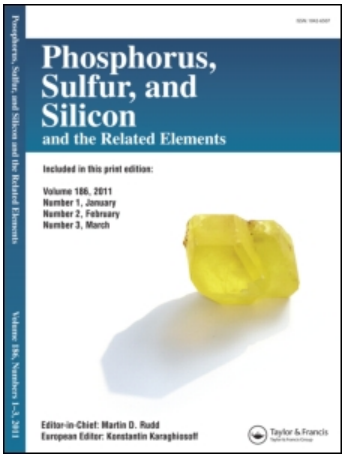


**Phosphorus, Sulfur, and Silicon and the Related Elements**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618290

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**To cite this Article** Landis, William J.(1999) 'The Nature of Calcium Phosphate in Mineralizing Vertebrate Tissues', Phosphorus, Sulfur, and Silicon and the Related Elements, 144: 1, 185 — 188
**To link to this Article: DOI:** 10.1080/10426509908546213
**URL:** http://dx.doi.org/10.1080/10426509908546213

*Phosphorus, Sulfur and Silicon*, 1999, Vol. 144-146, pp. 185-188
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# The Nature of Calcium Phosphate in Mineralizing Vertebrate Tissues

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This presentation will describe and summarize aspects of the chemical, biological and physical nature of the mineral deposited in the normal vertebrate skeletal and dental systems. Bone, calcifying cartilage and tendon, dentin, cementum and enamel mineral is a calcium phosphate, most often referred to as apatite and commonly denoted as $Ca_{10}(PO_4)_6(OH)_2$. The mineral is composed of crystals of a unique size and shape, having an unusual chemistry including ~3–5% carbonate and 5–10% $[HPO_4]^{2-}$, and serving as a reservoir for other cationic and anionic species. Apatite composition varies in the type and degree of ion substitution, number of atomic vacancies, crystallinity, and age and maturation. Nucleation, growth, and development of the crystals are mediated by organic matrix molecules specific for each tissue, notably collagen in all except enamel. Amelogenin protein modulates apatite formation in part in the latter tissue. The interactions between such organic molecules and the mineral crystals are complex and not entirely understood, but they ultimately lead to organic-inorganic composites with remarkable integrity, mechanical strength, distinct form and additional physical properties that are functionally representative.

*Keywords:* Calcium phosphate; apatite; mineralization; collagen; skeletal tissues; dental tissues

## INTRODUCTION

The normally mineralizing vertebrate tissues include bone, calcifying cartilage, calcifying tendon (especially among avian species), dentin, cementum, and enamel. All share the feature that their mineral component is a calcium phosphate salt identified as apatite or dahllite[1] and classically determined structurally as $Ca_{10}(PO_4)_6(OH)_2$. With the exception of enamel, all the tissues also have in common the presence of collagen as the principal constituent of their organic matrix. Enamel consists primarily of amelogenin protein in the early stages of this tissue formation. The nature of calcium phosphate and its growth and development in each of the vertebrate tissues, including enamel, are highly controlled and mediated by their respective organic matrix constituents. This report presents a summary overview of the mineralization of vertebrate tissues with

emphasis on the means by which calcium phosphate nucleates, grows, and develops in the vertebrate skeletal and dental systems.

