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ANALYSIS OF CANINE URINARY STONES USING INFRARED SPECTROSCOPY AND SCANNING ELECTRON MICROSCOPY

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Abstract

Infrared spectroscopic analysis of 741 canine urinary calculi revealed that struvite stones, 58 % of the total, were the ones most commonly to be found. Cystine stone disease, 21 %, is also of great significance for dogs, whereas calcium oxalate, urate and brushite calculi occur only seldom. 3 cases of xanthine stone formation were also noted.

SEM examination revealed structures similar to human stones such as bipyramidal weddellite, pseudomorphs from whewellite to weddellite, apatite deposits in cystine stones and characteristic mono-ammonium-urate needles. Other, unknown, structures were also discovered such as closely-knit intergrowths of cystine and brushite strata, mono-Na-urate and mono-K-urate intergrowths and Ca-urate. Of particular interest are the various forms of xanthine from compact spherical to lance-shapes in sheath-like arrangement.

KEY WORDS: Canine Urinary Calculi, Infrared Stone Analysis, Spectra Library, Scanning Electron Microscopy Examination, Xanthine Stones, Urate Stones, Bacterial Imprints.

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Introduction

According to the available literature, the incidence of canine urolithiasis lies between 0.4 and 3.3 % (1, 12, 15). However, these figures are based on statistics supplied by veterinary clinics, and representative data such as are available for human beings (14) have yet to be published. Analysis of canine urinary calculi can provide important information for therapeutic concepts designed to prevent recurrence. In addition to this, structural examination provides information on the genesis of the stones and reveals analogies to urinary stone diseases in human beings. 741 canine concrements were analysed using infrared spectroscopy. Selected samples were also examined under a scanning electron microscope.

Materials and Methods

741 canine concrements, the majority of which had been removed surgically, were examined using infrared spectroscopy and KBr press technique. A Type 598 Perkin-Elmer spectrometer with a wave-number range from 4,000 to 200 cm^{-1} was employed. For quantitative and qualitative analysis we used our own collection of spectra from pure and mixed substances (3, 4) which is stored in a PC-PE Professional 7300 computer as a spectrum library.

The scanning electron microscopical examinations were carried out on typical examples of canine urinary calculi. In addition to this, the fracture surfaces of certain concrements were sputter coated with silver and examined under a Cambridge Stereoscan 180 SEM using an EPS 1000 PGT x-ray microprobe analyser.

Results

Distribution by Breed:

The anamnestic data of the diseased dogs indicate that nearly every breed of dog is subject to urinary stones. However, certain breeds, such as dachshunds, terriers, cocker spaniels and poodles, are particularly at risk in this respect. 89 % of the stones examined had occurred in 14 breeds (see Table 1).

Infrared spectroscopy Analysis The IR spectroscopic quantitative analysis of the 741 concrements revealed great diversity of composition.

Table 1: Frequency of Urolithiasis in individual Breeds of Dogs (n = 741)

Breed	Number	%
Dachshund	204	27.35
Mongrel	102	13,76
Terrier	99	13.36
Cocker spaniel	69	9.31
Poodle	65	8.77
Dalmatian	35	4.72
Pekinese	24	3.23
Schnauzer terrier	16	2.15
Basset hound	14	1.88
Boxer	14	1.88
German shepherd	12	1.61
Münsterländer	12	1.61
Beagle	8	1.07
Chow Chow	7	0.94
Other breeds	60	8.36

The analytical precision provided by IR spectroscopy (proportions exceeding 5 per cent by weight will certainly be identified) enabled us to identify 63 % of the stones as being monomineral in composition. All the components occurring in human concrements were found in these. 233 stones were composed of 2 components, and 3 components were positively identified in 24 stones (Table 2).

Pure struvite stones, and those containing high proportions of struvite, mostly result from an infection of the urinary tract. Cystine stones, 21 % of the total, formed the second largest group of stone diseases in dogs. This was followed by a variety of urate stone and brushite, whereas Ca-oxalate stones (whewellite = 3 % and weddellite = 4.7 %) were very rare in comparison to the frequency of their occurrence (> 65 %) in human beings.

Ca-phosphate was seldom found, although brushite ($CaHPO_4 \cdot 2H_2O$) was found to be the major component of 18 concrements, and 3 stones were pure xanthine in composition.

