Urol Res (1985) 13:169–174

Urological Research

Crystallization Conditions in Urine of Patients with Idiopathic Recurrent Calcium Nephrolithiasis and with Hyperparathyroidism

J. M. Baumann, K. Lauber, F. X. Lustenberger, M. Wacker and E. J. Zingg

Clinic of Urology and Institute of Medical Chemistry of the University of Berne, Department of Urology, Regionalspital Biel, Switzerland

Accepted: July 11, 1984

Summary. (1) The highest degree of urinary supersaturation with respect to calcium oxalate monohydrate (COM) and brushite at which secondary nucleation and growth of small amounts of COM and hydroxyapatite (HAP) are inhibited was determined by new and simple methods. There were 39 subjects who produced 24 h-urine collections (11 idiopathic stone formers (ISF), 12 patients suffering from primary hyperparathyroidism (HPT) and 16 healthy controls (HC). These subjects had a moderate calcium and low oxalate intake. The results obtained were compared with the state of urinary saturation and with urine chemistry. The measurements of crystallization conditions with respect to COM were repeated in 26 subjects (11 ISF, 5 HPT, 10 HC) after a dietary oxalate load. (2) In 24 h-urines of HC diluted to 2.4 1/24 h the degree of supersaturation necessary to induce crystallization of COM and HAP was 2–5 times higher than the state of urinary saturation measured under the same test conditions. (3) ISF showed a decreased pyrophosphate concentration and a decreased inhibitory activity to HAP crystallization in their 24 h-urine. (4) The urinary inhibitory activity towards crystallization of HAP showed a positive correlation to urinary pyrophosphate concentration. (5) In the 24 h-urine of HPT hypercalciuria and increased saturation with respect to brushite which reached values to induce HAP crystallization were found. (6) After a dietary oxalate load urinary supersaturation with respect to COM reached values to induce COM crystallization in ISF and HPT but not in HC.

Key words: Calcium, Citrate, Hyperparathyroidism, Inhibitors, Magnesium, Nephrolithiasis, Oxalate, Pyrophosphate.

Abbreviations: COM = Calcium oxalate monohydrate; CP = Concentration product; CPR = Concentration product ratio; HAP = Hydroxy apatite; HC = Healthy controls; HPT = Patients suffering from primary hyperparathyroidism; ISF = Idiopathic stone formers

Introduction

Current concepts about the mechanism of stone formation assume that it is the result of crystallization processes for which urinary supersaturation with respect to stone forming salts is the driving force [8, 15]. The state of urinary saturation with respect to calcium salts depends on pH and ionic strength and on at least 9 ionized components forming from 19 to 24 complexes [17]. Calculation of urinary saturation therefore requires extensive chemical analyses and computer programs [19]. A simpler approach as used in our study is to determine the (calcium) x (oxalate) or the (calcium) x (phosphate) concentration product (CP) before and after incubation of the urine with a large crystal mass of calcium oxalate or calcium phosphate and to calculate the corresponding concentration product ratios (CPR) as a measure for the state of urinary saturation [17].

The absence of stone formation despite urinary supersaturation, which is also found in healthy people, may be explained by the effect of crystallization inhibitors [10] such as pyrophosphate, magnesium, citrate, phosphocitrate, acid glycosaminoglycans, acidic polypeptides or polyribonucleotide fragments [6]. It appears that crystallization conditions of stone forming calcium salts as well as the effect of inhibitors and of urine can best be studied in metastable solutions supersaturated with respect to these salts [15]. In these solutions crystallization processes are induced by a small amount of seed crystals and crystal growth rate [14] or degree of crystal growth and aggregation after a fixed incubation time [22] is measured. However, the true inhibitory potential of urine can hardly be shown since crystallization is almost prevented by the addition of only 3% of urine [1]. We have therefore developed another approach [2]: In order to overwhelm the high urinary inhibitory capacity 2 series of urine are gradually supersaturated by the addition of oxalic acid of increasing concentrations. One series is seeded with a small crystal mass and the other series with a large crystal mass of calcium oxalate monohydrate (COM) and after a fixed incubation time the maximum

(calcium) x (oxalate) CPs without measurable crystallization are extrapolated from the fall in calcium concentration. Inhibitory activity is expressed as the ratio of the CP of urine incubated with a small crystal mass and the CP of urine incubated with a large crystal mass. The state of urinary saturation is expressed as the ratio of the initial CP and the CP after incubation with the large crystal mass. A similar method for calcium phosphate is described in this paper. Both methods were used to examine urinary crystallization conditions in active normocalcaemic (idiopathic) stone formers (ISF), in patients with primary hyperparathyroidism (HPT) and in healthy controls (HC).

