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CRYSTALLURIA AND UROLITHIASIS IN A RELATIVELY STONE-FREE
POPULATION

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Abstract

The occurrence of renal stone in South African blacks is extremely rare. Whites however are prone to calculi to the same extent as that reported in other Western communities. The nature of the particulate material and crystalluria in urine samples from the two population groups were investigated using a Coulter Counter and scanning electron microscope. In addition, 10 calculi obtained from black patients over a 5 year period were analysed.

The particle size distribution curves obtained for normal black and white males were identical. The curves for normal black and white females were also identical but different from those for males. Black male stone formers had larger particles than their controls while the single black female stone former investigated had particles of the same size as female controls, but in greater numbers. Scanning electron microscopy revealed profuse amounts of crystalline NaCl, KCl and other salts in the urinary sediments of blacks. These were not observed in the specimens from whites nor in the black stone formers' urines. Analysis of the calculi identified chemical and ultrastructural features similar to those observed in stones from whites.

The hypothesis that the lower incidence of stone disease in blacks may be due to a high Na/Ca ratio is supported by our findings. It is suggested that various salts play a role in lowering the stone forming potential of such urines by a competitive substitution mechanism in which lattice calcium is displaced by sodium. It is also suggested that when urinary stone formation does occur in blacks, it does so via the same physico-chemical mechanisms as in any other race group.

KEY WORDS: Stone-free population; Coulter Counter; calculi; Scanning electron microscopy; urinary salts; crystalluria; particle size-distribution; ultrastructure; inhibitory mechanism; urinary Na/Ca ratio.

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Introduction

The occurrence of renal stone in South African blacks is extremely rare (11,13,16,18,25, 27). During the 9 year period 1971-1979, 1 in 510 white patients admitted to Groote Schuur Hospital, Cape Town, had a renal stone as compared with 1 in 44,298 blacks (14). Immunity to urolithiasis has also been reported for certain other race groups elsewhere in the world. For example, studies at the Darwin Hospital in Australia showed an absence of renal tract calculi in Aborigines despite the fact that this group constitutes 29% of the population served by that institution (1). The significantly low incidence of urinary calculi in the American Negro has been well documented (3, 6,7). Eskimos also appear to rarely form stones (13).

Modlin has reported extensively on the chemical composition of urine from black subjects and has suggested that their immunity to urolithiasis is due to their singular and immutable dietary habits which result in a urine the composition of which creates a milieu that is inimical to the formation of crystals (13,14). On the other hand, renal stone occurs in the white South African population with the same frequency as that reported in other Western communities (13). The presence therefore of a stone-free group (blacks) and a stone-prone group (whites) within one population provides an ideal situation for a comparative study.

Studies have shown that there is a qualitative and quantitative difference in the crystalluria of recurrent, idiopathic stone formers and their controls under the same conditions of dietary and fluid intake (5,21). We have postulated that differences also exist in the urine particle composition of a stone-prone and a stone-free group. In order to establish whether this is so and in the hope of obtaining some clues that might explain the difference in the stone forming potentials of urine from the two groups, we undertook to study the nature of the particles and crystalluria in urine samples from the black and white communities.

Materials and Methods

Subjects

The white subjects studied were 15 healthy stone-free males aged between 21 and 54 years (WM) and 12 healthy females aged between 21 and 45 years (WF) all of whom are members of the staff of the School of Chemical Sciences and the Department of Physiology, University of Cape Town. The black subjects were 20 males aged between 19 and 64 years (BM) and 20 females aged between 21 and 60 years (BF) who were randomly selected from those attending the general outpatient clinic at the Groote Schuur Hospital, Cape Town. Past medical histories were reviewed to ensure that no subject with a known metabolic or urinary disorder was included in the sample groups. Urine samples were collected in pre-heated vacuum flasks and were analysed at 37°C within a few hours of voiding.

Urine specimens from black stone formers (3 males and 1 female) were obtained from patients at the Department of Urology, Tygerberg Hospital, Cape Town and were collected either just prior to or just after surgery. The calculi removed from 3 of these patients (2 males, 1 female), as well as others collected over a 5 year period were used in the present study.

Particle size distribution analysis

A model TA II Coulter Counter coupled to a Population Accessory unit and fitted with a 100 μm diameter orifice was used for particle distribution analysis. Samples to be counted were pipetted into a double-walled glass vessel with an internal capacity 230 ml through which a low viscosity oil was pumped from a thermostatically controlled oil bath maintained at 37°C. The instrument was calibrated using Latex calibration beads (Coulter Electronics, Hertfordshire) of diameter 19 μm suspended in azide free ISOTON II solution.

Each urine sample was filtered through a 74 μm sieve to remove particles too large to be accommodated by the Coulter Counter. Thereafter, a 1 ml aliquot of filtered urine was pipetted into 150 ml of the thermostatted ISOTON II electrolyte and subjected to a trial count. Further aliquots were added in those cases where the concentration of particles was not sufficiently high to yield a statistically reliable particle size distribution. All samples were continuously stirred. The instrument was set to allow 2 ml of the ISOTON/urine solution to be drawn through the aperture for each counting procedure. Each sample was counted 3 times.

