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# The influence of hydroxyapatite and pyrophosphate on the formation product of calcium oxalate at different pHs

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**Summary.** The nucleating effect of hydroxyapatite (HAP) and the inhibitory effect of pyrophosphate (PPi) on calcium oxalate crystallization have been studied at different pH's in solution metastabely supersaturated with respect to calcium oxalate but saturated with respect to HAP. Crystallization was monitored by a decrease of calcium in the supernatant and formation products were calculated. At a pH above 6.0 already minimal HAP concentrations proved to be a suitable substrate for heterogeneous nucleation and growth of calcium oxalate. PPi showed a pronounced inhibitory effect on spontaneous as well as on HAP induced crystallization of calcium oxalate, this effect being highly pH dependent. HAP was found to neutralize the inhibitory effect of PPi in a molar ratio of 10:1.

**Key words:** Calcium oxalate – Heterogeneous nucleation and growth – Inhibitors – Pyrophosphate – Hydroxyapatite

## Introduction

The pathogenesis of calcium oxalate stones can not exclusively be explained by calcium oxalate crystallization in the pyelocalical system. Since the rate of crystal growth is slow, crystals do not reach dimensions large enough to obstruct collecting ducts or the pyeloureteral junction. Therefore they may not be trapped in the upper urinary tract during normal urinary transit time [3]. Already in the 1930's Randall postulated fixed particle growth on kidney calcifications as a possible mechanism for stone formation [8]. Epitactical growth of calcium oxalate on hydroxyapatite (HAP), the most frequent form of tissue calcification, has been demonstrated by several studies [4–6]. Pyrophosphate (PPi) is an important inhibitor of secondary nucleation and growth of HAP accounting for more than 50% of urinary inhibitor activity when tested with respect to small crystal concentrations in urine [1, 2]. The aim of this study was to examine the influence of HAP and of physiological concentrations of PPi on the crystallization of calcium oxalate at different pH's.

## Material and methods

In order to obtain stock solutions saturated with respect to HAP, 10 mg/ml commercially available crystals of HAP (Merck) were dissolved in 150 mmol/l NaCl buffered with sodium cacodylate at pH 7.4, 6.5 and 6.0 respectively. The final calcium concentration was kept between 0.9–1.4 mmol/l by the initial addition of 1.2 mmol/l calcium to the solution buffered at pH 7.4 and of 1.0 mmol/l phosphate to the solutions buffered at pH 6.5 and 6.0 respectively. Equilibration was performed at 37°C until pH and calcium concentration were stable. After equilibration, the solutions were centrifuged at 2,140 g and filtred (Millipore 0.45 μM).

One part of the stock solutions was used to prepare a crystal suspension of 10 mg/ml HAP stirred and aged for at least 1 week. From these stock solutions, series of solutions with constant calcium, pH, PPi and crystal content were prepared and gradually supersaturated with respect to calcium oxalate by the addition of increasing amounts of sodium oxalate. The series were then incubated in a shaking water bath at 37°C during 24 h and centrifuged at 9,950 g. Crystallization was monitored by a decrease of calcium in the supernatant, which was a linear function of the initial oxalate concentrations in the specimen (Fig. 1). From linear calcium decrease, the maximal oxalate concentration without measurable crystallization was extrapolated and the formation product $(Ca) \cdot (Ox)$ for calcium oxalate crystallization was calculated. Student's t-test was used for statistical analysis.

## Results

Figure 1 shows the results of an experiment performed at pH 7.4 with and without the addition of 0.075 mg/ml HAP crystals and/or 25 μM PPi, the latter being in the range of urinary concentration found in healthy controls [9]. Spontaneous nucleation and growth of cal-

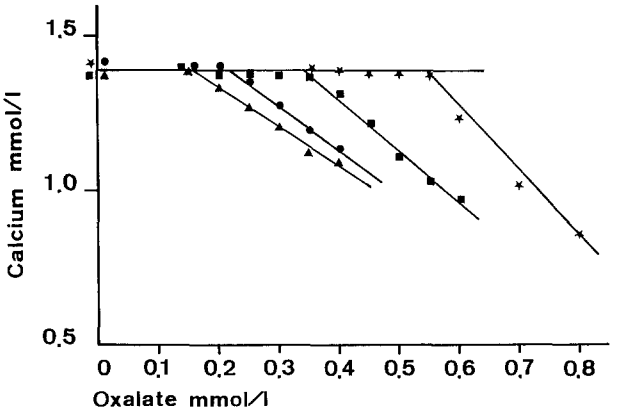


Fig. 1. Calcium versus oxalate concentration in HAP saturated solution at pH 7.4 after 24 h incubation with 25 µM PPi (*), 25 µM PPi and 0.075 mg/ml HAP (■), without any additives (●) and with 0.075 mg/ml HAP (▲)

