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X-RAY MICROANALYSIS OF URINARY STONES, A COMPARISON WITH OTHER METHODS

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Abstract

A previous study of urinary stones by a combined electron microscopy demonstrated the potential of scanning electron microscopy and X-ray analysis as an analytic tool for urinary stones. Electron diffraction was chosen for the final confirmation of crystals in the study. Although electron diffraction is highly accurate for this purpose, it is desirable to establish the sensitivity of X-ray analysis for the identification of stone components relative to the more commonly used methods. Eighty six consecutive urinary stones were analyzed by X-ray analysis and the findings were compared with those of X-ray diffraction, infrared spectrometry and chemical analysis. The results indicate that X-ray analysis exceeds X-ray diffraction and infrared spectroscopy in its sensitivity for the identification of stone components several fold. This was largely due to the inability of the latter methods to detect apatite in more than half of the apatite containing stones. The findings in X-ray analysis had the best correlation with chemical analysis,which was applied mainly to the detection of apatite. X-ray analysis is particularly suited for the detection of rare and minor inorganic components of urinary stones such as silica and gypsum, and is obviously one of the most powerful tools for the analysis of urinary stones. Further application of X-ray analysis to urinary stone is likely to discern rare inorganic components of urinary stones overlooked by other methods.

Key Words. Urinary Stones, Scanning Electron Microscopy, X-ray Analysis, X-ray Diffraction, Infrared Spectroscopy, Chemical Analysis.

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Introduction

Urolithiasis is highly prevalent. The prevalence of urinary stones in developed countries ranges from 4 to 15% of the adult males (Ljunghall et al, 1980; Robertson et al, 1984; Sierakowski et al, 1978; Vahlensieck et al, 1982). The stone incidence in females is half as common. Medical care for each stone incident costs approximately $2,000 in the United States. The projected costs of stone treatments of the entire national population of adult males are estimated to be $315,000,000 a year (Shuster and Scheaffer, 1984).

With the recent advances in technology, the surgical removal of many urinary stones is being replaced by non-traumatic methods such as the shock wave lithotripsy (Chaussey, 1982). For a stone which requires such removal or for an acute urinary obstruction caused by a stone, its composition is not overly meaningful. The major threat of urolithiasis is rather in its high recurrence rate. According to long term followup studies, the recurrence rate of urinary stones exceeds 50% among stone formers (Ljunghall et al, 1980). As a result of the progress made recently in the field of the pathogenesis and the prevention of stone recurrences, the recurrence can now be prevented in some 50% of stone formers by medical treatment (Scott and Lewi, 1984). Knowledge of the chemical composition is essential for the treatment and the prevention of urinary stones.

Of the various methods which have been attempted to analyze urinary stones, those currently in common use are polarization microscopy, chemical analysis, X-ray diffraction, infrared spectroscopy, and scanning electron microscopy. The methods vary in their sensitivity and reliability. Polarization microscopy measures the optical properties including the refractive index of a crystal. To experienced users, its accuracy is nearly as good as X-ray diffraction (Prien, 1974). However, interpretation of its results is frequently difficult and requires a special training. X-ray diffraction measures the interplanar spacings characteristic of each crystal. Because of the similarity of the interplanar spacings and intensities of the reflections in the diffraction patterns of various components of urinary stones, the method frequently fails to detect minor components (Beeler et al, 1964; Lagergren 1956; Otnes and Montgomery, 1980; Pollack and Carlson, 1969; Schneider, 1973; Sutor et al, 1971). Apatite composing less than 10% of a stone is as a rule overlooked by

the method. In view of its convenience and its ability to compensate for some of the deficiencies of crystallographic techniques, infrared spectrometry has gained an increasing acceptance in the last decade (Takasaki, 1971; Lloyd and Oldroyd, 1983). However, the method also has similar limitations in its sensitivity in the detection of minor components; as much as 20% of uric acid and struvite in a stone may be overlooked by the method (Pollack and Carlson, 1969). Thus, there is not yet a satisfactory method for the analysis of urinary stones, and, therefore, two or more methods are commonly used together.

SEM and X-ray Analysis Of Urinary Stones

In spite of its potential advantages, there have been rather limited applications of X-ray analysis to urinary stones (Chambers et al, 1972: Hesse et al, 1979; Kim, 1979, 1982; Leusmann, 1983, Rodgers, 1981). As a result of increasing utilization of scanning electron microscopes for the study of urinary stones, the potential of scanning electron microscopy (SEM) and X-ray analysis (XA) as a diagnostic tool for stone analysis has gradually become apparent (Kim, 1983b). Furthermore, when SEM morphology of the stones was tested for its accuracy in the analysis of 100 consecutive urinary stones, more than 95% of common stone components were identified by those who had no previous experience in this field but who had received only a few hours of training. The data indicated further a strong potential of the SEM as a tool for the analysis of urinary stones (Kim et al, 1984).