Patients

The group of active ISF consisted of 11 males (ages 30–60, mean 45.3 years) who had normal renal function, no urinary infection and normal serum values for calcium and uric acid. 6 of these patients were normocalciuric and 5 were known to be hyperabsorbers of calcium. Their active recurrent disease was defined by either the passage of at least 4 stones or 2 stone operations during the 2 years prior to the examination. The stone analyses revealed that 6 patients had produced pure calcium oxalate stones and 5 had mixtures of calcium oxalate and calcium phosphate. The group of HPT comprised 5 males and 7 females (ages 32–66, mean 48.9 years). Their disease had been diagnosed beforehand by serum calcium estimation and an inappropriately elevated value of parathyroid hormone in serum, and was confirmed after the study by surgical exploration. 5 of these patients had produced pure calcium oxalate stones, one a mixture of calcium oxalate and calcium phosphate, and one a struvite stone. In three of the remaining HPT there was no stone disease and in 2 patients no information about stone composition. All patients were free of stones at the time of the study. The HC were 10 male and 6 female healthy volunteers (ages 29–61, mean 42.1 years).

Material

All 3 groups were on the same diet with defined intakes of calcium (560 mg), phosphorus (1,160 mg), oxalate (70 mg) and fluid (1,500 ml). This diet was given the day before and during the 24 h-urine collection. After this collection the male HC, the ISF and the male HPT received a dietary load of 1,200 mg oxalate (rhubarb and spinach) and urine was collected during a further 14 h period overnight. Of every collected urine portion several samples were frozen. For the reconstitution of the 24 h-urine as well as of the 14 h-collection period aliquots proportional to the voided volumes were mixed and the sediment carefully dissolved as described previously [2]. For the tests on crystallization conditions these reconstituted urines were diluted to a standardized volume of 100 ml/h by the addition of twice distilled water.

Methods

1. Analytical Techniques

Calcium was measured with a Corning Calcium Analyzer, model 940, magnesium by atomic absorption UNICAM Sp 1900, citrate enzymatically (Boehringer kit Nr. 139,076), pH with a pH-meter Metrohm, Herisau, model E 603, phosphorus by the method of Bisaz et al. [4], pyrophosphate by the method of Heinonen et al. [11], uric acid by the method of Kageyama et al. [12] and oxalic acid by the method of Drawert et al. [7], which we adapted for urine by preparing the urine samples with EDTA to dissolve calcium oxalate precipitates.

2. Crystallization Conditions with Respect to Calcium Oxalate

According to the method previously described in detail [2], 2 series of urine aliquots were gradually supersaturated with respect to calcium oxalate by the addition of oxalic acid of increasing concentrations. In one series the maximum concentration product (CP) of (calcium) x (oxalate) which was tolerated without causing measurable secondary nucleation and growth of 0.02 mg of COM crystals/ml of urine during a $1^1/2$ h incubation time was determined. In the other series the CP of the saturated urine with respect to COM was measured after a 24 h equilibration with 10 mg COM crystals/ml of urine. Inhibitor capacity and the degree of the initial urinary saturation were calculated as described and given in the legends to Fig. 1.