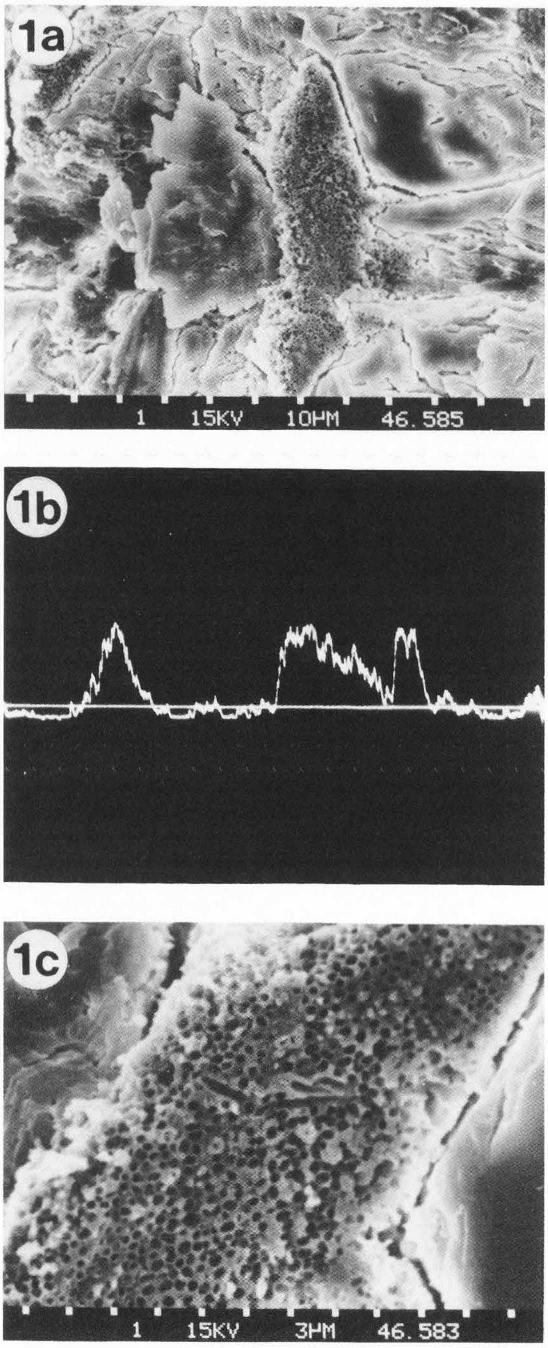
Scanning Electron Microscopical Examination

SEM (Scanning Electron Microscopy) micrographs and element-distribution pictures were taken of characteristic fracture surfaces on the stones in order to describe the structure of these canine urinary calculi. In this way a stone containing a high proportion of struvite with large struvite crystals was also found to contain clear areas of pseudoamorphous Ca-phosphate (Fig. 1a,b). The Ca-phosphate phase displayed a clearly perforated structure, probably attributable to the imprint of bacteria (Fig. 1c). Regular small nests of finely crystalline apatite were discovered in the majority of the cystine stones identified by IR analysis as monomineral (Fig. 2a). This apatite structure was confirmed by element analysis (Energy Dispersive X-ray Analysis) (EDXA) (Fig. 2b).

Table 2: Results of the infrared spectroscopic analysis of 741 canine urinary stones

Type of stone	n	%
I. MONOMINERAL		
Stru	255	34.44
Cys	155	20.92
NH_4U	27	3.65
Wed	7	0.94
Whe	1	0.14
Bru	11	1.48
Cap	3	0.40
Xan	3	0.40
SoU	3	0.40
Prot	4	0.54
	469	63.31
II. BINARY MIXTURE		
Stru/Cap	170	22.95
Stru/NH_4U	2	0.27
Stru/Whe	1	0.14
Stru/Prot	2	0.27
Cys/Stru	4	0.54
NH_4U/Stru	17	2.29
NH_4U/SoU	1	0.14
Wed/Stru	1	0.14
Wed/Whe	1	0.14
Wed/Cap	8	1.08
Whe/Wed	9	1.21
Whe/Cap	10	1.35
Bru/Stru	1	0.14
Bru/Wed	4	0.54
Bru/Whe	3	0.40
	234	31.60
III. TERNARY MIXTURE		
Stru/Whe/Cap	3	0.40
Stru/Cap/NH_4U	1	0.14
Wed/Whe/Bru	1	0.14
Wed/Whe/Cap	17	2.29
Whe/Wed/Cap	2	0.27
	24	3.24
IV. INDEFINABLE COMPOSITION		
Salts of uric acid	5	0.64
Unknown phosphate	4	0.54
Drugs	2	0.27
Insufficient material	3	0.40