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

Urine samples at 37°C were centrifuged for 5 minutes using a PICCOLO table-top low speed centrifuge operating at 2000 revolutions per minute. The deposited crystals were removed by repeated aspiration using a Pasteur pipette. Drop amounts were then filtered through a 0.2 μm Nuclepore filter (13 mm diameter) supported in a Sartorius membrane filter clamp (GMBH Gottingen). The filter papers, with deposited crystals, were then pasted onto aluminium stubs for SEM analysis. These were coated with approximately 100 nm of carbon at a pressure of about 1.3 mPa in a

Balzer's vacuum coater equipped with a planetary sample rotator. Specimens tilted at 35° to the collector were examined using a Cambridge S180 Scanning Electron Microscope operating at a nominal beam potential of 15 kV and beam current of 100 μA .

Images were recorded on Ilford FPA roll film at 60 second frame periods and 800 lines per frame. The SEM was equipped with an energy dispersive X-ray analyser system which was used for routine elemental analysis of the specimen (EDX).

Representative samples from various regions of each calculus were removed with a stainless steel probe and were ground to a fine powder with mortar and pestle. These were subjected to X-ray powder diffraction analysis (XRD). Diffraction patterns were recorded on KODAK DEF-392 film by the Debye-Scherrer method using a Philips powder camera of radius 28.65 mm and Ni filtered $\text{CuK}\alpha$ radiation of wavelength 1.5418 Å. In addition, grains from representative regions of each stone were mounted on graphite stubs and were carbon coated, as described above, prior to SEM analysis.

Results

Particle volume-size analysis

Volume-size distribution curves were obtained by plotting V_d against d where V_d is the volume of crystals of diameter d . The mean curves so obtained for the WM and BM samples (figures 1 and 2 respectively) are very similar and show a high incidence of particles at a diameter of $\sim 3 \mu\text{m}$. Both groups showed a second, smaller peak in the range 15-25 μm but this is somewhat more pronounced in the BM samples. On the other hand a distinct third peak is apparent at a diameter of 10 μm in the WM group.

The particle volume-size distribution curves for the WF and BF groups (figures 3 and 4 respectively) are similar but both are significantly different to those of the males. The former have a small peak at a diameter of $\sim 3 \mu\text{m}$ as well as a second much larger peak in the range 15-35 μm with the maximum particle volume corresponding to a diameter of 25 μm .

The mean particle distribution curve obtained for the BM stone formers (figure 5) has a dominant peak at a diameter of 10 μm . Two smaller peaks at 3 and 25 μm also occur. The distribution curve for the single BF stone former is shown as a dotted line in the same figure.

The histogram in figure 6 shows the mean percentage of the total volume due to particles having diameters $> 12 \mu\text{m}$. For females, the contribution from such particles is 60-70% while in males the figure is only 20%. In BM stone formers it is even less with a value of 12%.

Stone-formers' clinical profiles and urinalysis

Clinical profiles for each black stone-former as well as the composition of their respective calculi (as determined by XRD, SEM and EDX) are listed in Table 1. Urinalysis figures for 8 of these subjects are given in Table 2. In addition to the constituents listed, Na and K were determined in three patients (means 2323 ± 943 and $1838 \pm 469 \text{ mg/24 hr}$ respectively) and Mg in five patients (mean $97 \pm 24 \text{ mg/24 hr}$). All serum Ca,

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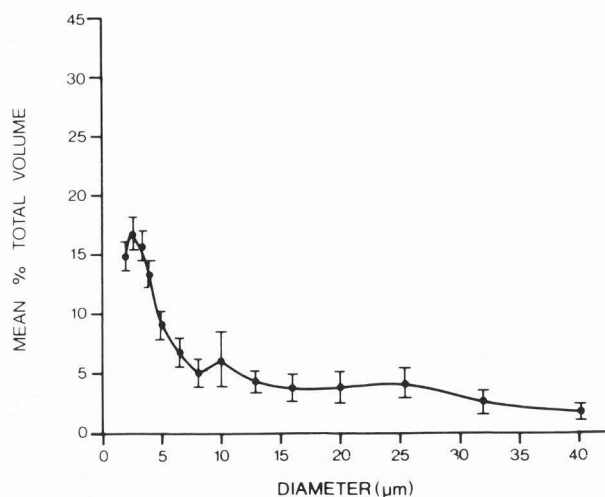


Fig 1: Mean volume-size distribution curve (\pm SEM) of particles in the urine at 37°C of 15 white male subjects.

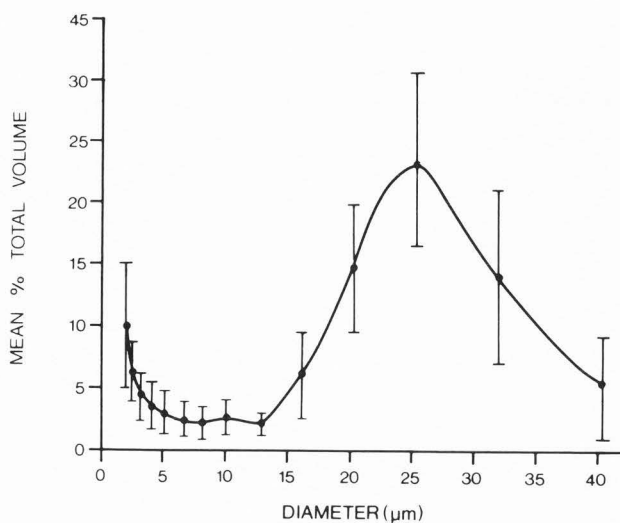


Fig 3: Mean volume-size distribution curve (\pm SEM) of particles in the urine at 37°C of 12 white female subjects.