3. Urinary Crystallization Conditions with Respect to Calcium Phosphate

We determined the inhibitory activity towards secondary nucleation and growth of hydroxyapatite (HAP) which is the most common calcium phosphate found in stones and tissue calcifications. The state of saturation was related to brushite as described by others [17] because a constant equilibrium between HAP as a solid phase and a solution supersaturated with respect to this salt (e.g. urine) may not be reached for months [16]. The crystal suspensions used were prepared with commercially available crystals (Merck) in a solution of NaCl 0.15 mol/l buffered at pH 6.60 with piperazine -N,N'-bis (2-ethane-sulfonic acid) ("Pipes") 50 mmol/l and containing NaN_3 0.1%. They were equilibrated for several days. The consistency of the seeding properties was followed up by running tests as described below in a standard solution buffered at pH 6.60 with "Pipes" 50 mmol/l and containing NaCl 0.15 mol/l, Na_2HPO_4 10 mmol/l, EHDP 2.5 µmol/l added as standard inhibitor and NaN_3 0.1% added as preservative. The urines were also buffered at pH 6.60 with "Pipes" (final concentration 50 mmol/l), contained NaN_3 (final concentration 0.1%) and were diluted to the equivalent to 100 ml/h with twice distilled water. Then three series of 1.0 ml aliquots were gradually supersaturated by the addition of 0.02 ml of $CaCl_2$-solutions of increasing concentrations to reach final calcium concentrations of 10 mmol/l. The first series, the "blanks" were acidified with 0.02 ml HCl 2N. The second series were seeded with 0.02 ml of HAP suspension to produce a final concentration of 0.2 mg HAP per ml of urine. The third series were seeded with 0.02 ml of brushite suspension to produce a final concentration of 10 mg brushite per ml of urine. The 2 series of seeded samples were then incubated at conditioned room temperature (22 °C) on a small shaker with pH being maintained at 6.60 ± 0.02. After 24 h they were centrifuged for 3 min at 9,980 *g*. The calcium concentrations in the blanks and in the supernatants of the seeded samples were then measured. The fall of calcium concentration in each seeded sample was calculated by subtracting the calcium concentration of the supernatant from the calcium concentration of the corresponding blank containing the same starting calcium. In each seeded series the regression line of these falls versus the calcium concentrations of the blanks was computed, the maximum calcium concentration without crystallization extrapolated and the maximum (calcium) x (phosphate) concentration product (CP) without brushite or HAP crystallization calculated. The degree of the initial saturation under the specific test conditions was expressed as CPR of the initial (calcium) x (phosphate) CP in urine and the maximum (calcium) x (phosphate) CP without brushite crystallization. Inhibitory capacity was expressed as the ratio of the maximum (calcium) x (phosphate) CP without crystallization of HAP and the maximum (calcium) x (phosphate) CP without crystallization of brushite.

Table 1. Results of chemical analyses ($\bar{x} \pm 2$ SE) of 24 h-urine

	♀		♂			Significances
	HC (a)	HPT (b)	HC (c)	ISF (d)	HPT (e)	
Number of patients	6	7	10	11	5	
Urine volume ml/h	55.4 ± 14.2	65.8 ± 11.0	57.1 ± 12.1	58.0 ± 8.5	64.2 ± 22.8	n.s.
pH	6.18 ± 0.26	6.09 ± 0.22	6.15 ± 0.18	6.08 ± 0.22	6.24 ± 0.62	n.s.
Calcium μmol/h	123 ± 24.2	418 ± 130	164 ± 27.9	200 ± 52.0	420 ± 102	(a) vs (b) $p < 0.005$ (c) vs (e) $p < 0.001$
Magnesium μmol/h	148 ± 24.2	167 ± 21.7	177 ± 40.0	157 ± 18.1	168 ± 44.2	n.s.
Phosphate mmol/h	1.06 ± 0.15	1.20 ± 0.08	1.49 ± 0.14	1.22 ± 0.15	1.35 ± 0.24	(a) vs (c) $p < 0.005$ (c) vs (d) $p < 0.02$
Oxalate μmol/h	15.2 ± 3.16	15.2 ± 2.05	15.5 ± 1.17	15.8 ± 1.81	17.3 ± 3.58	n.s.
Uric acid μmol/h	154 ± 15.8	156 ± 10.6	178 ± 20.1	169 ± 23.0	159 ± 42.6	n.s.
Citrate μmol/h	125 ± 27.3	175 ± 47.1	95.7 ± 18.2	98.5 ± 28.9	165 ± 34.2	(c) vs (e) $p < 0.005$
Pyrophosphate μmol/h	1.31 ± 0.40	1.88 ± 0.45	2.74 ± 0.56	1.29 ± 0.38	2.02 ± 0.82	(a) vs (c) $p < 0.005$ (c) vs (d) $p < 0.001$

