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Original Research Paper

Urology

CALCULOGENESIS- IS IT SIMILAR TO GEOLOGICAL ROCKS AN IN DEPTH. ULTRASTRUCTURAL STUDY

Dr. A. K. Sanwal* Assistant Professor, Department of Urology, M.L.B. Medical College, Jhansi. *Corresponding Author

Dr. Sanjay Chaube Associate Professor, Department of Surgery, M.L.B. Medical College, Jhansi

Dr. Surya Prakash Professor and Head, Department of Surgery, M.L.B. Medical College, Jhansi

ABSTRACT Urinary calculus disease is one of the oldest known medical disorders of mankind. Despite an enormous effort devoted to unravelling the mechanism of urolith formation and development, the subject is still wide open to discussion. Present work efforts to elaborate on this complex subject with reference to stone substance architecture with correlation of structure and composition, from nucleus to periphery. 30 "urinary stone's" were randomly picked up from the rockery of the division of urology, I.M.S., B.H.U. chemical composition is determined by "chemical spot test" and by X-ray diffraction techniques. Calcium oxalate stones had largely 'crystalline and microcrystalline' patterns though not uncommonly 'cryptocrystalline' habits were observed. Phosphatic calculi were largely in 'crypto-crystalline' form though large 'phenocrysts' were also demonstrated. Disparity between nucleus and shell has been shown in a large number of urinary calculi. Geological phenomenon of 'zoninning ', 'itching', 'fracture', 'columnization', and twinning were observed. A varying pattern of stone architecture of the same chemical composition indicates a constantly changing physico-chemical environment inside the kidney and pelvicalyceal system. Classical morphological habits of given chemical constituents, though seen, may not be the rule, due to changing crystallisation conditions. Findings indicate that the process of urinary stone formation and development is comparable to geological rock formation. Summary: Results support the 'Geological hypothesis' for urinary stone formation and development.

KEYWORDS : Urinary stone, ultrastructure, geological hypothesis stones.

INTRODUCTION

Urinary calculus disease is one of the oldest known medical disorders of mankind. Although the bladder stone was the typical urinary concrement of history, renal and ureteric stones are very common today.

Despite an enormous effort devoted to unravelling the mechanism of urolith formation and development in recent years, the subject is still wide open to discussion.

The investigation of the fundamental causes of urolithiasis involves both clinical and scientific approaches, the latter essentially embracing two areas: crystallisation experiments and stone analysis.

On the other hand stone analysis allows the investigator to characterise the chemical conditions prevailing at the time of nucleation and growth.

Accurate knowledge of the chemical composition of a calculus is important not only with regard to understanding its genesis, but also for the prescription of suitable prophylactic measures.

An indepth ultrastructural study also requires the knowledge of the chemical composition of stone for which crystallographic techniques are considered as gold standard, i.e. X-ray diffractometry and optical methods.

X-ray diffraction is used universally for analysis of a urolith, constituents within a particular stone can be identified by comparison with a reference set of values for known calculus. Though constituents in less than 10-20% concentration have very weak diffraction patterns. It remains the single most important method for stone analysis.

SEM is ideally suited to study the ultrastructure in depth, specially of human urinary calculi which are often irregular; Routine qualitative analysis was done on all stones removed.

Present work is to elaborate on this complex subject, with the objective of study of ultrastructure with reference to stone substance architecture from nucleus to periphery with correlation of structure and composition.

Our observations have supported the 'geologic stone formation theory of calculogenesis'

METHODS

The study involves determination of chemical composition of 30 urinary calculi by 'spot' test as well as x-ray diffraction. Ultrastructure of urinary calculi was determined by scanning electron microscopy. This work was carried out in the urological laboratories of the division of Urology in collaboration with Departments of Geology and Metallurgical Engineering, B.H.U. Material: Thirty calculi are- randomly selected out of Division's -rockery-.

METHODS:

These urinary calculi were washed in tap water followed by distilled water, dried in air and photographed for gross morphology. These stones where cut with the help of a hand saw and representative samples having nucleus and periphery in a single piece were selected from each stone for SEM. Chemical analysis and X-ray diffraction analysis were performed using the remaining part.

X-ray Diffraction Analysis Were Done.