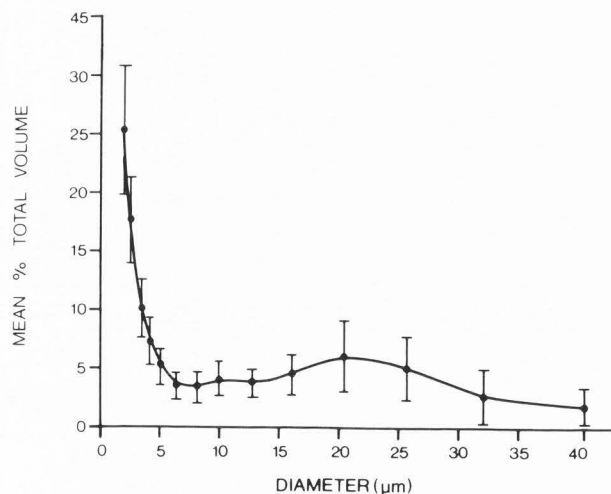


Fig 2: Mean volume-size distribution curve (\pm SEM) of particles in the urine at 37°C of 20 black male subjects.

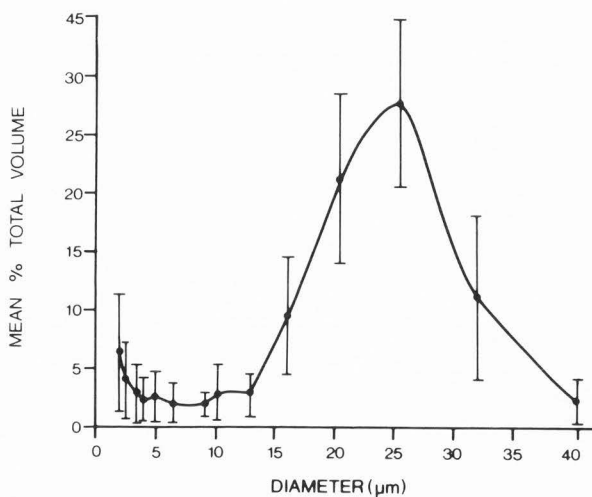


Fig 4: Mean volume-size distribution curve (\pm SEM) of particles in the urine at 37°C of 20 black female subjects.

P, uric acid, total protein, albumin and alkaline phosphatase were within normal limits.

SEM analysis

The urinary deposits from the BM controls did not contain significant quantities of any recognizable crystalline material. Occasionally single, isolated calcium oxalate dihydrate (COD) crystals (< 5 µm diameter) were observed. Small aggregates of COD were exceptionally rare. One such example however is shown in figure 7. A feature of the BM specimens was the presence of profuse deposits of NaCl, KCl and a number of other urinary salts containing variable combinations and concentrations of Na, P, S, Cl, K and Ca. Typical examples include clusters (diameter ~15 µm) containing Na, S, Cl and K (figure 8) and "spiky" aggregates (diameter ~40 µm) containing Na, P, S, Cl, K and

Ca (figure 9). These respective EDX spectra are shown in figures 10 and 11. Bread-loaf shaped entities (diameter ~12 µm) of purely organic composition (figure 12) and spherular deposits (diameter ~5 µm) containing Na, S and K (figure 13) were also observed in several samples.

Profuse deposits of NaCl and KCl were also a feature of the BF control urines. No COD crystals were observed in any of these samples. Deposits rich in K and S with small amounts of Na, P and Cl were frequently observed. Examples of these are shown in figure 14 with the corresponding EDX spectrum in figure 15. Chunks of debris (diameter 30-40 µm) containing small amounts of particulate matter were frequently observed (figure 16). Trace amounts of Na, P, S, Cl and K were found to be present in such material. The deposits

TABLE 1. CLINICAL PROFILE OF BLACK STONE-FORMERS

PATIENT NO.	SEX	AGE	CLINICAL PRESENTATION	PRE-DISPOSING FACTORS	MANAGEMENT	STONE COMPOSITION
276	M	12	FLANK PAIN	UTI, MALNUTRITION	PYELOLITHOTOMY (R)	AAU
306	M	23	FLANK PAIN	UNKNOWN	UPPER POLE HEMINEPHRECTOMY	COM
385*	M	55	-	-	-	COM, APA
397*	F	39	-	-	-	STR, APA
415	M	22	FLANK PAIN	UNKNOWN	PYELOLITHOTOMY (R), PERCUTANEOUS NEPHROLITHOTOMY	COM, COD
535	M	40	FLANK PAIN, HAEMATURIA	UNKNOWN	URETEROLITHOTOMY (R)	COM
573**	F	43	RENAL COLIC	RECURRENT UTI	URETEROLITHOTOMY (L)	STR, APA, COM, COD
626	M	60	RENAL COLIC	UNKNOWN	URETEROLITHOTOMY (L)	COM
645**	M	35	BILATERAL FLANK PAIN	IVP:PAPILLARY NECROSIS, MEDULLARY SPONGE KIDNEY	PYELOLITHOTOMY (R)	COM
646**	M	45	BACK PAIN	IMMOBILIZATION (ANKYLOSING SPONDYLITIS)	PERCUTANEOUS NEPHROLITHOTOMY (R)	COM

** Urine samples from these patients were subjected to particle counting and sizing using the Coulter Counter. (Urine from a third male stone former (patient No. 000, Table 2) was analysed in the same way. However this patient has not as yet undergone surgery for the removal of his stone).

