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Urological Research

Crystalluria in Marathon Runners

1. Standard Marathon – Males

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Summary. Epidemiological evidence suggests that marathon runners have a higher incidence of renal stone formation than occurs in the general population. Since crystalluria and stone disease are thought to be related, we subjected urine samples from a group of marathon runners to particle counting and sizing in a Coulter Counter equipped with a population accessory unit. The volume-size distribution curves so obtained were bimodal with one peak occurring in the 2–5 μm diameter range and a second in the 15–32 μm diameter range – a pattern that is remarkably similar to the distributions reported for recurrent idiopathic stone formers and distinctly different to those recorded for control subjects. Analyses by scanning electron microscopy and X-ray powder diffraction revealed other features which are regarded as typical of stone formers' crystalluria. These physicochemical data indicate that marathon runners may be at increased risk of urinary stone formation.

Key words: Marathon runners, Crystalluria, Calcium particles, Calcium oxalate crystals.

Introduction

The passage of crystals in the urine has long been considered a feature of renal stone disease [9]. 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 [10, 11]. It was found that crystals excreted by controls are small (3–4 μm in diameter) and belong to a unimodal distribution whereas those excreted by stone formers belong to a distribution which contains, in addition to the 3–4 μm peak, a second peak of much larger particles (20–40 μm in diameter). These particles were identified as calcium oxalate dihydrate (COD) crystals and were often found to occur in aggregates up to 200 μm in diameter [10, 11].

Recently, it has been found that marathon runners may be prone to renal stone formation [8]. A survey of entrants in the 1977 New York City Marathon found that the incidence of urinary stone formation in these runners was 4.5 times greater than in the matched population [8]. In an attempt to gain insight into this phenomenon we characterised the nature of the calcium crystalluria found in a group of marathon runners. We applied the criteria described above to determine whether our experimental group was at increased risk of urinary stone formation.

Materials and Methods

Controls, Marathon Runners

The controls were 15 randomly chosen healthy male members of staff of the School of Chemical Sciences, University of Cape Town and were aged between 21 and 54 years. Nocturnal urine specimens (i.e. the first voided urine after waking) were collected in pre-heated thermos flasks and were analysed at 37 °C within a few hours of voiding. Analyses were repeated at 27 °C and 47 °C for three samples so that the effect of temperature on the particle size distribution could be examined. To establish the effect of long term storage, three further samples were refrigerated at −10 °C for approximately 3 weeks after which the analyses were repeated. In these cases, the frozen samples were slowly warmed and were allowed to equilibrate at 37 °C for several hours before particle counting was commenced.

The 7 volunteer marathon runners were adged between 22 and 40 years. All were experienced runners whose training schedule prior to the race involved running between 40 and 160 km per week. All had previously completed at least 3 marathons. None had a history of urinary stone disease, unusual diet or excess Vitamin C intake. Nocturnal urine samples were obtained 2 days before the marathon and on race day itself, as well as before, during and immediately after completion of the course. In addition samples were collected on days 1, 3, 5 and 10 following the marathon. In all cases the urine was collected in preheated thermos flasks and subjected to particle counting at 37 °C within 3 h of voiding.

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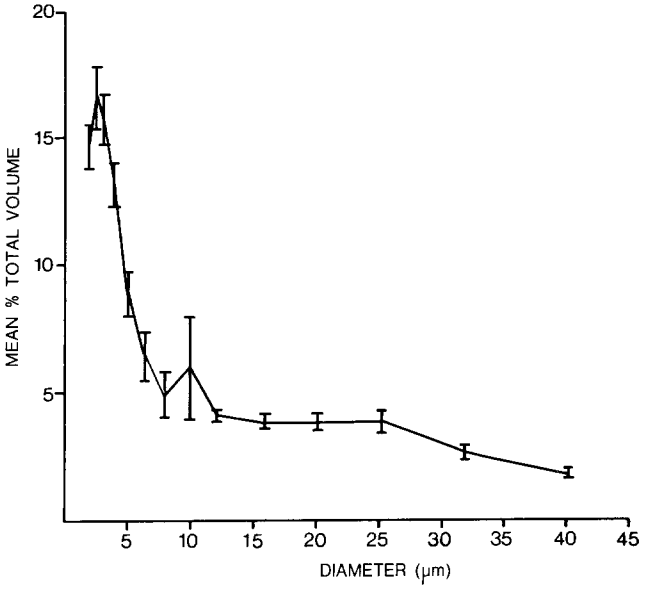

