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Heterogeneous Nucleation of Calcium Oxalate by Seeds of Monosodium Urate (38929)

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Calcium-containing renal stones may be formed by patients with hyperuricosuria, who present no obvious abnormalities of calcium metabolism such as hypercalciuria. These patients were shown to form less stones, when their renal excretion of uric acid was lowered by allopurinol (1). In an effort to determine whether there might be a causal relationship between hyperuricosuria and calcium stone formation, we performed studies of heterogeneous nucleation (2) *in vitro*, involving uric acid, monosodium urate, calcium oxalate, and calcium phosphate. We show that "seeds" of monosodium urate may initiate heterogenous nucleation of calcium oxalate and calcium phosphate.

Material and Methods. Studies were performed at 37° in the standard solution containing 5 m*M* sodium cacodylate (Sigma Chemical Company), which was adjusted to an ionic strength of 0.15 with NaCl and to varying pH with NaOH or HCl. For the studies of heterogenous nucleation of calcium oxalate, calcium (as calcium chloride), oxalate (as sodium oxalate), and a trace amount of [^{14}C]oxalate (New England Nuclear) were added to the standard solution. The activity product of Ca^{2+} and oxalate ($Ox^{=}$) in the solution was $1.57 \times 10^{-8} M^2$, which was approximately sixfold higher than the thermodynamic solubility product of calcium oxalate (3). This solution was metastably supersaturated (4) with respect to calcium oxalate, since the spontaneous precipitation of calcium oxalate did not occur during the period of the experiment. The solution was "seeded" by adding crystals of uric acid or monosodium urate (Sigma Chem. Co., 0.1–5 mg/ml). The uric acid crystals were composed mostly of rhomboidal plates, ranging from 4 to 6 μm in length. The preparation of monosodium urate consisted mostly of needles, 8–12 μm in length. At various times after seeding, aliquots of the suspension were filtered through 0.22 μm Millipore filters; the filtrates were assayed for ^{14}C-radioactivity in a liquid scintillation counter. At the conclusion of the experiments, the filtrates were also assayed for calcium by atomic absorption spectroscopy and oxalate by the method of Hodgkinson and Williams (5).

The heterogeneous nucleation of calcium oxalate was shown by the decrease in the concentration of calcium and oxalate and in ^{14}C-radioactivity in the filtrate. The decline in equimolar amounts of calcium and oxalate indicated crystallization of calcium oxalate. Only the radioactivity data are presented here, since the fractional change in ^{14}C-radioactivity of the filtrate was within 5% of the fractional change in oxalate concentration.

The heterogenous nucleation of calcium phosphate was examined by a similar technique. Sufficient amounts of calcium (as calcium chloride) and phosphate (as Na_2HPO_4) were added to the standard solution to obtain a solution approximately threefold saturated with respect to Ca^{2+} and $HPO_4^{=}$ (6). Solid seeds of uric acid or monosodium urate were then added to this metastably supersaturated solution. The heterogeneous nucleation of calcium phosphate upon seeding was measured from the decline in calcium concentration of the filtrate. In selected samples of the filtrate, phosphate was also measured (7). The decline in equimolar amounts of calcium and phosphate indicated the probable formation of a solid phase of calcium monohydrogenphosphate. Experiments were run in duplicate or triplicate, and the results were acceptable only if replicate values

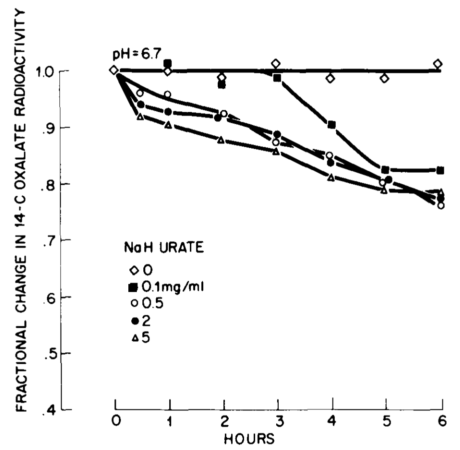

FIG. 1. Heterogeneous nucleation of calcium oxalate by seed of monosodium urate (Na H Urate). Whereas, there was no change in [^{14}C]oxalate radioactivity of the filtrate when no seed was added, a rapid decline in radioactivity occurred upon seeding with monosodium urate.

agreed within 5%. In the figures presented here, each point represents the mean of replicate experiments.

Results. At pH 6.7, seeds of monosodium urate (0.5–5 mg/ml) initiated rapid decline in [^{14}C]oxalate radioactivity of the filtrate, indicating crystallization of calcium oxalate (Fig. 1). At a low concentration of monosodium urate (0.1 mg/ml), there was a lag of 3 hr before ^{14}C-radioactivity declined. At pH 5.7, [^{14}C]oxalate radioactivity of the filtrate also decreased following seeding with monosodium urate, though to a smaller degree than at pH 6.7. At 6 hr after seeding with monosodium urate (2 mg/ml), the oxalate radioactivity decreased by only 10% at pH 5.7, whereas it decreased by 23% at pH 6.7. In contrast, seeds of uric acid (as high as 5 mg/ml) failed to cause crystallization of calcium oxalate at either pH 6.7 or 5.7; seeding of metastably supersaturated calcium oxalate solution by uric acid did not lower the [^{14}C]oxalate radioactivity of the filtrate.