1. Stone samples (including centre, interior and outer region) are crushed with a hammer and are ground to a fine powder with agate mortar and pestle. This included all stone constituents in the powder.
2. For twenty four samples, a Seifert-3000 X-ray diffractometer was used and analysis was done in the department of ceramics engineering, Institute of Technology, BHU.
3. Due to technical fault developed later in Seifert - 3000 diffractometer, for remaining six stones samples. Phillips X-ray diffractometer was employed.
4. Powder is dispersed onto a perplex sample holder to cover an area of 5 x 12 x 2 mm (-10-15 gm).
5. Sufficient depth of material (2 mm) allowed us not to use any adhesive binder e.g. cellotape required in previous

XRD studies in our division (Rawat et al., 1992; Narayan et al 1994) using Rigaku X-ray diffractometer.

6. Angle of incidence of X-rays is taken between 10° to 800 which is optimum for urinary calculus analysis.
7. Average time required for one powdered sample analysis was about 20 minutes.
8. Strip chart recording, thus obtained, is analysed by matching with standard reference values as described by Sutor et al. (1968).

For SEM a Jeol 840, A scanning electron microscope was used. Source is a heated tungsten hairpin filament. The emitted electrons were brought to focus or cross over with the aid of a wehnelt electrode assembly. Electron lenses were electrons employed to demagnify the cross over and focus the probe to the surface of the specimen. The electron beam is moved The method:

1. The representative piece of appropriate size containing nucleus to surface was grounded over a glass surface to make them smooth.
2. The above sample was mounted over aluminium stubs with the help of silver plate.
3. The mounted sample was coated with gold-palladium (Au-Pd) in a vacuum coater to make the specimen conductive.
4. The treated sample was introduced into the vacuum chamber and viewed through the -Jeol 840 A- scanning electron microscope at 10 KV.
5. Signals emitted from the specimen surface, when an electron beam hit it, were taken up by the deflector, amplified and picked up by the cathode ray tube of the display screen on which image was obtained.
6. The pictures were photographed in increasing magnification on Indu 120 roll film. 125 ASA/22 Din.

A total of about 250 prints were studied thoroughly through a hand lense under good illumination from the point of view of ultrastructure. The observations are recorded. The representative photographs, depicting classically an observation, were assembled, photographed and plates were obtained for the purpose of this work.

RESULTS

Table 1: Chemical Composition By Spot Test (n=30)

Type of Stone	UUT	LUT	Total	%
A. pure calculi				
1. Calcium oxalate	6	|	7	23.33
2. phosphate stone	6	|	7	23.33
a)triple phosphate	-	-	-	-
b)Double phosphate	-	-	-	-
c)Single phosphate	-	-	-	-
B. Mixed calculi	16	7	23	76.66
1. Ca oxalate ± Uric acid ± Ammonium acid urate.	5	2	7	23.33
2. Triple phosphate ± Ca oxalate ±Uric acid	-	2	2	6.66
3. Double phosphate ± Ca oxalate	5	1	6	20.00
4. Single phosphate ± Ca oxalate ±Uric acid	6	2	8	26.66

Table 2: Chemical composition by x-ray diffraction analysis (n=30)

Type of Stone	UUT	LUT	Total	%
Pure	18	4	22	73.33
Whewellite	17	1	18	60.00
Whewellite	-	-	-	-
Whewellite Whewellite	1	2	3	10.00
Struvite	-	1	1	3.33
Hydroxyapatite	-	-	-	-
Brushite	-	-	-	-
Whitlockite	-	-	-	-
Ammonium acid urate	-	-	-	-
Uric acid	-	-	-	-
Xanthine	-	-	-	-
Cystine	-	-	-	-

Table 3:

Sl. N0	I.N.	Spot test	XRD	Predominant Ultrastructure
1.	2/93	CaOX, PO_4 UA_2 NH_4	WHE, UA	Amorphous Porus/ massive
2.	11/93	CaOX, UA NH_4	WHE	Cryst,(plates)
3.	17/93	CaOX, UA, NH_4	WHE+WEDD	Cryst,(plates, prism)
4.	23/93	CaOX	WHE+WEDD	Cryst,(plates, phenocryst)
5.	25/93	CaOX, PO_4	WHE	AMOR (porus)
6.	26/93	CaOX, UA,	WHE+WEDD	Cryst,(plates and spherules)
7.	29/93	CaOX	WHE	Cryst,(plates)
8.	30/93	CaOX, UA,	WHE	Cryst,(plates, prism, plates)
9.	34/93	CaOX, UA NH_4	WHE+Sod. Ura	Cryst,(spherules, tabular)
10.	36/93	CaOX, PO_4 UA	WHE	Cryst,(plates, envelopes)
11.	39/93	CaOX, UA	WHE	Amor,(porus,uratie)
12.	43/93	CaOX, PO_4 UA	WHE	Cryst,(plates)
13.	44/93	CaOX,	WHE	Cryst,(rockery, fibrous)
14.	45/93	CaOX, PO_4	WHE	Cryst,(plates)
15.	03/94	CaOX, PO_4 UA NH_4	WHE	Amorphous,(porus)
16.	06/94	$CaPO^4$ UA NH_4	STR+AAU	Amorphous,(coffin lid inclusion)
17.	07/94	CaOX, PO_4 NH_4	WHE	Cryst,(needles, thin plates or blade)
18.	12/94	CaOX,	WHE	Cryst,(plates, blades)
19.	16/94	$CaPO^4$ UA NH_4	WHE	Cryst, (spherules)
20.	18/94	$CaPO^4$ UA NH_4	WHE	Cryst,(plates, pyramids, ribbon)
21.	20/94	CaOX,	WHE	Cryst,(blades, spherules,
22.	22/94	CaOX, PO_4	WHEDD+UA	Cryst,(rectangular, hexagonal , square)
23.	27/94	CaOX, PO_4	WHE	Cryst,(blocks, plates)
24.	29/94	CaOX, PO_4	WHE	Cryst,(plates)
25.	31/94	CaMg PO_4 UA NH_4	WHIT, AAU, SAU, UAD	Cryst,(acicular)
26.	32/94	CaOX, NH_4	STRUVITE	Cryst,(plates)
		P0+UA		
27	33/94	CaOX	WHE+ UA+WHIT	Cryst (Plates)
28	34/94	CaOX, UA	WHE	Cryst (Plates)
29	35/94	CaOX, PO_4	WHIT+UA	Amorphous (porus)
30	36/94	CaOX, PO_4	WHIT+UA	Cryst (Plates, spherules and blocker)

Table 4:

SN	I.N.	XRD	Geological phenomenon		Character	Inclusion	Remarks
			Primary	Secondary			
1.	11/93	Pure oxalate WHE	Et, Col, Twin, EP	Ac, St, Ev	-	-	Phenocryst
2.	17/93	WHE+ WEDD	Epi, Col	Cr. C	-	-	Collo material is M2&P2

3.	23/93	WHE+ WEDD	Epi, Col	Cre, St	F	-	
4.	25/93	WHE	-	CrC, We, St	F	-	Microcysta from rapid formation
5.	26/93	WHE+ WEDD	Tw, Ep, Col	We	F	-	Psedumost
6.	23/93	WHE	Col, St	CrC, Acc, St	-	-	/ultratin
7.	30/93	WHE	Tw, Epi	CrC, We	V	-	-
8.	36/93	WHE	Epi	CrC St	-	-	-
9.	39/93	WHE	Col, Ep, ET	St	-	-	Phenocryst
10.	43/93	WHE	Col, Ep	St, We, Cry	F	-	-
11.	44/93	WHE	Epi, Et	St, We, Cry	F	-	Phenocryst
12.	45/93	WHE	-	We, St, Crye, We	F	-	-
13.	3/94	WHE	-	We. Z, St	F	-	-
14.	7/94	WHE	Ep, Tw	Z, St, Crye	-	-	-
15.	12/94	WHE	Tw, Ep	We, St, CryC	F	-	-
16.	16/94	WHE	Tw, Ep	Aee	-	-	-
17.	18/94	WHE	Tw, Ep, Et	CryC, Acc, We, St	-	-	-
18.	20/94	WHE	Tw, Ep, Col	Z, Cry, St	-	-	-
19.	27/94	WHE	Tw, Ep	CryC, Acc	F	-	-
20.	29/94	WHE	Epi, Col	We, St	F	-	-
21.	39/94	WHE Mixed oxalate	Col, Ep	We, CryC	F	-	-
22.	2/93	WHE+ UA	-	We	V+F	+ve	In periphery large irregular mass wh
23.	34/93	WHE+ Sod Epi monohy	Epi	Cye, We St	-	-	Multiple communic ble channels disocuphas
24.	22/94	WEDD +UA Epi. Pure Phosph ate	Epi	-	-	-	-
25.	32/94	Str+A AU Ei. Ep	Epi, Et	We. St	F	-	-
26.	6/94	Str+A AU Ei. Ep	Et, Ep	St, We	V	-	Phenocryst
27	31/94	White +AAU Tw SAU+ UAD	Tw	We, St, Cry	-	-	-
28	33/94	WHIT= UA Ep	Ep	We, St, Cry	-	-	-
29	36/94	WHIT= UA Tw	Tw	We, St	F	-	-
30	39/94	WHIT= UA Ep	Ep	St, We, Cry	-	-	-