Fig. 1. Mean volume-size distribution curve (+ S.E.M.) for 15 control urines at 37 °C. (Note that the distribution is essentially unimodal with the major peak occurring at a particle diameter of 3 μm)

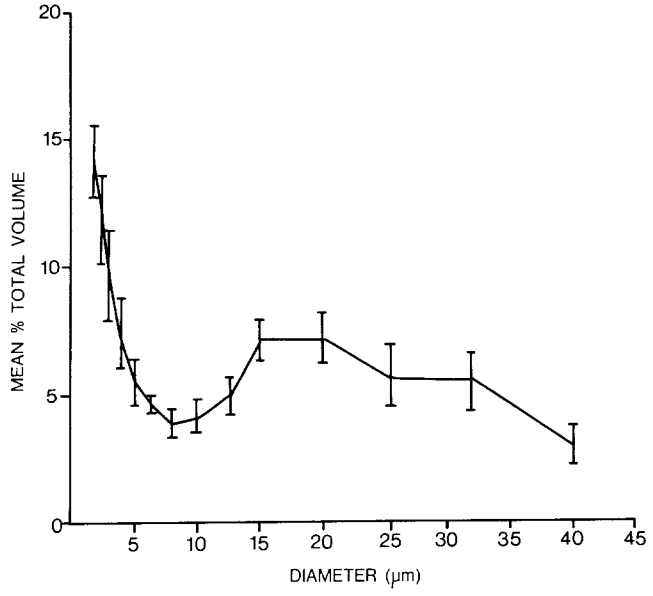

Fig. 2. Mean volume-size distribution curve (+ S.E.M.) for 7 marathon runners' urines (day +3) at 37 °C. (Note that the distribution is distinctly bimodal with the second peak occurring in the 15–32 μm diameter range)

Particle Size Distribution Analysis

A Model TA II Coulter Counter with 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 of 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 19.00 μm suspended in azide free ISOTON II solution.

The pH of each urine sample was measured prior to filtering 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 thermostated 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.

Calcium crystals were determined in some of the urine samples by selective complexation with EDTA, according to the method described by Robertson [9].

Scanning Electron Microscopy (SEM) and X-Ray Powder Diffraction (XRD)

Urine samples at 37 °C were centrifuged for 5 min using a PICCOLO table-top low speed centrifuge operating at 2,000 revs 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 Sartorrius 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 °C 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 FP4 roll film at 60 s frame period 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 (EDAX).

Deposited crystals from a few of the centrifuged samples were subjected to XRD analysis. 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 Mn filtered FeK_2 radiation of wavelength 1.937 Å.

Statistical Methods

Statistical analyses were performed by two way analysis of variance (ANOVAR). Significance was determined at the $p < 0.05$ and $p < 0.01$ levels.

Results

Particle Volume-Size Analysis

Volume-size distribution curves were obtained for all controls and all marathon runners by plotting V_d against d where V_d is the volume of crystals of diameter d (μm). The 15 controls all displayed a major peak in the 3–10 μm diameter range while peaks of a very minor nature in the 15–30 μm diameter range were observed in several of the samples. The mean volume-size distribution curve for the 15 control urines at 37 °C is shown in Fig. 1.

