Size Distribution Characteristics of Mineral Phase in Renal Stones

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Abstract □ The size distribution of the mineral phases in three renal stones (whewellite, struvite, and whitlockite) was determined using a particle-counting technique after removal of the organic matrix. The multisized crystallites of the investigated stones revealed close similarity in size distribution characteristics. Whewellite size parameters were in good agreement with the parameters of calcium oxalate monohydrate crystals formed in the kidneys of rats injected with L-4-hydroxyproline. However, these parameters differed significantly from the values calculated from the size distribution of calcium oxalate crystals voided in the urine of recurrent stone formers. The data obtained suggest that critical size distribution characteristics may be instrumental in causing the mineral phase to agglomerate and adopt a close packing in renal stones.

Keyphrases □ Renal stones—size distribution characteristics of mineral phase □ Size distribution—mineral phase of renal stones □ Urinary stones—size distribution characteristics of mineral phase □ Minerals—size distribution characteristics in renal stones

The study of crystal deposits in freshly voided urine and their relation to stone formation has received considerable attention (1–4). The ultrastructure of renal calculi was investigated by various methods to understand stone etiology and growth mechanisms (5–7). However, the size distribution and geometrical characteristics of individual crystals forming the renal calculus were overlooked. The basic question of whether there exists a critical dimension or a critical size range favoring stone construction in humans has not been answered. The quantitative evaluation of these parameters promises a better understanding of the clustering process and the packing stability of the mineral phase in urolithiasis.

The purposes of the present study were to determine the crystal size distribution of calcium oxalate, ammonium magnesium phosphate, and tribasic calcium phosphate in stones using different measuring techniques and to compare the data obtained with data on calcium oxalate crystals formed in the kidneys of rats injected with L-4-hydroxyproline.

EXPERIMENTAL

Three urinary stones[1] characterized as whewellite (calcium oxalate monohydrate), struvite (ammonium magnesium phosphate hexahydrate), and whitlockite (tribasic calcium phosphate) were obtained from recurrent stone formers.

A series of experiments was conducted using different solvents and conditions to remove the organic matrix and to deaggregate the individual crystals from the polycrystalline mass without acting on the mineral phase or changing the crystal morphology. Ten milliliters of an aqueous solvent was added to ~10 mg of the stone and left at room temperature for 7–14 days. The best results were obtained when whewellite, struvite, and whitlockite stones were digested in aqueous 80% ethylenediamine, 30% NaOH, and 12% sodium hypochlorite solutions, respectively. The digested stones were filtered, and the mineral phase residue was washed repeatedly with the respective solvent and subjected to size analysis.

The deaggregated mineral phase of the different stones was analyzed by a particle counter[2] with a 16-channel analyzer and a 100-μm tube. A "clean" electrolyte solution (0.9% NaCl) saturated with the stone material was used as the suspension medium to ensure that no part of the mineral phase dissolved in the electrolyte solution during the size analysis.

RESULTS AND DISCUSSION

Mineral Phase in Renal Stones—Renal stones generally consist of coherent compact mineral phases of very low voidage. The mucoproteinous matrix accounts for 2–2.5% (w/w) (8, 9). The particle-size distribution of the deaggregated mineral phase of the investigated stones is given in Figs. 1 and 2. Particle-size distributions based on the number and volume showed a close similarity among the investigated stones. For example, the number distribution curves (Fig. 1) showed that 50% of the particle population was <5 μm. On the other hand, the volume distribution curves (Fig. 2) indicated that the median sizes (r_{50}) of the investigated stones was 25–30 μm.

The size data in Figs. 1 and 2 were used to estimate the particle population parameters of the mineral phase. The Rosin–Rammler distribution (10), which is given by $G_r = 100[\exp - (r/r_{mean})^{\gamma}]$, was used to describe the multisized particle population, where G_r is the total cumulative volume of the mineral phase oversize in percent, r is the particle size in micrometers, r_{mean} is the statistical mean particle size in micrometers derived graphically (corresponding to the coordinate G_r = 36.8%), and γ is the degree of uniformity of the size distribution, which