Because of a possible involvement of subjective judgments in the morphological interpretations and the tendency of crystals to form multiple crystal habits, SEM is not yet accepted as a routine method of stone analysis. However, SEM has been utilized as an auxiliary method and has been shown to have a special place in stone analysis (Berg and Szabo-Foeldvari, 1982; Dosch and Koester, 1975; Cifuentes Delatte, 1983; Kim, 1982). The subjectiveness of SEM in stone analysis is readily compensated by a simultaneous application of XA. The demonstration of elemental contents within seconds in a crystal visualized by SEM is uniquely suited for the analysis of urinary stones. Combined with the SEM morphological characteristics of the crystalline as well as non-crystalline components of the stones, XA multiplies the accuracy of the method in the analysis of these concrements. Furthermore, the method has many advantages, including its minimal sample preparation, use of small specimen sample, expeditiousness, economy, the ability to observe the spatial relationships of the stone components and to detect non-crystalline components of urinary stones (Kim, 1982; Kim and Johnson, 1981; Kim et al, 1984).

Methods of Stone Preparation for SEM

In previous applications of X-ray analysis to the analysis of urinary stones, the stones have been embedded in araldite, cut with a saw and the surface polished (Chambers et al, 1972; Rodgers, 1981). The polished surfaces of the stones are perhaps better suited for the concentration mapping of the elements. However, the specimen preparation is more time consuming and yields two dimensional images, thus eliminating the main advantage of SEM, imaging of the three dimensional morphology. Simple cracking of the stones eliminates the problem and reduces the cost of specimen preparation significantly (Kim et al, 1984). Because of the cohesive property of crystals, fracture of the stones preserves their three dimensional morphology.

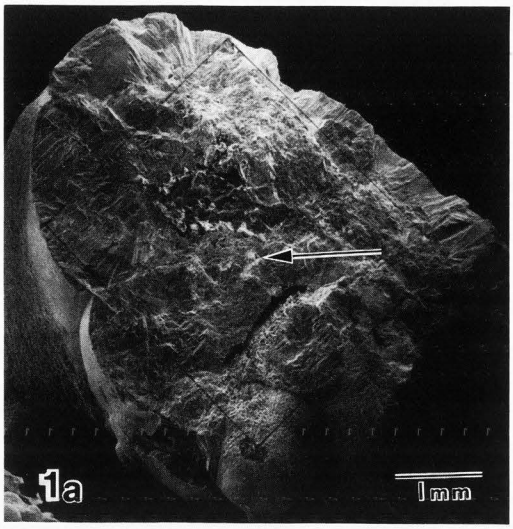
Stones in this study were cracked with a razor blade and hammer, glued onto aluminum stubs using carbon conductive glue and sputter coated with either carbon, aluminum, silver, or gold. In order to ensure an entire examination of the stone components, representative fragments of stones were mounted with the fractured surfaces up under a dissecting microscope. Certain crystals, particularly scanty weddellite, tend to develop on the natural surface of the stone. For such stones, additional fragments were mounted with the natural surfaces up. Carbon coating has a theoretical advantage since it does not add a detectable element to the samples to be analyzed by XA. However, carbon or aluminum coating is inconvenient to use for the following reasons; both are more time consuming, frequently cause a charging effect, and make it very difficult to photograph the samples. The spectrum of gold overlaps the phosphorus peak and is not suited for XA of those stones containing small amounts of phosphorus. Silver coating is thus the method of choice for XA of urinary stones (Leusmann, 1983). Scanning electron microscopy and x-ray analysis were performed using an ETEC Autoscan scanning microscope equipped with a Kevex detector and a Tracor Northern NS880.

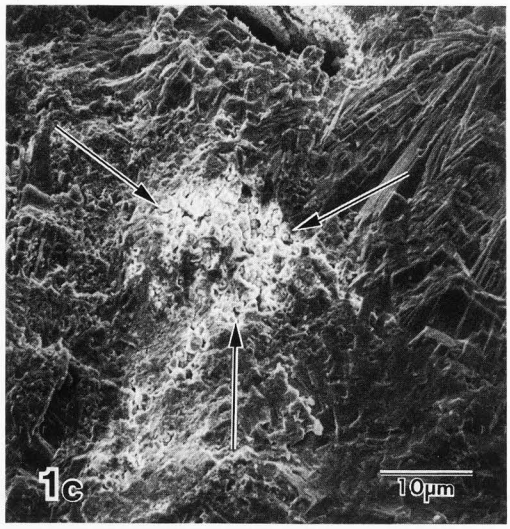
It is very time consuming and costly to scan and analyze an entire stone at high magnifications. Fortunately, the components of urinary stones occur in patches and layers, which minimize the need for an exhaustive analysis. XA of an entire stone at a low magnification and a few representative crystals at higher magnifications are usually sufficient (Fig. 1). It usually requires less than 15 minutes to complete XA, including photographic recordings of a stone. XA is the only method which allows us to identify discrete crystals at desired magnifications and is one of the most powerful tools available for the study of urinary stones.