Table 5

S.N.	I.N.	XRD	Phenomenon Seen
1.	11/93	WHE	Erosion Etching
2.	23/93	WHE+WEDD	Weathering, fracture
3.	25/93	WHE	Weathering, fracture
4.	26/93	WHE+WEDD	Fracture
5.	29/93	WHE	Weathering
6.	30/93	WHE	Vein
7.	39/93	WHE	Etching, weathering
8.	43/93	WHE	Fracture, Veins
9.	44/93	WHE	Etching Weathering, fracture
10.	45/93	WHE	Weathering, fracture
11.	3/93	WHE	Fracture Weathering
12.	12/94	WHE	Weathering, fracture
13.	18/94	WHE	Etching, weathering
14.	27/94	WHE	Fracture
15.	29/94	WHE	Fracture Weathering
16.	34/94	WHE	Fracture Weathering
17.	21/93	WHE+UA	Fracture, Veins, fractures
18.	34/93	WHE+Sod. Urate, Mono	Weathering
19.	32/94	Pure Phosphates Struvite phosphates With admixture	Etching Weathering, fracture
20.	6/94	Struvite+AAU	Etching Weathering, veins
21.	31/94	Whit+AAu+SAU+UAD	Weathering
22.	33/94	Whit+UA	Weathering
23.	36/94	Whit+UA	Weathering, fracture
24.	39/94	Whit+HA	Weathering

DISCUSSION

Despite years of intensive research, the mechanisms of a urinary calculus formation and development remains a largely unexplained phenomenon and, thus, cannot be the basis for prophylaxis of stone disease. However the overall chemical composition does help a clinician to give therapeutic instructions to minimise the recurrences.

Ultrastructure:

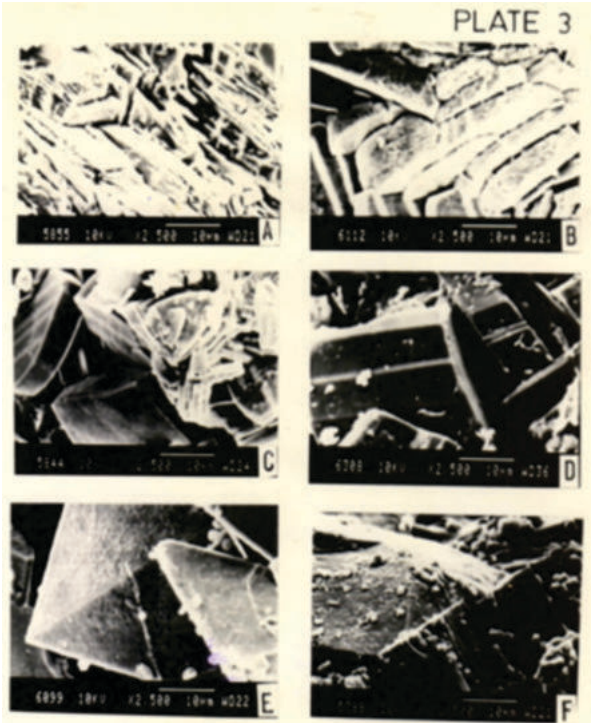
It is a common observation in scanning electron microscopy that the stone constituents of proven chemical compositions may appear in well crystalline, semi crystalline or poorly crystalline or amorphous forms. The geologists find similar observations with respect to substances in solid state in nature. Geological phenomenon, seen in human urinary calculus like crystal complex ing, syntaxy and epitaxy as well as etching, erosion or weathering is akin to the crystallographic substances found in nature, but still, has to be suitably explained. Of course, a specific depositional environment will determine the sediment texture, well crystallised/poorly crystallised fraction ratio and its overall geometry. In geological terminology crystals may be euhedral, subhedral or anhedral as defined with glossary later. In our attempt to understand the complex process of calculogenesis and to find suitable explanations of geological phenomena seen in the urinary calculi depositional environment of the 'human mineral assemblages' (stones) in the kidney has to be taken into account. It appears that the basic physico-chemical process is the same as in the nature- i.e. 'nucleation' and 'growth'. In nature, drastic circumstances of supersaturation are required for crystal nucleation then for crystal growth. Such extreme situations, while explainable in crevices and depth of mother earth, are not likely to be found in the pyramidal depths of kidney, yet the nucleation is a necessity to initiate any substance to stone form whether in nature or in kidney. It requires a supersaturation state.