The effect of temperature on the distribution curves was studied by repeating the analyses for 3 controls at 27 °C and 47 °C. In all cases the major peak occurring in the 3–10 μm diameter range suffered a decrease in height (i.e. there was a decrease in the number of particles having this diame-

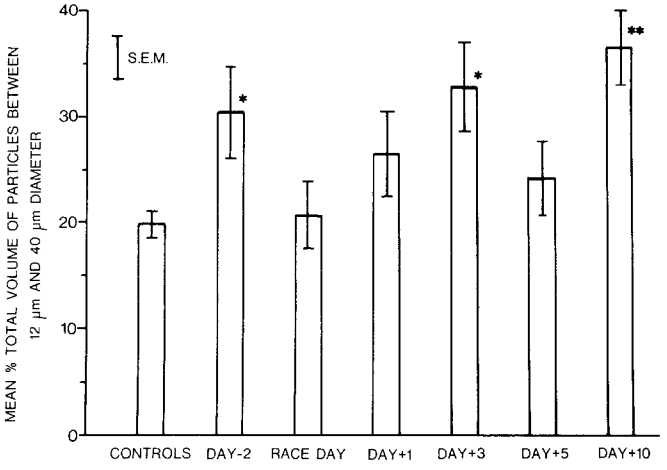

Fig. 3. Histogram of the mean percentage total volume (+ S.E.M.) of particles in the diameter range 12–40 μm for all the marathon runners on the various days of the experiment. (*: $P < 0.05$ compared with control group; **: $P < 0.01$ compared with control group)

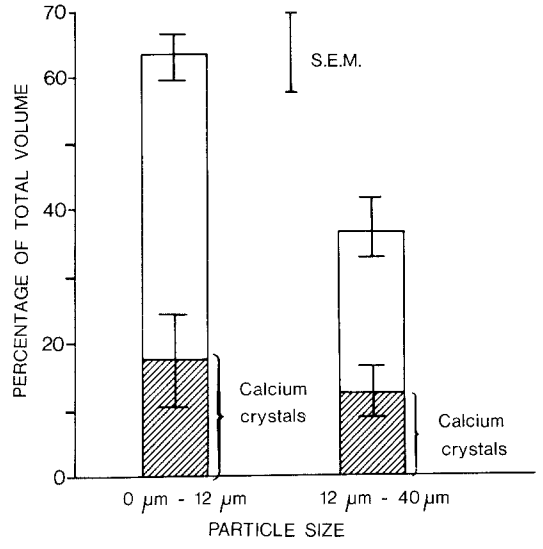

Fig. 4. Histogram of the mean percentage total volume (+ S.E.M.) of all particles in the diameter ranges 0–12 μm and 12–40 μm for all the marathon runners (day + 10). The *shaded areas* represent Ca particles

ter) as the temperature was increased from 27 to 37 °C. In addition, the minor peaks occurring in the larger diameter ranges were shifted by about 5 μm to smaller size ranges when the temperature was increased. The refrigerated samples showed an increase in the percentage of the total volume of larger particles (> 12 μm diameter) from 18.03 ± 0.91 to 25.87 ± 1.7% ($P < 0.01$).

The particle volume size distribution curves for the urines of the marathon runners all displayed a major peak in the 2–5 μm diameter range *as well as* a second (sometimes bimodal) peak of significant magnitude in the 15–32 μm diameter range. These features were present in the distribution curves recorded *before* the marathon and *remained throughout the period of the experiment.* The mean volume-size distribution curve for all the runners on day +3 is shown in Fig. 2 while a histogram of the mean percentage total volume of the larger particles on the various days of the experiment is shown in Fig. 3. The histogram in Fig. 4 shows the percentage volumes of the differently sized particles in the runners' urines obtained on day +10 of the experiment and the fraction of these which are due to Ca particles (shaded areas). Ca crystals constitute 28% of all the particles in the small diameter range (0–12 μm) and 34% of those in the larger range (12–40 μm). When Ca crystals alone are considered, it is found that 50.4 (± 8.4%) occur in the larger diameter range.