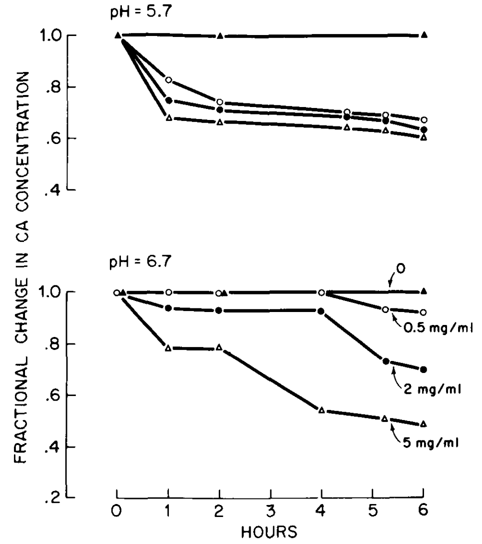

FIG. 2. Heterogeneous nucleation of calcium phosphate by seed of monosodium urate. The filtrate concentration of calcium declined following seeding with 0.5–5 mg/ml of monosodium urate at both pH 5.7 and 6.7. In the control experiment (indicated by solid triangles), calcium concentration of the filtrate did not change.

Seeding by monosodium urate of solutions metastably supersaturated with respect to calcium phosphate at either 5.7 or 6.7 was followed by a rapid decline in the calcium concentration of the filtrate, indicating heterogeneous nucleation of calcium phosphate (Fig. 2). At pH 5.3, monosodium urate also caused a decrease in the filtrate concentration of calcium, though to a lesser extent than at higher pH. At 6 hr after seeding with monosodium urate (2 mg/ml), the filtrate concentration of calcium declined by 24% at pH 5.3, whereas it decreased by 52% at pH 6.7. Seeding by uric acid caused only small decrease in calcium concentration of the filtrate at pH 5.7 and 6.7, and no significant change at pH 5.3 (Fig. 3).

Discussion. The p*K* for the dissociation of uric acid into monohydrogen urate has been reported to be 5.47 (8). In aqueous solutions containing Na$^+$, uric acid begins to be converted to monosodium urate as the pH is raised above 5.7 (9).

Unlike those who form uric acid stones, the urinary pH of patients with hyperuricosuria and calcium stones usually exceeds 5.7 (10). Further, their urine samples are usually supersaturated with respect to monosodium urate, because of high urate content (9). Thus, the physicochemical environment of urine is favorable for the nucleation of monosodium urate (9), even

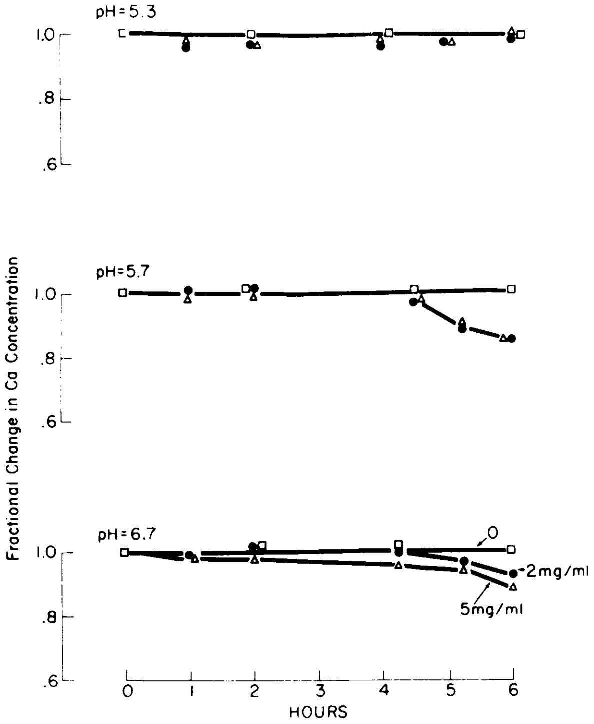

FIG. 3. Heterogeneous nucleation of calcium phosphate by seed of uric acid.

though such a crystallization has not yet been directly demonstrated.

This study suggests that monosodium urate when so-formed may contribute to the formation of calcium stones. Because of normal calcium content, the urine samples from patients with hyperuricosuria and calcium stones are often metastably supersaturated with respect to calcium oxalate and brushite ($CaHPO_4 \cdot 2H_2O$) (11), but seldom oversaturated (4). From such metastable urine samples, monosodium urate may serve as seeds for the heterogeneous nucleation of calcium oxalate and calcium phosphate. As little as 0.1 mg/ml of monosodium urate, an amount that could be potentially formed from urine, caused heterogeneous nucleation of calcium oxalate *in vitro*.

Summary. Seeds of monosodium urate caused heterogeneous nucleation of calcium oxalate at pH 5.7 and 6.7, and of calcium phosphate at pH 5.3, 5.7, and 6.7 from metastably supersaturated solutions *in vitro*. Seeds of uric acid had a small or no effect. The results could account for the formation of calcium stones among patients with hyperuricosuria and normocalciuria.

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