crystal in question; it is a kind of structural molecule.

In general, the unit cell is defined by three edges labelled a, b, and c (or x, y, and z in the same order), and the corresponding angles: $\alpha = b\lambda c$, $\beta = a\lambda c$, $\gamma = a\lambda b$. The three planes are labelled (001) or C, (100) or B, and (010) or A as shown in Fig. 1.

The atomic or ionic positions are located in reference to the edges or *axes*. Depending upon the length of the axes, their inclination to one another, and the atomic

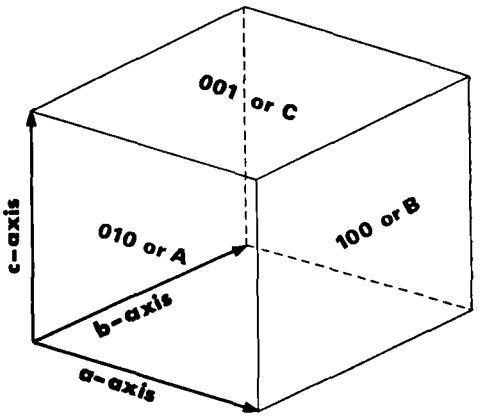

Fig. 1. General features of a unit cell.

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positions, a total of seven major, different unit cells is possible, corresponding to the seven *crystal* Bravais *systems:* triclinic, monoclinic, orthorombic, tetragonal, cubic (regular, isometric), trigonal (rhombohedral), and hexagonal systems. By considering the symmetry relations between the atomic positions, the seven systems may be subdivided into 32 *crystal classes*, and taking account of the possible arrangements within the atomic positions themselves, a total of 230 *space groups* may be established. Each of these space groups has its own notations some of which will be referred to later.

The chemical composition of a compound is reflected in the content of the unit cell. The calculation is:

1. Every atomic position within the boundaries of the unit cell counts one;
2. Every ion on a surface of the unit cell counts 1/2, since this ion is shared by two unit cells;
3. Every ion on an edge of the unit cell counts 1/4, since this ion is shared by four unit cells;
4. Every ion on a corner of the unit cell counts 1/8, since this corner ion is shared by eight unit cells.

The total sum of counts gives the net chemical content of one unit cell. As a check the positive and negative charges must balance.

In general, the chemical composition of a compound is not expressed in terms of the chemical content of the unit cell, but in terms of conventional, empirical formulas. The relation between the unit cell content and the corresponding formula is expressed by a factor z, which will give the number of formula-units in one unit cell. Examples:

Compound	Unit cell content	Empirical formula	z
Hydroxyapatite	$Ca_{10}(PO_4)_6(OH)_2$	$Ca_5(PO_4)_3OH$	2
Brushite	$Ca_4H_4(PO_4)_4(H_2O)_8$	$CaHPO_4 \cdot 2H_2O$	4
Whitlockite	$Ca_{24}(PO_4)_{16}$	$Ca_3(PO_4)_2$	8

The formulas are used only for the sake of convenience and simplicity. They give just the proportion between the chemical constituents in a compound, and do not inform about the chemical character, the bond strengths, etc. in the compound. It is a misconception when the formation of hydroxyapatite is regarded as an »18th order reaction» because the unit cell contains 18 ions or ionic groups, whereas the formation of brushite is a »2nd order reaction» because the empirical formula has one Ca^{2+} and one HPO_4^{2-} ion (*Neuman & Neuman*, 1958).

Sometimes formulas more fanciful than those given above have been used. So hydroxyapatite has been described by 3 $Ca_3(PO_4)_2 \cdot Ca(OH)_2$ instead of $Ca_{10}(PO_4)_6(OH)_2$. This usage is unfortunate because it conveys the notion that hydroxyapatite consists of three moles of tricalcium phosphate and one mole of calcium hydroxide. Hydroxyapatite has also been viewed as a hydroxide and not as a phosphate, by being given the formula $(Ca_5(PO_4)_3)^+ - OH^-$ (*Kurmies*, 1953). Such complex cations do not exist in the lattice.

The arrangement of atoms and ions

The structure assumed by any solid is such that the atomic nuclei and electrons tend to arrange themselves in a form with a minimum energy content. In so-called anisodesmic compounds like phosphates and carbonates this results in the formation of discrete groups which act as units within the structure.

The PO_4^{3-} group (Fig. 2) forms a regular tetrahedron with 0^{2-}ions at the four corners and one P^{5+} ion in the center. The P—O distance in these tetrahedra is 1.5.—1.54 Å, and therefore considerably shorter than for instance the Ca—O distances, which may vary between 2.46—2.82 Å in phosphates. The result is that PO_4^{3-} groups appear in the structure.

In the same way the C^{4+} ion and the 0^{2-} ions form CO_3^{2-} groups in carbonates. These groups assume the form of equilateral triangels (Fig. 2).

On a volume basis, the oxygen ions far exceed all other elements in phosphates and carbonates. These other elements may therefore be regarded as fitting in the interstices between the large oxygen ions. The determining factors for the positioning are the size and the charge of the ions.

The radius of the commoner ions encountered in biologic minerals are:

Ba^{2+}	1.34 Å	Cl^-	1.81 Å
K^+	1.33 Å	OH^-	1.40 Å
Sr^{2+}	1.12 Å	O^{2-}	1.40 Å
Ca^{2+}	0.99 Å	F^-	1.36 Å
Na^+	0.97 Å	P^{5+}	0.35 Å
Mg^{2+}	0.66 Å	C^{4+}	0.16 Å

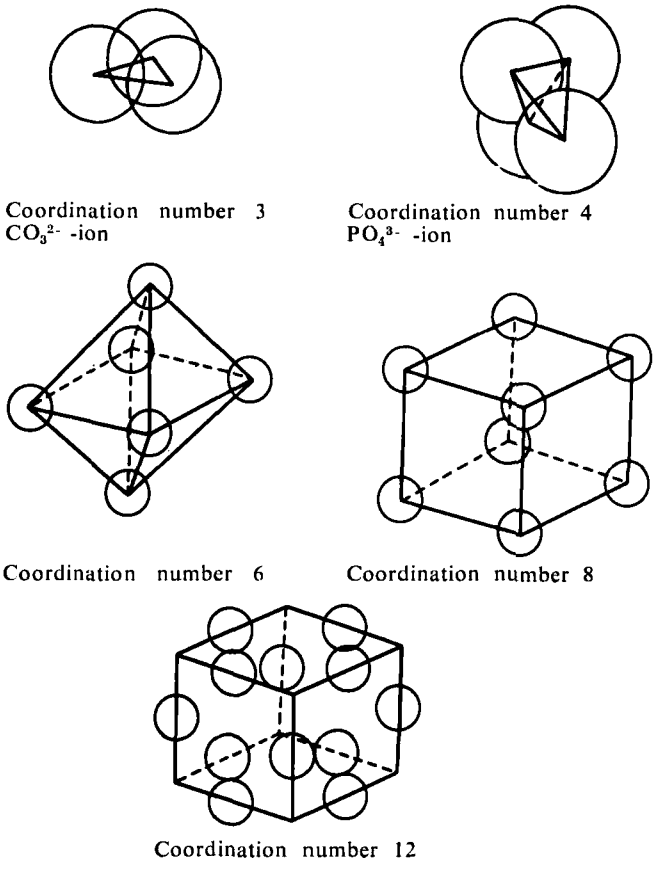


Fig. 2. Packing of atoms at various coordination numbers.

The hydrogen ion, the proton, is essentially dimensionless (cp. the radii of OH^- and O^{2-}).

The size of the ions imposes geometric restrictions on the whole arrangement. By the coordination number is meant the number of oxygen ions which can possibly be accomodated around a cation. This number is dependent on the ratio between the radius of the cation and that of oxygen.

If the radius ratio is below 0.22, the closest packing will arise when three oxygens are arranged in an equilateral triangel around the cation, e.g. the CO_3^{2-} in Fig. 2.

If the ratio is between 0.22 and 0.41, four oxygens can be placed in a regular tetrahedron as has been shown for the PO_4^{3-} in Fig. 2.

When the ratio is between 0.41 and 0.73 ($Ca^{2+}/O^{2+} = 0.99/1.40 = 0.7$), the geometrically most feasible arrangement would be six oxygens in an octahedron. With still higher ratios the oxygens can be visualized as being placed at the corners of a cube (coordination number eight), or on the edges of a cube (coordination number twelve). These arrangements have also been pictured in Fig. 2.

When the radius ratio lies near the theoretical boundary, the coordination may vary. Thus Ca^{2+} and Na^+ may have coordination number of either six or eight. Such variations may occur even within the same structure. In hydroxyapatite for instance the so-called »columnar Ca» has a total coordination number of nine, whereas the so-called »hexagonal Ca» is coordinated irregularly to six oxygens (in addition to OH^-).

In the following drawings all the oxygens have been left out for the sake of clarity. The individual ions are of the correct order relative to one another, but not relative to the unit cell axes. Consequently, the representations will appear much less dense than the real structures. These simplifications are justified in order to bring out those points which are of more importance from the practical point of view.