Table 2. Results of chemical analyses ($\bar{x} \pm 2$ SE) of urine collected during the 14 h-overnight period after a dietary oxalate load of 1,200 mg

	♂			Significances
	HC (a)	ISF (b)	HPT (c)	
Number of patients	10	11	5	
Urine volume (ml/h)	51.2 ± 9.8	50.5 ± 10.6	60.8 ± 22.6	n.s.
pH	6.08 ± 0.23	6.14 ± 0.28	6.07 ± 0.51	n.s.
Calcium (μmol/h)	113 ± 24.1	121 ± 30.0	371 ± 111	(a) vs (c) $p < 0.001$
Magnesium (μmol/h)	162 ± 30.0	120 ± 22.0	161 ± 46.0	(a) vs (b) $p < 0.05$
Phosphate (mmol/h)	1.49 ± 0.16	1.26 ± 0.15	1.51 ± 0.28	n.s.
Oxalate (μmol/h)	48.4 ± 10.1	51.2 ± 6.61	59.8 ± 6.05	n.s.
Uric acid (μmol/h)	166 ± 12.8	161 ± 19.4	171 ± 53.2	n.s.
Citrate (μmol/h)	80.7 ± 19.4	82.8 ± 26.8	143 ± 48.8	(a) vs (c) $p < 0.02$
Pyrophosphate (μmol/h)	2.22 ± 0.45	1.22 ± 0.36	2.05 ± 0.68	(a) vs (b) $p < 0.005$

Results

1. Chemical Analyses

Since the female HC showed lower urinary 24 h-excretions of phosphate and pyrophosphate than the male HC, the results obtained in the two sexes are shown separately in Table 1. The main disturbance found in 24 h-urine of the ISF was a diminished excretion of pyrophosphate. Both, male and female HPT showed an increase of urinary calcium. In addition the few male HPT also had an elevated excretion of citrate. After a dietary oxalate load (see Table 2) urinary oxalic acid increased in all 3 groups ($p < 0.001$) and in ISF and HC calcium excretion decreased ($p < 0.02$ each) compared with the corresponding values in the 24 h-urine. Furthermore ISF showed hypomagnesuria and hypopyro-

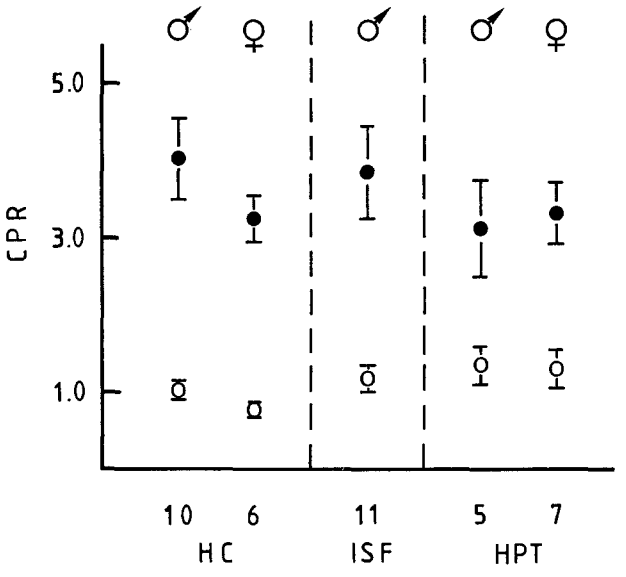

Fig. 1. Crystallization conditions with respect to COM in 24 h-urine diluted to 100 ml/h and buffered at pH 6.0: Inhibitory activity (Inhibition) is expressed as the ratio of the maximum CP of (calcium) x (oxalate) without crystallization of 0.02 mg COM/ml of urine and the CP of (calcium) x (oxalate) in urine equilibrated with 10 mg COM/ml of urine. The degree of urinary saturation under the test conditions (Saturation) is expressed as the ratio of the initial CP of (calcium) x (oxalate) in the urine and the CP of (calcium) x (oxalate) of the urine equilibrated with 10 mg COM/ml of urine. CPR = 1 denotes saturated, CPR < 1 undersaturated and CPR > 1 supersaturated urine. Values are given as $\bar{x} \pm 2$ SE, the number of subjects and the groups are indicated in the 2 lines at the bottom. • = Inhibition; ○ = Saturation