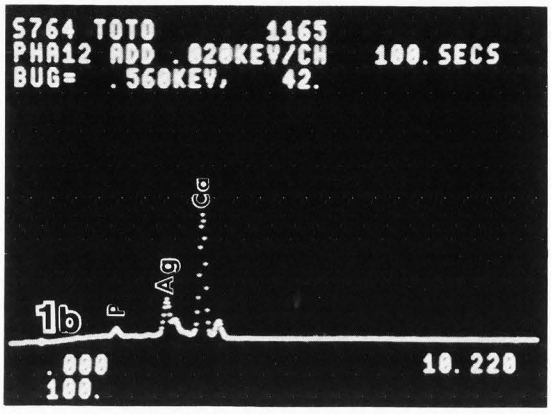
XA of Urinary Stones

In an attempt to establish electron microscopic criteria for identification of the composition of urinary stones, a few hundred urinary stones were examined by combined electron microscopy, i.e., SEM, XA, transmission electron microscopy (TEM), and electron diffraction (ED). SEM combined with XA was most suitable for the purpose. TEM was used mainly to locate the crystals by their recognizable silhouettes. The combined method enabled us to perform ED of crystals observed by SEM under direct vision in a TEM. Simultaneous applications of XA and ED to the samples in an analytical STEM further facilitates an identification of crystals observed by SEM. Prior knowledge of the elemental contents of a crystal significantly reduces the time and effort needed for analysis and multiplies the accuracy of crystal identification by ED.

Since XA is applied to the stone components identified by SEM, some knowledge of crystal







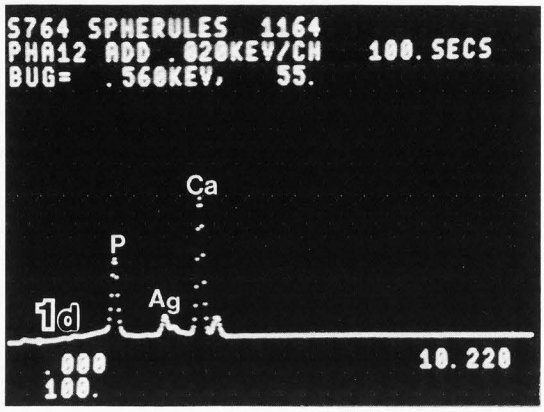


Fig. 1a. A hackly fracture surface of a whewellite stone. The area marked by an arrow contained calcium and phosphorus.
Fig. 1b. X-ray analysis of the rectangular area in Fig. 1a shows a tall peak of calcium and a small peak of phosphorus.
Fig. 1c. A closer view of the area marked by an arrow in Fig. 1a. Amid the tabular crystals of whewellite, a small cluster of apatite is seen (arrows). Crystals in urinary stones occur in clusters and layers enabling an expeditious analysis of urinary stones by X-ray analysis.
Fig. 1d. X-ray analysis of the area marked by arrows in Fig. 1c yields the peaks of calcium and phosphorus. Such scanty calcium phosphate in a stone is usually overlooked by X-ray diffraction and infrared spectroscopy.

morphology is required. The morphology and elemental contents of crystals commonly encountered in urinary stones are listed in table 1. Readers are referred to other reviews for further details of the crystal morphology (Berg and Szabo-Foeldvari, 1982; Cifuentes Delatte, 1983; Dosch and Koester, 1975; Kim et al., 1984).

Calcium Oxalate

Two forms of calcium oxalates occur in urinary stones, monohydrate (whewellite) and dihydrate (weddellite). The higher incidence of the monohydrate can be attributed to its greater stability in urine. However, the relatively high incidence of the dihydrate in crystalluria and urinary stones is not yet fully explained.

One of the major problems in XA of urinary stones is its inability to distinguish the two hydrates of calcium oxalate. Fortunately, both hydrates have highly characteristic crystal habits. Occasionally described pseudomorphism of the bipyramid shaped crystals causes some concern (Berg et al, 1979). The pseudomorphism may be due in part to the partial transformation of weddellite into whewellite, complex mutual growth of weddellite and whewellite (Kim and Johnson, 1981), and certain similarities of the two hydrates at the light microscopic level (Kim et al, 1984). The extent of such pseudomorphism is probably very limited and is mainly of academic interest.

Calcium Phosphates

Six types of calcium phosphates are known in the human body; calcium hydroxyapatite, carbonate apatite, brushite, whitlockite, octacalcium phosphate (OCP), and amorphous calcium phosphate (ACP). Although it is theoretically possible to distinguish

Table 1. Crystals Encountered in a Series of Combined EM Study of Urinary Stones.

Crystal Names	Habits	Elemental contents
Whewellite	Tabular	Ca
Weddellite	Bipyramids	Ca
Apatite	Needles and Plates	Ca, P
Brushite	Arrowheads	Ca, P
Whitlockite	Laminated Spherules	Ca, P
Struvite	Trapezohedral	Mg, P
Uric Acid	Polymorphic	None
NH_4-Urate	Pincushions	None
Sodium Urate	Acicular	Na
Cystine	Hexagonal Plates	S
Gypsum	Sphenoid	S, Ca
Silica	Anhedral Leaf	Si
Na-Phosphate	Empty shells	Na, P

each calcium phosphate by the Ca:P ratio derived from its stoichiometry; such is not the case in practical XA for the following reasons. The Ca:P ratio of a particular calcium phosphate in any single stone as determined by XA tends to vary significantly. This appears to be due mainly to the variations in the angular relationships between the incidental electron beam, the crystal surface, and the detector. Crystals in nature, particularly biological crystals, are seldom, if ever, ideal. A variety of crystal defects and substitutions of the lattice ions are known to occur. Furthermore, calcium phosphates as well as other phosphates are frequently mixed in the same stone and the coexistence may further disturb the Ca:P ratio.