The biological phosphates

belong to the orthophosphates, i.e. they are built around the PO_4^{3-} group, and they are predominantly calcium orthophosphate.

These orthophosphates have been the subject of study for mineralogists and geologists long before their biological importance was appreciated, and many of the phosphates are still better known by their mineral names than by their chemical designations. These compounds were synthetically prepared by treating calcium oxide (CaO) and phosphorus-pentoxide (P_2O_5) thermally, which fact is still reflected in the commonly used nomenclature:

Theoretical equation	Compound	Chemical name	Basicity
1 $CaO+P_2O_5+2\ H_2O$	$Ca(H_2PO_4)_2$	Monocalcium phosphate	1.00
2 $CaO+P_2O_5+H_2O$	$2CAHPO_4$	Dicalcium phosphate	2.0
3 $CaO+P_2O_5$	$Ca_3(PO_4)_2$	Tricalcium phosphate	3.0
4 $CaO+P_2O_5$	$Ca_4O(PO_4)_2$	Tetracalcium phosphate	4.0
5 $CaO+1\frac{1}{2}P_2O_5+\frac{1}{2}H_2O$	$Ca_5(PO_4)_3OH$	Pentacalcium phosphate	3.33
8 $CaO+3P_2O_5+6H_2O$	$Ca_8H_2(PO_4)_6 \cdot 5H_2O$	Octacalcium phosphate	2.67

Monocalcium phosphate is too soluble to be of interest, and tetracalcium phosphate does not form under aqueous conditions.

The basicity is expressed in number of Ca equivalents pr. P atom according to the convention of *Bjerrum et al.* (1936). It is a measure of the stability trend. The stability among the biological phosphates would therefore be expected to increase in the order: dicalcium phosphate, octacalcium phosphate, tricalcium phosphate, and pentacalcium phosphate.

Sometimes the chemical composition of a phosphate is expressed in a % CaO and b % P_2O_5. This would correspond to 0.71 · a % Ca and 0.44 · b % P. Expressing the composition in terms of calcium and phosphorus is preferable to the old usage of CaO and P_2O_5.

In recent years, single crystals of phosphates have been produced by hydrothermal procedures or by growing them in solution. This advancement has made it possible to use all the powerful instruments of modern crystallography and to obtain far more detailed information about the properties of phosphates than can be done from mineral and microcrystalline specimens (for review, see *Elliott*, 1969).

Pentacalcium phosphate — hydroxyapatite — $Ca_5(PO_4)_3OH$—Hap

This phosphate belongs to the apatite group of minerals which consists of two series.

The apatite series contains fluorapatite (Fap), $Ca_5(PO_4)_3F$, chloroapatite (Clap), $Ca_5(PO_4)_3Cl$, and Hap. Carbonate-containing apatites are included in this series under the name of carbonate-apatite (Cap), often being assigned the somewhat incorrect formula $Ca_{10}(PO_4)_6CO_3$. These apatites are practically isostructural, and extensive substitutions may take place. The naturally occurring minerals may be regarded as mixed apatites. They often contain other cations than Ca. Mineral names for mixed Hap and Fap are pyroclasite and sombrerite; for mixed Hap and Cap — dahllite, monite, ornithite; for mixed Fap and Cap — francolite, staffelite, nauruite.

The pyromorphite series includes pyromorphite, $Pb_5(PO_4)_3Cl$, mimetite, $Pb_5(AsO_4)_3Cl$, and vanadite, $Pb_5(VO_4)_3Cl$. This series is not of biologic significance.

Apart from a very few exceptions, the existence of Hap as a distinct entity has not been recognized in the dental and physiological literature up to the early sixties. Today it is generally accepted that Hap is the backbone mineral in the hard tissues.

The crystal structure of apatite (Fap) was determined independently by *Naray-Szabo* (1930) and *Mehmel* (1930) in the same issue of the same journal. The only difference was that Naray-Szabo placed the F-ions at c-heights 1/4, 3/4, whereas Mehmel placed them at c-heights 0 and ½. The latter positions are correct for Clap, whereas the former apply for Fap and Hap (*Posner et al.*, 1958; *Kay et al.*, 1964). See Fig. 3.

The structure belongs to space group $P6_3/m$. The letter P (for primitive) signifies that the unit cell is characteristic of the hexagonal system, i.e. the axes a and b are equal and different from axis c, angles α and β are 90°, and angle γ is 60°. The symbol $6_3/m$ means that there is a six-fold (hexad) axis parallell to the c-axis so that equivalent points will be brought into coincidence when the whole structure is rotated 1/6 of 360° (= 60°) around the c-axis. However, this symmetry operation is of a special kind. In addition to the

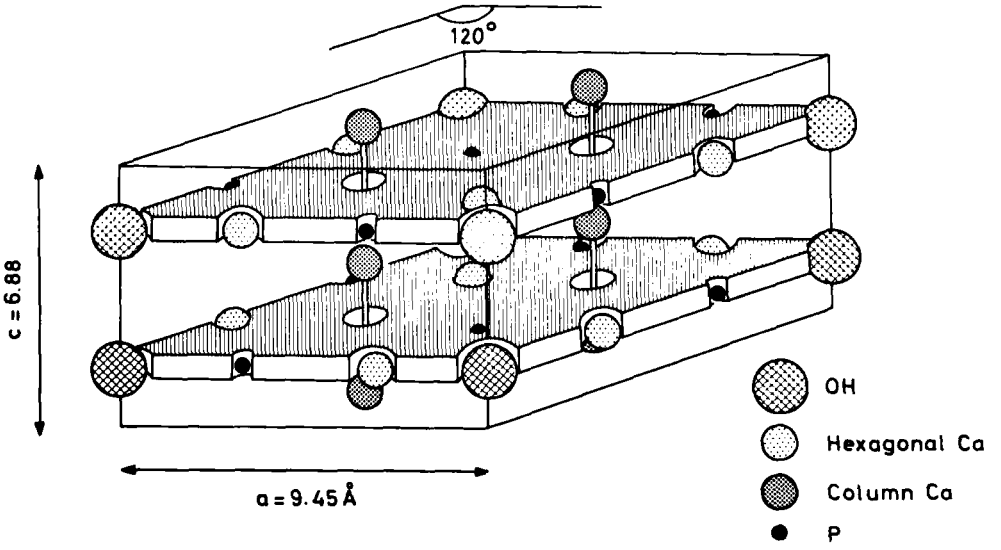


Fig. 3. Unit cell of hydroxyapatite. System: hexagonal. Space group: P $6_3/m$. z:2. Empirical formula: $Ca_5(PO_4)_3OH$

60° rotation, the symbol 6_3 specifies that the structure should be translated along the c-axis for a distance which is one half (3/6) of the length of the c-axis. The whole operation is a combined rotation-translation, and 6_3 denotes a *screw axis*. Lastly, the symbol /m tells that the lattice points are symmetrically distributed about a mirror plane which is perpendicular to the screw axis (and to the c-axis).

The lattice contains two kinds of calcium positions. In the one position, Ca^{2+} is located at c=0 (or c=1.00) and at c=½, and with the coordinates 1/3 and 2/3 for both the a and the b axes. These calcium ions make up a column extending from the basal to the top (001) plane, and they are termed *columnar calcium* or Ca_I. There are a net number of four columnar calciums in each unit cell.

The remaining six Ca^{2+} ions (*hexagonal calcium* or Ca_{II}) are situated on planes parallel to the basal plane at c = 1/4 and c = 3/4. The six PO_4^{3-}-tetrahedra are also on these planes. The halogen ions are located on the c-axes through the corners of the unit cell. The arrangement is shown in Fig. 5, and can be viewed as a column of Ca-triangles (or PO_4^{3-} triangles) making up a channel with halogen ions located inside.

The pure Hap, Fap, and Clap — and other end-members of the apatite series — have their distinct a-axis and c-axes (Fig. 4). These variations may largely be explained by the different bond lengths between the halogens in the channel and the calcium in the calcium triangels in Fig. 5. According to *Young & Elliot* (1966) there is a good correspondence between the typical bond lengths and the a-axes:

Bond	Typical length	a-axes
Ca—F	2.288 Å	9.364 Å
Ca—OH	2.355 Å	9.422 Å
Ca—Cl	2.73 Å	9.634 Å

It therefore seems that the halogens will contract or distend the calcium triangels until normal bond lengths are