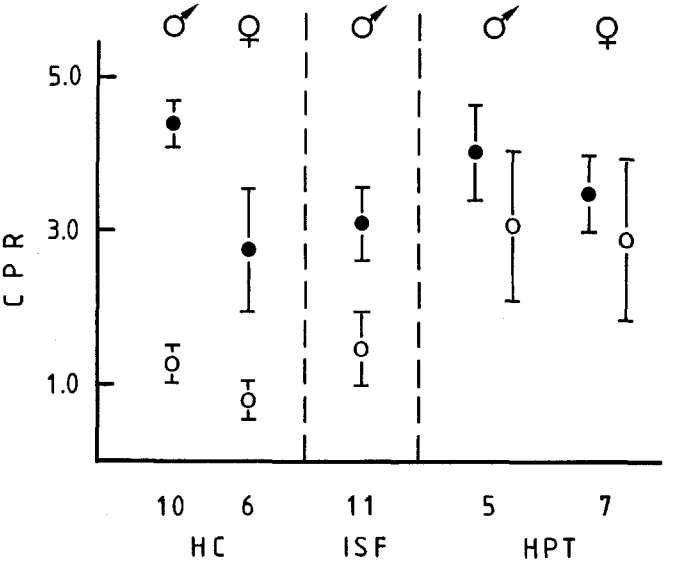

Fig. 3. Crystallization conditions with respect to calcium phosphate in 24 h-urine, diluted to 100 ml/h and buffered at pH 6.60: Inhibitory activity is expressed as the ratio of the maximum CP of (calcium) x (phosphate) without crystallization of 0.2 mg HAP/ml of urine and the CP of (calcium) x (phosphate) in urine equilibrated with 10 mg brushite/ml of urine. The degree of saturation under the test conditions is expressed as the ratio of the initial CP of (calcium) x (phosphate) in the urine and the CP of (calcium) x (phosphate) in urine equilibrated with 10 mg brushite/ml of urine. Other details as in Fig. 1

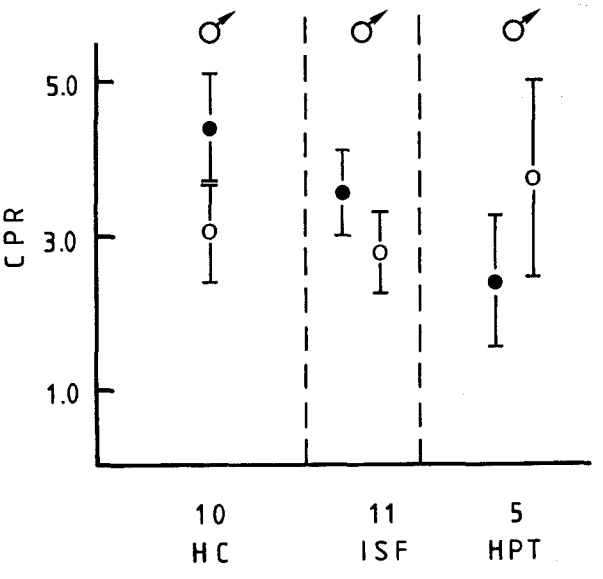

Fig. 2. Urinary crystallization conditions with respect to COM in the 14 h-collection period after dietary oxalate load of 1,200 mg: Other examination conditions and details as in Fig. 1

phosphaturia. In HPT, hypercalciuria with an elevated excretion of citrate persisted.