* hospital records incomplete

AAU: ammonium acid urate; APA: apatite; COD: calcium oxalate dihydrate; COM: calcium oxalate monohydrate; STR: struvite; UTI: urinary tract infection; IVP: intravenous pyelogram.

typically observed in the BM control samples, figures 8, 9, 12 and 13) were not observed in the female controls.

The deposits from the BM stone formers' urines were surprisingly featureless. Several isolated, single COD crystals (diameter ~10 μ m) were observed in one of the specimens together with some KCl deposits. The other two samples showed only KCl. Other urinary salts such as those described for the BM controls (figures 8, 9, 12 and 13), were not observed.

The urinary deposits obtained from the single BF stone former contained debris particles similar to the example shown in figure 16. However no KCl, NaCl or deposits rich in K and S typical of those occurring in the BF controls, were observed in this sample.

The WM and WF control urines did not contain any of the urinary salt deposits which typically characterised the specimens from black subjects. Occasionally envelope-shaped COD and dumb-bell-shaped calcium oxalate monohydrate (COM) crystals were observed. An example of the former is shown in figure 17. Non-descript debris particles were

invariably present.

SEM examination of the calculi in this study revealed well-known ultrastructural features. Radially striated COM crystals were frequently observed (figure 18) in the oxalate-containing stones as were clusters of COM plates (figure 19). Tiny apatite (APA) spherules enmeshed in a mucoid glue were common (figure 20) in the STR/APA and COM/APA stones. Figure 21 shows the presence of small holes in the APA substrate surface while the well known "Y" shaped cracks associated with struvite deposits were also observed (figure 22). Stone 276 contained aggregates of fibrillar ammonium acid urate (AAU) crystals (figure 23).

Discussion

Particle volume-size distribution studies by other workers have shown that there is a qualitative and quantitative difference between recurrent idiopathic stone formers and their controls (5,21). The distribution pattern in the latter shows a preponderance of small crystals in the 3-4 μ m diameter range while in stone formers there

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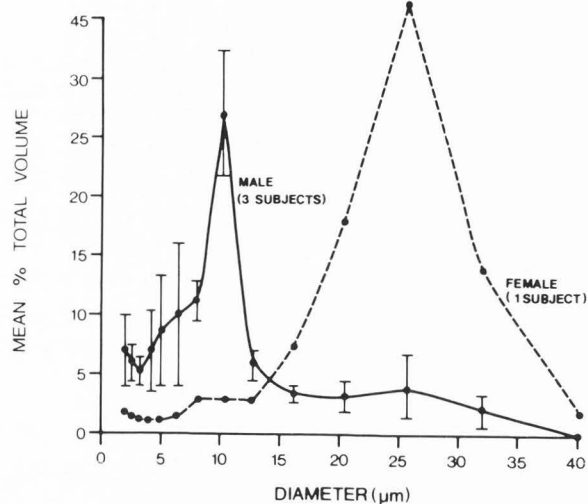


Fig. 5: Mean volume-size distribution curve (\pm SEM) of the particles in the urine at 37°C of 3 black male stone formers (solid line) and 1 black female stone former (broken line).

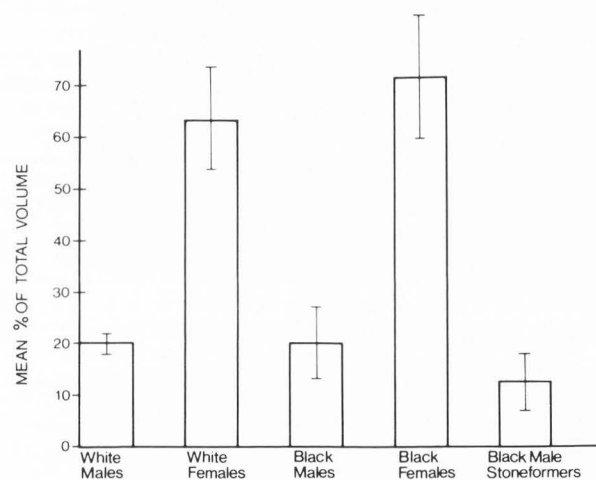


Fig. 6: Histogram of the mean percentage total volume (\pm SEM) of particles with diameter > 12 µm.

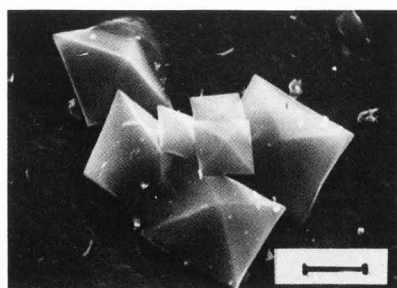


Fig. 7: Aggregate of COD crystals, each in the size range 2-10 µm. Such deposits were extremely rare in the urines of black subjects. Bar = 3 µm.

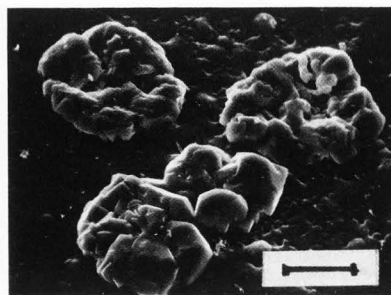


Fig. 8: Typical deposits observed in BM urines. Bar = 10 µm.