Although OCP has been described in urinary stones, the morphology of naturally occurring OCP has yet to be portrayed. Synthetic OCP is either blade shaped or columnar (LeGeros et al, 1984). Amorphous calcium phosphate with the stoichiometry of tricalcium phosphate has been described in calcified tissues and urinary sediments (Eanes et al, 1965; Cifuentes Delatte, 1983). Presumably because of the difficulty in detecting ACP, its presence in urinary stones has not been conclusively demonstrated. Brushite, having a highly characteristic arrowhead shaped morphology, can be readily identified by SEM (Dosch and Koester, 1975; Kim, 1982). Whitlockite is rather rare in urinary stones. Multilayered concentric laminations appear to be characteristic of whitlockite (Kim, 1982). Blade shaped and cubic whitlockite has been described as well (Dosch and Koester, 1975; Kim, 1983a; Santos and Gonzales Diaz, 1980). Thus, even without the Ca:P ratio, the distinction of various calcium phosphates in most urinary stones is possible by SEM and XA. However, XA is unable to distinguish hydroxyapatite from carbonate apatite. More work is needed in the morphological characterization of rare calcium phosphates, i.e., ACP, OCP and whitlockite.

Struvite

Struvite is one of the largest crystals and is easily recognized by its trapezohedral or coffin-top shape. Even when the crystal is partly buried in a stone, it can be identified by the characteristic Y-shaped cracks on the crystal surface. Struvite yields Mg and P upon XA, which has led to the establishment of its morphological criteria (Kim, 1982). Struvite always coexists with apatite, since both crystals share similar optimum pH ranges for their precipitations; no exception has yet been encountered in the EM series. Newberyite and ammonium magnesium phosphate monohydrate have been described but are very rare. Needle shaped ammonium magnesium phosphate monohydrate was observed in a canine urolith (Cheng et al, 1981). The morphology of newberyite is, thus far, unknown.

Uric Acid and Urates

Of all the components of urinary stones, the identification of uric acid poses a particularly difficult problem. A small amount of uric acid is commonly overlooked by X-ray diffraction as well as infrared spectrometry. Uric acid is also the most difficult crystal to identify by XA and SEM, since the crystal does not contain identifiable elements. Furthermore, uric acid crystals are very polymorphic; equant, columnar, prismatic, fibrillar, tabular, and blade shaped uric acid crystals have been described (Kim, 1982; Kleeberg et al, 1981). One of the major problems in the SEM identification of uric acid is its remarkable similarity to whewellite (Kim et al, 1984). XA distinguishes these two crystals within seconds. By exclusion of crystals containing inorganic elements, uric acid thus can be identified with a high degree of accuracy by SEM. It is noteworthy that XA of uric acid crystals frequently yields elements which are not members of the lattice ions of crystals in urinary stones such as K, Al, Cl, and S. The relationship between these elements and uric acid is not yet known. Two different types of uric acid, anhydrous and dihydrate, occur in urinary stones. The distinction of the two currently requires crystallography.

Two uric acid salts occur in urinary stones, ammonium acid urate and sodium urate. Both crystals have characteristic crystal habits. Ammonium acid urate is recognized by its pincushion shaped appearance and does not contain detectable elements by XA (Ambruster, 1979, Kim, 1982). Sodium urate occurs very rarely and only two cases have been encountered in this series. Crystals of this component are very long and slender with pinacoid tips (Kim, 1982). The crystals were arranged loosely and haphazardly in urinary stones. Presumably because of the loose arrangement of the crystals and lower sensitivity of XA to this element, sodium in the crystals was difficult to detect.

Gypsum

Gypsum is so rare that its presence in urinary stone has been questioned. However, XA has unequivocally demonstrated crystal clusters containing Ca and S in two stones which were subsequently confirmed to be gypsum by electron and X-ray diffractions. Gypsum crystals are sphenoidal in appearance which are formed by the stacking of lamellar crystals (Kim, 1982).

Cystine

The hexagonal plate shape of cystine crystals has been reported (Fuss et al, 1976). However, two of the cystine stones in this series yielded rectangular plates as well. XA of cystine stones yields a surprisingly tall peak of sulfur (Kim, 1982).

Silica

One of the advantages of XA is its ability to detect any amount of crystalline and amorphous inorganic components, i.e., silica in urinary stones. Silica calculi occur usually in patients taking aluminum trisilicate for the treatment of peptic ulcers. Thus far, less than 20 cases have been reported (Medina et al, 1981). However, XA of human urinary stones disclosed a relatively frequent occurrence of amorphous silica and crystalline silicon dioxide in human urinary stones (Alpaugh and Johnson, 1984; Kim et al, 1983). Amorphous silica in urinary calculi escapes detection by X-ray diffraction. No other method currently in use is better suited than XA for the detection of such amorphous inorganic components of urinary stones.