2. Crystallization Conditions with Respect to Calcium Oxalate

24 h-urines of female HC were undersaturated with respect to COM and showed a lower state of saturation ($p < 0.02$) then 24 h-urines of male HC (Fig. 1). HPT had an elevated saturation compared to HC ($p < 0.005$ in women, $p < 0.05$ in men). In the 24 h-urine all groups revealed an inhibitory activity towards secondary nucleation and growth of COM which was 2–4 times higher than the degree of saturation measured under the same test conditions. After the dietary oxalate load the degree of saturation tripled in all 3 examined groups (Fig. 2) yet in HC it remained significantly below ($p < 0.01$) the supersaturation necessary to induce crystallization, whereas in ISF and HPT it reached and exceeded the inhibitory capacity. In HPT inhibitory capacity was significantly lower than in HC ($p < 0.005$). When plotting the urinary inhibitory capacity to crystallization of COM versus the measured urinary concentrations of each of the low molecular weight inhibitors pyrophosphate, citrate and magnesium, we did not find any significant correlation.

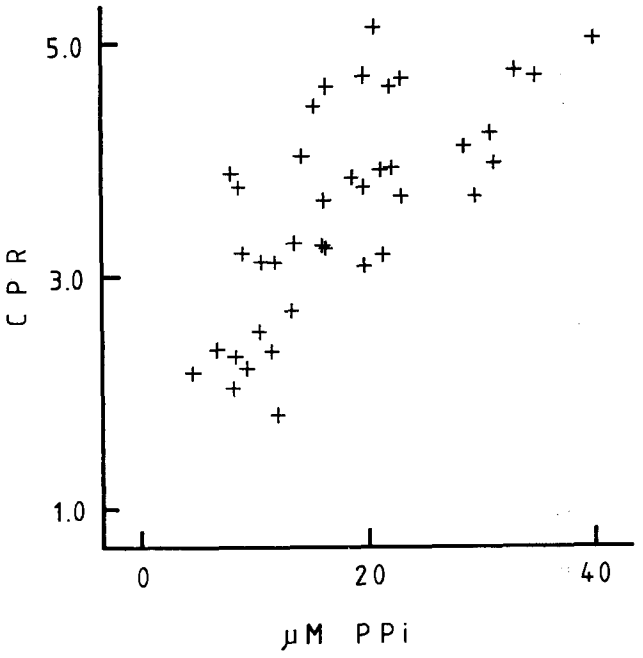

Fig. 4. Urinary inhibitory activity towards HAP crystallization (CPR) plotted versus urinary pyrophosphate concentration in the testsystem (PPi): The correlation between the 2 parameters calculated as linear regression is defined as $y = 0.0745 \times + 2.2330$, $r = 0.7056$ ($p < 0.001$); $n = 39$

3. Crystallization Conditions with Respect to Calcium Phosphate

The urine of female HC was undersaturated with brushite and had a significant lower degree of saturation than urine of male HC ($p < 0.02$) (Fig. 3). In urine of male HC the inhibitory capacity towards secondary nucleation and growth of HAP was 4 times higher than the degree of urinary saturation measured under the test conditions. In ISF and female HC this inhibitory capacity was significantly lower ($p <$ 0.001 each). In HPT urinary saturation reached values which could induce the crystallization of HAP. The CPR for HAP inhibition plotted versus urinary pyrophosphate concentration showed a significant correlation between these 2 parameters (Fig. 4). Such correlations could not be demonstrated in the case of citrate or magnesium.

Discussion

This study has been performed on a moderate calcium- and a low oxalate intake because such a diet is common in calcium stone therapy and has been recommended together with a high fluid intake to our recurrent stone formers. In order to assess the effect of a high oxalate ingestion we also investigated the male patients, who are much more subject to non infectious calcium stone disease than women, under a dietary oxalate load. The chemical analyses of the urine passed under our dietary conditions revealed that compared to the HC ISF excreted by 50% less pyrophosphate in their 24 h-urines, a finding which agrees with our previous results [3]. This diminished pyrophosphate excretion was also found after the dietary oxalate load. Hypopyrophosphaturia was also demonstrated by a recent study done on calcium stoneformers with short term recurrence [25] but is not observed in calcium stone disease in general [23]. Among the other low molecular weight inhibitors magnesium was decreased in ISF after the oxalate load and citrate was elevated in our few male HPT. With regard to the stone forming ions we found on our moderate calcium intake an elevated calcium excretion only in HPT and in the 5 ISF who were known to be hyperabsorbers of calcium but not in ISF as a group. After the dietary oxalate load there were no significant differences between ISF and HC with respect to calciuria and oxaluria in contrast to the results obtained from examinees who received a basal diet of 1,000 mg calcium and sodium oxalate 5 mg/kg body weight [20].