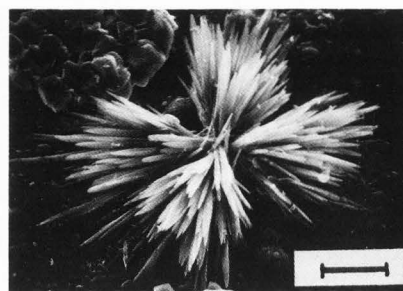


Fig. 9: Aggregates of "spiky" crystals frequently observed in BM urines. Bar = 10 µm.

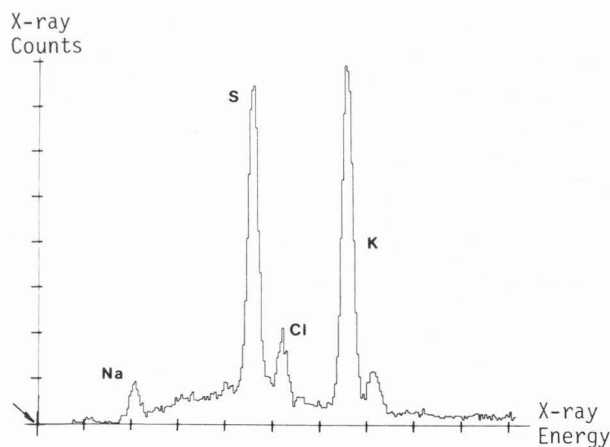


Fig. 10: EDX spectrum of deposits in figure 8.

is, in addition, a second peak of much larger particles in the 20-40 µm diameter range. It is tempting to speculate that such differences might also exist in the crystal distribution patterns of urines from populations with different stone forming potentials. However, if the peak occurring at 10 µm in the WM urines is disregarded, the particulate population curves obtained in the present study for urine from these 2 groups are identical and show no differences (figures 1 to 4).

TABLE 2. MEAN TWENTY-FOUR-HOUR URINARY CONSTITUENTS IN BLACK STONE-FORMERS

PATIENT NO.	VOLUME ml	Ca mg	PO ₄ mg	UA mg	CYSTINE μ mol	ORNITHINE μ mol	LYCINE μ mol	ARGININE μ mol
306	2164	124	2347	622	105	83	128	47
415	4640	232	1824	353	71	99	88	negligible
535	830	200	2584	454	81	53	125	negligible
573	1783	223	3487	319	cystine screen test negative			
626	1700	308	1824	-	88	26	269	125
645	2360	248	1967	235	cystine screen test negative			
646	1070	72	1734	420	cystine screen test negative			
000	1740	208	3154	235	cystine screen test negative			

Particle size distribution analysis does not therefore provide any obvious insight into the stone protective mechanism inherent in the urine of black subjects.

Of some interest is the particle distribution pattern obtained for females which is clearly significantly different to that of the male subjects. Previous distribution studies reported by other workers do not make mention of this difference. We therefore conclude that such studies have been confined to male subjects only. Indeed Robertson et al. (21) used 6 male idiopathic stone formers and 6 male controls for their study while Crassweller et al. (5) do not indicate whether their subjects were male or female. There exists a host of reasons which might explain this difference. These include, for example, cyclic hormonal influences on the female urogenital tract and on oxalate metabolism. Another reason might be associated with the more powerful urinary stream that has been reported for females at all ages (15). We suggest that as a result thereof, larger particles (debris and/or crystals) are more readily flushed from the female urinary tract than from their male counterparts giving rise to a particle distribution that is shifted to the larger diameter range. It must be emphasised that the Coulter Counter does not distinguish between crystalline and non-crystalline material. As such, the "particles" counted by the instrument could indeed be epithelial debris which has sloughed off, say, the bladder wall.

Epidemiological studies have shown that the incidence of renal stone is at least twice as common in males as in females (2). In essence therefore, comparison of male/female crystalline features is analogous to white/black comparisons in that both cases involve a stone-prone and a relatively stone-free group. If this is so, then the marked difference in the volume-size distribution patterns of males and females observed in this study might well be of some significance in understanding the processes which determine urolithiasis. The occurrence of a dominant peak at 25 μ m in the distribution curve of females might

indicate, for example, an upper limit of particle size imposed by some, as yet unidentified, inhibitors.

The particle volume-size distribution curve obtained for the BM stone formers is somewhat surprising in that it shows a peak at a diameter of 10 μ m (figure 5). Other workers have reported peaks in the 3-4 μ m and in the 20-40 μ m diameter range for stone formers (5,21). Our curve does indeed show a small peak at a diameter of 25 μ m as well as a moderately sized one at a diameter of 3 μ m. It is therefore apparent that while the urine of BM stone formers is characterised by larger particles than those which occur in BM controls, these particles are significantly smaller than those in WM stone formers.

Robertson and Peacock have suggested that the dividing line between small and large crystals be drawn at a diameter of 12 μ m (20). They have accordingly shown that stone formers have a significantly higher percentage of crystals with diameters > 12 μ m than do controls. However, figure 6 shows that in the present study, particles in the BM stone formers' urines with diameters > 12 μ m contribute less towards the total particulate volume than do those of the controls. Clearly, if the dividing line is drawn at 10 μ m, the ratio would be dramatically altered. It is moreover noted that a small peak occurs at a diameter of 10 μ m in the distribution curves of the controls. It is therefore suggested that 10 μ m might be the critical particle diameter in evaluating BM urolithiasis.

Although we managed to obtain urine from only one BF stone former, we nevertheless wish to tentatively comment on the particle distribution pattern determined for this sample (figure 5). The curve is very similar to the mean curve for BF controls (figure 4) and shows a large peak at a diameter of 25 μ m. However, there are many more particles of this size in the stone former with a contribution of 40% to the total particulate volume as compared to 27% in the controls.