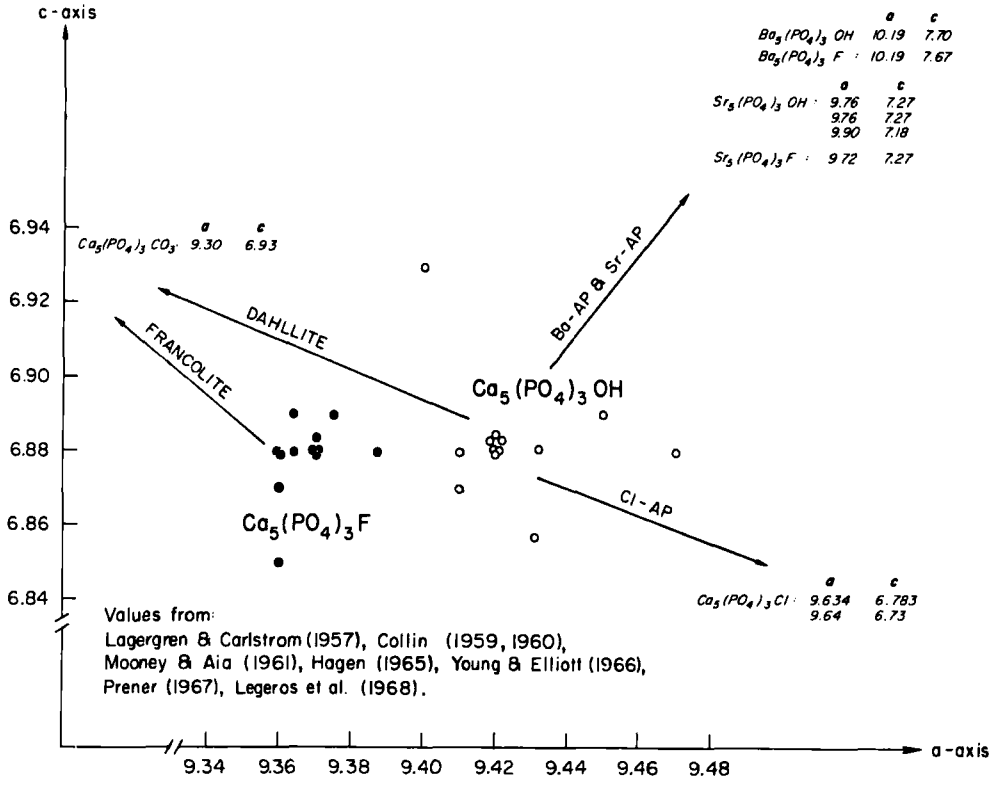


Fig. 4. Unit cell parameters for the end-members of the apatite series, Angstrom units.

established. It would be expected that the F^- ion and the OH^- ion would have about the same attraction for the calcium in the next-nearest calcium triangle, wherefore the c-axes in Hap and Fap would be alike. The much larger Cl^- ion will be forced to be part way between neighbouring triangles, and will reduce the c-axis by its attraction forces.

Present structure determinations place the F^- ion exactly at c = 1/4 and 3/4, and in the center of the calcium triangles in Fig. 5. However, the OH^- ion assumes an asymmetric character because of the proton attached to the oxygen, and cannot geometrically be placed at the intersection of a triad axis and a symmetry plane. By neutron diffraction *Kay et al.* (1964) were able to show that the OH^- is, on an average, located 0.3 Å above or below the plane of the calcium triangles. In order to avoid too close a contact between adjacent hydroxyls, the OH^- ions must either be arranged in an »ordered column» with all the hydrogens pointing in the same direction (OH-OH-OH- etc. along the c-axis), or the arrangement is one of »disordered column» with the direction reversed at various places along the c-axis. This disorder could be caused by vacancies or fluoride impurities in the Hap lattice (OH-OH-vacancy-HO-HO-F-OH-OH-etc.). *Kay et al.* (1964) suggested that the »disordered column» is to be preferred because it faciliates the introduction of the fluoride ion into the Hap lattice.

The two above-mentioned arrangements

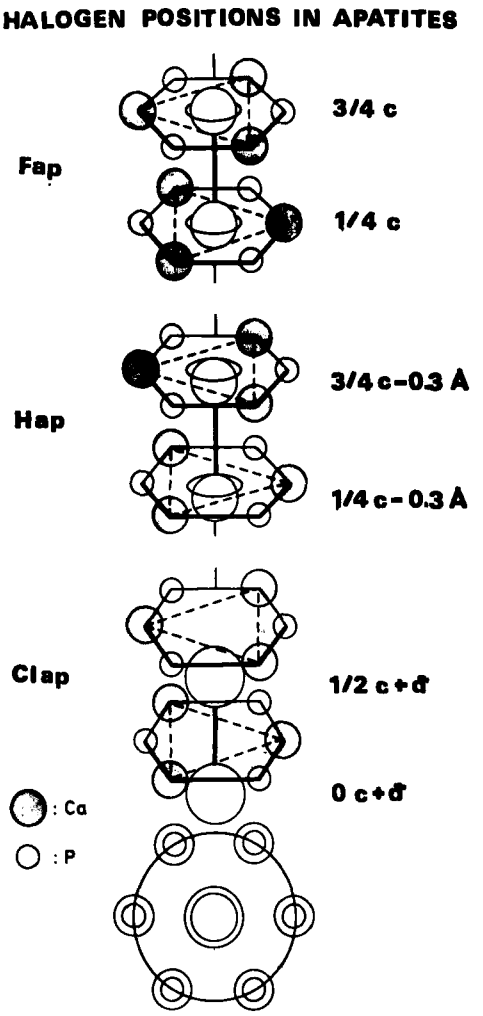


Fig. 5. The F-ion is located at the intersection of the symmetry plane and the triad axis. The OH^- ion is shifted slightly above or below the symmetry plane. The Cl^- ion lies approximately midway between neighbouring symmetry planes.

have no significant consequences for the structure itself; Hap will still belong to the $P6_3/m$ group. However, a third possibility exists.

If all the OH^- ions in one (100) plane point in one direction (say »downwards») and all the OH^- ions in the next-nearest (100) plane point in the opposite direction (say »upwards»), the structure will lose its hexagonal symmetry. As an example Fig. 6 shows that the trigonal axis is no longer present. In the same way there will be no hexad axes and no plain mirror planes, and the structure cannot belong to space group $P6_3/m$.

Instead new symmetry features will arise. If the unit cell of Hap is doubled in the a (or b) direction, the »downward» OH^- ions may be brought into coincidence with the »upward» OH^- ions by first letting the ions glide along half of the b-axis, and then by reflecting the »downward» ion across a mirror plane (Fig. 6). This symmetry element is termed a *glide plane*, in this case a b-glide, and is denoted by »b». In addition, the special arrangement of the OH^- ions will produce a diad *screw axis* (2_1), which is also represented in Fig. 6. By a combined translation and rotation part I in the structure may, first, be brought into co-incidence with part II and, secondly, with part III.

The new unit cell may be obtained by cutting the Hap unit cell at $c = 3/4$ and at $c = -1/4$, and then turning the remaining cell in the way shown in Fig. 6. The new c-axis will correspond to the doubled b-axis of Hap, and the new angel β will correspond to the angel γ of Hap. The unit cell will be monoclinic (inclined in one direction) belonging to space group $P2_1/c$. The stroke denotes that the screw axis is perpendicular to a glide plane with the glide in the c direction of the unit cell.

This *monoclinic hydroxyapatite* has been prepared by *Elliott* (1971), and it has been shown that Clap crystallizes in the monoclinic system (*Young & Elliott*, 1966; *Prener*, 1967). It is to be noted, however, that small amounts of impurities, e.g. F^-, will stabilize the hexagonal structure in both these apatites. If Hap is genuinely monoclinic, then the occurrence of OH^- ions in statistical disorder would imply

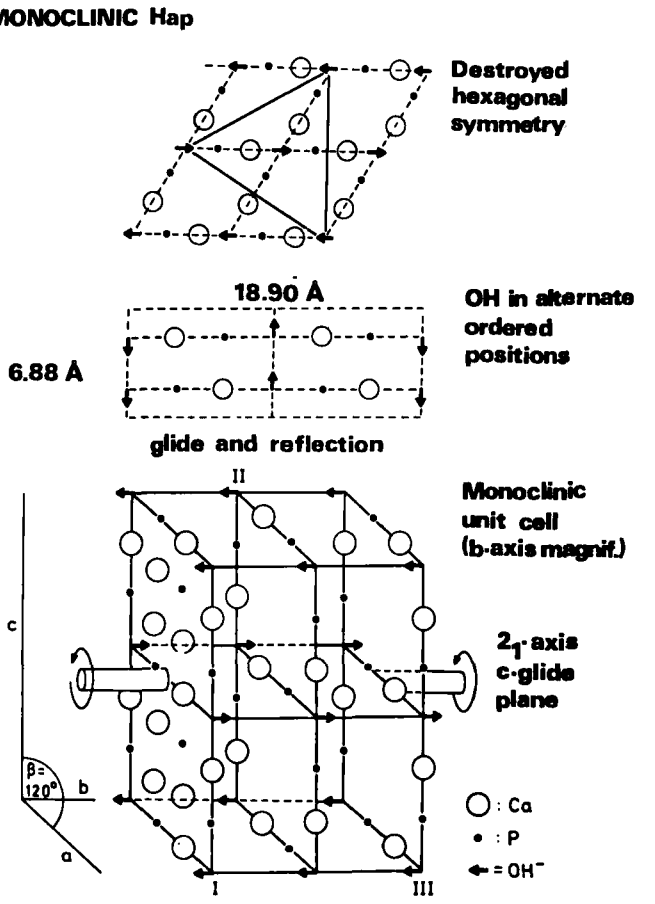


Fig. 6. The relationship between hexagonal and monoclinic hydroxyapatite.

that these ions are not in their lowest possible energy state, and that the energy of the crystal would be reduced if an ion were substituted in an ordered position. This fact might be an explanation why OH^- ions are preferentially replaced by F^- ions, which are always located at a = b = 0 and at c = 1/4 and 3/4 in the unit cell.

