Volume 1986 | Number 4 Article 44

9-19-1986

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SCANNING ELECTRON MICROSCOPY/1986/IV (Pages 1713-1720)
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0586-5581/86$1.00+05

EXPERIMENTAL INVESTIGATION OF THE GENESIS OF STRUVITE STONES IN CATS

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(Received for publication April 03, 1986, and in revised form September 19, 1986)

Abstract

Infrared spectroscopy of feline urinary stones revealed that struvite was the main constituent in 77.6 % of all concrements. However, only in 30.8 % (16/52) of struvite stone patients were any infections of the urinary tract detected.

Scanning electron microscopical comparison of non-infected feline struvite stones and human struvite concrements which had grown in the presence of infection revealed clear differences. All the feline struvite concrements were of coarse crystalline construction with the crystalline form typical of struvite. Traces of partial solution and stratification were frequently detected on the crystalline surfaces. The human struvite stones whose growth had been accompanied by infection did not display these features; the predominant structures in these concrements revealed very little evidence of any ordered growth. Examination of the urine and calculation of the relative supersaturation showed that where physiological pH values and physiological concentrations of lithogenic substances were present sterile urine can become supersaturated with struvite.

The morphological peculiarities of the feline concrements and the results of urinary analysis indicate slow crystalline growth rates. Phases of growth alternate with periods of stagnation. This process may be influenced by dietary factors. In contrast to this, struvite stone formation in the presence of infection is characterised by rapid growth in continually supersaturated urine.

KEY WORDS: Feline Urinary Calculi, Urinanalysis, Infrared Stone Analysis, Spectra Library, Scanning Electron Microscopy Examination, Relative Supersaturation, Infection-free Struvite Formation

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Introduction

A major epidemiological study (33) showed that the incidence of urolithiasis in cats was 0.57 %. The literature describes a growing tendency to urinary stones in cats (1, 36). All breeds of cats are affected by this disease (3, 34, 35, 36), but the degrees to which the breeds are at risk differs (3, 34, 36). The disease was commonest in the domesticated cat (European short-haired cat) and the Persian cat (3, 36). Apart from cystine, all the most important substances involved in urolithiasis are formed in cats (3, 14, 15, 26, 30). One particular feature of the frequency distribution is the high proportion of struvite, up to 97 % (3, 13, 14, 20, 30). This indicates the presence of special conditions favouring struvite stone formation. Although human struvite stones are almost all associated with infection, this mineral can be formed in cats even without infection through high concentrations of mineral matter, referred to the digestible energy (magnesium, phosphorus).

The following study examines the connection between the genesis and structure of struvite stones in cats.

Materials and Methods

Of the 67 feline urinary stones examined, 52 contained struvite as their main constituent. These were used for investigating the genesis of feline struvite stones.

External Structure and Surface

The surface structure and colour of the concrements were investigated and documented.

Infrared Spectroscopical Urolithic Analysis

The feline uroliths were examined using an infrared spectrometer and the KBr press technique. A Perkin-Elmer 598 dispersive IR photospectrometer with a wave-number range from 4,000 to 200 cm^{-1} was employed. For qualitative and quantitative analysis we used our own collection of spectra from pure and mixed substances (11, 12).

SEM Investigations

In order to study the morphology of the concrements their surfaces and fracture surfaces were examined by scanning electron microscopy (SEM). Feline struvite stones which were free from infection and which had been passed as pure after examination through an infrared spectrometer were selected for this along with human struvite stones affected by disease. A Cambridge Stereoscan 180 equipment was used in conjunction with the EPS 1000 x-ray microprobe Analyser.

Urinary Investigations

Spontaneous urine samples from 17 cats with struvite stones were examined. The following parameters were determined:
pH value; spec. gravity; magnesium; inorganic phosphate; ammonium; sodium; potassium; calcium; uric acid; inorganic sulphate; oxalic acid; citric acid, and chloride.
The relative supersaturation with struvite was calculated using the EQUIL programme (7). The measured values were used for this purpose.