Sodium Orthophosphate Hydrate

A sodium phosphate stone deserves a comment since it further illustrates the high sensitivity of XA in the identification of inorganic components in urinary stones. Sodium phosphate crystal deposition in the human body has been virtually unknown. Because of its high affinity for calcium, phosphate in mammals precipitates as calcium salts with the exception of struvite in urinary stones. A stone containing Na and P was detected by XA and was subsequently identified as sodium orthophosphate hydrate ($Na_3PO_4.12H_2O$) (Kim et al, 1985). The discovery of a new type of stone in a small series of stone analyses further demonstrates the high sensitivity of XA for the identification of rare or scanty inorganic components of urinary stones.

Comparison of XA with Other Methods of Stone Analysis

The confirmation of the crystalline components of a few hundred urinary stones studied by a combined EM has been accomplished by ED. Although ED is highly accurate in crystal identification, the method is not generally utilized for the analysis of urinary stones. Since it is desirable to establish the sensitivity of XA for the analysis of urinary stones relative to other commonly used methods, the results of 86 additional urinary stones analyzed by SEM and XA were compared with those obtained by X-ray diffraction (XRD), infrared spectroscopy (IR), and chemical analysis. XRD, IR, and chemical analysis were performed independently at a separate institution. X-ray powder diffraction was performed routinely using a Diano Semiautomatic Diffractometer, Model XRD 8535 (Diano Corp., Woburn, Mass). When the results were questionable, an additional diffraction using a Debye-Sherrer diffraction camera and infrared spectroscopy with a Perkin Elmer 727 Infrared Spectrometer using the KBr pellet technique were applied. Forty three stones were cracked and analyzed first by XA and SEM and the remaining 43 stones by XRD. Unused portions of the stones were exchanged between the two institutions. Upon completion of the studies, the data were similarly exchanged. Chemical analysis and IR were applied mainly to those stones for which the disagreeing XRD and XA results were obtained.

Table 2. Comparison of 86 stones Analyzed by X-ray Analysis(XA) and X-ray Diffraction(XRD).

Total	Missed by Incidence by XA + XRD	Missed by XRD*	SEM+XA*
Whewellite	53	7	2
Weddellite	34	8	5
Apatite	50	36**	-
Struvite	12	5	-
Uric Acid	10	4	1
NH_4-Urate	3	3	-
Brushite	2	2	-
Cystine	2	-	-
Unidentified	1	1	1
Total	167	66	9

* These are all minor components of the stones.
** The presence of calcium phosphate was further confirmed by chemical analysis.

The comparisons of 86 stones analyzed by XA and XRD are listed in Table 2. Some of the minor discrepancies between the data acquired through the two different methods may be attributed to sampling errors. For instance, minor components of the stones may have been included only in the samples analyzed by XA or vice versa. We are in the process of confirming the occurrence of such sampling errors by exchanging the specimens subjected to the two different methods. Most crystals missed either by XA or XRD were only minor components of the stones. The missing of weddellite in two samples by SEM and XA may be attributed to the emphasis given to the fractured surfaces of the stones in this study. Only the fractured surface and adjacent natural surface of the stone were routinely examined by SEM and XA. Weddellite has a propensity to deposit on the surface of whewellite stones.

It should be noted that the distinctions between weddellite and whewellite, apatite and brushite, and uric acid and its salts by SEM were made on the basis of the morphological criteria. Occasional pseudomorphism of weddellite described in the literature is ignored in this study. Brushite, ammonium urate and sodium urate are highly characteristic in their morphology. Although our preliminary study indicates a possible morphological distinction between anhydrous uric acid and uric acid dihydrate, no such distinctions were attempted in this study.

The study demonstrates that XA exceeds XRD in its sensitivity several fold. This is attributed mainly to the failure of detecting apatite by XRD in more than 70% of the apatite containing stones. The presence of calcium and phosphate in these stones

Table 3. Comparison of 31 Stones analyzed by X-ray Analysis(XA) and Infrared Spectroscopy (IR).

	Total Incidence	Missed by IR	Missed by XA+SEM
Whewellite	16	4	-
Weddellite	7	2	-
Apatite	20	12	-
Struvite	9	3	1
Uric Acid	6	2	-
NH_4-Urate	3	3	-
Cystine	1	-	-
Total	62	26	1

was subsequently confirmed by chemical analysis. A significant finding of the data is the sensitivity of XA combined with SEM to the identification of uric acid and NH_4-urate, since the urates do not contain elements detectable by XA. Uric acid missed by XRD in four cases was also a minor component of the stones. The presence of uric acid was subsequently confirmed by chemical analysis and electron diffraction.