Biological apatites are all built on the unit cell given in Fig. 3. However, their chemical and physical properties often differ from those of pure Hap. This variation is evidently caused by the fact that the biological mineralization takes place in an environment which — from the point of view of crystal formation — is always polluted.

The structural problems arising from this variation may be grouped under three headings: (1) non-stoichiometric apatites, (2) hydrated apatites, and (3) carbonate-containing apatites.

Non-stoichiometric apatites

By this term is meant apatites whose Ca/P mole ratio differs from the theoretical 1.67. The biological apatites — dental enamel, bone, cementum, and dentin minerals — have ratios lower than this value, with dental enamel most closely approximating to the unit cell content.

The non-stoichiometry may be caused by a low calcium content or a high phosphorus content, or a combination of

both. In addition, the biological minerals may contain admixtures of less basic phosphates.

a) The Ca-positions in the unit cell may be occupied by other cations without appreciably affecting the structure. However, the biologically most likely cations do not seem to be very active in this respect; magnesium has been found to be strongly rejected by the apatite lattice (*Neuman & Mulryan*, 1971), and substitution by strontium is restricted (*Lagergren & Carlstrøm*, 1957). The mono-valent Na^+ has an ionic radius (0.97 Å) which is comparable to that of Ca^{2+} (0.99 Å), but the charge does not balance. The K^+-ion is much too large (1.33 Å) to fit into the structure without significant disturbancies.

A low Ca/P ratio may also arise because of random absence of Ca^{2+} in the Hap structure. Some substitution of H_3O^+ or similar ions for Ca^{2+} must then be postulated in order to maintain electrical neutrality. Apatites with such vacancies have been termed »Ca-deficient apatites», (*Posner & Perloff*, 1957).

b) An increased P content may show up in the analyses because of substitution of phosphate ions in the crystal surfaces and of adsorption on the surfaces. In these cases the lattice itself is unaffected. In the surface there is a greater latitude for substitutions than in the interior, and the surface has a net electrical charge which must be balanced by ions of the opposite charge.

Neuman & Neuman (1958) have postulated that the apatite crystals are surrounded by a layer of water, the so-called hydration layer. Ions present in this water shell may also be included in the chemical analyses although the ions have no bearing upon the composition of the structure proper.

These surface processes will be dependent upon the magnitude of the surfaces, and will therefore assume a greater importance for bone mineral (specific surface up to 200 m^2/gm) than for dental enamel (specific surface of 1—3 m^2/gm).

Some attempts have been made to explain a low Ca/P ratio by changes in the PO_4^{3-}-positions. If some trivalent PO_4^{3-} is replaced by divalent HPO_4^{2-}, the phosphorus content would be the same, but some Ca^{2+} would possibly be lost because of the lowered counter-charge. *Simpson* (1968) has suggested that the PO_3F^{2-} ion may effect such a reduction in the minus charge.

c) The observed Ca/P ratio would decrease if Hap were coexisting with some other phosphate (Hap: 1.67, TCP: 1.50, OCP: 1.33, DCP: 1.0). Some of the possibilities will be mentioned later. Note that the presence of Ca-salts other than phosphates will raise the ratio above 1.67, which is generally not observed in biological minerals.

Hydrated apatites

If all the hydroxyl positions in Fig. 3 were filled with OH^- ions, an ideal 1.8 % of H_2O would be obtained. All biological apatites have been found to contain an excess of water which has been a matter of considerable controversy. »Water» in this connection means water which cannot be removed by the usual desiccation procedures.

Some of this water is thought to be extraneously located on the crystal surfaces (hydration layer) or entrapped on internal surfaces. This would especially apply to dental enamel where the water content is only 3—4 %. Some of the water is also considered as essential, i.e. it is an integral part of the lattice itself.

Theoretically, water can be placed as neutral H_2O-molecules in the OH^- sites and elsewhere in the lattice, H_3O^+ ions in the Ca^{2+} sites, and »tetra hydroxyls», $H_4O_4^{4-}$, in the PO_4^{3-} positions (*McConnell*, 1965; *Simpson*, 1968).

Several authors have postulated that the inclusion of water leads to the formation of definite, stoichiometric compounds which are not just substituted apatites. Thus *Dallemagne et al.* (1949) have maintained that bone mineral consists of a hydrated »α-tricalcium phosphate», which has a Ca/P ratio of 1.50. Like bone mineral this phosphate is said to convert to β-tricalcium phosphate upon heating while Hap is unaffected. All the same, »α-tricalcium phosphate» gives the X-ray diffraction diagram typical of apatites, presumably because some water substitute in the lattice according to the formula $3\ Ca_3(PO_4)_2 2H_2O$ or $3Ca_3(PO_4)_2 \cdot H_2(OH)_2$. Note that the designation »α-tricalcium phosphate» is a misnomer since this is the name commonly given to the high temperature form of tricalcium phosphates (cp. later).

Eanes et al. (1965) found that co-called »amorphous calcium phosphate» was first precipitated in carbonate-free, alkaline solutions. This non-crystalline product had a Ca/P ratio of 1.50 which slowly rose to 1.67 when in contact with the precipitation solution. »Amorphous calcium phosphate» seems to have much in common with calcium phosphate minerals classified under »collophane» (*Frondel*, 1943). The biological importance of »amorphous calcium phosphate» is somewhat obscure for the present.

Neither »α-tricalcium phosphate» nor »amorphous calcium phosphate» do possess specific crystallographic properties, and they are not assigned structures of their own. It is possible to estimate the percentage crystallinity, i.e. the distribution of non-crystalline and crystalline phosphate (*Termine & Posner*, 1966), but this percentage would not necessarily indicate that different phosphates were involved. In the same way the conversion of »α-tricalcium phosphate» to β-tricalcium phosphate upon heating is hardly a specific reaction for the former, but rather a reaction common to acidic phosphates containing HPO_4^{2-} groups, and Ca/P ratios lower than 1.67. At about 600° C these groups will react

$$2\ HPO_4^{2-} \xrightarrow{\text{heat}} P_2O_7^{4-} + H_2O$$

The pyrophosphate will in turn react with remaining phosphate to give β-tricalcium phosphate.

Carbonate-containing apatites

Unless special precautions are taken, carbonate is probably always present in synthetic low-temperature apatites. Carbonate-bearing minerals are termed dahllite, monite, ornithite (Hap with CO_2) and francolite, staffelite, nauruite (Fap with CO_2).

Biologic apatites are always formed in CO_2 environments, and chemical analyses show that they contain from 1 % and upward to 7—8 % CO_2. The general effects of carbonates are of two kinds (*Legeros et al.*, 1968). In the first place the carbonate will limit the crystal size, which results in higher dissolution rates on account of increased specific areas. In the second place the crystal morphology is changed. This is important because some faces in an anisotropic crystal are more susceptible to dissolution than others. These disturbancies are enhanced by the presence of Mg^{2+}, and minimized by the presence of F^-.

The carbonate content may be attributed to carbonate adsorbed as HCO_3^- or CO_3^{2-} on the crystal surfaces or entrapped in the interior of the crystals. Such adsorbed or chemisorbed ions will go undetected by X-rays, but may be demonstrated by virtue of their preferential solubility. *Jensen & Møller* (1944) found that all the carbonate in biological apatites could be explained in this way.

In many natural phosphates carbonate is present as $CaCO_3$, calcite (see Fig. 9). Infrared absorption spectroscopy has revealed bands characteristic of calcite in the hard tissues (*Posner & Duyckaerts*, 1954; *Underwood et al.*, 1955), but diffractograms showing calcite lines have apparently not been produced, possibly because the calcite content is below the detection limit of about 3 %.

An old controversial question is whether carbonate can substitute in the apatite lattice or not. It was originally held that a CO_3^{2-} group could replace 2 OH^-ions on the hexagonal axis of the apatite lattice (Fig. 3), giving the formula $Ca_{10}(PO_4)_6CO_3$. In the case of Fap, a location of CO_3^{2-} in the positions mentioned was refuted by *Gruner & McConnell* (1937). However, this kind of substitution is held to be possible by *Winand* (1968).