X-Ray Powder Diffraction

Crystals isolated from the randomly selected urines of 6 marathon runners and one control were subjected to XRD analysis. The presence of COD was established in all 7 samples. In addition, COM was identified as the minor component in 4 of the runners' urines. Significant amounts of brushite (BRU) were found in the control urine as well as in 2 of the runners' samples. Uric acid dihydrate (UAD) was identified as a very minor component in 3 of the marathon runners' urines.

Scanning Electron Microscopy and Energy Dispersive X-Ray Analysis

COD crystals, less than 10 μm in cross section, were commonly observed in the control urines (Fig. 5) while COM (Fig. 6) was rare. In the marathon runners' urines, much larger COD crystals occurred. These were typically 15–20 μm in cross section (Fig. 7) although larger ones were often observed (Fig. 8). Frequently, 2 or more crystals were found fused together, yielding a "particle" measuring over 40 μm in cross section (Fig. 9). Rod and platelike crystals of BRU occurred as randomly deposited entities (Fig. 10) and as rosette-like aggregates (Fig. 11) of cross section 20–30 μm. The presence of Ca and P in such deposits was confirmed by EDAX (Fig. 12). Another common observation during SEM examination of the marathon runners' urinary deposits was that of large globular and irregular particles rich in K, Cl, S, P and Na (EDAX) as shown in Figs 13 and 14 respectively.

Discussion

The particle volume-size distribution profile for our control urines agrees with that reported by Robertson and Peacock [10] in that it is unimodal with the major peak occurring in the small (< 10 μm) diameter range (Fig. 1). Of some considerable interest is the clearly observed difference between this distribution profile and that recorded for the marathon runners' urines (Fig. 2). The latter contains 2 peaks, one in