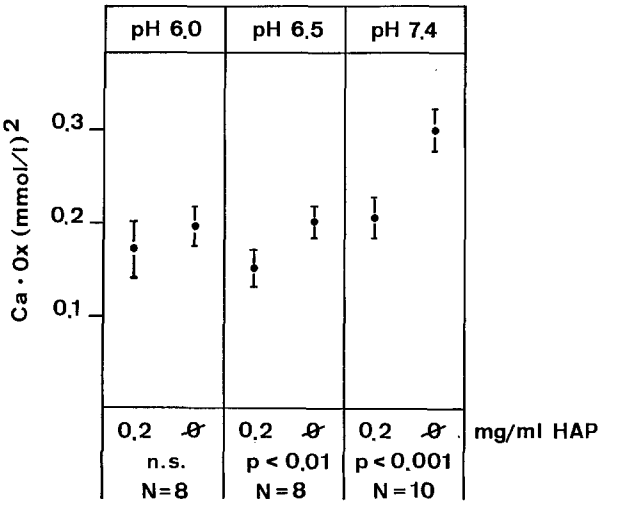


Fig. 2. Formation products for spontaneous and HAP-induced calcium oxalate crystallization at different pHs ($\bar{x} \pm 2SE$)

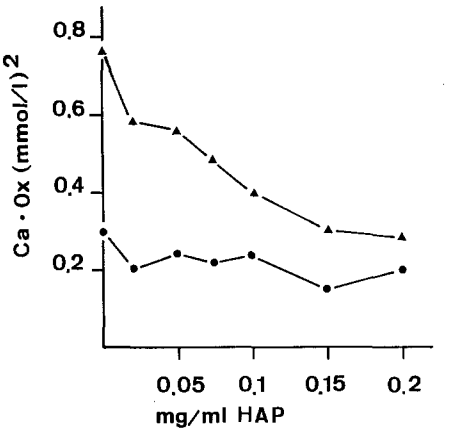


Fig. 3. Formation products for HAP-induced calcium oxalate crystallization at pH 7.4 with (▲) and without (●) PPi plotted versus HAP-concentration. ▲ = 25 µM PPi; ● = control

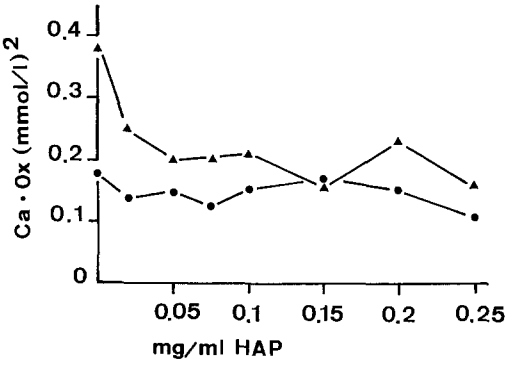


Fig. 4. Formation products for HAP-induced calcium oxalate crystallization at pH 6.0 with (▲) and without (●) PPi plotted versus HAP concentration. ▲ = 25 µM PPi; ● = control

cium oxalate started with the addition of 0.24 mmol/l oxalate. The critical oxalate concentration was reduced in the presence of HAP. HAP proved therefore to be a nucleator of calcium oxalate crystallization. The addition of 25 µM PPi more than doubled the critical oxalate concentration for spontaneous crystallization. This increase was partially abolished by the simultaneous addition of 0.075 mg/ml HAP.

The decreasing effect of HAP on the formation product of calcium oxalate was tested at different pHs and was found to be statistically significant at pH 7.4 and pH 6.5 but not at pH 6.0 (Fig. 2). At pH 7.4 HAP induced nucleation was already visible with the addition of only 0.02 mg/ml HAP and was almost independent from the seed concentration up to 0.2 mg/ml HAP (Fig. 3).

Figure 3 also demonstrates the influence of different HAP concentrations on the inhibitory effect of 25 µM PPi. Already 0.02 mg/ml HAP were able to reduce the inhibitory effect of PPi which was nearly abolished by the addition of 0.15 mg/ml HAP. A similar effect of HAP on PPi inhibited crystallization was observed at pH 6.0 where again about 0.15 mg/ml HAP supressed the inhibitory effect of 25 µM PPi (Fig. 4). The neutralization of PPi by HAP was also studied at fixed HAP but variable PPi concentrations at pH 6.0 (Fig. 5). Comparison of the two curves of the formation products obtained with and without HAP revealed that 0.075 mg/ml HAP neutralized about 15 µM PPi.

The inhibitory effect of different concentrations of PPi on HAP-induced calcium oxalate crystallization was tested at pH 7.4 and 6.0 (Fig. 6). The figure clearly shows that alkalinization of the solution from pH 6.0 to 7.4 doubled the inhibition on the whole range of PPi being studied. At both pHs a linear dose/response curve was found.

