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STRUVITE CRYSTALLURIA AND UROLITHIASIS IN CROSS LABRADORS

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Abstract

Recurrent struvite crystalluria and urolithiasis in a Cross-Labrador bitch was studied using a combined Coulter-Counter and scanning electron microscope (SEM) approach. Staphylococcus bacteria were cultured from the patient's urine as well as from the calculi themselves. Urine samples were subjected to particle counting and sizing during active and non-active periods of stone formation. Size distribution curves so obtained were identical as were those derived from sterile and non-sterile specimens. These showed a peak incidence at a diameter of 5μm. Particle sizes for 6 controls were also determined and showed an even distribution over a much wider range with small peaks occurring at 3,10, and 20μm diameters.

SEM studies of urine sediments revealed the presence of struvite crystals in all the controls as well as in the stone-former. These occurred in a variety of shapes and sizes but were generally larger in the controls. SEM also revealed intimate admixtures of struvite and apatite in calculi surgically removed from the patient.

The results of this study indicate that crystal numbers are of greater significance than crystal size. It is also suggested that Cross-Labradors may be unusually predisposed to struvite crystalluria. The repeated recurrence of struvite urolithiasis in the subject indicates a possible inherent physiological malfunction in the animal's ability to cope with this crystalluria. The absence of a nucleation inhibitor in the stone-former's urine is also postulated

KEY WORDS: Struvite crystalluria, struvite urolithiasis, Cross Labradors, particle size distribution, scanning electron microscopy, ultrastructure, inhibitory mechanisms, crystal predisposition, Coulter Counter.

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Introduction

Urolithiasis has been reported in many breeds of dogs and several different constituents have been identified in calculi from such animals (1,2,7,21). These include calcium oxalate monohydrate (COM), calcium oxalate dihydrate (COD), apatite (APA), struvite (STR), cystine and uric acid and urates. The relative incidence of these shows that 60-65% of canine stones are composed of phosphates, 11-16% of oxalates, 8-12% of urates and 11-18% of cystine (2,20,21).

As with human calculi different aetiological factors give rise to the different stone types. In the case of STR stones, urinary tract infection caused by urea splitting bacteria, in particular Staphylococci and Proteus, are regarded as playing a key role in the pathogenesis thereof (2,3,7,9,18). Streptococcus and Escherichia coli have also been identified (2,18). The bacteria liberate ammonia from urea thereby causing an increase in urine pH(6,10). Since STR is insoluble in alkaline solution (5), precipitation and urolith formation ensues.

We undertook to investigate and characterise the crystalluria in a Cross Labrador bitch in which recurrent struvite urolithiasis had occurred over a 3 year period. A combined Coulter-Counter and scanning electron microscope approach was used for this purpose. These techniques have been used with much success in the study of human crystalluria (4,14,15, 19). The former however has not been utilized for the determination of crystal size distributions in canine urine specimens.

Materials and Methods

The subject was a Cross-Labrador bitch of welfare origin in which struvite stones were first detected at the age of 5 years. Apart from the urolithiasis, the dog was normal in all other respects.

During the 9 year period that we have attended this animal, only minor complaints such as infectious laryngo-tracheobronchitis (kennel cough), mild flea-bite dermatitis and anal gland impaction have required treatment. The control dogs were 6 healthy Cross-Labrador bitches with no history of urolithiasis.

Clinical profile and bacterial cultures

Blood urea nitrogen levels were determined in the subject using an Ames Blood Analyser with an Ames Bun Kit 6250. Plasma sodium, potassium, chloride,bicarbonate, urea, creatinine, total protein, albumin, calcium inorganic phosphate, cholesterol, urate, total bilirubin, conjugated bilirubin, gamma glutemile transaminase, alkaline phosphatase, aspartate transaminase, lactate dehydrogenase and alanyl transaminase were determined using a Technicon SMA Autoanalyzer by means of standard methods.