Results

External Structure and Surface

Macroscopically, a coarse crystalline surface structure was identifiable on all the solitary feline struvite concrements. The human struvite stones, by contrast, were fine crystalline.

IR-Spectroscopical Urolithic Analysis

Of the 67 feline uroliths examined, 52 (77.6 %) contained struvite as their main constituent, and 47 of these 52 concrements were identified as pure struvite by infrared spectroscopy. In 15 cases (22.4 %) other types of urolith (ammonium hydrogen urate, carbonate apatite, weddellite, whewellite and protein) were identified as blend components.

Only in 20 (29.8 %) of the total of 65 cats examined could any infection of the urinary tract be positively identified. Infection of the urinary tract was present in 16 (30.8%) of the 52 struvite stone cats.

SEM Examination

SEM examination of typical struvite concrements of these cats revealed large, sometimes coffin-lid shaped (Figs. 1, 2, 3) and envelope-shaped (Fig. 4) single crystals, some of which had coalesced.
In the case of the human struvite concrements which had grown in association with infections, comparative magnification showed no single-crystal-like structures. No typical struvite crystal form could be identified (Figs. 5, 6, 7).
The feline struvite crystals displayed a particular surface structure which was not found in the human concrements. In the Figures 8 and 9 clear traces of partial solution can be seen on the surfaces of the crystals.
Ordered, clearly defined stratification on the surfaces of one crystal is visible in Figure 10. In contrast to this, the structures of the human stones appeared to be random in nature (Fig. 6). Figure 11 shows the subtle, regular structures of one of the feline concrements. Human IR-spectroscopically pure struvite concrements very often display layers of fine crystalline apatite (carbonate apatite) (Figs. 5, 6, 7, 13, 14), whereas apatite colonies occur only seldom in the feline struvite concrements (Figs. 12, 15). The chloride peak in Fig. 15 indicates the presence of chlorapatite.

Urinary Examination

The spontaneous urine produced by 17 cats with struvite concrements was examined. The measured and calculated figures obtained display great variation (Table 1).
Figure 16 gives the distribution of the calculated figures for relative supersaturation of struvite in diagrammatic form.
6 of the 17 values lie in the region of supersaturation. The differentiation between infection-free urines and those infected with bacteria shows clearly that where physiological concentrations of magnesium, inorganic phosphate and ammonia are present the urine can become supersaturated even without any accompanying infection of the urinary tract.

Discussion

The proportion of struvite (77.6 %) and other urolithic substances in cats agree with the results of similar investigations reported in the literature. The frequency of struvite stones is given as 70 - 97 % (3, 5, 14, 26, 30). Infections of the urinary tract are less common, proportions of 3 - 66 % have been described in the literature (3, 8, 9, 17, 29). In our study we found 29.8 % (total, n = 67) and 30.8 % (struvite, n = 52).
The SEM-investigations showed clear distinctions existing between the struvite concrements originating in a sterile milieu and those in association with infections.

The coarse crystalline structures of the feline concrements with their typically crystal form indicate slow crystal growth, and very high concentrations of lithogenic substances and/or high pH values are unlikely here. In the case of the human struvite stones originating from an infected milieu, on the other hand, no coarse crystalline, single-crystal-like structure could be detected.

This result implies that no slow growth had occurred and that the urine had been continually supersaturated with struvite. The conditions required for this could be met through the rise in the concentrations of ammonia and inorganic phosphate, and through the rise in the pH value as a direct or indirect consequence of the infection