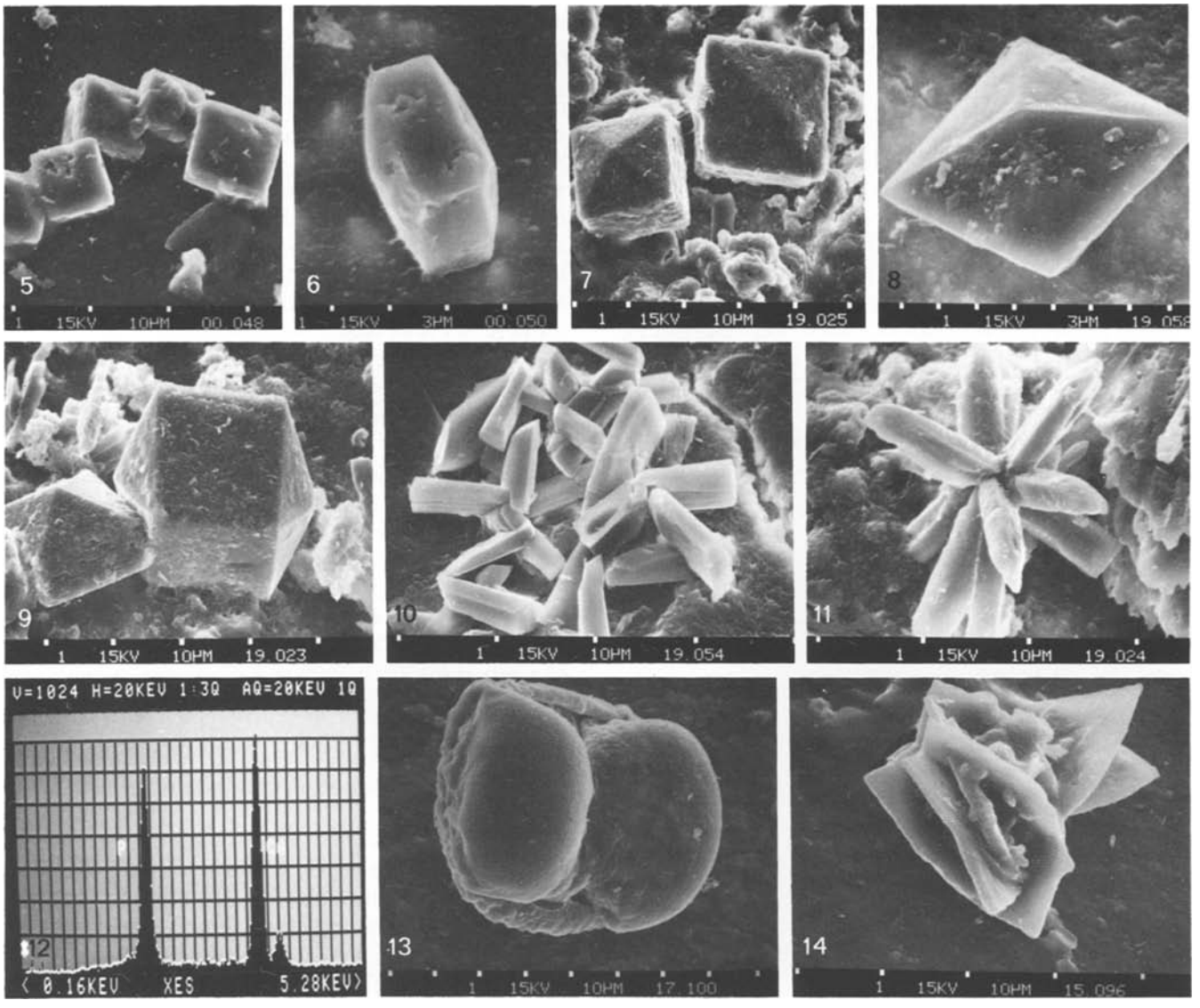

Fig. 5. Five COD crystals, typical of those observed in the control urines (Note the cross section dimensions are < 10 μm)

Fig. 6. COM crystal occasionally observed in the control urines (Note the cross section < 10 μm)

Fig. 7. COD crystals typical of those observed in the marathon runners' urines (Note cross section 15–20 μm)

Fig. 8. Large COD crystal (cross section > 25 μm) commonly observed in marathon runners' urines

Fig. 9. Two large COD crystals fused together yielding a "particle" with a cross section > 40 μm

Fig. 10. Random deposits of BRU crystals observed in some of the marathon runners' urines

Fig. 11. Rosette-like aggregate of BRU crystals (cross section > 20 μm)

Fig. 12. Energy dispersive X-ray analysis spectrum recorded for the deposits shown in Fig. 11. The presence of Ca and P is clearly indicated

Fig. 13. Large globular deposit (cross section ~ 60 μm) frequently observed in the marathon runners' urines (Elemental composition K, Cl, S, P, Na)

Fig. 14. Large irregular deposit (cross section ~ 30 μm) frequently observed in the marathon runners' urines (Elemental composition Ca, K, Cl, S, P, Na)

the 2–5 μm diameter range and a second in the 15–32 μm range, and thus bears a remarkable resemblance to the bimodal distribution reported by Robertson and Peacock for recurrent idiopathic stone formers [10].

The identification by XRD of the crystalline particles in the marathon runners' urines as predominantly COD, with only trace amounts of COM, also mimics the crystal characteristics of the typical stone former [10]. Yet another feature of the marathon runners' urines which closely resembles that of the typical stone former is crystal aggregation [6, 11]. This was frequently observed (Figs. 9 and 11) in our samples. Particle size measured during SEM matched the Coulter Counter determinations as seen in Figs. 5–11.

The presence of large particles in the urines of marathon runners could arise as a result of increased growth and aggregation of crystals in a urine that may become supersaturated due to the dehydration that occurs during marathon running [2]. On the other hand, it must be remembered that the Coulter Counter does not distinguish between crystalline and non-crystalline material. As such, the "particles" counted by the instrument could, to some extent, be epithelial debris which has sloughed off during the physical trauma of long distance running. Indeed, the voiding of such debris (together with crystals) could continue for several days after the marathon even should the athlete rest during this period. Deposits which may be examples of this debris are shown in Figs. 13 and 14. The elemental composition of these deposits suggests the presence of various urinary salts. Such salts could have occurred as a result of in vivo crystallization in which epithelial debris provided a framework for crystal deposition (discussed later) or they could be artefacts arising during centrifugation and filtration of the urine sample.