Infrared spectroscopy was applied to 31 stones which had not been unambiguously identified by XRD (Table 3). Although limited in number, the results show again a higher sensitivity of XA compared with IR. IR frequently fails to detect small amounts of apatite and urate. The superiority of XA in the identification of the uric acid is rather surprising. In addition to calcium oxalates overlooked in 6 stones, IR was unable to specify the two different types of calcium oxalate in ten additional stones. Of the three methods compared with XA, the chemical method had the best correlation in the detection of small amounts of calcium phosphate (Table 4).

Table 4. Comparison of 71 Stones Studied By X-Ray Analysis and Chemical Analysis.*

	Total Incidence	Missed by Chem. Anal.	Missed by XA
Apatite	62	2	4
Uric Acid	9	3	-
Total	71	5	4

* These stones contained many other components. Since chemical analysis was performed specifically for apatite and uric acid, the other components were not included.

Summary

The results of this study, although still preliminary, demonstrate that XA combined with SEM has a higher sensitivity than other commonly used methods in many areas of stone analysis. Of the specimen preparations for XA of urinary stones, the method described in this study, i.e. simple cracking followed by sputter coating, which requires only a few minutes of preparation time, is not only economical but highly accurate. A minor disadvantage of the method is its inferiority to the polishing method in the concentration mapping of elements, which is better suited for the quantitation of stone components. Although the quantitation of stone components was not attempted in this study, an objective quantitation using a computerized morphometry should yield highly accurate results. The method of XA described, namely XA of the entire specimen at a low magnification followed by analyses of the representative areas of the stone components at desired magnifications, is also highly accurate. This method takes less than 15 minutes on the average for the complete analysis of a stone.

XA is particularly useful in the detection of minor inorganic components of the stones. For instance, no other method exceeds XA in its sensitivity to the detection of apatite and silica. The results also demonstrate a surprising accuracy of the method in the identification of organic crystals as well. It should be noted that the usefulness of SEM and XA in the identification of rare crystals, particularly organic crystals, remains to be ascertained. An extensive use of the method is likely to discover rare stone components overlooked or unidentified by other methods.

References

Alpaugh HB, Johnson FB (1984) Silicon dioxide calculi in humans in the absence of silicate antacid medication. Scanning Electron Microsc. 1984; II:969-972.

Ambruster T (1979) Ammonium acid urate in human urinary calculi. Naturwissenschaften, 66:115-116.

Beeler MF, Veith DA, Morris RH, Biskind GR (1964) Analysis of urinary calculus. Comparison of methods. Amer. J. Clin. Pathol. 41:553-560.

Berg W, Lange P, Bother C, Rossler D (1979) Pseudomorphosen in Kalzium Oxalat-Harnstein. Z. Urol. Nephrol. 72:351-357.

Berg W, Szabo-Foeldvari E (1982) Das Kristalline Harnsediment in: Seiner Bedeutung die Aetio-Pathogenese und Diagnostik des Harnsteinleidens, Friedrich-Schiller-Universitat, Jena, pp.28-119.

Chambers A, Hodgkinson A, Hornung G (1972) Electron probe analysis of small urinary tract calculi. Invest. Urol. 9:376-384.

Chaussey Ch. (1982) Extracorporeal shock wave lithotripsy. New Aspects in the Treatment of Kidney Stone Disease, S. Karger, Basel, pp.96-101.

Cheng P-T, Pritzker KPH, Tausch J, Pittaway A, Millard J (1981) Analytical scanning electron microscopy and x-ray microdiffractometry of renal calculi using etched plastic sections. Scanning Electron Microsc., 1981; III:163-168.

Cifuentes Delatte L (1983) Crystalluria, in:Stones, Clinical Management of Urolithiasis, RA Roth, B