Fig. 1: Feline bladder stone, large crystals, IR Analysis: 100 % struvite

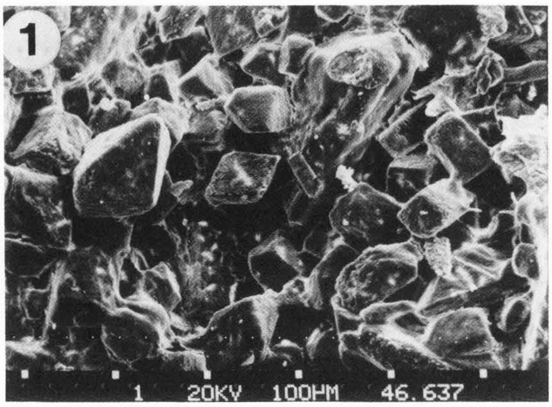
Fig. 2: Feline bladder stone, large, partly coffin-lid shaped individual crystals IR Analysis: 100 % struvite

Fig. 3: Feline bladder stone, large, partly coffin - lid-shaped individual crystals. IR Analysis: 100 % struvite

Fig. 4: Feline bladder stone, envelope-shaped individual crystals. IR Analysis: 100 % struvite

Fig. 5: Human bladder stone, struvite with apatite colonies, no typical struvite crystal forms. IR Analysis: 100 % struvite

Fig. 6: Human bladder stone, struvite with apatite colonies, no typical struvite crystal forms. IR Analysis: 100 % struvite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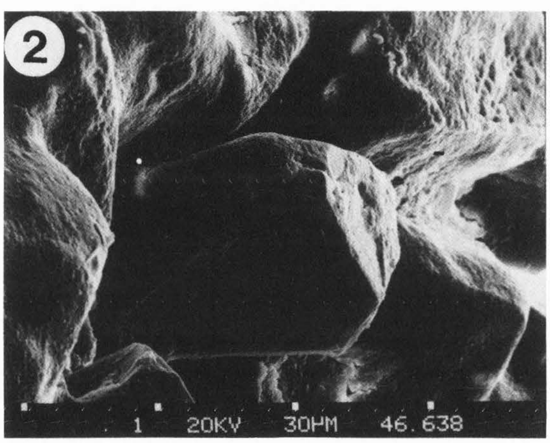
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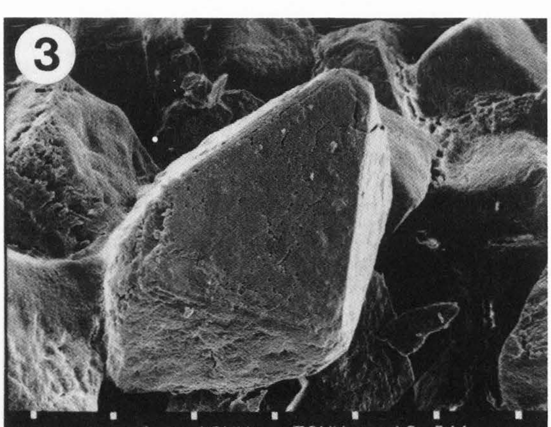
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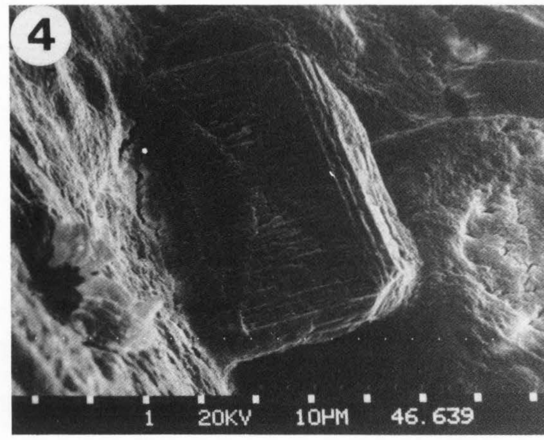
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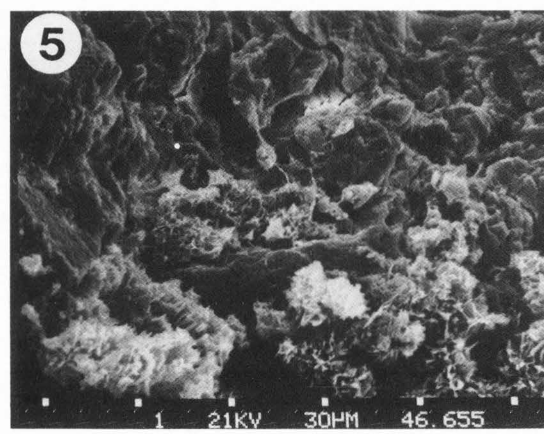
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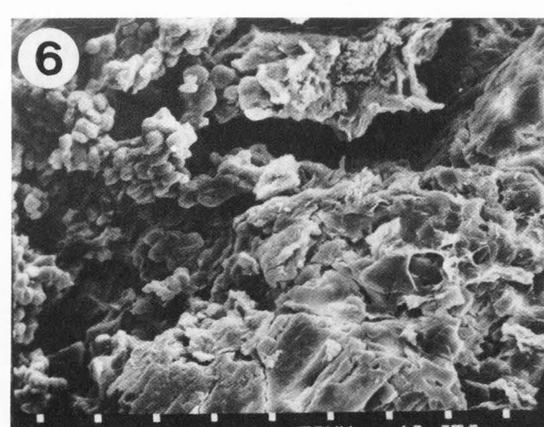
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Table 1: Quantitative urinary analysis of spontaneous urines of struvite stone formers without infection; in brackets ( ): with infection, n = 13; (n = 4)