## REMARKS

### Mineral Composition, Size, and Shape and their Biological Implications

Biological mineralization is a complex series of events occurring between certain organic and inorganic components of a tissue or organism at various orders of its structure. Such mineralization is specific in the manner in which crystals are deposited, grow, and progress in number, size and mass. For the vertebrate tissues in general, mineral nucleation is located outside the confines of a cell and it is compartmentalized in regions of restricted fluid (serum) flow and small volume. Fluid levels in these and other extracellular regions are supersaturated in $[Ca^{2+}] \times [(PO_4)^{3-}]$ with phosphate, rather than calcium, acting as the critical factor limiting the biological activity of many mineralization processes. The nucleation events are the result of heterogeneous reactions[2] in the biological tissues and lead to mineral formation in which ~3-5% carbonate is substituted for $[PO_4]^{3-}$ or $[(OH)_2]$ groups of the apatite[3]. Compositionally, then, the structure of apatite may be varied and can become complicated even more with substitutions for either of these groups by citrate, chloride and additional anions and for $Ca^{2+}$ by sodium, magnesium, and many other cations. Further, the phosphate may take the form of $[H_2PO_4]^-$ or $[HPO_4]^{2-}$ in addition to $[PO_4]^{3-}$, and those $[HPO_4]^{2-}$ groups, at least in bone, are spectroscopically unique when compared to other calcium phosphates of the vertebrates[4]. Indeed, in young bone, the $[HPO_4]^{2-}$ groups resemble a synthetic $[HPO_4]^{2-}$ species that has a brushite-like ($CaHPO_4{\cdot}2H_2O$) structure[5]. The content of $[HPO_4]^{2-}$ amounts to ~5-10% of bone mineral and, like carbonate, changes with both the age and maturation of the mineral and the tissue it comprises[5,6]. An added level of structural complexity of vertebrate mineral occurs in the identification of non-apatitic or labile carbonate or phosphate groups in early mineral deposits of bone[7,8], forming enamel[7,8] or calcifying cartilage[9] and in the failure to detect hydroxyl groups in bone apatite[10]. While subtle, these compositional and structural features of the mineral are extremely important since they affect its physicochemical reactivity and ultimately the biological function particular to the tissue that is calcified.

Besides having the non-stoichiometric, defect structure just described, apatite is also unusual in its crystal size and shape. In the collagenous-based tissues, the crystals principally are irregularly-shaped, thin platelets, among the smallest found in nature, on the order of 45 x 30 x 2-4 nm[11-13]. Enamel crystals vary in length and have their other dimensions about an order of magnitude larger than those just given; thus, enamel takes the shape of a long, narrow ribbon. Such platelets and ribbons possess extensive sur-

face areas (~150-200 $m^2/g$ for the former[1]), which serve as locations for the substitution and exchange of ions mentioned earlier. Ion substitution and exchange directly affect the solubility character of the mineral, its capacity to store or release the ions into the extracellular fluid, and its mechanical properties[2,10]. In addition, the large surface areas greatly facilitate adsorption and desorption of proteins and other organic constituents of the tissues[2]; these and related surface interactions between crystals and organic molecules and macromolecules also influence the functional properties of the tissues, including, for example, their mechanical nature and their ability to control the mineralization process through osteoclast resorption or osteoblast deposition of matrix. The crystallographic c-axes of platelets and ribbons generally follow the longest crystal dimension[14] and, in the collagenous-based tissues, are parallel to one another and to the collagen long axes associated with them (see below)[2,12]. This feature underlies the strict specificity between the crystals and collagen that consequently dictates the singularly distinct mechanical, biological and physicochemical properties of the tissues.



Mineral Formation

The formation (nucleation, growth, and development) of mineral in all vertebrates is carefully controlled by certain organelles or components particular to the organic matrices of the tissues. As noted previously, mineral formation is generally confined to extracellular portions of the tissue and compartmentalized within regions having restricted fluid flow and small volume. Amelogenin protein appears to mediate early enamel mineral formation[15] and collagen mediates mineralization in the other normally calcifying vertebrate tissues[2,10,12,16]. Proteins other than amelogenin and collagen may also be important in the vertebrate mineralization processes[17]. The role of collagen is currently the best understood with respect to mineral formation. Here, specific regions of collagen, its so-called hole and overlap zones[16], appear strictly in register and form narrow channels throughout the protein assemblages[12,18]. These meet the criteria of compartmentation having limited fluid flow and small volume and apatite crystal nucleation and growth would occur within these sites[12]. Development of crystals within the constraints of the collagen structure results in their preferential growth lengthwise along the long axis direction of the protein and widthwise along the channels[12,18]. Accommodation by collagen in this manner yields thin crystal platelets having preferred c-axial orientation along the collagen long axis. Progressive development of the crystals is suggested to produce parallel sheets of mineral that putatively overgrow the collagen chains and provide a mineral-organic matrix composite[12]. In this manner, the crystal size and shape and its location, orientation, alignment, and distribution with respect to collagen can be modeled. In a similar structural analysis of forming enamel, interrela-

tions among the early mineral ribbons and organic matrix components in this tissue are being examined[19]. These studies combined with compositional details should provide added insight into the character of calcium phosphates during vertebrate mineralization

ACKNOWLEDGMENT

The author is grateful to Mrs. K. Hodgens (Children's Hospital, Boston) for technical assistance over many years and to the National Institutes of Health for support of research through grants AR 41452, AR 34078, and AR 34081.

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