Between the ages of 5 and 9 years the subject was treated for 12 separate episodes of STR urolith formation. Radiology revealed the presence of uroliths in the bladder only with no renal involvement. Surgery (cystotomy) was necessary on 5 occasions while medical treatment sufficed on the others. Particular care, especially after the first recurrence, was exercised to ensure that no urolithic debris remained after surgery and that urinary tract infection was irradicated. Medical treatment consisted of a variety of antibiotics which were selected on the basis of in-vitro sensitivity and in-vivo response and were continued for periods up to 2 months in duration. The treatment also included urinary acidifiers administered intermittently, increased dietary salt to induce polydipsia, urease inhibitors and a low protein diet to maintain blood urea nitrogen below 10mg%. Bacterial cultures were obtained from a swab from the bladder mucosa, from urine samples obtained by cystocentesis or mid-stream collection, and, on one occasion, from a crushed urolith. Urine samples were divided in two. One half was cultured for bacterial content while the other half was subjected to particle counting and sizing using the Coulter-Counter. In both instances these were effected immediately after collection.

Particle size distribution analysis

A model TAII Coulter Counter coupled to a Population Accessory unit and fitted with a 100μm diameter orifice was used for particle counting and sizing. The method has been described in detail elsewhere (11). Essentially, samples to be counted were pipetted into a double walled glass vessel through which a low viscosity oil was pumped from a thermostatically controlled oil bath maintained at 38-39°C. Each urine sample was filtered through a 74μm sieve to remove particles too large to be accommodated by the Coulter Counter. Thereafter an aliquot of filtered urine was pipetted into 150ml of the thermostatted ISOTON II electrolyte for counting. All samples were counted 3 times.

Scanning electron microscopy (SEM) and X-ray powder diffraction (XRD)

Details of these procedures have been described elsewhere(11). Deposited crystals were removed from centrifuged urine samples by means of a Pasteur pipette and filtered through a 0.2μm Nucleopore filter. The filter papers were pasted onto aluminium stubs for carbon coating and SEM analysis.

Representative samples of the calculi removed from the subject on different occasions were pulverised using mortar and pestle and were subjected to XRD analysis. Diffraction patterns were recorded on KODAK DEF -392 film by the Debye-Scherrer method using a Philips powder camera of radius 28.65mm and Ni filtered CuK_α radiation of wavelength 1.5418Å.

Statistical analysis

This was performed by two way analysis of variance (ANOVAR). Significance was determined at the 0.05 and 0.01 levels.

Results

Bacterial cultures and urinalysis

During episodes of stone formation a coagulase positive staphlyococcus was cultured from urine samples and one of the calculi, while during inactive stone formation periods urine bacterial cultures were negative. Results of plasma analysis, obtained on 2 occasions using the Technicon SMA Auto analyzer were within the normal range.

Particle volume-size analysis

Crystalluria was always present in the subject and no significant difference in particle size and distributions was found between sterile and non-sterile urine specimens.

The mean particle population curve for the controls is shown in Figure 1. It is seen that particles occur over a wide range of sizes with peak incidences at diameters of 3,10 and 20μm. Their respective contributions to the total particulate volume are 12.46%, and 8.30% and 11.18%. On the other hand the size-volume distribution curve for the subject shows the presence of a large domi-peak at a diameter of 5μm (figure 2). These particles contribute 31.33% of the total particulate volume. Statistical analyses confirmed significant differences in total volume between the subject and controls at diameters of 3μm ($P<0.05$), 5μm ($P<0.01$) and 20μm ($P<0.05$).

The number of particles per unit volume in the subject's urine($34.8\times10^6 \pm$

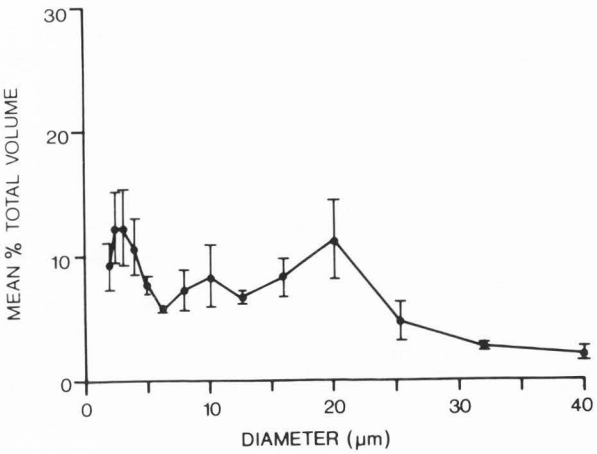

Fig. 1. Mean volume-size distribution curve (± S.E.M.) of particles in the urine at 37-38°C of 6 female Cross - Labradors.