Parameter	Max	Min	$\bar{x}$	SD
pH	7.40 (7.40)	5.73 (6.03)	6.56 (6.69)	0.492 (0.643)
spec. gravity	1.040 (1.030)	1.005 (1.025)	1.023 (1.028)	0.007 (0.003)
magnesium (mmol/l)	7.56 (6.96)	0.30 (0.90)	2.71 (2.73)	1.92 (2.89)
inorganic phosphate (mmol/l)	121.80 (49.00)	21.20 (4.74)	50.26 (34.31)	7.09 (20.63)
ammonia (mmol/l)	126.40 (84.70)	8.30 (13.30)	47.82 (60.20)	35.07 (33.22)
rel. supersaturation, struvite	1.896 (1.555)	0.0084 (0.0805)	0.7338 (0.7326)	0.6504 (0.6108)

of the urinary tract. The partial solution evident in the struvite crystals (Figs. 8, 9) of the feline struvite concrements and their clear stratification (Fig. 10) prove that in the course of growth, periods of growth had alternated with periods of stagnation. These could have been caused by fluctuations in the concentrations of the lithogenetic ions, and fluctuations in pH values caused by the "alkaline tide" due to dietary reasons.

The major significance of the mineral substance concentration in food in feline lithogenesis, and especially magnesium, has been demonstrated by experiments in which the formation of magnesium phosphate or struvite could be initiated simply by adding magnesium or magnesium, phosphorus and calcium to the diet (6, 10, 16, 21, 27, 31).

The greater frequency with which apatite colonies occurred (Figs. 5, 6, 7) is probably due to the raised pH values associated with infection. These would favour the formation of carbonate ions containing apatites.

The concentrations of phosphate (4.74 - 121.80 mmol/l) and ammonia (8.30 - 126.40 mmol/l) agree with the literature (10, 19, 21, 28, 32, 37). On the other hand, the figures in the literature for magnesium (0.33 - 25 mmol/l) and the specific gravity (1.005 - 1.084) (6, 10, 19, 32) lie well above those we obtained. The calculated figures for struvite supersaturation show that under physiological conditions concentrations of lithogenic substances may occur which will facilitate the crystallization of struvite (4).

The concept of struvite stone formation without infection will continue to be supported by the special bactericidal properties of feline urine (18, 25). One of the reasons for this is that due to the high protein requirement natural for cats (10, 20) greater quantities of urea are excreted renally (18, 25).

It is generally accepted that infections of the urinary tract with urease-forming bacteria in cats are not the cause of lithogenesis (2, 17, 22, 23, 24) but are a consequence of it (17, 22, 30).

