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An investigation into the architecture and composition of a urinary calculus

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A bladder stone of diameter of 16 mm was sectioned to reveal an internal structure showing some complexity. Visually apparent were concentric rings divided into two principal zones. The outer zone comprised large single interlocking crystals, but the inner zones consisted of a much more fragile environment in which large single crystals were embedded in a much finer matrix. More detailed analysis was performed on the stone's layers using X-ray diffraction which is capable of identifying the crystallites but may also be used to elucidate some of the crystallite characteristics and thereby provide evidence for the growth environment. Proton induced X-ray emission was carried out at a number of points along a radius of the sample to examine the distribution of key trace elements. A possible mechanism for the influence of this banding on the fragility of the stone is discussed.

1. Introduction

In humans, the presence of urinary stones disease is a substantial burden to the health service [1] and is a major contribution to the morbidity of a population. Apart from severe discomfort, in extreme cases uroliths can lead to renal dysfunction and for this reason an understanding of the genesis and development of urinary stones is highly desirable in that it may thereby become possible to restrict or prevent urolith growth *in vivo*.

The architecture of uroliths in humans [2] and small animals is very similar and hence comparable comments to those above apply equally to veterinary applications [3, 4] where the incidence of stones may exceed the average of 2–3% found in humans. The incidence of stone formation in canines is very sex and species specific, as is the type of stone formed. One important difference between human and canine stone growth is that there is evidence to suggest that the growth of canine stones can be retarded by the choice of an appropriate dietary regime [3, 4]. Only anecdotal accounts imply that the same is true for humans.

Detailed analysis of the crystalline constituents of uroliths has been used by some authors to infer stages in the growth of uroliths [5–7], and this paper is a step toward establishing a link between diet and stone formation.

2. Method

Two techniques were selected for this study, namely X-ray diffraction (XRD) and proton induced X-ray Emission (PIXE). XRD is a sensitive technique for unambiguously identifying the phase composition of the uroliths, and PIXE is a powerful tool for identifying the presence of trace elements. A further consideration was also relevant when the techniques were chosen. The XRD technique chosen was powder diffraction and requires the removal of a small amount of the urolith which is subsequently ground to a fine powder in an agate mortar and placed on a rotating stage following the familiar powder diffraction technique. This has the disadvantage that precise spatial localization is difficult and information pertaining to the orientation of the crystallites in the sample is lost.

PIXE analysis was performed on the intact sample and hence the spatial orientation was not lost although the disadvantage in this case was that the technique reveals the elements present, not the identification of a particular phase.

A complete canine urolith was obtained by surgical means and was subsequently sterilized using a high dose-rate cobalt source. This method was chosen since chemical or thermal methods of sterilization may effect the presence of the phases or introduce significant errors in the PIXE analysis. The PIXE technique uses accelerated protons which impact on the target material being analyzed. The impact of the protons causes X-ray emission, the energy of which are characteristic of the elements comprising the target. The beam diameter used for this study was 0.5 mm.

Sectioning of the stone was achieved by use of a high-speed rotary saw, but despite precautions the stone did

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partly fragment during this stage of processing. However, samples were obtained which consisted of complete segments thus permitting a detailed study across the stone radius whilst maintaining the spatial arrangement of the constituent layers.

3. Results

XRD of a sample removed from the stone is shown in the upper section of Fig. 1. Comparison with the archive XRD pattern for struvite in the lower section of Fig. 1, indicates that the primary constituent is struvite. The presence of additional peaks on the upper diffraction pattern indicated by the solid arrows which are either broader or substantially taller than the struvite peaks on reveals that a second phase is present which can be identified from archive data to be hydroxyapatite.

An optical micrograph of one of the urolith segments is shown in Fig. 2. Visual inspection at this magnification revealed a core zone comprising large crystals ($\approx 500\,\mu\mathrm{m}$) loosely embedded in a powdery matrix resulting in a composite of very low strength. The overlying banded region could be easily seen to be discrete from the core region.

It is possible to hypothesize the nature of the layering observed in the outer zone of the urolith. Either the same crystalline material is present throughout the stone, and the variation in appearance is as a function of crystallite order, size and incidence of organic matrix, alternatively, there are differences in the materials present. PIXE can be used to differentiate between these two possibilities. Of particular interest to this investigation was the presence of calcium, phosphorous and magnesium since these are the major constituents of the materials commonly found in uroliths.

Fig. 3 shows the PIXE analysis of Ca, P and Mg at equally spaced points (pitch of 0.5 mm) along a radius and include the stone center and stone edge.

The calcium phosphorous ratio provides some information about the form in which the calcium is present, so, for instance, this ratio would be different if the calcium is present as hydroxyapatite rather than oxalate. XRD has indicated that oxalates, although a common constituent of uroliths, are not present in a measurable quantity in this sample. Table I shows the mass percentages of Ca, Mg and P for struvite and hydroxyapatite and also the calcium oxalate ratio for each.

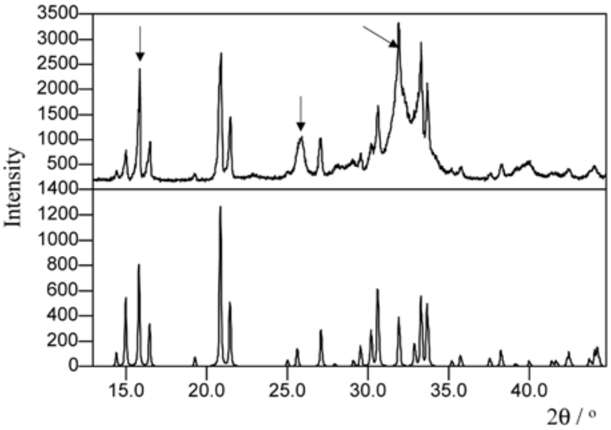

Figure 1 The X-ray diffraction pattern for a sample removed from the urolith under discussion. The arrowed peaks in the upper portion include intensity from hydroxyapatite.