Abbreviations: Stru = Struvite; Cap = Carbonate apatite; NH_4U = NH_4-hydrogen urate; Whe = Whewellite; Wed = Weddellite; Prot = Protein; Cys = Cystine; Bru = Brushite; SoU = Sodium hydrogen urate



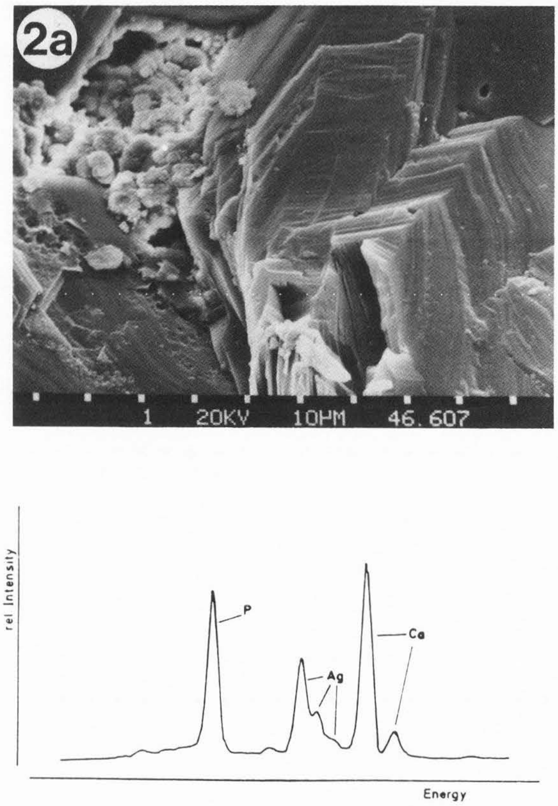


Fig. 2a: Hexagonal compact cystine crystals with fine crystalline apatite colonies.

Fig. 2b: Ca and P identified with EDXA in the apatite colonies shown in Fig. 2a.

Fig. 1a: Struvite stone. Large, compact struvite crystals with pseudoamorphic apatite showing clear bacterial imprints.

Fig. 1b: As Fig. 1a with superimposed y-modulated EDXA signal of Ca; apatite detection.

Fig. 1c: Section of Fig. 1a. Bacterial imprints in the apatite zones.

A rare phase combination was discovered in one cystine stone with a dense brushite envelope (Fig. 3a). The brushite crystals in the boundary layer were intermeshed with cystine crystals, although no mixed phases of the other component appeared in either the cystine or the brushite phases. This was confirmed by element analysis (Fig. 3b, c, d).

A typical shell-like growth structure was found in the pure brushite stones (Fig. 4a). The individual brushite crystals displayed their typical basalt prism structure (Fig. 4b).

Canine weddellite stones display well formed bipyramidal crystals with numerous adhesions (Fig. 5).

One pure whewellite stone still displayed the characteristic bipyramidal weddellite surfaces after IR analysis. However, the marked signs of wear on it were indicative of dehydration, and hence this was in fact a pseudomorph of whewellite to weddellite (Fig. 6).