While the Coulter-Counter studies did not reveal any significant differences in the urinary

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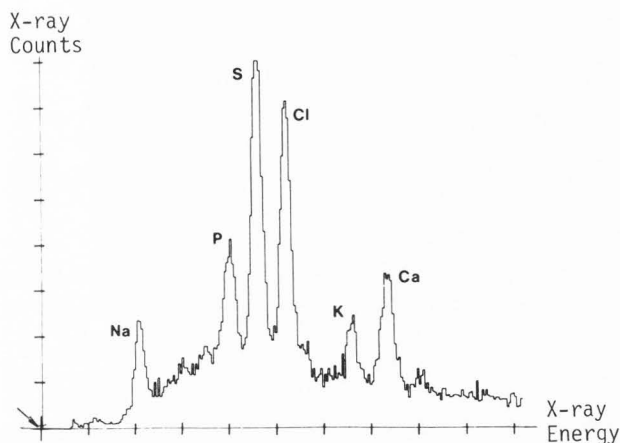


Fig. 11: EDX spectrum of "spiky" crystal aggregate in figure 9.

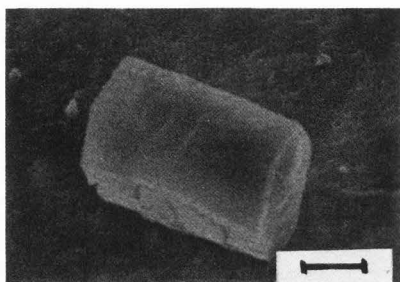


Fig. 12: "Bread-loaf" shaped deposit frequently observed in BM urines. Bar = 3 μ m.

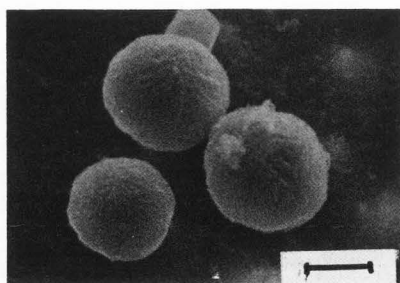


Fig. 13: Spherular deposits frequently observed in BM urines. Bar = 3 μ m.

particle size-distribution patterns of black and white subjects, our SEM investigations identified qualitative differences.

Conventional crystalluria as reported by other workers (26), does not seem to occur in black subjects. Instead, profuse deposits of sodium and potassium chloride, which are not observed in the filtered urinary deposits of white subjects, are common. In addition, BM urines contain other salts which were not detected in the BF specimens nor in any of the deposits from the white group. The BF group in turn seems also to

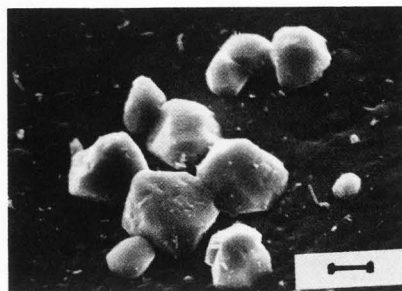


Fig. 14: Deposits frequently observed in BF urines. Bar = 3 μ m.

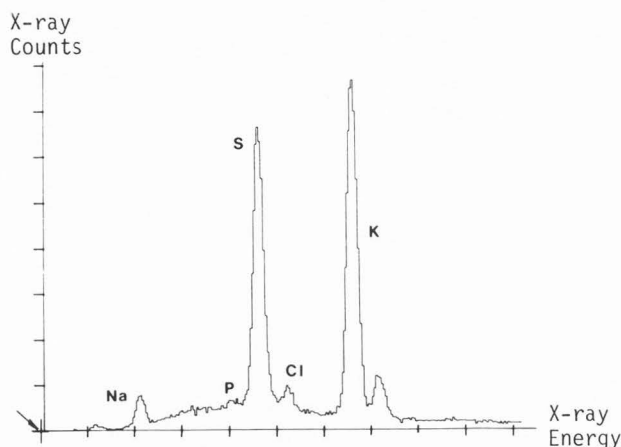


Fig. 15: EDX spectrum of deposits in figure 14.

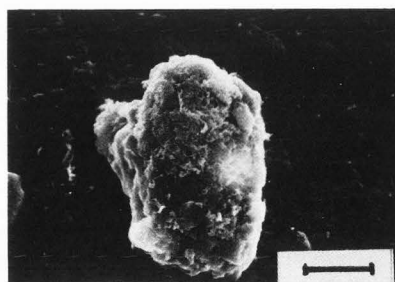


Fig. 16: Chunk of debris containing small amounts of particulate matter frequently observed in BF urines. Bar = 10 μ m.

have its own unique salt, containing mainly K and S. It is unlikely that these salts were deposited as a result of erroneous experimental procedures (such as rapid cooling of urine, for example). All procedures and experimental conditions were constant throughout the investigation and were equally applicable to the urine samples from black and white subjects.