Most authors hold that PO_4^{3-} may be replaced by CO_3^{2-} (*McConnell*, 1952, 1965; *Simpson*, 1964; *Legeros et al.*, 1968). McConnell proposes that 3 PO_4^{3-} are replaced by 4 CO_3^{2-}. Some H_2O and »tetrahedral hydroxyls», $(OH)_4^{4-}$, are called upon to balance the electrical charge together with H_3O^+ ions. The exact location of the CO_3^{2-} groups is not known. McConnell has suggested that 3 planar CO_3^{2-} may be distributed symmetrically around the hexagonal axis perpendicular to the basal plane, and that the fourth CO_3^{2-} is located on the hexagonal axis parallel to the basal plane, with the disappearance of one OH. If then one H_3O^+ occupies a Ca-position and one »tetrahydryl hydroxyl» a PO_4^{3-} position, the over-all reaction would be given by the following equations:

$$\begin{array}{lllllll} 3\ PO_4^{3-} & + & Ca^{2+} & \rightarrow & 4\ CO_3^{2-} & + & H_3O^+ \\ 9e & - & 2e & = & 8e & - & e \end{array}$$

and

$$\begin{array}{lllllll} PO_4^{3-} & + & OH^- & \rightarrow & (H_4O_4)^{4-} & + & H_2O \\ 3e & + & e & = & 4e & & \end{array}$$

Newesely (1963) holds that the incorporation of carbonate into apatites is not an ordinary substitution according to crystal-chemical rules, but that the incorporation is dependent upon the development of structural faults which must be so diminitive that the structure as a whole is maintained. *Hayek et al.* (1966) and *Newesely* (1967, 1968) have later given vent to the view that carbonate substitutes in the structure of octacalcium phosphate by partially replacing acidic phosphate groups. The final structure has been given the formula $Ca_4(PO_4)_2(HPO_4)_{0.4}(CO_3)_{0.6}$, and is supposed to possess all the characteristica of bone and dentine minerals.

Octacalcium phosphate — $Ca_8H_2(PO_4)_6 \cdot 5H_2O$ — OCP

The existence of OCP as a specific crystalline entity was long obscure, mostly because OCP and Hap have very similar diffraction pattern. They can be distinguished from each other chiefly by the d_{khl} 18.86 Å and d_{hkl} 2.67 Å in the powder diagrams. The crystallographic properties have been determined by *Brown et al.* (1957), *Brown* (1962), and *Brown et al.* (1962).

The biologic importance of OCP depends upon the fact that it is more easily precipitated than Hap although

the latter has the higher, general stability. In the presence of small amounts of fluoride (0.005 ppm) the apatite structure is preferred (*Newesely*, 1960). *Simpson* (1966) observed that aerating the equilibrating systems with 1 and 10 % CO_2 in mixtures of gases, enhanced the formation of OCP. Left to itself in the mother solution OCP would be expected to transform to Hap.

Jensen & Hansen (1957) detected OCP as a minor constituent of calculus, and this observation has been confirmed many times since. *Schroeder* (1969) found that OCP occurred in 95 % of the calculus samples he studied, which was a much higher incidence than any of the other phosphates showed. He regarded OCP as a regular constituent of dental calculus, and not merely »a transitory mineral». These findings are somewhat surprising in view of the fact that Mg-containing solutions like saliva have generally been found to favour the formation of tricalcium phosphate over that of OCP (*Newesely*, 1960).

The evidence for the existence of OCP in hard tissues is largely of a circumstantial nature. *Brown* (1966) has summarized the evidence in seven points:

1) The platy shape of some bone and dentin crystallites favours the view that OCP at least plays some part in the mineralization of these tissues;
2) A Ca/P ratio lower than 1.67 in mineralizing tissue is in accord with the presence of OCP;
3) OCP, but not Hap, is partially converted to pyrophosphate upon heating. It has been observed that the amount of pyrophosphate is greatest in tissues in early stages of mineralization, indicating that OCP is present;
4) In vitro calcifications of cartilage have given products with Ca/P ratios of 1.33 after correcting for the presence of $CaCO_3$;
5) Solubility diagrams have indicated that child bone has a Ca/P of about 1.33, whereas adult bone has a ratio close to 1.67, suggesting that OCP is an intermediary in the formation of Hap;
6) Two diffuse lines in the pattern of fetal enamel have been found to correspond to lines in OCP;
7) The physico-chemical properties of OCP would favour this phosphate as the initial precipitate.

Quicker & Dulce (1967) estimated that the content of OCP in dental enamel was of the order of maximum 1—2 %, and they did not observe any difference between enamel from carious and non-carious teeth.

OCP belongs to space group $P\bar{1}$. This symbol means that the structure is triclinic (inclined in three directions); all the three axes and all the three angles in Fig. 1 are unequal. In addition, the unit cell contains an inversion axis ($\bar{1}$, bar one, or minus one bar), i.e. any atomic group is inverted across a centre.

The unit cell shown in Fig. 7 indicates that the structure may be divided into a water layer and an apatite layer.

The water layer explains the ease with which OCP loses water, and why the compound is so kinetically active. Formulas with 2 moles of water are sometimes given (*Hayek et al.*, 1960) or with 3 moles of water (*Brown et al.*, 1957). This variation reflects the difficulty of establishing a definite degree of hydration. For the present the generally accepted formula is $Ca_4H(PO_4)_3 \cdot 2\frac{1}{2}\ H_2O$ or $Ca_8H_2(PO_4)_6 \cdot 5H_2O$. The latter should probably be preferred because it is consistent with the unit cell content of 5 moles of essential

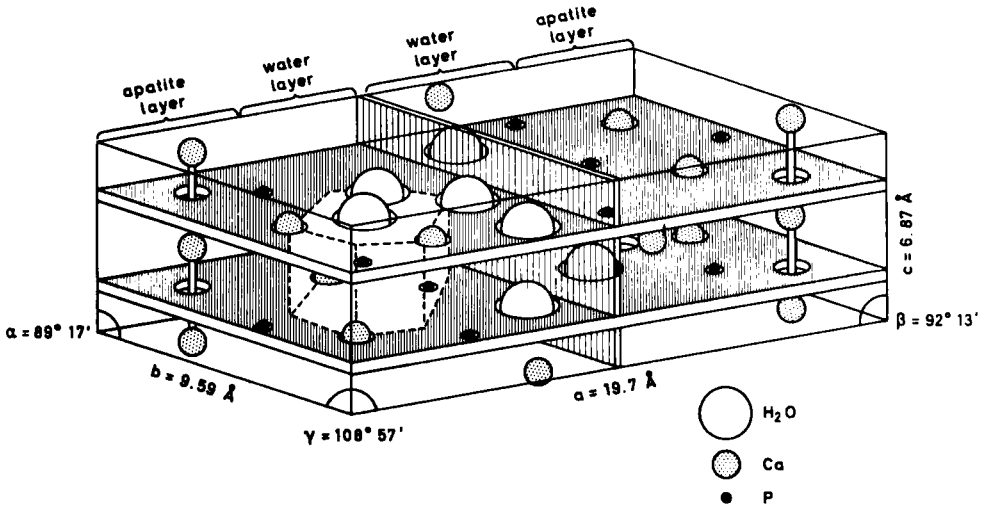


Fig. 7. Unit cell of octacalcium phosphate. System: triclinic. Space group: $P\bar{1}$. z:2. Empirical formula: $Ca_8H_2(PO_2)_6 \cdot 5H_2O$.

water, and because it clearly distinguishes from tetracalcium phosphate, $Ca_4O(PO_4)_2$, which is a member of the two-component system CaO-P_2O_5 and therefore not found in aqueous systems. In Fig. 7 the H_2O molecules are represented by spheres which are not drawn to scale.

The apatite layer has many similarities to the apatite structure. The two columns of Ca_I in apatite are virtually the same in OCP, but the calcium ions are surrounded by seven oxygens rather than the nine they would have in Hap. The most interesting feature is found in that part of the structure which corresponds to the hexagonal axis in apatite. These parts are shown in Fig. 8 projected on the basal plane. In apatite, the three Ca^{2+} at c = 1/4 and the three Ca^{2+} at c = 3/4 form a regular hexagon, with the two OH^- on top of each other in the center of the hexagon. In OCP, two of these hexagonal Ca_{II} are lacking, and the two OH^- are replaced by one H_2O molecule and one oxygen from a PO_4^{3-} group. The resulting arrangement is shown in Fig. 7.

The close structural correspondence between OCP and Hap indicates that they may show epitaxial overgrowths. *Taves* (1963) and *Brown* (1966) have expressed somewhat different views as represented in Fig. 8.

According to Taves the structure will grow as Hap if the corner position of the Hap unit cell is filled with OH^-, and as OCP if filled with oxygen from PO_4^{3-} groups. Under physiologic conditions phosphate ions would be present in higher concentrations than hydroxyl ions, which would make it likely that the formation of OCP is preferred. Phosphate groups are also regarded as crystal poison for the lateral growth of Hap.