Finlayson (Eds.), Williams and Wilkins, Baltimore, pp. 8-20.
Dosch VW, Koester C (1975) Rasterelektronenmikroscopie von Harnsteinen. Z. Urol. 68:35-41.
Eanes ED, Gillessen IH, Posner AS (1965) Intermediate states in the precipitation of hydroxyapatite. Nature 208:365-367.
Fuss M, Vanlanduyt P, Jedwab J (1976) A routine method for the integrated study of microcrystals in urine by light and scanning electron microscopy, electron microprobe and micro-x-ray diffraction, in: Urolithiasis Research, H. Fleisch W.G. Robertson, L.H.Smith (Eds.), Plenum Press, New York, pp.269-272.
Hesse A, Lange P, Berg W, Bother C, Hienzsch E (1979) Scanning electron microscope and microprobe investigation of phosphate phases in urolithiasis. Urol. Int. 34:81-94.
Kim KM (1979) X-ray analysis of pathological calcification including urinary stones. Scanning Electron Microsc. 1979; II:739-744.
Kim KM (1982) The stones. Scanning Electron Microsc. 1982; IV:1635-1660.
Kim KM (1983a) Mulberry particles formed by red blood cells in human weddellite stones. J. Urol. 129:855-857.
Kim KM (1983b) Diagnostic electron microscopy of urinary stones, in: B.F Trump, R.T. Jones (Eds.), Diagnostic Electron Microscopy, Plenum Press, New York, Vol. IV; pp.227-247.
Kim KM, Resau J, Chung J (1984) Scanning electron microscopy of urinary stones as a diagnostic tool. Scanning Electron Microsc. 1984; IV:1819-1831.
Kim KM, Alpaugh HB, Johnson FB (1985) Sodium orthophosphate hydrate ($Na_3PO_4.12H_2O$): A new type of human urinary stone. Urol. Res. In Press.
Kim KM, David R, Johnson FB (1983) Siliceous deposits in human urinary calculi - an E. M. study. Urol. Res. 11:155-158.
Kim KM, Johnson FB (1981) Calcium oxalate crystal growth in human urinary stones. Scanning Electron Microsc. 1981; III:146-154.
Kleeberg J, Gordon T, Kedar S, Dobler M (1981) Studies on the morphology of human uric acid stones. Urol. Res. 9:259-261.
Lagergren C (1956) Biophysical investigation of urinary calculi. Acta Radiol. (Stockh.), 133(Suppl.):1-71.
LeGeros RZ, Kijikowska R, LeGeros JP (1984) Formation and transformation of octacalcium phosphate, OCP: A preliminary report. Scanning Electron Microsc. 1984; IV:1771-1777.
Leusmann DB (1983) Routine analysis of urinary calculi by scanning electron microscopy. Scanning Electron Microsc. 1983; I:387-396.
Ljunghall S, Bachmann U, Danielson BG (1980) Epidemiology of renal stones in Sweden, in: G. Brockis, B. Finlayson (Eds.), Urinary Calculus: Proceedings of the International Urinary Stone Conference. Perth. Australia, 1979, Littleton, Mass.:PSG, pp.25-33.
Lloyd DT, Oldroyd NO (1983) Analysis of Urinary calculi, in: Stones, Clinical Management of Urolithiasis, R.A. Roth, B. Finlayson (Eds.) Williams and Wilkins, Baltimore, pp.8-20.
Medina JA, Sanchidrian JR, Cifuentes Delatte L (1981) Silica in urinary calculi, in: Urolithiasis, Clinical and Basic Research, Smith LH, Robertson WG, Finlayson C (Eds.), Plenum Press, New York, pp.923-927.
Otnes B, Montgomery O (1980) Method and reliability of crystallographic stone analysis. Invest. Urol. 17:314-319.
Pollack SS, Carlson GL (1969) A comparison of X-ray diffraction and infrared technics for identifying kidney stones. Amer. J. Clin. Pathol. 52:656-660.
Prien EL (1974) The analysis of urinary calculi. Urol. Clin. N. Amer. 1:229-240.
Robertson WG, Peacock M, Baker M, Marshall DH, Pearlman B, Speed R, Sergeant V, Smith A (1984) Epidemiological studies on the prevalence of urinary stone disease in Leeds, in: Urinary Stone, R. Ryall, J.G. Brockis, V. Marshall, B. Finlayson (Eds.) Churchill Livingstone, Melbourne, pp. 6-10.
Rodgers AL (1981) Analysis of renal calculi by X-ray diffraction and electron microprobe analysis: A comparison of two methods. Invest. Urol. 19:25-28.
Santos M, Gonzales Diaz PF (1980) Ultrastructural study of apatites in human urinary calculi. Calcif. Tiss. Int. 31:93-108.
Schneider HJ (1973) Comparative urinary stone analysis, quantitative chemical, X-ray diffraction, infrared spectroscopy and thermoanalytic procedures. Int. Urol. Nephrol. 5:9-17.
Scott R, Lewi H (1984) The medical prophylaxis of recurrent renal stone disease, in: Urinary Stone, R. Ryall, J.G. Brockis, V. Marshall, B. Finlayson (Eds.), Churchill Livingstone, Melbourne, pp. 148-154.
Shuster J, Scheaffer RL (1984) Economic impact of kidney stones in white male adults. Urology 14:327-331.
Sierakowski R, Finlayson B, Landes RR, Finlayson CD, Sierakowski N (1978) The frequency of urolithiasis in hospital discharge diagnoses in the United States. Invest. Urol. 15:438-441.
Sutor DJ, Woley SE, Mackenzie KR, Wilson R,Scott R, Morgan HG (1971) Urinary tract calculi. A comparison of chemical and crystallographic analysis. J. Urol. 43:149-153.
Takasaki E (1971) An observation on the analysis of urinary calculi by infrared spectroscopy. Calcif. Tiss. Res. 7:232-240.
Vahlensieck EW, Bach D, Hesse A (1982) Incidence, prevalence and mortality of urolithiasis in the German Federal Republic. Urol. Res. 10:161-164.

Discussion with Reviewers

P-T. Cheng: What were the difficulties encountered by XRD that prompted you to study those 31 stones by IR (Table 3)?

Authors: IR was performed to resolve the discrepancies frequently encountered between the results of XRD and XA. However, the results of IR frequently disagreed with those of XA combined with SEM as well. The accuracy of the XA results was eventually confirmed by chemical analysis (Table 4).