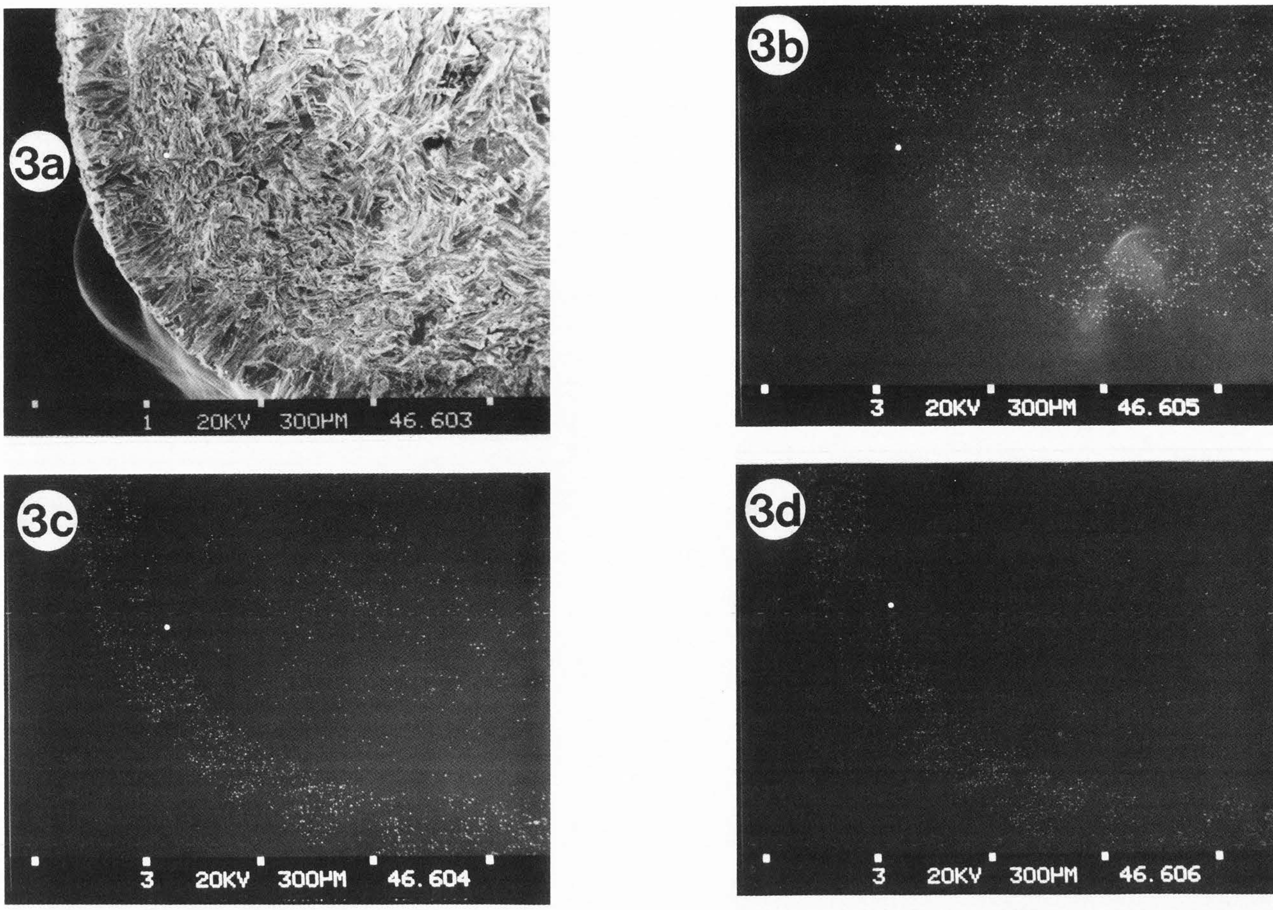


Fig. 3a: Cystine stone with firmly superimposed, evenly grown brushite envelope. Figs. 3b, 3c and 3d show elemental analysis of the area in Fig. 3a. Fig. 3b: S distribution. Detection of cystine. Fig. 3c: Ca distribution. Fig. 3d: P distribution.

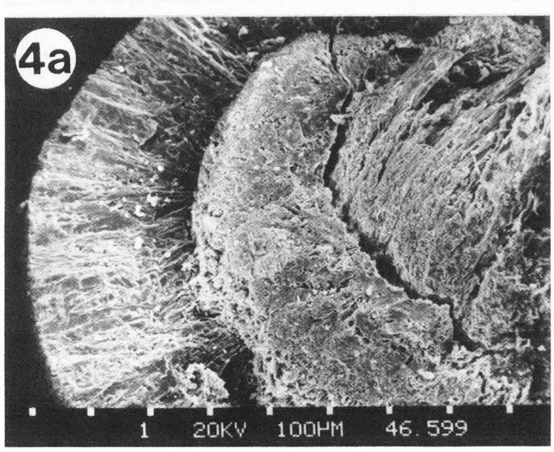


Fig. 4a: Stratified growth in a brushite stone. Diverse structures in the individual strata.