In summary, it may be stated that the particular structures of feline urinary calculi, the composition of the urine and certain special physiological features provide important indications that struvite stone formation in cats occurs for the most part without any accompanying infection of the urinary tract.

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Fig. 7: Human bladder stone, struvite with apatite colonies, no typical struvite crystal forms. IR Analysis: 100 % struvite

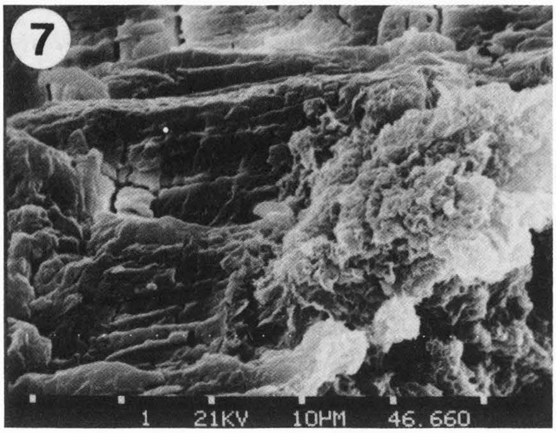
Fig. 8: Feline bladder stone, evidence of partial solution on the crystalline surfaces. IR Analysis: 100 % struvite

Fig. 9: Feline bladder stone, evidence of partial solution on the crystalline surfaces. IR Analysis: 100 % struvite