The examination of crystallization conditions was performed in our study on a constant urine dilution of 100 ml/h and at pH of 6.0 for calcium oxalate and at pH 6.6 for calcium phosphate. The constant urine dilution seems to be important to correct for variations of stone forming ions, inhibitors, chelators and ionic strenght due to different states of diuresis, since the urine volumes even under the defined fluid intake of this study (1.5 l/day) showed variations of up to 100% (see tables). A diuresis which exceeds 2 litres per day is included in our stone therapy and therefore seems to be normal for our stone patients with short term recurrence. The pH of 6.0 used in the calcium oxalate system was in the range of the average pH observed in this study. In the calcium phosphate system a pH of 6.6 was chosen for reasons of the stability of the HAP crystals [16]. The incubation times used for the inhibitor tests comply with the rapid crystallization of COM [14] and the discontinuous crystallization of HAP [15]. Although we had chosen those crystal concentrations for our test systems which were most sensitive to physiological variations of low molecular weight inhibitors (pyrophosphate, citrate, magnesium) pyrophosphate was the only inhibitor which showed a good correlation between its concentration and the urinary inhibitor activity; this was only evident in the calcium phosphate system. Pyrophosphate seems to be an important inhibitor of the crystallization of small amounts of HAP. However, when HAP-seed crystals are used in a concentration range of several mg/ml pyrophosphate contributes 9%, citrate 48%, and magnesium 20% towards inhibitory activity of urine [5]. A large surface of seed crystals may therefore increase the relative efficiency of a weak inhibitor present in high urinary concentrations (e.g. citrate) and suppress the action of potent inhibitors present in low urinary concentrations (e.g. pyrophosphate).

In this study 24 h-urine of HC showed a high inhibitory activity to secondary nucleation and growth of COM and HAP, which was in the range of 2–5 times the degree of urinary saturation as measured under the same test condi-

tions. With respect to calcium phosphate this favorable relation was disturbed in ISF by a decreased inhibitory activity to HAP and in HPT by an increased saturation. The 24 h-urine of ISF and HPT showed no marked disturbance in the calcium oxalate system. However, after an oral oxalate load urinary supersaturation in ISF reached, and in male HPT even exceeded the values necessary to induce crystallization of COM. ISF as well as HPT, although calcium oxalate prevailed in their stone analyses, showed a basic disturbance in the calcium phosphate system as found by others [24], and revealed a pathological reaction to an oral excess of oxalate as demonstrated with respect to crystalluria [21]. If we assume that calcium oxalate stones can originate from calcifications of the renal medulla [9, 18], our findings may be interpreted as follows: A primary disequilibrium in the calcium phosphate system may have enhanced the formation of medullary HAP-deposits which were not sufficiently protected by crystallization inhibitors. These deposits together with a relative hyperoxaluria after oxalate ingestion may have induced heterogenous nucleation and growth of calcium oxalate. HAP has been found to be a suitable substrate for COM growth [13].

Acknowledgements. We thank Prof. R. G. G. Russell for helpful advice, Mrs. B. Känzig for technical assistance and Mrs. M. Härri for preparing the manuscript. This work has been supported by the Swiss National Science Foundation 3.878-079 and 3.873-081.