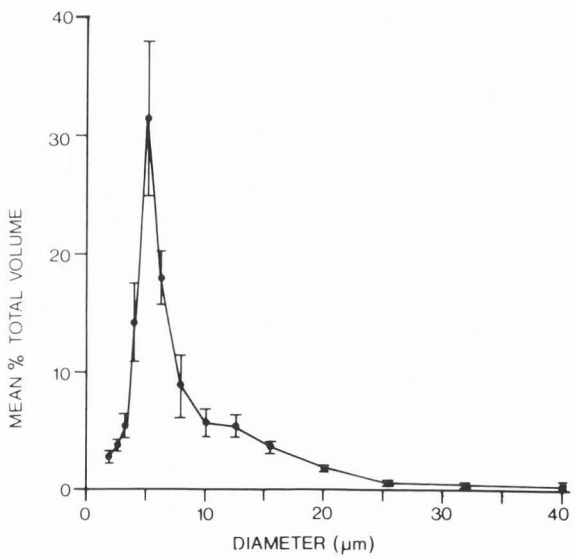

Fig.2. Mean volume-size distribution curve (± S.E.M.) of particles in the urine at 37-38°C of a Cross-Labrador bitch with recurrent struvite urolithiasis.

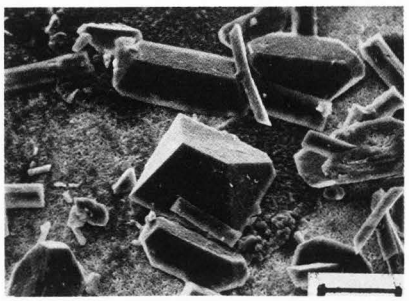

Fig. 3. Struvite crystals typically observed in the urinary deposits of the control dogs. Bar = 30μm.

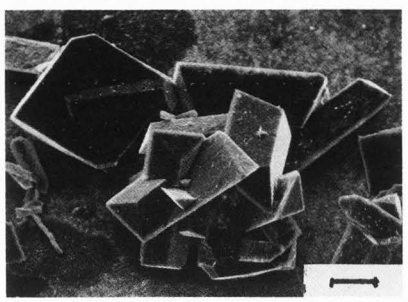

Fig. 4. Aggregate of struvite crystals observed in the urinary deposits of the control dogs. Bar = 30μm.

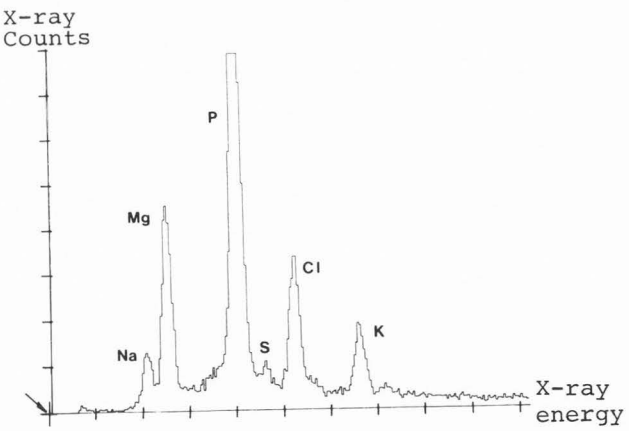

Fig. 5. EDX spectrum of crystal aggregate shown in Figure 4.

10.4×10^6) was at all times higher ($P = 0.05$) than that occurring in the controls ($9.2 \times 10^6 \pm 1.9 \times 10^6$).

SEM and XRD analyses

SEM revealed the presence of STR crystals in all the control urines. These were of a variety of shapes and sizes and occurred singly as well as in aggregates. Typical examples are shown in figures 3 and 4 respectively. In both cases the EDX spectrum was identical and showed large Mg and P peaks as expected. However, smaller peaks of Na,S,Cl and K were also recorded (figure 5) suggesting the presence of minute amounts of urinary salts. Crystals in the urine of the subject were slightly smaller (figure 6) but large ones, sometimes in association with other crystals of different size and morphology, were also observed. These showed the same microchemical composition as mentioned above for the control samples. XRD analyses of the calculi from the subject identified STR and APA

as the main constituents. This was confirmed by SEM which showed the presence of large STR crystals in intimate admixtures with tiny spherular deposits of APA (figure 7). The EDX spectrum recorded for the former is shown in figure 8.