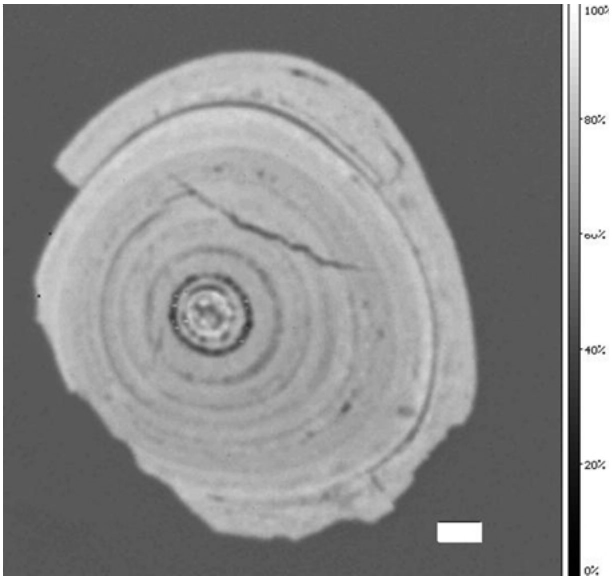

Figure 2 Optical micrograph of the urolith. The core region is visible in the top left corner. The white bar represents 1 mm.

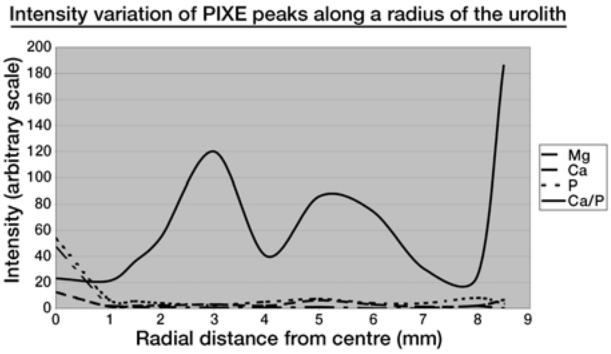

Figure 3 The PIXE data for calcium, magnesium and phosphorous along the radius of the urolith segment. The calcium/phosphorous ratio is shown as a percentage.

TABLE I Summary of the percentage by mass of the three elements of interest

Mineral name	Stochiometric formula	%Ca	%Mg	%P	Ca/P
Struvite	$MgNH_4PO_4 \cdot 6H_2O$	0	9.8	12.7	0
Hydroxyapatite	$Ca_{10}(PO_4)_6(OH)_2$	39.8	0	18.5	2.15

4. Discussion

The X-ray diffraction data shown in Fig. 1 indicates that there are two phases present in this urolith, but does not provide any evidence for where these materials are to be found. The PIXE data and the data contained within Table I can be used to derive this valuable information. Ca/P ratios close to 0% indicate that there is little hydroxyapatite in that location and conversely ratios tending toward 2 indicate that hydroxyapatite does occur. Examination of Fig. 3 clearly shows that the Ca/P ratio varies between extremes of around 20% at radii of 0–1 mm, 7.5 mm and values of 100% or greater at radii of 3, 5 and 8.5 mm. The implication from this is that hydroxyapatite is not common in the urolith core, but is found in three bands; at 3, 5 mm and the outer surface. A low Ca/P ratio does not, however, exclusively indicate the presence of struvite in the core since this region could comprise the organic matrix at the exclusion of crystalline components albeit this is unlikely. Further evidence for the presence of struvite in the core is derived from the Mg PIXE signal since stochiometric hydroxyapatite contains no magnesium. It is evident from Fig. 3 that there is an elevated signal from Mg in the core region in comparison to the rest of the stone thus strongly suggesting the prevalence of struvite.

The very high Ca/P ratio at the edge of the urolith, i.e at 8.5 mm can only be explained by the presence of hydroxyapatite. Indeed such a high value for this ratio at this point strongly implies that hydroxyapatite is the primary phase since the XRD results indicate a low overall incidence hydroxyapatite.

A tentative interpretation of the data in Fig. 3 suggests the following architecture.

Stone center (0–1 mm)	Primarily struvite
(1–2.5 mm)	Increasing hydroxyapatite
(2.5–3.5 mm)	High hydroxyapatite proportion
(3.5–4.5 mm)	Primarily struvite
(4.5–6.5 mm)	High hydroxyapatite proportion
(6.6–8 mm)	Primarily struvite
Stone edge (8–8.5 mm)	Primarily hydroxyapatite

5. Conclusion

The presence of adjacent layers implies that these layers may form weak zones within the stone [8] and thus this analysis provides insight into the mechanism of certain lithotriptor techniques where high-intensity shock-waves are used to destroy the uroliths *in vivo*. The presence of struvite in the core is reasonably common but the alternating layers of hydroxyapatite is less so and may provide evidence for the environment in which this urolith grew. Both the issue of the influence of architecture on fragility and the significance of the layer compositions as an indicator of growth environment are areas of active investigation and form the subject of further papers. This combination has, however, revealed a powerful method of extending our knowledge of the composition and architecture or urolith.

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