Brown (1966) holds that OCP is first formed by a precipitation process, and that unit cell thicknesses of OCP are subsequently converted to Hap by a hydrolysis process. Depending upon the relative rates of precipitation and hydrolysis, the final crystal may vary from pure OCP to pure Hap in a layered structure. Crystals formed in this way will retain the shape of OCP crystals i.e. they will exhibit a platy or blade-like appearance, although

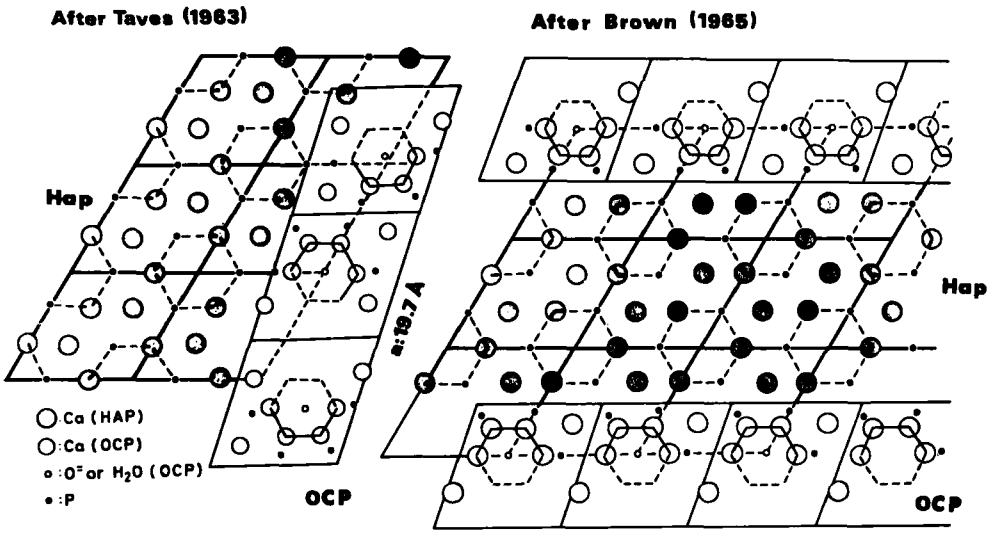


Fig. 8. Hap-OCP epitaxis.

other properties will be those of Hap. Hap crystals formed without OCP as an intermediary will have a needle-like morphology (»acicular»). The presence of small amounts of fluoride is thought to speed up the hydrolysis with formation of apatite. Too high concentrations of fluoride are suggested to accelerate the hydrolysis so much that premature alterations in the precipitated OCP could limit the length and breadth of the final crystal, resulting in mottling of the enamel.

Brown et al. (1962) found that fluoride-treated OCP was very soft and smeared easily. They suggested that fluoride treatment may increase the chemical stability of dental enamel, but decrease its mechanical strength, provided that OCP is present in enamel.

Tricalcium phosphate — β—$Ca_3(PO_4)_2$ — TCP

Water-free phosphates corresponding to the chemical composition $Ca_3(PO_4)_2$ have long been known to be formed in the melt. *Trømel* (1932) found TCP to be dimorphous. The high temperature modification, α-tricalcium phosphate, was produced at about 1200 °C, and changed upon cooling into β-tricalcium phosphate. Note that these phosphates are different from the hydrated »α-tricalcium phosphate» (*vide supra*).

Frondel (1941) reported a Mg-containing TCP mineral which he termed whitlockite. This name is often used as a general designation for TCP without implying that Mg is a constituent. Maritinite, zeugite, and pyrophosphorite are also TCP minerals. These phosphates can form from water solutions under atmospheric conditions of temperature and pressure (*Frondel*, 1943).

Jensen & Rowles (1957a) prepared synthetic TCP by ignition of stoichiometric amounts of $CaHPO_4 \cdot 2H_2O$ and $CaCO_3$. By including $MgCO_3$, they observed a regular contraction of the lattice when the Mg content increased up to about 2.3%. They suggested that Mg^{2+} impeded the growth of Hap crystals by substituting in the surface, whereas this ion promoted the formation of TCP. *Ando*

(1958) found that magnesium stabilized β-tricalcium phosphate against both the transformation to α-tricalcium phosphate and against dissolution in citric acid.

In aqueous systems TCP, instead of the normal Hap, may precipitate in the presence of Mg^{2+} (*Hayek & Newesely* 1958). A similar effect is obtained when Fe^{2+}, Mn^{2+}, and Al^{2+} are added.

TCP is a normal constituent of dental calculus (for references, see *Schroeder* 1969). It is more frequent in subgingival than in supragingival calculus possibly because the former has a higher Mg content. *Schroeder* (1969) found that the incidence of whitlockite was higher in old than in young calculus. He suggested that TCP was formed from initially precipitated $CaHPO_4 \cdot 2H_20$ (brushite) by the induction effect of Mg^{2+}. *In vitro* experiments have indicated that $CaHPO_4$ (monetite) is an intermediary in the formation of TPC (*Hayek & Newesely* 1958).

TCP is quite common in calculi from the sublingual gland (*Jensen & Danø* 1952) and may be observed in concrements of the urinary tract (*Jensen & Thygesen*, 1938; *Jensen*, 1941). It is occasionally present in other pathological calcifications (*Albertini et al.*, 1946; *Brandenberger & Schinz*, 1945; *Frondel & Prien*, 1946; *Morris & Beeler*, 1967). The so-called »caries crystals» observed in dentinal caries are considered to be whitlockite (*Newesely*, 1972).

The chemical composition and the crystal structure of TCP have presented some difficulties. *Keppler* (1963, 1965, 1968) has produced hydrated phosphates with a whitlockite structure, and he has suggested that Fe^{2+} or Mg^{2+} is connected to acidic phosphate groups according to the formula $3\ Ca_3(PO_4)_2 \cdot MgHPO_4$. However, *Newesely et al.* (1964) have confirmed that whitlockite is essentially water-free, and *Wallace & Brown* (1971) found a Ca/P mole ratio of 1.50 for whitlockite indicating a formula of $Ca_3(PO_4)_2$.

The structure has a rhombohedral lattice which is denoted by the letter R. The rhombohedral unit cell may be regarded as being produced from a regular cube which has been drawn out in the way illustrated in Fig. 9 for $CaCO_3$. The parameters are given by the edge length ($=a_r$) and the inter-edge angle ($=\alpha_r$). As indicated in Fig. 9 this rhombohedron may also be regarded as being part of a usual hexagonal unit cell characterized by the hexagonal parameters. The c-axis is the same for the rhombohedral and hexagonal structures.

The rhombohedral lattice has always a triad, vertical axis which may be of different kinds. *Frondel* (1941) originally found that whitlockite has a so-called *inversion axis* ($\bar{3}$) which means that the three equivalent point groups may be inverted across a center of symmetry to give enantiomorphous point groups. However, whitlockite has later been shown to possess piezoelectric and pyroelectric phenomena which are possible only when centers of symmetry are lacking. The axis in whitlockite must therefore be a regular triad axis denoted by 3.

Whitlockite has also a glide plane, which was described in Fig. 6. In monoclinic Hap the glide plane is perpendicular to the screw axis, and this relationship was symbolized by $2_1/c$ (with a stroke). In whitlockite, however, the glide plane is parallel to the triad axis, and the symbol becomes 3c (without a stroke).

Whitlockite — β-tricalcium phosphate — is therefore referred to space group R3c.

The unit cell of TCP, whitlockite, has been drawn with hexagonal coordinates

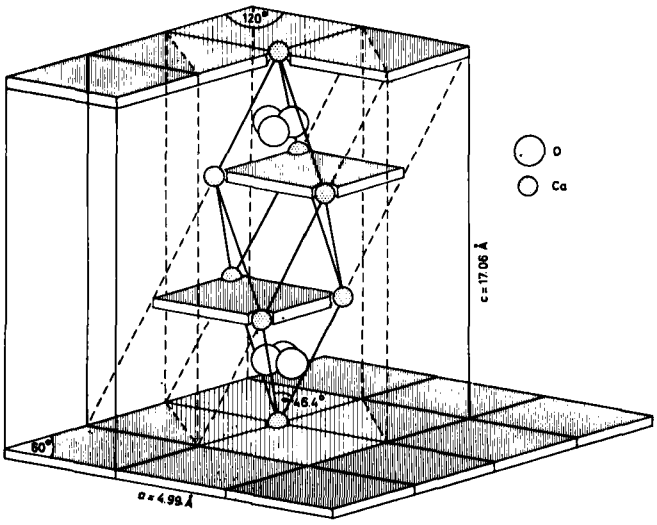


Fig. 9. Unit cell of calcite. System: rhombohedral. Space group: $R\bar{3}$ 2/m. z:2. Empirical formula: $CaCO_3$.

in Fig. 10. Lattice parameters have been given by *Frondel* (1941) and *Jensen & Rowles* (1957b). The structure is extremely complex, and it is so difficult to simplify that even the main features are not readily comprehended. The structure may be though of as consisting of twelve columns parallel to the c-axis, each of them containing Ca^{2+} ions and PO_4^{3-} tetrahedra in a somewhat irregular fashion. Available evidence indicates that about 10% of the Ca^{2+} ions are replaced by Mg^{2+}. The unit cell has generally been found to contain 21 Ca^{2+} and 14 PO_4^{3-} tetrahedra, corresponding to 7 $Ca_3(PO_4)_2$. However, when glide planes are present in the structure, the number of atoms must be even, i.e. the formula must be 8 $Ca_3(PO_4)_2$. The explanation offered for the discrepancy is that some type of statistical distribution takes place. The atomic positions given in Fig. 10 may be regarded

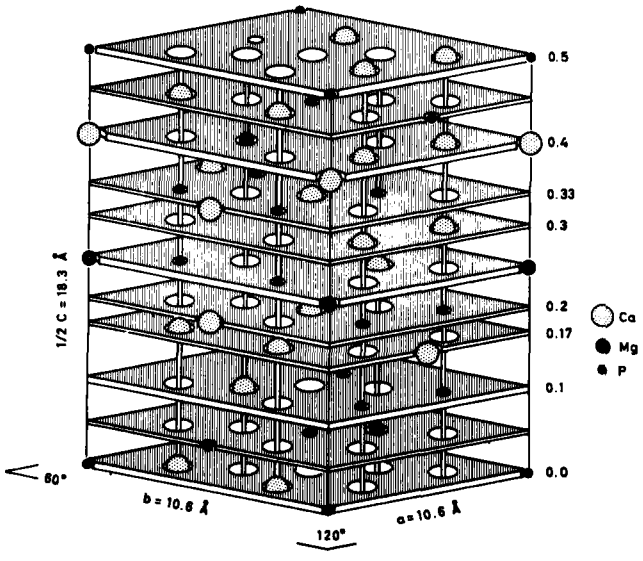


Fig. 10. Unit cell of whitlockite. System: rhombohedral. Space group: R3c. z:8. Empirical formula: β-$Ca_3(PO_4)_2$. The unit cell has been constructed from the parameters determined by Dr. Bryan Dickens, National Bureau of Standards, Md., USA.

as possible sites for occupancy, a large number of the positions being vacant in the actual crystal.