Discussion

Crystalluria has long been considered a feature of renal stone disease in humans (14). Studies have shown that there is a qualitative and quantitative difference in the crystalluria of recurrent idiopathic stone formers and their controls (4,15). It is tempting to speculate that crystalluric differences might also exist in normal and stone-forming canines. The particle size-volume distribution curves shown in figures 1 and 2 tend to support this. Whereas the crystalluria in controls occurs over a wide size range with no particular diameter dominating the distribution, the recurrent stone former's particle volume-size curve is characterized by one very large peak at 5µm. This is somewhat surprising in that human urinary stone disease is associated with large particle crystalluria (8). Indeed, Robertson et al report crystals in the size range 20-40µm in the recurrent stone former as opposed to 3-4µm in controls (15,16). It would therefore appear that crystal size per se, is not an important factor in canine urolithiasis. Perhaps crystal numbers are of greater significance. The results of the present study in which the number of particles per unit volume was found to be nearly 4 times greater in the stone former than in the controls clearly demonstrate that this is a real possibility.

Struvite crystalluria was found in all the controls as well as in the subject at all times, even during non stone-forming periods when the urine was sterile. Although STR calculi are generally associated with urinary tract infection, stones of this type have been found in dogs with sterile urines (1,2,18). As such, it is perhaps not surprising to find STR crystalluria in non-infected urine samples as well.

It is of some interest to note that the mycoplasma, Ureaplasma Urealyticum, has recently been identified as a urea splitter in human struvite stone disease. No test for mycoplasma was applied in this study. However, the fact that the stone former was treated with a wide range of antibiotics including tetracycline and erythromycin over a prolonged period suggests that mycoplasma would have been irradicated.

The presence of crystals in all the subjects of this study (stone former and controls) suggests that Cross- Labradors may be unusually predisposed to STR crystal formation. A predisposition to STR stones has been reported for Minature Schnauzer dogs (13) while in another study involving Beagles, one ancestral line was more predisposed to these calculi than another (12). The latter authors conclude, as we do, that certain as yet unidentified factors may predispose animals to urolith formation.

Despite the fact that STR crystalluria was present in all controls as well as in the recurrent stone former, their respective distribution patterns were nevertheless distinctly different. This would seem to indicate an inherent physiological malfunction in the latter's ability to cope with crystalluria. Perhaps, for example, a nucleation inhibitor is absent from the stone-former's urine resulting in very large numbers of small crystals which ultimately aggregates into calculi. The presence of such an inhibitor in normal urine would, in turn, result in fewer crystals per unit volume and a more even distribution of crystal sizes over a wider diameter range. The results of the present study tend to support this concept.

As far as the calculi themselves are concerned, SEM revealed ultrastructural features which are well known and have been reported for both human and animal calculi. In particular, the intimate association of large trapezoidal STR crystals with small apatite spherules as shown in figure 7 is a common observation (17).

In reporting this case study we recognise that Cross-Labradors are not a homogeneous population of dogs and that only one subject was investigated. As such, our conclusions are only speculative and are tentatively presented. Nevertheless we feel that the findings will be of some interest to other investigators of canine urolithiasis.

Acknowledgments

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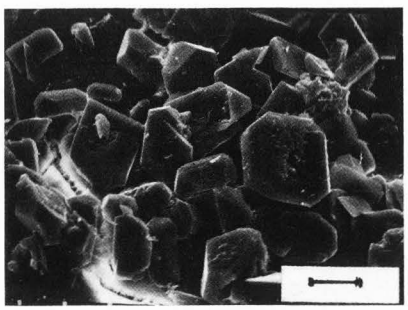

Fig. 6. Survey picture of struvite crystals in the urinary deposits of the recurrent stone-former. Bar = 10μm

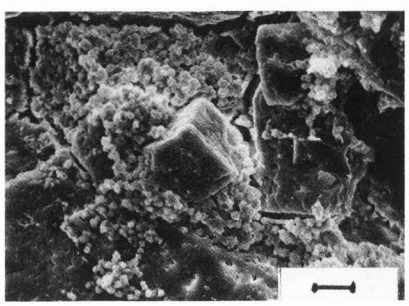

Fig. 7. Large struvite crystals in intimate contact with spherular apatite deposits observed in one of the calculi from the recurrent stone-former. Bar = 10μm