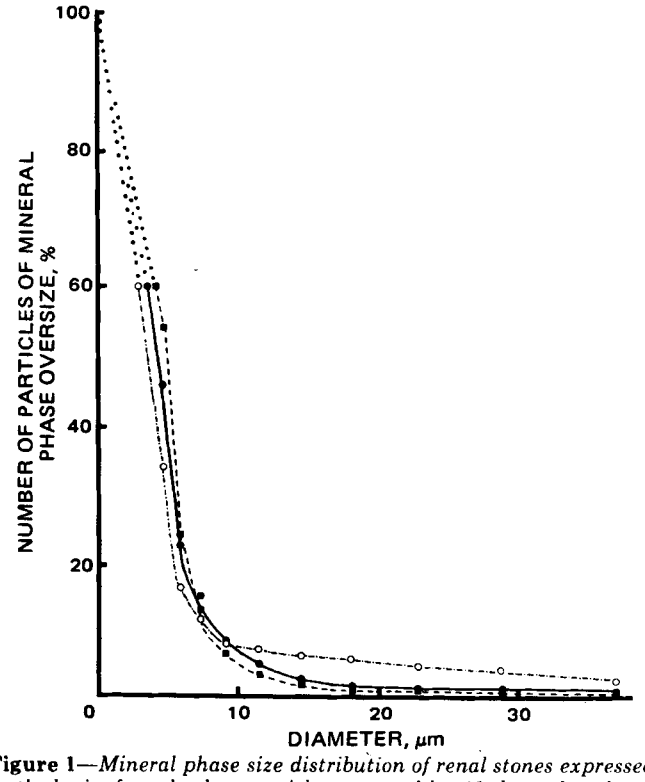

Figure 1—*Mineral phase size distribution of renal stones expressed on the basis of number by a particle counter with a 16-channel analyzer. Key:* ○, *calcium oxalate monohydrate;* ●, *calcium phosphate; and* ■, *ammonium magnesium phosphate.*

[1] Provided by Dr. M. El-Hilali, Department of Urology, University of Sherbrooke, Sherbrooke, Quebec, Canada.

[2] Model TAII, Coulter Electronics, Hialeah, FL 33010.

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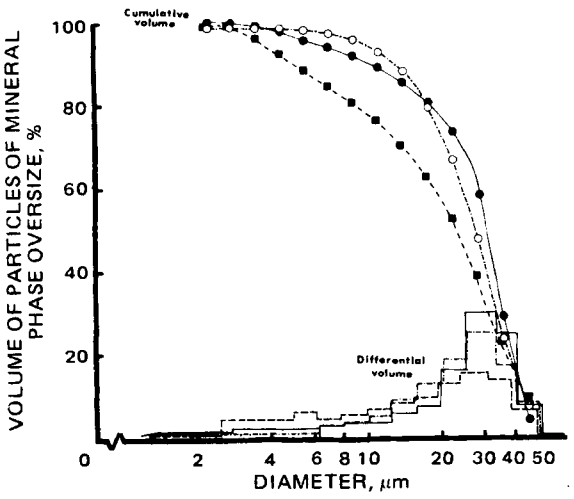

Figure 2—*Mineral phase size distribution of renal stones expressed on the basis of volume by a particle counter with a 16-channel analyzer. Key: ○, calcium oxalate monohydrate; ●, calcium phosphate; and ■, ammonium magnesium phosphate.*

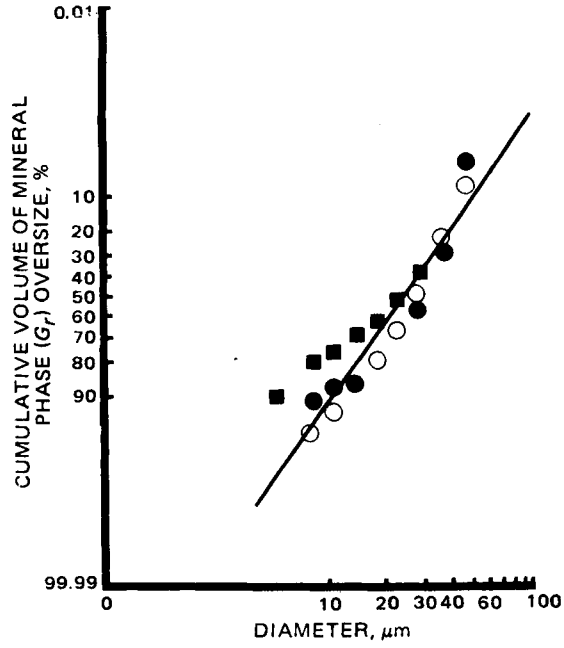

Figure 3—*Scattergraph of the mineral phase size distribution of the investigated renal stones based on the Rosin–Rammler extrapolation. Key: ○, calcium oxalate monohydrate; ●, calcium phosphate; and ■, ammonium magnesium phosphate.*

also is obtained graphically as the slope of the linear relation between log (log $100/G_r$) and log r. The most probable crystal size in the size (10) distribution (r_m) is obtained from $r_m = r_{mean}(\gamma - 1/\gamma)^{1/\gamma}$.