Dicalcium phosphate dihydrate — $CaHPO_4 . 2H_2O$ — DCPD

DCPD occurs in nature as the minerals brushite, epiglaubite, and stoffertite. These minerals may vary somewhat as to the degree of hydration.

Neuman & Neuman (1958) maintained that DCPD plays an all-important role in nucleation processes, and this view has been supported in many quarters, e.g. *Sobel & Laurence* (1960), *Brudevold et al.* (1965). However, the existence of DCPD has never been shown in normal hard tissues.

DCPD may occasionally be found in extopic mineralizations (*McCarty & Gatter*, 1963) and in urinary calculi (*Morriss & Beeler*, 1967), and it is fairly common in dental calculus, especially in young and supragingival specimen (*Schroeder*, 1969).

DCPD has a somewhat narrow stability field around pH 5—6, which fact might contribute to explaining that the compound is not normally formed from the more alkaline tissue fluids. The precipitate will slowly convert to OCP and eventually to Hap, and, in the presence of Mg^{2+}, possibly to TCP. *Legeros et al.* (1967) maintain that brushite would be easily transformed to Cap in biological systems.

Lattice parameters are given by *Beevers & Raistrick* (1954), and a complete structure determination has been made by *Beevers* (1958) who assigned the structure to the monoclinic space group 12/a.

The monoclinic lattice was referred to in Fig. 6. The three axes in the unit cell are all unequal in length, and the angles α and γ are 90°. The angle β is by convention always obtuse so that the plane (001) is sloping towards the observer. Whereas in the other six crystal systems the symmetry axes are usually regarded as being parallel to the c-direction of the unit cell, the symmetry axes in the monoclinic system are regarded as parallel to the b-direction. The b-axis is often termed the »orthoaxis», and the a-axis the »clinoaxis». The symmetry planes in the monoclinic system are also vertical, and not horizontal as they are generally depicted.

The symbol 2/a therefore means that the structure of DCPD has a rotation diad axis along the b-direction, crossing a vertical symmetry plane. It is implied in this arrangement that also a center of symmetry is present. Furthermore, the symmetry planes are glide planes, i.e. the point groups are first moved a distance equal to half of the a-length of the unit cell and then reflected across a vertical, regular mirror plane. The letter »I» denotes that the lattice belongs to the monoclinic system with a point group in the center of the unit cell. These main features of the structure are brough out in Fig. 11.

Jones & Smith (1962) observed positive piezoelectric effects in DCPD which means that a center of symmetry is absent. The diad rotation axis must therefore be lacking, and the space group is Ia with only a glide plane. The departure of atoms from centrosymmetric positions is probably not large.

A prevalent misconception has been that DCPD has a much less complex structure than Hap and that, as a consequence of this presumed simplicity, DCPD is more likely to form initially in biological systems. A comparison of Figs. 3 and and 11 will show that Hap and DCPD are not much different with regard to complexity.

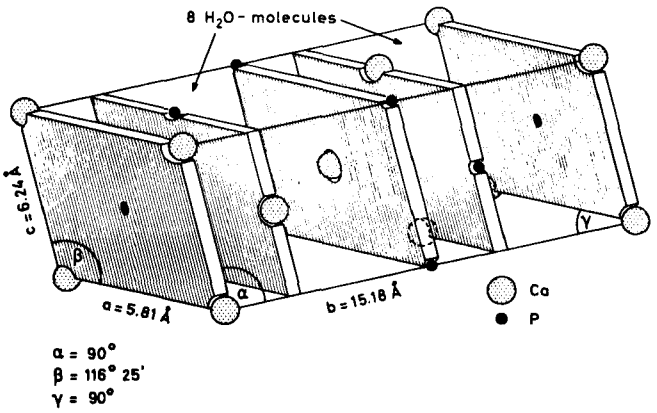


Fig. 11. Unit cell of brushite. System: monoclinic. Space group; I2/a, z:4. Empirical formula: $CaHPO_4$: $2H_2O$.

Dicalcium phosphate anhydrous — $CaHPO_4$ — DCPA

DCPA occurs in nature as the mineral monetite. Like all phosphates it is used as a constituent in superphosphate fertilizers.

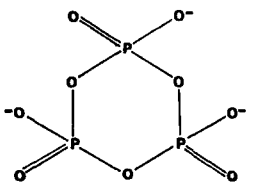
Its occurrence in the human body is uncertain. *Westerden & Little* (1958) seem to have observed DCPA in dental calculus, and *Jones & Cruickshank* (1961) suggest that it is involved in the mineralization of bone. It may have some biological significance that DCPA is transformed to carbonate-containing apatites in the presence of CO_2 (*Legeros et al*, 1967). If OCP takes part in mineralizing processes, it is noteworthy that DCPA may form as a separate phase in the hydrolysis of OCP (*Brown et al.*, 1962). *Hayek & Newesely* (1958) suggested that DCPA may be more stabile than DCPD at temperatures above 35°C. It may be noted that DCPA is easily overlooked in powder diffractograms of apatitic materials because many of the lines coincide with those of Hap and OCP.

The crystallography of DCPA has been studied by *Smith et al.* (1955) and *Mac Lennan & Beevers* (1955). *Jones & Cruickshank* (1961) have made further refinements of the structure. All agree that DCPD belongs to the triclinic class with space group $P\bar{1}$, and that it thus possesses the same symmetry as OCP. A schematic representation of the unit cell is given in Fig. 12.

Condensed phosphates and fluorite

Condensed phosphates have PO_4^{3-} tetrahedra linked together by oxygen bridges. They are divided into three categories: polyphosphates, metaphosphates, and ultraphosphates (*Mooney & Aia*, 1960). The last-mentioned category has probably no biological significance.

Metaphosphates have the general formula $M_nP_nO_{3n}$, where M is a monovalent cation. Sodium metaphosphate, $(NaPO_3)_n$ has been used in dentifrices as an abrasive. The metaphosphates make up ring (cyclic) structures:



Trimetaphosphate, $M_3P_3O_9$

Polyphosphates, sometimes ambiguously termed polymetaphosphates, have the general formula $M_{n+2}P_nO_{3n+1}$, where M

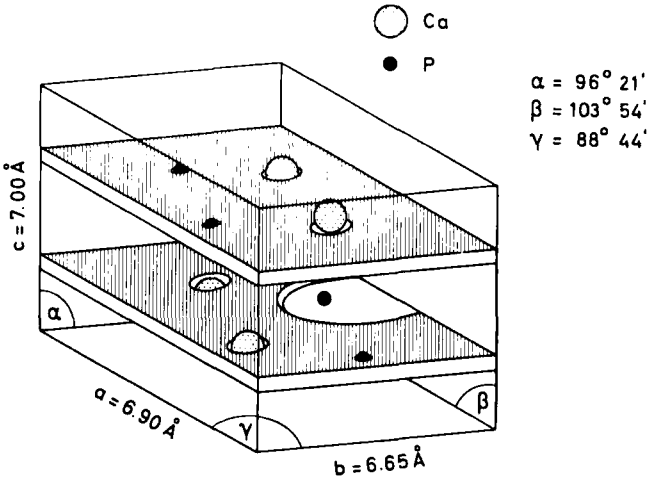
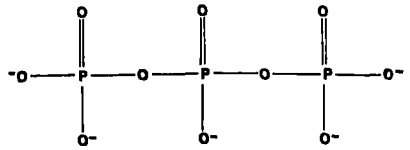


Fig. 12. Unit cell of monetite. System: triclinic. Space group $P\bar{1}$. z:4. Empirical formula: $CaHPO_4$.

is a monovalent cation. The polyphosphates have attracted some biological interest as a possible source (»phosphagen») for the formation of adenosin triphosphate. Pyrophosphate, $Ca_2P_2O_7$, has been proposed to act as a coating on apatite crystals impeding their growth as well as their dissolution (*Fleisch et al.*, 1966), and might therefore be of some consequence for the carious process. The content of pyrophosphate in the hard tissues is exceedingly small (*Newesely*, 1967).