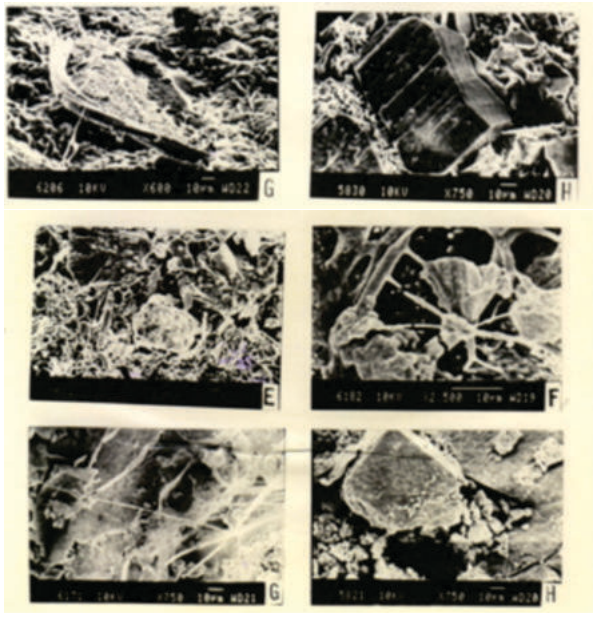
Then how does this state of supersaturation encountered in renal depths? Bodily earthquakes are one explaination for supersaturation. In nature, disturbances of isostatic equilibrium cause earthquakes. In the same way, sudden

disturbances in ionic equilibrium and sudden efflux of heavy matabolities in tubules of kidney, can bring out the drastic changes in ionic equilibrium resulting in urinary calculi. 'Earthquake Phenomenon 'applies in this sense to indicate sudden efflux of heavy metabolites through the ducts of Bellini leading to a very high supersaturation level due to dietary or metabolic assaults or a disease process. Experiments done by Vermeulen and Lyon (1968) using oxamide rich diets in rats seem to support this view.

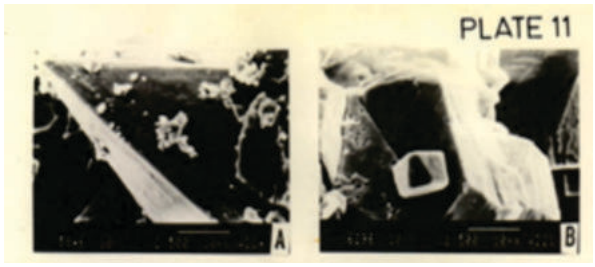
Formation of a rock may occur due to consolidation of lava in an airy atmosphere, hence not applicable to the 'milieu interior' of our body. However, the same phenomenon exists in deep sea explosions of earth where lava contacts with sea water and leads to sudden cooling. An analogy can be drawn between pyramidal depths and pelvic urine to the oceanic depth and sea water. But extremes of temperature and pressure in such explosions resulting in formation of eglestial rocks may not form in the body. Then how do we proceed? Basic processes of crystal nucleation and rate of growth in sedimentary rock in the sea, as well as in human kidney, are a function of the degree of supersaturation of the poor water/surface water. At low levels of supersaturation crystal nucleation is very slow and excess dissolved material is consumed by crystal growth in a limited number of nuclei. This results in high degree of crystallinity of mineral phase which also tends to favour existing mineral phases as syntax ial and/or epitaxial overgrowths - the former expalning 'pure' and the latter 'mixed' stone genesis. Demonstration of multiple enucleation of oxalate stones in rats by Vermulen et al. (1988) and epitaxial theory of Lansdale (1968) on uric acid-cum-oxalate stones are initial efforts for our sedimentary rock hypothesis proposed here.