Figure 3 shows a scattergraph and a linear representation of the mineral phase size distribution of the investigated stones. From this plot, the median size (r_{50}) is 25.5 μm, the most probable size in the distribution (r_m) is 26.9 μm, the uniformity factor of the size distribution (γ) is 2.8, and the coefficient of variation (CV) is 63.7%. The coefficient of variation is calculated from $CV\ (\%) = 100[(r_{16} - r_{84})/2r_{50}]$, where r_{16} and r_{84} are the particle sizes when $G_r = 16$ and 84%, respectively.

The examination of the calcium oxalate crystals from the whewellite stone using a scanning electron microscope supported these findings. The mean projected diameter was 28 μm (Fig. 4). This mean projected diameter correlated well with the median size (r_{50}) and the most probable size in the mineral phase size distribution (r_m) obtained by the counter.

***In Vivo* Crystallization of Calcium Oxalate Monohydrate**—The detailed growth history of urinary stones is unknown, and no information is available on the size distribution of the individual crystallites that occur immediately after *in vivo* crystallization. In this study, the size distribution data of calcium oxalate crystallites in renal stones were compared with data for the calcium oxalate crystals produced in rat kidneys after

Table I—Size Characteristics of Calcium Oxalate Monohydrate Crystallized *In Vivo*

Source of Calcium Oxalate Crystals	Median Size (r_{50}), μm	Mean Size (r_{mean}), μm	CV, %	Uniformity Factor (γ)	Most Probable Crystal Size in Size Distribution (r_m), μm
Urinary stone	25.5	31.5	63.7	2.8	26.9
Rat kidneys (experimental urolithiasis)	26.0	31.0	57.7	2.7	26.1
Urine of recurrent stone formers	9.7	14.0	63.8	3.5	8.8

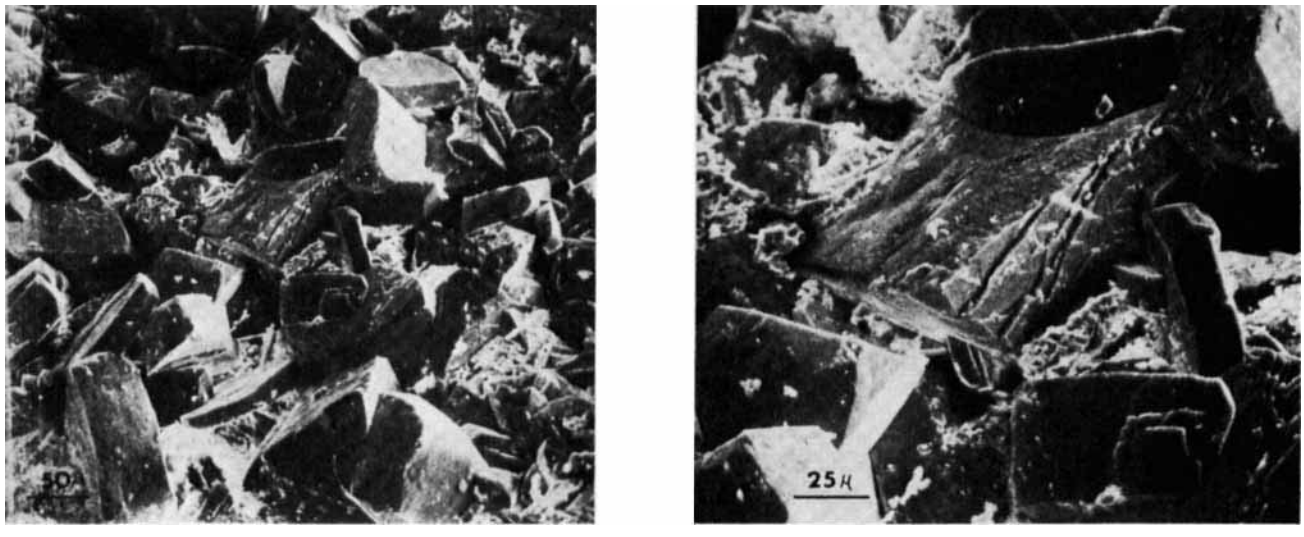

Figure 4—*Scanning electron micrograph of calcium oxalate monohydrate crystals in a calcium oxalate renal stone. The mean projected diameter is 28 μm.*