References

1. Baumann JM, Wacker M (1979) Experiences with the measurement of inhibitory activity of urine and crystallization inhibitors by different techniques. Urol Res 7:183–188
2. Baumann JM, Wacker M (1980) The direct measurement of inhibitory capacity to crystal growth of calcium oxalate in undiluted urine and in other inhibitor containing solutions. Uro. Res 8:171–175
3. Baumann JM, Bisaz S, Felix R, Ganz U, Russell RGG (1977) The role of inhibitors and other factors in the pathogenesis of recurrent calcium-containing stones. Clin Sci 31:365–374
4. Bisaz S, Russell RGG, Fleisch H (1968) Isolation of inorganic pyrophosphate from bovine and human teeth. Arch Oral Biol 13:683–696
5. Bisaz S, Felix R, Neuman WF, Fleisch H (1978) Quantitative determination of inhibitors of calcium phosphate precipitation in whole urine. Min Electrol Metab 1:74–83
6. Breslau NA, Pak CYC (1980) Urinary saturation, heterogenous nucleation, and crystallization inhibitors in nephrolithiasis. In: Brenner BM, Stein JH (eds) Nephrolithiasis, contemporary issues in nephrology, vol 5. Churchill Livingstone, New York Edinburgh London, pp 13–36
7. Drawert F, Paul H, Hagen W (1981) Enzymatische Bestimmung von Oxalsäure und Ameisensäure im Bier. Brauwissenschaft 3:57–61
8. Finlayson B (1978) Physicochemical aspects of urolithiasis. Kidney Int 13:344–360
9. Finlayson B, Reid F (1978) The expectation of free and fixed particles in urinary stone disease. Invest Urol 15:442–448
10. Fleisch H (1978) Inhibitors and promoters of stone formation. Kidney Int 13:361–371
11. Heinonen JK, Honkasalo SH, Kukko EI (1981) A method for the concentration and for the colorimetric determination of nanomoles of inorganic pyrophosphate. Anal Biochem 117: 293–300
12. Kageyama N (1971) Direct colorimetric determination of uric acid in serum and urine with uricase-catalyse system. Clin Chim Acta 31:421–426
13. Koutskoukos PG, Sheehan ME, Nancollas GH (1981) Epitaxial considerations in urinary stone formation. II. The oxalatephosphate system. Invest Urol 18:358–363
14. Meyer JL, Smith LH (1975) Growth of calcium oxalate crystals. II. Inhibition by natural urinary crystal growth inhibitors. Invest Urol 13:36–39
15. Nancollas GH (1976) The kinetics of crystal growth and renal stoneformation. In: Fleisch H, Robertson WG, Smith LH, Vahlensieck W (eds) Urolithiasis research. Plenum Press, New York London, pp 5–23
16. Neuman WF, Neuman MW (1958) Solubilities. In: Bay EB, Coggeshall LT, De Bruyn PPH, Dragstedt LR, Park T, Taliferro WH (eds) The chemical dynamics of bone mineral. The University of Chicago Press, Chicago 37, pp 23–38
17. Pak CYC, Hayashi Y, Finlayson B, Chu S (1977) Estimation of the state of saturation of brushite and calcium oxalate in urine: A comparison of three methods. J Lab Clin Med 89:891–901
18. Prien EL (1975) The riddle of Randall's plaques. J Urol 114: 500–507
19. Robertson WG, Peacock M, Nordin BEC (1968) Activity products in stone-forming and non-stone-forming urine. Clin Sci 34:579–594
20. Robertson WG, Peacock M, Nordin BE (1971) Calcium oxalate crystalluria and urine saturation in recurrent renal stone-formers. Clin Sci 40:365–374
21. Robertson WG, Peacock M (1972) Calcium oxalate crystalluria and inhibitors of crystallization in recurrent renal stone-formers. Clin Sci 43:499–506
22. Robertson WG, Knowles F, Peacock M (1976) Urinary acid mucopoly saccharide inhibitors of calcium oxalate crystallization. In: Fleisch H, Robertson WG, Smith LH, Vahlensieck W (eds) Urolithiasis research. Plenum Press, New York London, pp 331–334
23. Russell RGG, Hodgkinson A (1966) The urinary excretion of inorganic pyrophosphate by normal subjects and patients with renal calculus. Clin Sci 31:51–62
24. Smith LH, Jenkins AD, Wilson JWL, Werness-Rochester PG (1984) Is hydroxyapatite important in calcium urolithiasis? Fortschr Urol Nephrol 22:193–197
25. 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

Prof. Dr. J. M. Baumann
Clinic of Urology
Regionalspital Biel
CH-2500 Biel
Switzerland