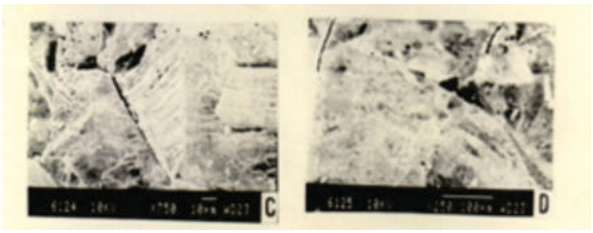
Hence better analogy for a urolith formation is to the methodology of formation of a sedimentary rock rather than an eglestial one briefed above. The morphological appearances observed in SEM such as massives, phenocrysts, macrocrysts etc.) are the vivid examples of such a mechanism **(Plate no. 3 E,F,H).** This connotes a state of high crystallinity under lower degree of saturation (yet supersaturation). All stones with uniform or nearly so crystalline structure, best exemplified by oxalates depict the same ultrastructure **(Plate 3 B-E)**.





Therapeutic implications of SEM studies: It is well known in mineralogy that "minerals are in equilibrium with their surroundings only in the environment in which they form (Law of mineral instability, Keller 1989) . If extrapolated in human conditions, in the ambient ionic atmosphere of human kidney, the urinary calculi will also be in metastable conditions or water i.e. urine in the with respect to their interstices of calculi water i.e. (urine) leading to either deposition of and surface precipitates or dissolution depending reactions taking place. Diagenesis is the sum of those processes by which originally sedimentary deposits attempt to reach equilibrium with their environment. Though owing to the influence of organic substances, matrix and bacteria, the process of diagenesis may be arrested or modified. If suitably applied this process can be potentially manipulated to 'weather' or dissolve a human urolith. Let us see whether our scanning electron microscopic observation on urolith supports the above concepts in hand. Table XII shows evidence of etching, erosion, or dissolution in the calculi studied. Etching was demonstrated with crystals of pure oxalate calculi **(Plate 11 A)**, as well as pure phosphate calculi (**Plate 11** F-H), comparing to Dissolution (Plate 11 weathering in rocks, calculi of 'pure oxalates' was seen in 11/22 , 2/2 calculi of 'mixed oxalates' and all calculi (6/6) of pure oxalates or mixed phosphates. The actual process of dissolution of solids by an aqueous solution involves the removal of ionic species by either diffusion transport or by surface reaction, observation of evidence of dissolution of crystals with SEM help to elucidate the dissolution rate controlling mechanism. Slow may the partial dissolution of mineral phases by will result in formation of crystallographically controlled features such as "Etch pits' ' surface reaction in contrast, 'weathering' or 'corrosion' results from more rapid transport controlling mechanisms. Though it is not possible to indicate exactly what change in the milieu interior has caused these changes in the calculi. It does suggest that potentially these procedures can be utilised for dissolution of calculi if exactly known. It also indicates that calculus in the milieu interior involves itself in dynamic chemical processes whether leading to deposition or erosion from time to time.