It is noted that the above-mentioned salts, with the exception of a small amount of KCl in one specimen, were found to be absent from the stone formers' urinary deposits. It is therefore

GROUP	Na	Ca	PO ₄	K	Mg
BLACK SUBJECTS	6716 (2438)	81 (56)	614 (314)	3198 (1314)	113 (63)
WHITE SUBJECTS	4899 (1817)	138 (74)	940 (236)	3425 (1212)	122 (40)
WHITE STONE-FORMERS	3450 (1633)	162 (88)	752 (286)	-	105 (39)
BLACK STONE-FORMERS	2323 (943)	202 (73)	2365 (662)	1838 (469)	97 (24)

CONCENTRATIONS EXPRESSED IN mg PER 24 HOURS

Data for black subjects, white subjects and white stone formers are from Modlin (13); data for black stone formers are from the present study. Standard deviation values are presented in brackets.

suggested that they might play some role in lowering the stone forming potential of urine from black subjects thereby providing them with a protective mechanism against urolithiasis.

Modlin has reported that the urine of South African blacks has a low concentration of both Ca and P and a high concentration of sodium (the latter due to their high dietary intake of table salt), relative to South African whites (13,14). He has postulated that the resultant high urinary Na/Ca ratio constitutes a physiological crystallization inhibitor mechanism and argues that even if a crystal nucleus of an insoluble calcium compound such as APA is formed in the urine, the concentration of Na relative to Ca in solution would tend to prevent further deposition by say, epitaxy, by the competitive substitution of lattice ions, the Ca²⁺ ions being displaced from the solid by the entering Na⁺ ions. It is unlikely however that epitaxy is involved as this is energetically unfavourable. A phase change and/or short- as well as long-range lattice disorder are much more likely events.

The urinalysis data of the present study are somewhat interesting when compared with Modlin's data (Table 3). It is seen that the mean Ca value for black stone formers is significantly greater than that reported by Modlin for normal black subjects (13). Indeed, it even exceeds Modlin's value for white controls and white stone formers. A similar inversion of results occurs with our Na values. Whereas Modlin's mean value for black subjects is high, that recorded for our black stone forming patients is low. We have also recorded very much higher levels of PO₄³⁻ in the stone formers' group as compared to normal black subjects (13).

It thus appears that the urine of black stone formers is characterised by a high concentration of Ca (and PO₄³⁻) and a low concentration of Na. As a consequence, the Na/Ca is low (Table 4). In the present study this ratio had a value of 20 (concentrations expressed in mmol/24 hr). Not surprisingly this value is significantly lower

than the values of 105 and 43 reported by Modlin for black and white subjects respectively, but agrees very well with the value of 17 for white stone formers (13) (Table 4).

BLACK SUBJECTS	105 (72)
WHITE SUBJECTS	43 (29)
WHITE STONE-FORMERS	17 (8)
BLACK STONE-FORMERS	20 (8)

(CONCENTRATIONS EXPRESSED IN mmol PER 24 HOURS)

Our results therefore support Modlin's hypothesis in that we have shown the converse to be true viz urolithiasis in South African blacks is associated with a low Na/Ca ratio.

Our SEM observation of profuse Na deposits in the BM and BF controls coupled to the fact that these deposits are absent in the stone formers' urines lend further support to Modlin's hypothesis. Moreover, it is likely that K⁺ ions may also play some role in the protective mechanism of normal blacks since K levels in our black stone-forming patients are somewhat suppressed relative to normal black subjects (Table 3). The observation of KCl and other potassium-containing deposits in our samples tends to support this idea.

Although Mg deficiency is regarded as playing a key role in calcium oxalate urolithiasis (12), no significant differences were observed in the values between the black stone formers of the present study and the black and white normal

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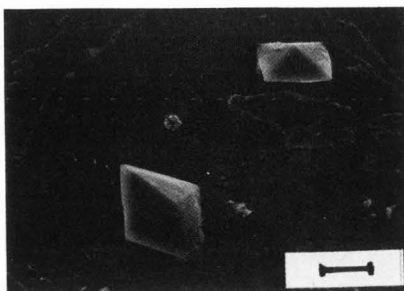


Fig. 17: Two envelope-shaped COD crystals occasionally observed in the urine of white subjects. Bar = 10 μm .

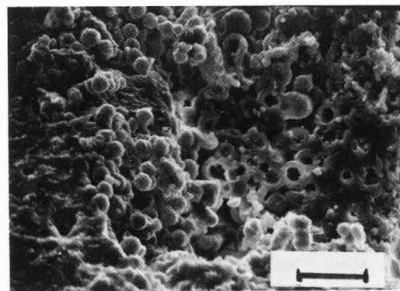


Fig. 21: Holes in APA surface might be the sites from which calcified bacteria or APA spherules have become dislodged (stone 397). Bar = 10 μm .

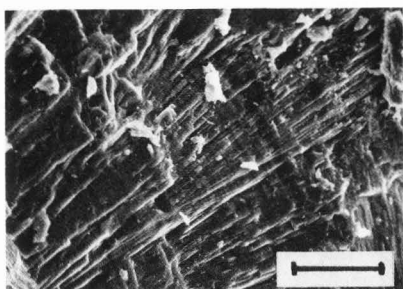


Fig. 18: Radially striated COM crystals (stone 306). Bar = 30 μm .

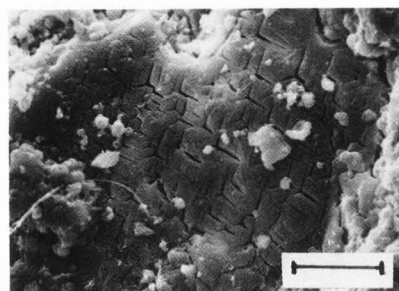


Fig. 22: Characteristic "Y" shaped cracks in struvite surface (stone 573). Bar = 10 μm .