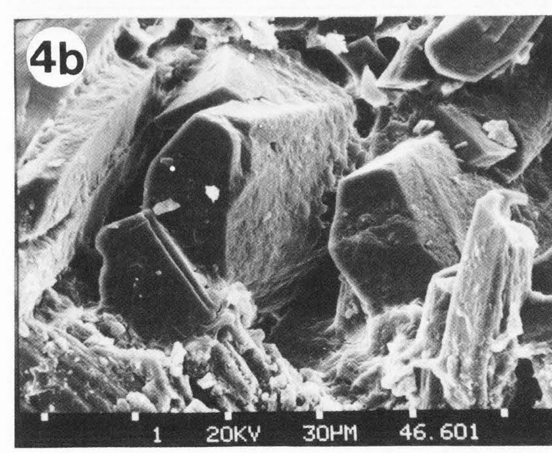
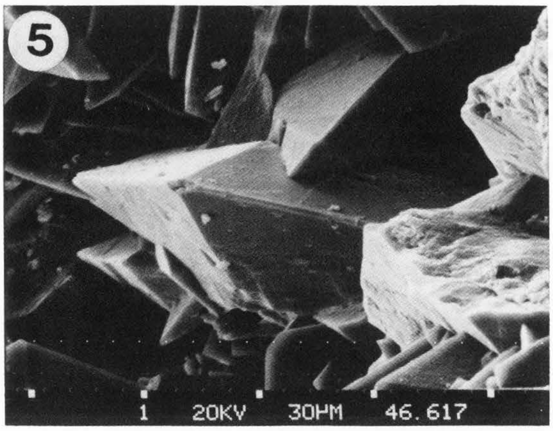
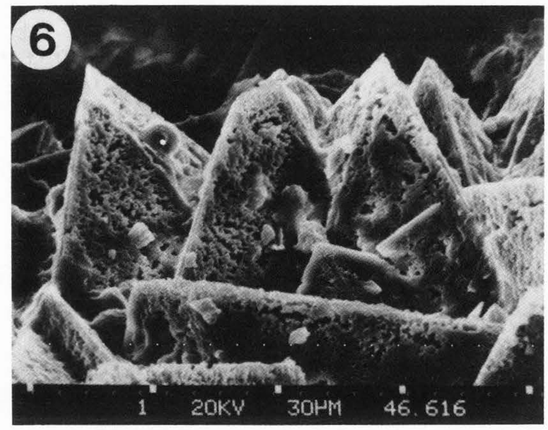


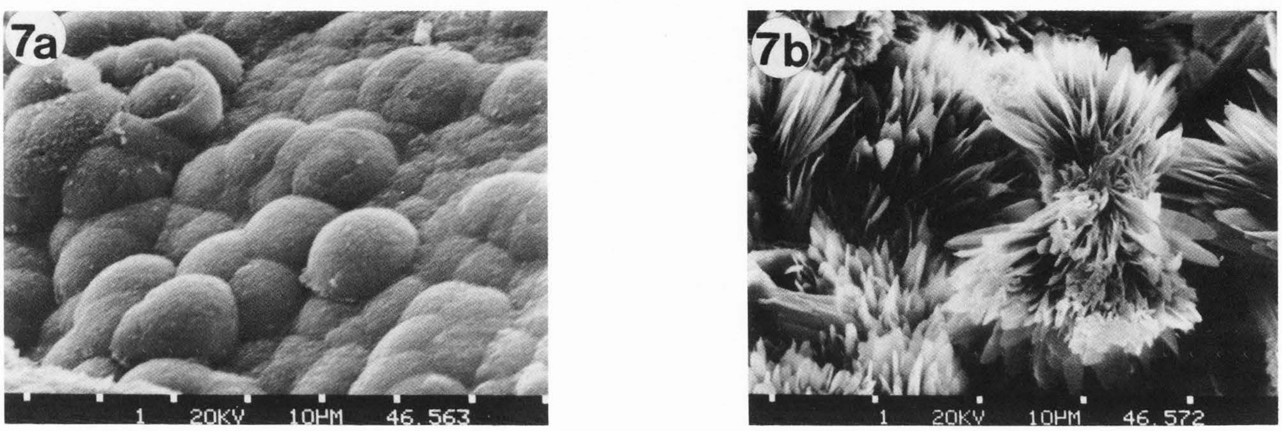
Fig. 4b: Characteristic basalt column-like crystals of brushite.