Polyphosphates have a linear structure:



The unit cell parameters and the space group of $Ca_2P_2O_7$ have been determined by *Corbridge* (1957). The structure belongs to the tetragonal system with a tetrad screw axis ($P4_1$). The unit cell contains 8 $Ca_2P_2O_7$-groups, but the atomic positions have not been determined.

A number of crystalline forms have from time to time been reported to be present in the hard tissues. So *Torell* (1960) observed the occurrence of cubic $KCaF_3$ in areas of arrested caries, and *Berndt* (1970) reported that special crystalline products were formed when Hap was added to SnF_2 solutions.

Fluorite, CaF_2, seems to have more than sporadic interest since it is frequently formed on the enamel surface after topical applications of fluoride. Fluorite is the preferred solid phase in biological environments when either of two conditions are met: 1) the fluoride concentration must be high relative to the phosphate concentration, 2) the pH is low, i.e. below 5.0. These conditions are probably only obtained in the oral cavity, and may be of advantage with a view to the initial fixation of fluoride to the enamel surface. The crystal structure of CaF_2 is well known. It crystallizes in space group Fm3m (Fig. 13). The unit cell is face-centered (F) and has both horizontal and vertical mirror planes, with triad axes going diagonally through the unit cell.

The hard tissues

All hard tissues give an apatitic pattern upon diffraction, but the lines are so dif-

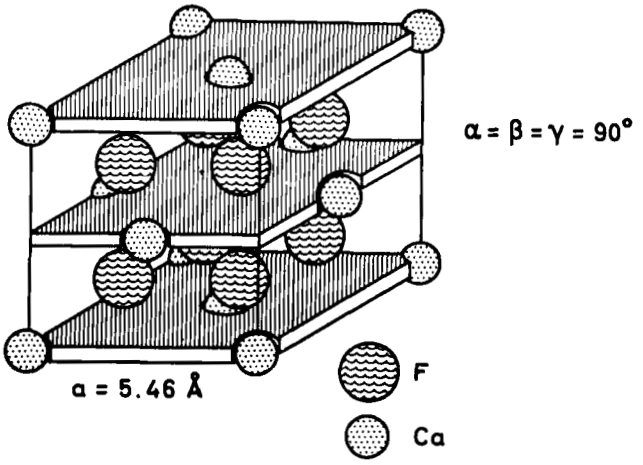
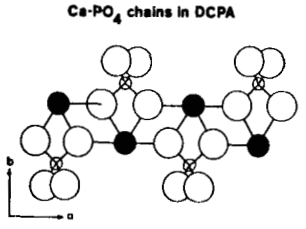


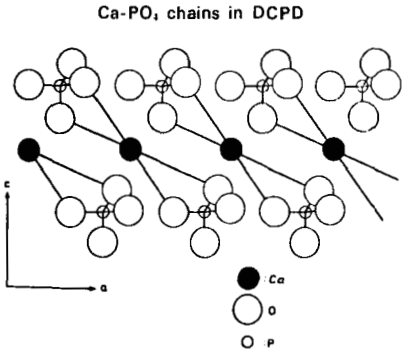
Fig. 13. Unit cell of fluorite. System: isometric. Space group: Fm 3m. z:4. Empirical formula: CaF_2.

fuse that they can hardly be conclusive as to what kind of phosphate is present. An additional difficulty is the structural similarities between the phosphates. Although the similarities probably lead to true epitaxial overgrowth only in the case of Hap and OCP (Fig. 8), they may nevertheless have a mutual orientation effect, because of the layered character of the phosphates.

In DCPA the Ca^{2+} ions and the PO_4^{3-} tetrahedra make up double chains extending along the a-axis. These chains are linked together to form distorted sheets of atoms in the horizontal (001) plane (Fig. 12). Projected on this plane (»viewed from above») the arrangement will be (*MacLennan & Beevers*, 1955):



Similar sheets of Ca^{2+} ions and PO_4^{3-} tetrahedra are present in DCPD which may be inferred from Fig. 11. The arrangement within these sheets, which are parallel to the (010) planes, are (*Beevers*, 1958):



Brown et al. (1962) point out that certain structure elements in OCP and DCPA are so closely similar that these two phosphates have a definite orientation effect upon each other. In both Hap and OCP calcium-phosphate chains stretch out along the crystallographic c-axis.

The identification of the minerals in hard tissues is also hampered by the possibility that layers of good crystallinity may intermingle with layers of poor crystallinity. This arrangement has been termed paracrystalline.

Under these circumstances the chemical composition of the hard tissues will

Table I. *Composition of extracted bovine cortical bone and dental enamel as compared to Hap*

	Cortical bone*			Dental enamel**			Hap	
	%	mEq/gm »Hap»	mEq/gm »No Hap»	%	mEq/gm »Hap»	mEq/gm »No Hap»	%	mEq/gm
Cations								
Ca	26.20	12.90	0.200	36.19	18.10	—	39.84	20.00
Mg	0.42	—	0.348	0.47	—	0.38	—	—
Na	0.99	—	0.432	0.57	—	0.25	—	—
K	0.31	—	0.080	0.30	—	0.08	—	—
Fe,Zn,Mb					appr.	0.08		
TOTAL		12.90	1.060		18.10	0.79		20.00
Anions								
P	12.06	—	—	17.23	—	—	18.52	—
as PO_4^{3-}	—	11.57	—	—	16.29	—	—	18.00
as HPO_4^{3-}	—	—	—	—	—	0.26	—	—
CO_2	4.58	—	—	2.77	—	—	—	—
as HCO_3^-	—	—	1.040	—	—	0.45	—	—
Cl	0.05	—	0.013	0.25	—	0.07	—	—
F	(1-200 ppm)	—	—	0.02	—	0.01	—	—
$Cit^{=}$	0.04	—	0.004	—	—	—	—	—
OH^- estim.	2.20	1.33	—	2.83	1.81	—	3.39	2.00
TOTAL		12.90	1.057		18.10	0.79		20.00
mEq.cations/ mEq.anions	—	*1.00*	*1.003*	—	*1.00*	*1.00*	—	*1.00*
Ca/P weight	2.16	—	—	2.10	—	—	2.15	—
O^{2-} in PO_4^{3-}	24.90	—	—	35.57	—	—	39.84	—
O^{2-} in HCO_3^-	6.35	—	—	3.84	—	—	—	—
% analysed	78.10			100.04			100.00	
Max. % as Hap	64.89			83.50			100.00	
Max. % as Cap	78.62			47.55			0.0	
Max. % as Clap	0.76			3.71			0.0	
Max. % as Fap	(0.50)			0.53			0.0	

* Calculated from data of *Armstrong & Singer, loc.cit*

** From *Hagen* (1965) *loc.cit.*, covering the literature up to 1960.

throw valuable light on the question about the nature of the mineral phase.

In Table I tabulations regarding bovine bone and human, permanent dental enamel have been given. *Armstrong & Singer* (1965) made a very thorough analysis of KOH-ethylene glycol extracted bone from bovine cortex, and the data for dental enamel are the means of practically all determinations made on that tissue up to 1960 (*Hagen*, 1965).

A comparison brings out the large dif-

ferences between the mineral phase of bone and dental enamel. Considerable parts of the bone chemistry is apparently unaccounted for, whereas the means for dental enamel make up a neat 100% figure.

On the supposition that the backbone mineral in both tissues is Hap, 83% of dental enamel and 65% of bone mineral may possibly consist of this phosphate. Assuming the presumably extraneous ions to be in electrical balance, the remaining Ca/P ratios compare favourably with that of Hap. In general, the ratio for bone has been found to be closer to that of TCP, namely 1.94, than is the case here.

Theoretically, 78% of the bone mineral may be Cap — a higher percentage than if Hap only were present — and 47% of the enamel tissue.

The fluorine content is so low that a maximum of about 0.5% of Fap can be present, possibly rising up to as much as 10% in the outermost layers of dental enamel. From a structural point of view, it therefore seems unlikely that the pronounced effect on hard tissue metabolism by fluoride can be referred to a formation of Fap at the expense of Hap. A more likely explanation seems to be that fluoride acts as a catalyst in the structuring of the minerals such as has been mentioned above, or that fluoride effects a diffusion blockage when present in the calcium triangles of Hap (*Young et al.*, 1969).

The content of Cl- is apparently much higher in dental enamel than in bone mineral, the theoretical content of Clap being 3.7% and 0.8%, respectively. *Dykes & Elliott* (1971) actually proposed that Clap is present in dental enamel.

As for dental enamel, there seems to be a good agreement between the chemical composition and the hypothesis that this tissue is essentially Hap with adsorptively bound, extraneous ions. Other pieces of evidence point in the same direction. The large crystallites in dental enamel have in general been found to be rod-like such as is the case for Hap, and the unit cell parameters are indistinguishably similar to those considered typical of Hap (Fig. 4).

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