Fig. 10: Feline bladder stone, clear stratification on the crystalline surfaces. IR Analysis: 100 % struvite

Fig. 11: Feline bladder stone, subtle, regular structure of a crystal. IR Analysis: 100 % struvite

Fig. 12: Feline bladder stone, struvite with apatite deposits. IR Analysis: 100 % struvite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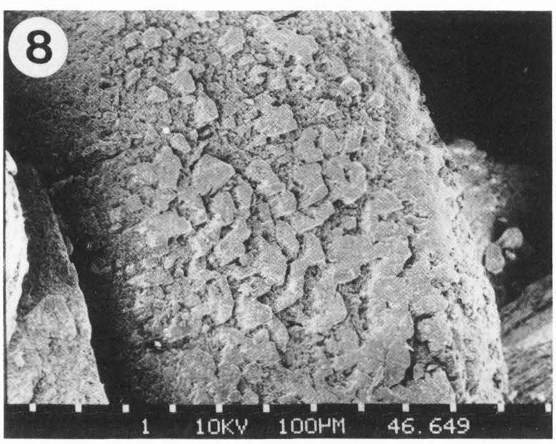
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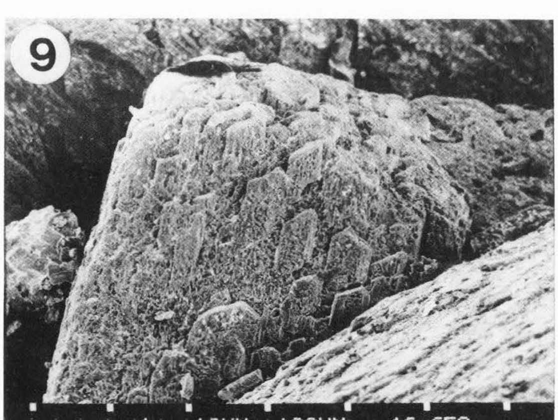
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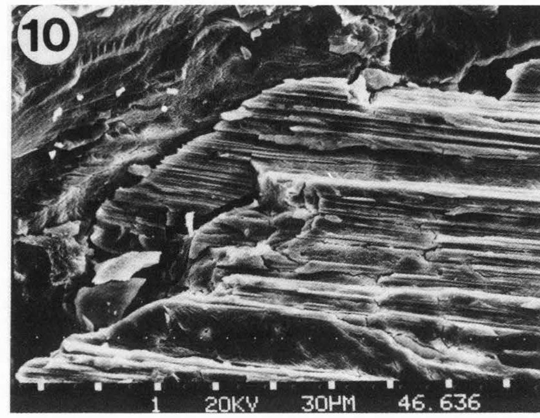
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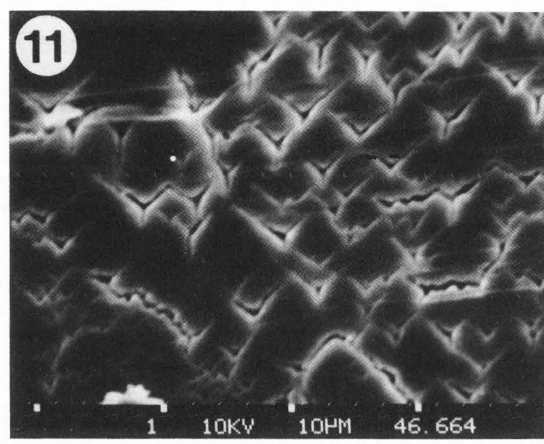
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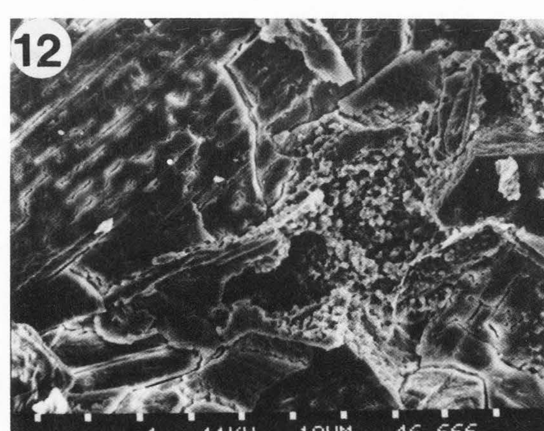
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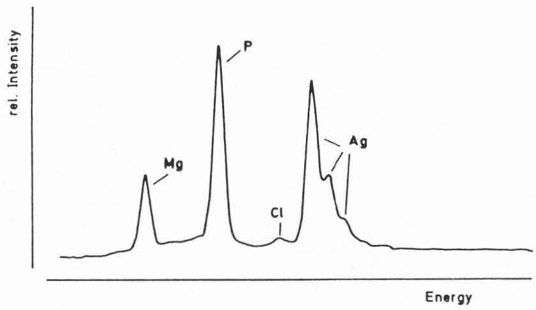