Xanthine calculi are also very rare in human beings. The SEM examination revealed two very different structures in the same stone. One was a compact sphere whose individual elements had grown in a series of shells (Fig. 7a) and the other displayed lance-shaped crystals of sheath-like arrangements (Fig. 7b).

Mono-ammonium urate appeared in the same fascicular form as it does in human stones (Fig. 8a, b), although the monomineral form is commoner in dogs than in human beings.







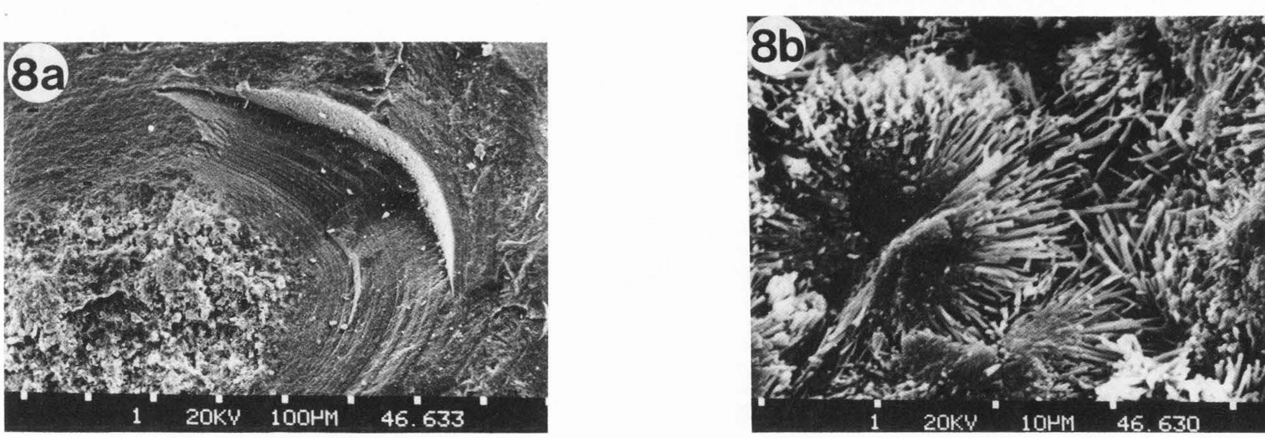


Fig. 5: Typical bipyramidal weddellite crystals with adhesions. IR analysis: weddellite.

Fig. 6: Pseudomorphs of whewellite to weddellite. IR analysis: whewellite.

Fig. 7: Xanthine stone. Fig. 7a: spherical, compact structures; Fig. 7b: Sheath-like crystalline arrangement.

Fig. 8a: Fracture surface of a mono-NH_4-acid urate stone. Loose core with associated compact, concentric layers.

Fig. 8b: Fascicular ammonium acid urate crystals.