D. Leusmann: Did you ever find Ca-oxalate trihydrate in your investigation?

Authors: Calcium oxalate trihydrate occurs frequently in in vitro experiments and has been considered to be a possible precursor of calcium oxalate dihydrate and/or monohydrate (Tomazic BB, Nancollas H: A study of the phase transformation of calcium oxalate trihydrate-monohydrate. Invest. Urol. 16:329-335, 1979). The trihydrate has a polyhedral tabular habit with a propensity to form parallel stacks. Morphologically similar crystals containing only calcium by XA were occasionally encountered in

human stones. However, the trihydrate was not unequivocally identified by XD or ED. Possible difficulty of the crystallographic identification of the trihydrate due to its instability should be considered.

D. Leusmann: Did you find any epitactic relationships between stone forming substances, and do you think that epitaxy plays a dominant role in the formation of urinary calculi?

Authors: Epitaxy refers to a form of heterogeneous nucleation of a crystal occurring on the surface of another crystal, which approximates its atomic spacings to those of the newly seeded crystal. Certain similarities in the unit cell dimensions of crystals in urinary stones have frequently led to the belief that epitaxy predominantly accounts for the stone growth. Although surface nucleation of various crystals was frequently observed in urinary stones, it is not yet possible to determine the extent of epitaxy in the stones with the present state of the art. Furthermore, the crystal growth in urinary stones is too complex to be explained by such a simple phenomenon (Kim and Johnson, 1981, text reference).

D. Leusmann: You mention hydroxyapatite and carbonate apatite as two different kinds of components. Do you think that these are possibly mixed crystals with different contents of carbonate?

Authors: The determination of the degree of substitution of the lattice ions in apatite by carbonate or any other molecules is not an easy task. However, it has been known that apatite formed in carbonate-containing solutions in vitro is frequently 'carbonated.' It is, therefore, conceivable that biological apatite is 'carbonated' to some degree.

D. Leusmann: Were the cystine stones you analyzed pure or did they contain further components such as apatite?

Authors: Every cystine stone examined by XA and SEM contained small amounts of apatite. We have yet to see an exception.

D. Leusmann: After my knowledge and experience, nearly all substances occurring in urinary calculi are more or less altered by the electron beam during electron diffraction. Which beam current and tension did you use in your investigation to avoid the damage to the specimens?

Authors: In view of the high voltage used in ED, some beam damage to the samples is inevitable. However, by turning down the condensers and thus reducing the beam intensity, much of the damage can be avoided, and measurable diffraction patterns can be obtained in most cases. ED is routinely performed at an accelerating voltage of 80 kV and various camera lengths. Poorly crystallized weddellite and urates are particularly difficult to determine by ED.

G.M. Roomans: Would the use of the 'Statham- Small' method for quantitative x-ray analysis of particles (J. A. Small et al: Scanning Electron Microsc. 1978; I:445-454 and P.J. Statham, J.B. Pawley: Scanning Electron Microsc. 1978; I:469- 478) be an improvement over the use of a simple ratio method in the analysis of the calcium phosphates?

Authors: The quantitative XA methods, including those which are cited, may be useful to quantitate various stone components. The methods, in my opinion, are of little value in identification of stone components because of the complexity of the stones.

G.M. Roomans: Would the use of a windowless ED detector, allowing light element detection, be of value in the analysis of stones? Alternatively, do you see a use for EELS of sectioned crystals in analysis of stones?

Authors: The windowless ED detector or any other light element detecting EM analytic method has a theoretical potential in the analysis of urinary stones. The sensitivity and limitations of the method, however, remain to be determined. Our limited attempts to analyze organic components of the stones by wave length dispersive analysis have not been rewarding. Energy loss spectroscopy of sectioned crystals certainly has a place in stone research. However, any method which involves thin sectioning is difficult to apply to a large number of stones. It is also noteworthy that various solutions used in embedding tend to dissolve stone components.

A. Rodgers: You have commented that one of the major problems in XA of urinary stones is its inability to distinguish the two hydrates of calcium oxalate. What scientific or clinical significance is attached to such ability?

Authors: One of the most extensively studied but still unsolved problems in urolithiasis is the relatively high incidence of calcium oxalate dihydrates, whereas the dihydrate is known to be less stable than the monohydrate. In fact, the precipitation and growth of the pure dihydrate in vitro is extremely difficult. Successful elimination of the mechanism of the nucleation and growth of the dihydrate in urinary tract will undoubtedly be a major breakthrough in this field.

A. Rodgers: Is there likely to be any morphological difference between hydroxy- and carbonate-apatite?

Authors: Synthetic carbonate apatite was shown to be vacuolated by TEM (LeGeros RZ, Trantz OR, LeGeros JP (1967) Apatite crystallites: Effects of carbonate on morphology. Science 155:1409-1411), whereas hydroxyapatite is known to be needle or lamellar shaped. Thus far, the distinction of the two apatites by SEM has not been possible.

A. Rodgers: How do the characteristic Y-shaped cracks on the surface of struvite crystals originate?

Authors: The Y-cracks of struvite result from the cyclic twinning growth of hexagonal columns. Cleavage planes of trapezohedral struvite crystals frequently display stacks of hexagonal columns (Kim, 1982, text reference).