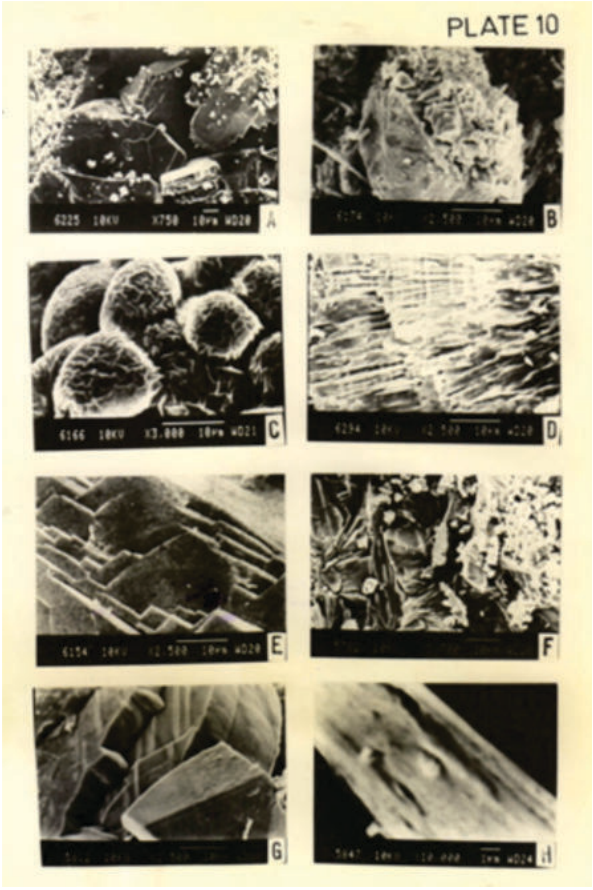




These dissolution phenomena are also influenced by surface free energy properties of the crystal (Hurst 1981). Euhedral crystal faces possess low surface free energy and are least likely to undergo surface corrosion or dissolution whilst high index crystal faces (subhedral or anhedral) are more likely to be influenced by dissolution influences. Thus SEM may help to indicate the detection of easily susceptible or non-susceptible calculi to dissolution processes. Abraded, anhedral surfaces and fractures of whatever origin, all have high surface free energies, therefore,' are susceptible to dissolution and corrosion.

This can be applied in post ESWL dissolution trials for residual calculi

Growing state will probably require modulation of the ionic atmosphere to stop its progress. The measures may be entirely different than one should recommend for decaying stones. In the end we like to conclude that ultrastructural studies on surface and depth by virtue of compactness of stone substance laid down as depicted in plate (**Plate IA and Plate 10**) indicated process/consolidation of stone, not a decay, infact, classical surfaces studies singh's work (1988) showing accretion is depicted even in depth (Plate 10 B). It needs elaborate studies on such issues.



CONCLUSIONS

SEM studies revealed that oxalate stones are in general predominantly crystalline/ semi crystalline. Same could not be determined for phosphate stones due to presence of large number of admixtures in these stones, though in some cases, amorphous pattern observed even in pure oxalate calculi. Side by side amorphous and crystalline material was seen in a large number of calculi. This study could demonstrate classical uric acid crystals even if present as 'inclusions.

Crystallinity of a 'stones type is influenced by physico-chemical atmosphere as well as other minor constituents. Amorphous looking material in our magnification range may prove to be crystalline if higher magnification range is possible.

Specificity of crystal morphology with respect to its ultrastructure was observed in some calculi, but was not composition, the rule. It requires microprobe analysis to enhance specificity.

To a clinician, an ultrastructural knowledge may tell whether stone is growing or is undergoing natural decay. It appears that specificity of a crystal morphology with composition of a stone, though, may be a relation observed in some cases, should not be given too much. Important factor seems to be the physico-chemical time of laying down of stone substance. The observation of various geological phenomena in in depth study of ultrastructure of urinary calculi supported the 'Geological Theory° of calculogenesis. An analogy is drawn between geological earthquakes and bodily metabolic assaults leading to supersaturated conditions optimum for laying down stone substance (i.e. nucleation). An analogy is again drawn between human and sedimentary rocks to explain growth of calculi, evidence of which were found in this ultrastructural study **(Plate 10)**. Dissolution phenomenon seen in urinary calculi **(Plate 11),** can be potentially manipulated for therapeutic application.

Geological theory of calculogenesis is supported by our in depth ultrastructural observations of various geological phenomena. A compatible analogy can be drawn between human rocks and sedimentary rocks formation in nature. This needs to be substantiated by future work in direction

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