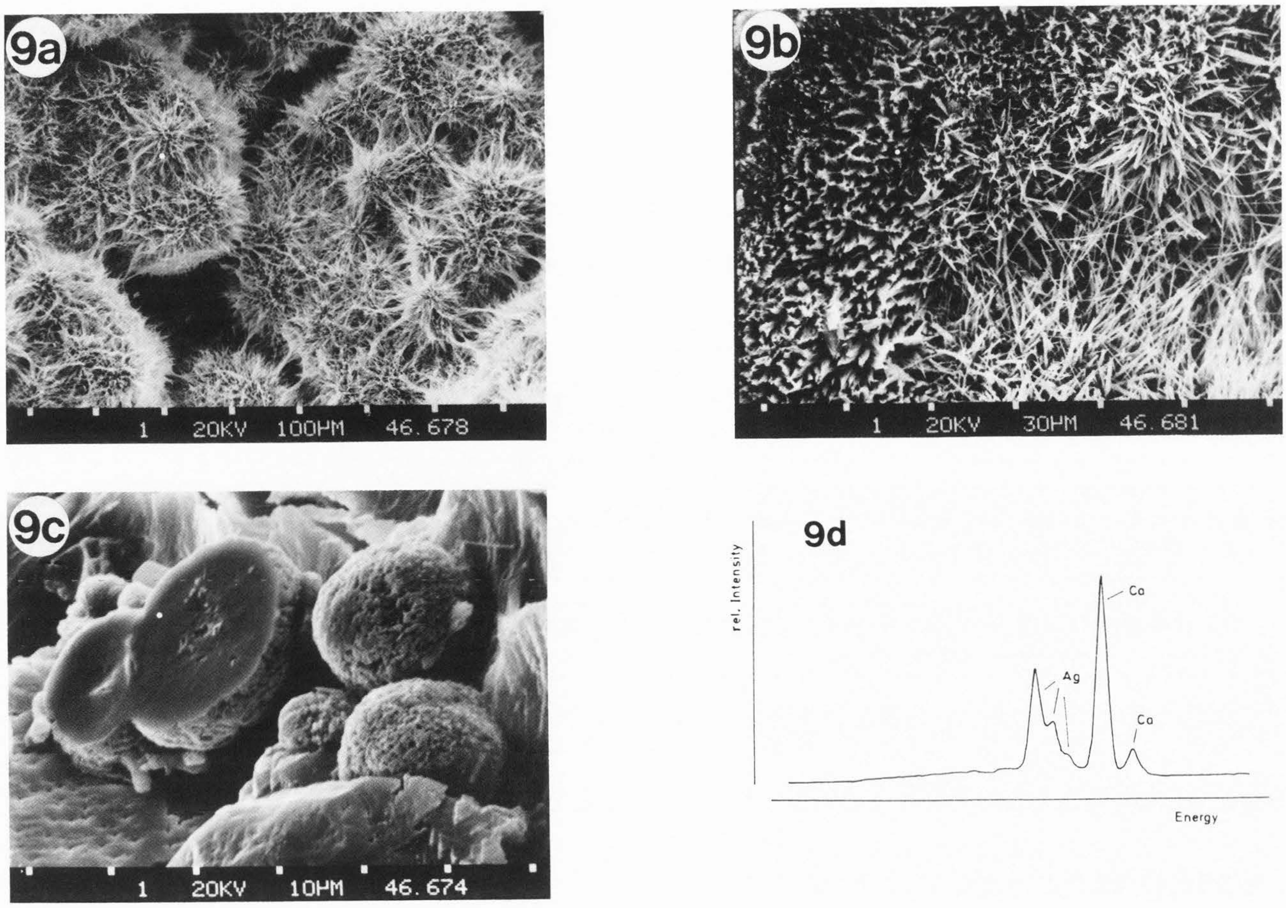


Fig. 9: Mixed acid urate stone. Fig. 9a: Fine needles of Na-acid urate in the centre; Fig. 9b: Boundary zone between needle-form Na-acid urate (right) and fibrous K-acid urate (left); Fig. 9c: Compact, spherical form of Ca-urate; Fig. 9d: EDXA of fig. 9c. Except for Ca, no other elements have been identified in the stone material (Ag due to sputter layer).

One group of urate stones proved impossible to classify precisely using IR spectroscopy and X-ray diffraction. During the SEM examination EDXA analysis revealed mixtures of urates in these concrements. Thus one and the same stone was found to have a mono-Na-acid-urate core (Fig. 9a), adhesions between mono-Na-acid-urate and mono-K-urate (Fig. 9b) and Ca-urate (Fig.9c). The cations were identifiable in each case using EDXA (Fig. 9d).

Discussion

The reason certain breeds of dogs are more vulnerable to urolithiasis than others remains a mystery. The main contributory factors, however, are most probably the popularity of individual breeds and, especially, genetic predisposition consequent upon the conditions under which the dogs have been bred. This would explain the remarkable frequency with which cystine diseases occur in dachshunds. IR spectroscopic analysis showed that of the 741 canine urinary stones examined, 21 % were cystine stones; under SEM-examination these displayed a remarkable similarity to their human counterparts. Although, in the literature, cystine stones are always described as being particularly pure in composition (9), we were constantly finding colonies of Ca-phosphate (3). The majority of canine stones consisted of struvite, and from anamnestic data it may be inferred that infection plays an important role in the formation of these stones. This proposition was also supported by the fact that we were able to identify bacteria imprints on such stones. Similar structures have also been described in human calculi (2, 11).