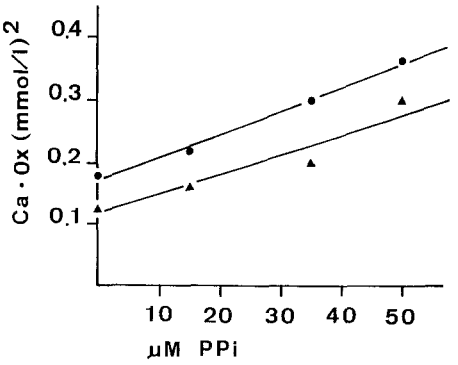


Fig. 5. Formation products for spontaneous (●) and HAP-induced (▲) calcium oxalate crystallization at pH 6.0 plotted versus PPi concentration. ● = control; ▲ = 0.075 mg/ml HAP

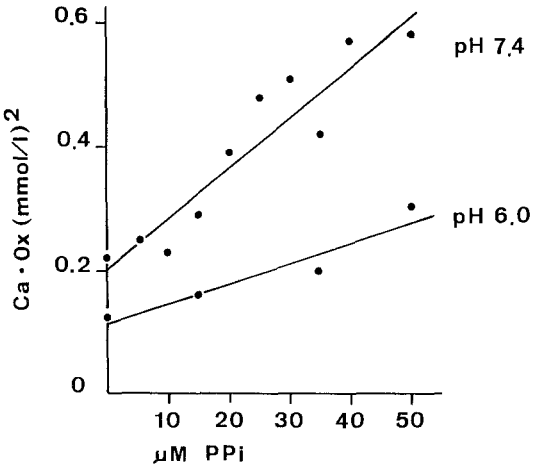


Fig. 6. Formation products for HAP-induced calcium oxalate crystallization (0.075 mg/ml HAP) at pH 6.0 and 7.4 plotted versus PPi concentration

## Discussion

This study confirmed that at a pH above 6.0 HAP is a suitable substrate for heterogeneous nucleation and growth of calcium oxalate, which has been demonstrated by several kinetic tests in equimolar solutions of calcium and oxalate [4–6]. Our test system determines formation products and does not allow kinetic analysis. But it has the advantage that measurements can be performed at low oxalate concentrations and more or less physiological calcium/oxalate ratios. Under these conditions a nucleating effect was already detected at the lowest HAP concentration used. Contrary to the findings of others [5], this nucleating effect could not be raised by the increase of the HAP concentration.

Under our test conditions, PPi showed a pronounced inhibitory effect on the spontaneous as well as on the HAP induced crystallization of calcium oxalate. This inhibitory effect was extremely pH dependent. An alkalinization of the solution from pH 6.0 to 7.4 doubled the inhibitory activity with respect to spontaneous as well as to heterogeneous nucleation and growth. This observation may serve as a further explanation for the therapeutic effect of alkalicitrate in the metaphylaxis of calcium oxalate stones, which has partially be attributed to an increase of urinary inhibitor activity [7].

The inhibitory effect of PPi on calcium oxalate crystallization was supressed in the presence of HAP, which has been found to neutralize PPi in a molar ratio of about 10:1. This neutralizing effect was almost independent from pH, probably because HAP and PPi have similar dissociation constants. From Fig. 4 some conclusions might also be drawn for the calcium oxalate stone formation. The figure shows that at a pH of 6.0 which is most often found in urine of stone formers [1], small amounts of HAP are already able to destabilize a solution which with respect to calcium oxalate is metastabely supersaturated and inhibited by PPi. A similar effect may be postulated for kidney calcifications in the urine of calcium oxalate stone formers. However, crystallization experiments in urine are required to give further evidence for this hypothesis.

## References

1. Baumann JM, Lauber K, Lustenberger FX, Wacker M, Zingg EJ (1985) Crystallization conditions in urine of patients with idiopathic recurrent calcium nephrolithiasis and with hyperparathyroidism. Urol Res 13:169–174
2. Baumann JM, Ackermann D (1988) Inhibition of calcium phosphate crystallization. In: Martelli A, Buli P, Marchesini B (eds) Inhibitors of crystallization in renal lithiasis and their clinical application. Acta Medica pp 83–88
3. Finlayson B, Reid F (1978) The expectation of free and fixed particles in urinary stone disease. Invest Urol 15:442–448
4. Koutskoukos PG, Sheehan ME, Nancollas GH (1981) Epitaxial considerations in urinary stone formation. II. The oxalate phosphate system. Invest Urol 18:358–363
5. Meyer JL, Bergert JH, Smith LH (1975) Epitaxial relationships in urolithiasis: the calcium oxalate monohydrate hydroxyapatite system. Clin Sci Mol Med 49:369–374
6. Pak CYC, Hayashi Y, Arnold LH (1976) Heterogenous nucleation with urate, calcium phosphate and calcium oxalate. Proc Soc Exp Biol Medi 153:83–87
7. Pak CYC, Fuller C, Sakhaee K, Preminger GM, Britton F (1985) Long-term treatment of calcium nephrolithiasis with potassium citrate. 134:11–19
8. Randall A (1937) The origin and growth of renal calculi. Am Surg 105:1009
9. Wikström B, Danielson BG, Ljunghall S, McGuire M, Russell RGG (1983) Urinary pyrophosphate excretion in renal stone formers with normal and impaired renal acidification. World J Urol 1:150–154

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