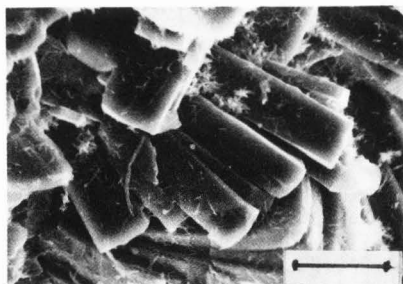


Fig. 19: Cluster of oblong, plate-like COM crystals (stone 535). Bar = 10 μm .



Fig. 23: Aggregate of fibrillar AAU crystals (stone 276). Bar = 10 μm .

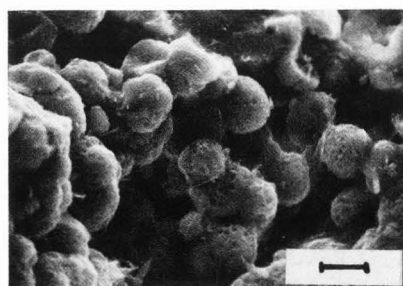


Fig. 20: Spherular deposits of APA enmeshed in a mucoïd glue (stone 573). Bar = 3 μm .

subjects investigated by Modlin (13). Nevertheless the trend shown in Table 3 clearly indicates that black stone formers appear to have the lowest Mg levels. Heterozygous cystinuria is also known to increase the risk of calcium oxalate stones (19). However, Table 2 shows that normal levels of cystine were measured in the black stone-formers' urines.

As far as stone formation itself is concerned our SEM studies have revealed well known ultrastructural features commonly observed in urinary calculi. Radially striated directional growth of COM similar to that shown in figure 18 has been described by Ogbuji and Finlayson (17) while the spherular nature of APA, as shown in figure 20, has been widely reported (8,22,24,26). The binding role of mucoïd material, shown in figure 20,

has often been described (4,17,23). Small holes in APA deposits (figure 21) have previously been reported for urinary and other calculi and are thought to arise from the dislodging of calcified bacteria (10,22). The "Y" shaped cracks in struvite (figure 22) have been described by Kim (8) and are thought to occur as a result of the cyclic twinning growth of hexagonal columns (9). Fibrillar AAU crystals similar to those shown in figure 23 have also been reported by Kim (8). Since ultrastructural features, such as those described above, are due to numerous physico-chemical factors, we suggest that when urinary stone formation occurs in South African blacks, it does so via the same mechanisms as it does in any other race group.

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We wish to record our thanks to the University of Cape Town and South African Medical Research Council for the award of research grants. We are extremely grateful to the Plant Protection Research Institute (Stellenbosch) for the loan of the Coulter Counter as well as Coulter Electronics SA (Pty) Ltd through whose good offices the loan of the instrument was made possible. All SEM studies were conducted in the Electron Microscope Unit of the University of Cape Town.

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Discussion with Reviewers

K.M. Kim: How did you continuously stir the samples to prevent the sinking of crystals in the Coulter Counter?

Authors: The Coulter Counter is equipped with an electronic stirrer which was kept in operation throughout the sampling and counting procedure.

K.M. Kim: Although human urine contains large quantities of NaCl and KCl, these salts do not normally precipitate in urine because of their solubility. How were the samples prepared for SEM? Were the crystals washed properly after filtration through the Nucleopore membranes?

Authors: All urine samples were treated in the same way. Specimens at 37°C were centrifuged after which deposited crystals were removed using a Pasteur pipette. These were filtered through 0.2 µm Nucleopore filters which were then pasted on to aluminium stubs for SEM investigation. Deposited crystals were not washed after filtration.

K.M. Kim: How would high concentrations of NaCl and KCl in urine reduce the incidence of urinary stones?

Authors: High concentrations of sodium (and perhaps potassium) may prevent the deposition of calcium salts by a competitive substitution mechanism in which Ca²⁺ ions are displaced from their lattice positions by entering Na⁺ ions.

G.S. Mandel: What was the distribution of particles greater than 74 µm, i.e., those not included in this study?

Authors: Particles with diameters greater than 74 µm were not included in this study as they are too large to be accommodated by the instrument. Such particles have also been excluded by other workers in their respective Coulter Counter studies (5,21).

G.S. Mandel: I was intrigued by the presence of NaCl crystals in the urine of blacks. How much salt do they ingest? This dietary effect might explain the very low incidence of stones in South African blacks, but really could not be extrapolated to American blacks who also have a reduced incidence of stones.

Authors: The average daily intake of sodium chloride in South African blacks is 17 gm as compared to 10 g in Western communities (13). It should be noted that it is not the high dietary salt intake per se that provides the black population with a protective mechanism against urolithiasis but the fact that this dietary feature is coupled to relatively low urinary calcium levels. It is thus the high Na/Ca ratios that appear to be of importance. Investigation of urinary calcium levels in American blacks would thus be of some considerable interest.

P.T. Cheng: Do you think the chunks of debris (30-40 µm) as observed by SEM in BF samples contribute significantly to the dominant peak at 25 µm as measured by Coulter Counter? Is there any information on the internal ultrastructure of

these particles?

Authors: We believe that these chunks do indeed contribute significantly to the dominant 25 µm peak as no other particles such as crystal aggregates or salt deposits were observed at this diameter. The internal ultrastructure of the debris particles was not investigated in this study.

S. Deganello: What relationship, if any, would you expect to find between high salt content and the presence of a high molecular weight inhibitor?

Authors: We are not aware of any such relationship.