Ca-oxalate stones occur relatively seldom in dogs (5, 15), but when they do they have the same crystalline shapes and products of transformation as human stones (3, 6, 7, 8, 10, 13). They display well formed weddellite crystals, and also pseudomorphs from whewellite to weddellite.

Of special interest in canine urinary stones is the combination of cystine and brushite. This occurred in one stone as firmly connected layers (Fig. 3). Until now we have not yet examined xanthine in a urinary stone using SEM. The change from compact spheres to lance-shaped crystals in sheath-like arrangements was typical of the stones investigated. Also of interest in these canine stones were the mixtures of various urates, in which sodium and potassium urates occurred in the familiar form of fine neddles (3, 10), in contrast to Ca-urate, which occurred in compact spherical structures. The reasons underlying the formation of these various urates in dogs have not been elucidated.

As we had expected, urate stones occurred most frequently in Dalmatians, although they were also found in certain of the other breeds (including terriers, spaniels, boxers). This may be explained by the fact that impaired functioning of the liver accompanied by reduced rates of transformation of uric acid to allantoin produces an increase in uric acid-serum concentrations, which in its turn leads to increased excretion of uric acid in the kidney.

SEM examination of canine urinary stones revealed a whole series of structures familiar in human stones. This enabled us to identify certain structures which had not previously been observed.

Acknowledgement

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Discussion with Reviewers

A. Rodgers: Identification of only one constituent in 63 % of the stones is surprising. Did you analyse different regions of the same stones or was only one representative sample tested?

Authors: A representative sample was examined. Concrements < 3 mm were completely ground down, larger stones were sawn through the middle and the saw-dust analysed.

A. Rodgers: Could you comment on the occurrence of pure struvite stones in dogs as opposed to struvite/apatite calculi in humans?

Authors: The clear difference in the proportions of monomineral struvite concrements in dogs is proably due to specific conditions affecting the infection in the bladder. However, no concrete grounds for this have yet been identified.

K.M. Kim: Infrared spectroscopy, or any known method of crystal identification, is known to have limitations particularly in the detection of minor components in urinary stones. Scanning electron microscopy and X-ray microanalysis of selected samples in your series appeared to have demonstrated some minor components missed by infrared spectroscopy. Could you comment on the sensitivity of the two methods in the stone analysis?

Authors: The greater sensitivity of X-ray microanalysis arises from the method of its employment. Whereas in the case of microanalysis a microscopically small area is examined, in the case of IR spectroscopy and other chemo-physical methods of investigation a comparatively large sample of the substance is analysed. Thus using microanalysis, it is possible to detect the presence of subsidiary components in the minutest quantities when these are distributed nonhomogeneously in the concrement. With IR spectroscopy, however, proportions of (<2 %) cannot be detected in struvite/carbonate apatite mixtures. Hence, it appears to be sensible to employ a combination of both these methods when doing basic research.

K.M. Kim: How accurate is infrared spectroscopy in distinguishing weddellite from whewellite?

Authors: 10 % weddellite or 10 % whewellite can be identified as subsidiary components of whe/wed or wed/whe mixtures by IR-spectroscopy.

<u>K.M. Kim:</u> On the "pseudomorphism" of calcium oxalates, what, in your opinion, is the mechanism of the formation of whewellite with bipyramid habit?

<u>Authors:</u> "Pseudomorphism" describes the phenomenon whereby whewellite displays apparently bipyramidal structures. This arises when the weddellite originally present has been transformed into the more thermodynamically stable whewellite has been preserved.

<u>K.M. Kim:</u> With regard to urate crystals containing calcium and potassium, is there any evidence that these elements constitute lattice ions of the crystals? Could they be surface contaminants?

<u>Authors:</u> As the electron micrographs stem from the fracture surface of the concrement contamination by urine constituents would appear to be unlikely. In addition to this, the clear differences in the surface structures (fig. 9b) strengthen the assumption that we are here concerned with various urates.

<u>S. Deganello:</u> In your experience the "signs of wear" noticed in the weddellite pseudomorph are usually representative of dehydration. If so why?

<u>Authors:</u> Weddellite is stabilized by urine constituents (e.g., magnesium). If this stabilization is not complete, dehydration can in vivo produce the more stable whewellite.