Notwithstanding these considerations, our studies using the complexing agent EDTA with day +10 urine samples clearly show that a large proportion of the particles present are calcium crystals. We have also distinguished which of these are small and which are large by taking a dividing line at 12 μm as suggested by Robertson and Peacock [10] and have calculated the percentage of the total volume (of calcium particles) on each side of this critical diameter. Our results show that 50.4% occur in the larger range whereas the values reported for the stone formers lie between 5 and 40% [10]. This highlights the crystal similarities in the urines of marathon runners and stone formers.

The bimodal particle size distribution (characteristic of the recurrent stone former) is present in the marathon runners' urines not only after the marathon but even before. This could be because the period immediately preceding a marathon represents the climax of a strenuous and rigorous training schedule during which the above effects (crystal growth, epithelial sloughing) could occur.

Finlayson [4] has pointed out that all theories of the aetiology of stone disease can be classified according to the state in which the particles grow, as either fixed particle disease, such as Randall's plaques and foreign body encrustation, or free particle disease when crystalluric particles remain unattached but are prevented by their size from passing unhindered through the urinary tract. In a more recent paper, however, the same author presents calculations which suggest that free particles cannot in fact grow fast enough to cause stone disease in the upper urinary tract and advances strong arguments for the fixed particle approach [5]. As far as the present study is concerned it seems likely that the latter mechanism is indeed operative in that sloughing of debris from epithelial walls during long distance running could produce sites at which crystal particles could become attached or trapped. The in vivo residence time of such particles might be sufficiently increased to allow crystal growth to proceed unhindered resulting in obstruction of a duct. This process corresponds with Carr's theory of the aetiology of urinary stone disease, described by Finlayson [4], which requires the trapping of "large free crystalluria particles" in pericalyceal lymphatics followed by fixed particle growth. In support of this theory, Finlayson [4, 5] quotes published SEM micrographs showing individual calcium oxalate particles adhering to renal tubule walls in a man with hyperoxaluria and lithiasis. The effect has also been observed in in vivo rat studies [12].

Cellular debris itself can increase the risk of stone formation by providing nucleating centres for crystallization. Many workers believe that an organic matrix is a prerequisite for stone formation in that it presents a framework, or matrix, for crystal deposition processes [1, 7, 13].

Although it appears that fixed particle growth occurs in marathon runners (as described above), we suggest that the abnormally fast growth rates required for a free particle mechanism might be possible either as a result of dehydration or as a result of other unidentified factors.

Our results show that an increase in temperature has 2 effects. Firstly, the height of the peak occurring in the 3–10 μm range decreases, indicating dissolution of crystals. Secondly the minor peaks in the large diameter ranges shift to slightly smaller ranges (Δ shift $\sim$ 5 μm) indicating partial dissolution of crystals (i.e. decrease in particle size) as well as the breaking up of aggregates.

Our studies of freezing and long term storage effects show that particles undergo a significant increase in size during the refrigeration period. It is interesting to note that other workers studying *short* term storage effects of urine samples at various temperatures showed that no change in mean crystal size occurred in 44% of the specimens [3]. Decreases in mean crystal size occurred in 30% while small increases were registered for the remainder.

Our results therefore indicate that particle counting should be conducted at 37 °C and should be concluded within a few hours of sample collection.

In conclusion, we wish to draw attention to Finlayson's observation "that large particle crystalluria is associated with stone disease" [4]. We have observed such crystalluria in the urines of 7 marathon runners in this study. Moreover, the particle volume-size distribution profile for these samples has been shown to be remarkably similar to that reported for recurrent idiopathic stone formers [10]. In the

light of the above, therefore, we conclude that the results of this study suggest that marathon runners are at risk of urinary stone formation.

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