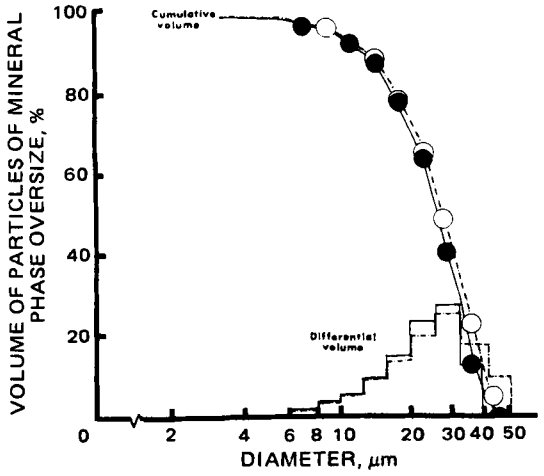

Figure 5—*Size distribution curves of calcium oxalate monohydrate crystals in the renal stone and in experimental urolithiasis. Key:* ○, *calcium oxalate stone; and* ●, *calcium oxalate crystals in rat kidneys.*

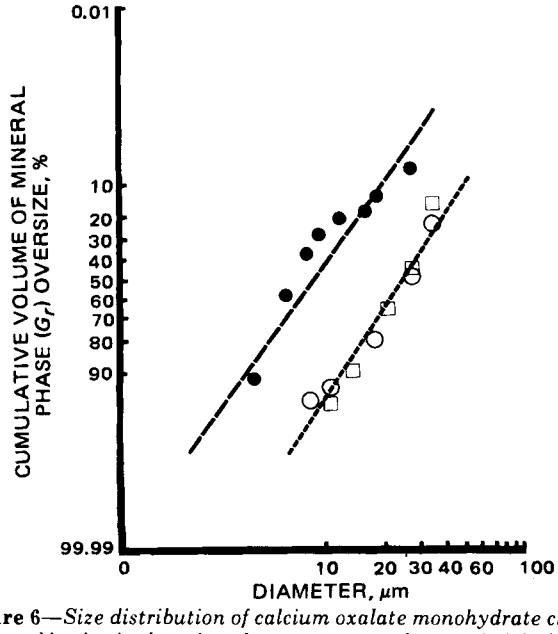

Figure 6—*Size distribution of calcium oxalate monohydrate crystals produced* in vivo *in the urine of recurrent stone formers* (●) *(12), in the urinary stone* (○), *and in rat kidneys (experimental urolithiasis)* (□).

the injection of L-4-hydroxyproline. The experimental details on the formation of calcium oxalate crystals in rat kidneys by injection of L-4-hydroxyproline was described recently (11).

Figure 5 illustrates the size distribution curves of calcium oxalate crystals in the renal stone and calcium oxalate crystals formed in rat kidneys, clearly showing a close similarity in the size distribution of the mineral phase in the rat kidneys and in the human stone. This observation suggests that crystal growth in rat kidneys (experimental urolithiasis) and in the urinary stones may be controlled by similar biological and physicochemical variables.

On the other hand, Robertson and Peacock (12) studied the size distribution of calcium oxalate crystals voided in the urine by recurrent stone formers (those having a history of stones over a period of years). The recurrent stone formers excreted calcium oxalate crystals that gave a bimodal distribution. The examination of the data reported by Robertson and Peacock and data on urinary stones revealed wide variation. To compare the size distribution parameters of calcium oxalate crystals passed in the urine of stone formers with those of calcium oxalate crystals of both renal and rat kidney stones, the Robertson and Peacock data were recalculated and plotted (Fig. 6). The size parameters of calcium oxalate crystals were determined and are given in Table I. The values given in Table I indicate that while the size parameters of the calcium oxalate crystals in both the renal stone and rat kidneys were very close, significant differences existed with calcium oxalate crystals voided in the urine of stone formers. For example, the r_{50} value for crystals in the renal stone was 25.5 μm, and in rat kidneys it was 26.0 μm; for the crystals passed by the recurrent stone formers, it was 9.7 μm.

The uniformity factor (γ), a measure of the uniformity of the distribution, also showed a significant difference between the crystals of the stone and those voided in the urine of stone formers.

Some investigators (9, 13) stated that the coherence of the particles in stones is a function of the surface and shape of the mineral phase particles as well as the presence of the organic matrix. The contribution of the grain size and size distribution characteristics in particle retention and packing still is unclear. More studies are necessary for complete characterization of this problem and for the eventual understanding of stone genesis.

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