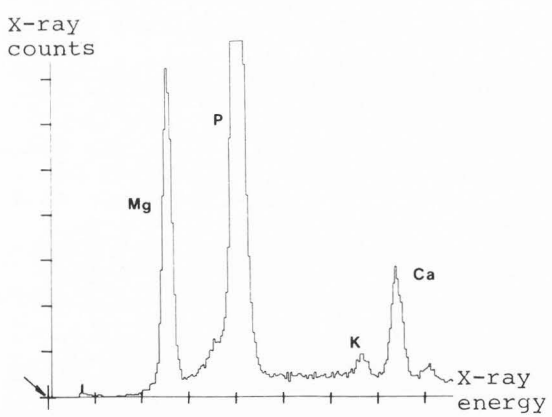

Fig. 8. EDX spectrum of deposits shown in figure 7. The occurrence of Ca,Mg and P confirm the presence of apatite and struvite. (The peak due to K probably arises as a result of the presence of a small amount of KCl, with the Cl peak being too small to be detected).

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Discussion with Reviewers

K.M. Kim: In the results, SEM is said to have revealed STR crystals. The findings should be specified. What were the SEM criteria used to identify STR?
Authors: STR crystals were identified on the basis of their morphology (trapezoidal and dipinacoidal) and the appearance of strong Mg and P peaks in the EDX spectra.
K.M.Kim: X-ray analysis of the crystals in urine revealed exceptional elements such as K,Cl and Na. Could these be crystal surface contaminants? How were the crystals for x-ray analysis prepared? Have they been washed?
S.R. Khan: Your x-ray microanalysis spectrum of struvite crystals shows a pretty decent peak for potassium. Is it possible that some of the magnesium in these crystals is substituted by potassium? The reason for my asking is that in our experience with struvite crystalluria in rats (Urol. Res.11:199; Urol Res. 12:271) we always got large peaks for potassium. In your Fig. 5 too, potassium is a pretty decent peak and the magnesium peak is correspondingly a lot smaller than in Fig. 8.
Authors: These questions raise an interesting point. Crystals were simply aspirated directly from centrifuged urine specimens and filtered through 0.2 µm Nucleopore filters without any washing. This suggests that K,Cl and/or Na are crystal surface contaminants. On the other hand however, the unusual habit of some struvite crystals (see following question) might be indicative of some substitution mechanism involving K and Mg.
S.R. Khan: The crystals from control dogs in Figs. 3 and 4 appear morphologically different form the crystals of the recurrent stone former in Figs. 6 and 7. The crystals in Figs. 3 and 4 are typical struvite while those of Figs. 6 and 7 are not. Their habit is different. Is this true or am I seeing too much in selected micrographs?
Authors: The micrographs selected for this paper were typical of those recorded for the various specimens. If morphological differences do indeed exist, they might possibly be due to substitution of one element by another as suggested in the previous set of questions.
K.M. Kim: The number of crystals rather than the size is said to be more important in the STR stone formation. How are the small sized particles better suited for stone formation than the larger sized particles?
P.T. Cheng: What do you think is the mechanism that would make crystal numbers more important that crystal size in canine urolithiasis?
Authors: Excessive crystal numbers could lead to an "overload" situation in which these particles could become entrapped and/or aggregate into larger entities. The mechanisms usually associated with large particles would then become operative.

S.R. Khan: What percentage of canine urinary stones are struvite stones?
Authors: Struvite is the major mineral in 80% of calculi removed from dogs in the U.S.A. (Klausner JS,Osborne CA, Griffith DP (1981). Animal model of human disease: canine struvite urolithiasis, Am J Path, 102, 457-458).
S.R. Khan: Was there a difference between the urinary pH of controls and the stone former and did the urinary pH change during the stone forming episodes?
Authors: There was no pH difference between control urines and the subject's urine during periods of non stone-formation. During stone forming episodes however, the subject's urinary pH increased.
S.R. Khan: Is it possible that the urea splitting organisms present in the Labradors are anaerobic and that they just did not grow in your culturing media?
Authors: The test for the presence of anaerobic bacteria was performed on only one specimen. This proved negative.
S. Deganello: Did you determine whether the urine contained any compound which could be a specific inhibitor of struvite formation?
Authors: No tests for the presence of inhibitors were performed. Such a study might have been expected to reveal the presence of a nucleating inhibitor in the control urines.
S. Deganello: Would you expect such an inhibitor to be the same as the one expected to inhibit the growth of calcium oxalates? If not, how would you expect such an inhibitor to differ?
Authors: This is difficult to answer. It seems unlikely that a single inhibitor could be effective on both calcium oxalate and struvite growth. On the other hand, perhaps, "broad spectrum" inhibitors do in fact exist.