Fig. 13: Distribution of elements, struvite, vide Fig. 7

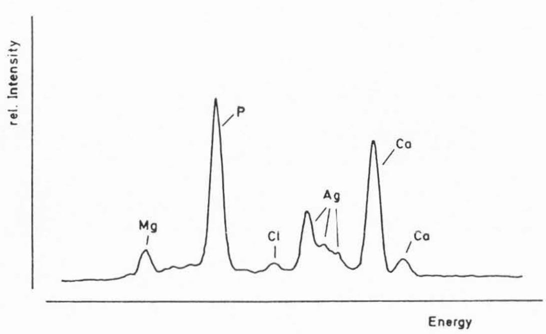

Fig. 14: Distribution of elements, apatite colony, vide Fig. 7

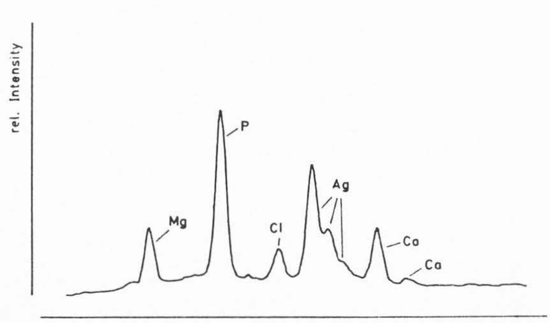

Fig. 15: Distribution of elements, apatite colony, vide Fig. 12

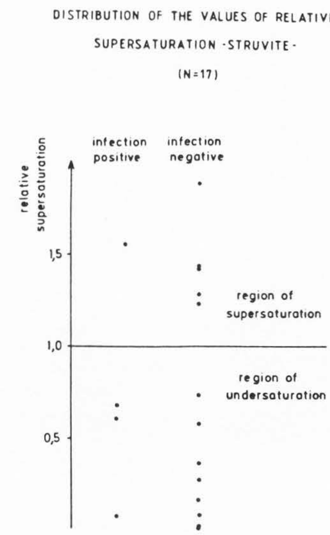

Fig. 16: Distribution of the values of relative supersaturation - struvite -

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Discussion with Reviewers

Y.M.F. Marickar: How was infection identified in the cats? Was it by culturing organisms or finding pus cells in urine or both?

Authors: Differentiation between animals with infections of the urinary tract and those without was based on information supplied by the veterinary surgeons involved in their treatment, who normally prepared a Uricult-culture. No microbiological examinations were undertaken.

Y.M.F. Marickar: Was a study made between uninfected feline concrements and infected feline concrements? How can you say that the differences seen between the feline concrements and human stones are not produced by differences in the feline and human urinary environments rather than infection alone?

Authors: No separate examinations of the concrements from animals with infections of the urinary tract and those without were made. We are not maintaining that the structural differences between human and feline urinary stones are independent of differences in the composition of the urine. Quite the contrary, it is just these differences in the urinary composition and the predominantly sterile milieu found with cats which are responsible for the structural differences in the struvite.

Y.M.F. Marickar: Fig. 3 (560x) showing individual crystal and Fig. 10 (410x) showing clear stratification of crystal surface have comparable magnification. But appearances are totally different. Were the different appearances seen in the same concrement or in different concrements? Could Fig. 10 be the edge of a crystal or a broken edge?

Authors: We are talking here about scanning electron micrographs of two different urinary stones. The surfaces of the stones were not treated, and hence it is unlikely that a broken edge (fig. 10) would occur.

Y.M.F. Marickar: Is it possible that struvite has tendency to take up different habits based on environmental factors? Our studies have identified coffin lid shaped habit in in vitro crystal growth studies in the absence of infection. Did you observe different habits of struvite in a single concrement?
Authors: We cannot say anything about the in vitro habits of struvite. We were able to observe variations in surface structure at different locations on the same concrement during our investigation.
Y.M.F. Marickar: Of the 17 urinary samples of cats examined, how many had urinary infection? Have you split up the values into two groups - infected urine and uninfected urine and studied the statistical significance of the difference in the parameters mentioned in table 1?
Authors: 4 of the 17 animals had urinary infections. Table 1 shows the results of the urine samples for these animals in brackets. There seemed to be little point in making any significant calculations where $n < 10$ ($n = 4$).
S.R. Khan: How did you identify chlorapatite?
Authors: Clear chloride peaks occurred in the analysis of the distribution of the elements in the apatite zone which we interpreted as indicating the presence of chlorapatite. However, we cannot exclude the possibility that the chloride ions identified might also originate from small portions of other compounds in the mixture.
S.R. Khan: You have beautifully illustrated differences between infected human stones and non-infected cat stones. Did you notice any difference between infected and non-infected cat stones?
Authors: Only non-infected feline stones were examined under the SEM.
S.R. Khan: In our experiments, male Sprague-Dawley rats consistently formed struvite crystals in sterile urine but some of these crystals had potassium substituting for magnesium. Did you find any such crystals in feline struvite stones?
Authors: No indications of any substitution of potassium for magnesium in struvite concrements were detected in our material.
A. Rodgers: Is there any special significance associated with the identification of chlorapatite in the feline stones?
Authors: The mere detection of chloride in the apatite zone (fig. 15) of a concrement does not permit any conclusions to be drawn regarding any special significance for feline struvite concrements.
A. Rodgers: Why was apatite (shown by SEM to be present) not detected by IR in some of the stones?
Authors: In the case of 5 of the 52 feline struvite concrements carbonate apatite was detected by IR. Our method does not permit very small proportions of carbonate apatite under 2 % to be identified in struvite concrements by IR.