Urol Res (1991) 19:151–158

Urological Research

Original articles

Crystalluria determined by polarization microscopy*

Technique and results in healthy control subjects and patients with idiopathic recurrent calcium urolithiasis classified in accordance with calciuria

U. Herrmann[1], P. O. Schwille[1], and P. Kuch[2]

[1]Departments of Surgery and Urology, and [2]Department of Mineralogy, Mineral Metabolism and Endocrine Research Laboratory, University of Erlangen-Nürnberg, Erlangen, FRG

Accepted: August 29, 1990

Summary. A retrospective study was done on the nature and degree of crystalluria in spontaneously voided fasting and postprandial urine of patients with recurrent idiopathic calcium urolithiasis (RCU) divided into normocalciuria (20 males, 20 females) and hypercalciuria patients (20 males, 20 females), and controls (20 males, 20 females). The crystals were obtained using a filter technique and identified by microscopy. In addition, individual data, clinical chemistry variables and indices reflecting the risk of calcium phosphate and calcium oxalate crystallization were evaluated. In contrast to findings of other investigators of crystalluria we observed only a few crystals on the filters. The most frequently occurring phases were (in this order) a urate-containing phase (tentatively termed uric), an amorphous calcium phosphate phase (tentatively termed isotropic) and a phase of spheroid-like particles, not yet definitely characterized (tentatively termed spheroid). Calcium oxalate crystals were found only exceptionally. There was no relationship between the degree of calciuria (normo- versus hypercalciuric RCU) and crystalluria. Among RCU, males generally had a predominance of the isotropic, females of the spheroid phase, as compared with controls. Also, RCU females were generally obese, and their spheroid score and lean body mass correlated negatively and significantly. The calcium phosphate and calcium oxalate risk indices were always low in normal individuals, higher in RCU. Patients of both sexes with urinary stones had normal parathyroid gland function, but higher total calcium in fasting serum and higher urinary pH as compared with controls. From these data we concluded that (1) crystalluria is a regular finding in human urine, but is more pronounced in RCU; (2) in males, the isotropic phase, in terms of frequency and its score, may be causally related to the development of urolithiasis; (3) the spheroid phase, more frequently observed in RCU females, may reflect an as yet unknown metobolic disorder; (4) the rareness of calcium oxalate crystals despite a high calcium oxalate risk index suggests that such crystals may be adherent to upstream tissue.

Crystalluria is considered to represent microurolithiasis, although urinary stone formation does not necessarily follow [9]. On the other hand, macrourolithiasis may only approximately reflect those processes controlling the liquid-solid transition of crystallizing substances, i.e., homogeneous and heterogeneous nucleation [8, 19, 24]. Viewed in this way crystalluria should be a constant finding in patients with frequently recurring urolithiasis and, conversely, it should be a rare event or never occur in healthy humans. Until now this hypothesis has not been examined systematically, although there is a long tradition among physicians of investigating crystals in urine. Most available studies report more and larger crystals and crystal aggregates in patients with urinary stones than in normal individuals [for overview see 22, 24], but the extremely divergent examination conditions hardly allow a valid comparison of published studies.

Crystal formation and dissolution are governed by the laws of thermodynamics and also the prevailing kinetic conditions (e.g., dependence on the time, concentration of nucleation inhibitors, etc.). Especially the constancy of temperature has to be carefully monitored when collecting urine and during further processing. Decreasing temperature accelerates nucleation, and processes such as centrifugation and filtration through artificial membranes may influence the concentration of inhibitors and promotors, all resulting in uncontrollable changes in the physicochemical state of the sample; similar remarks apply to the addition of substances to (e.g., antimicrobial agents) or removal (e.g., water) from urine [27, 12, 26]. Therefore, the measurement of crystalluria should be

* Part of this work was published as an abstract in Urol Res (1988) 16:235

Table 1. Study participant data: anthropometric (individual) and associated clinical chemistry (blood, urine) variables

	Males			Females		
	Controls	NC	IHC	Controls	NC	IHC
Number of patients	20	20	20	20	20	20
Patient data						
Age (years)	39 (19–61)	39.5 (22–69)	38 (22–61)	40.5 (20–65)	41.5 (27–67)	38.5 (26–62)
Weight (kg)	82 ±2	78 ±2	77 ±2	62 ±2	67 ±3*	63 ±2
Height (cm)	179 ±2	175 ±2	177 ±2	166 ±1	162 ±1*	160 ±1**
Body mass index [kg/(height; m)^2]	25.95 ± 1.04	25.87 ± 0.83	25.29 ± 0.73	22.21 ± 0.70	25.48 ± 1.03**	24.37 ± 0.88*
Lean body mass (kg)	61.85 ± 0.89	59.37 ± 0.96	59.23 ± 0.83	45.86 ± 0.77	45.57 ± 0.93	42.97 ± 0.77*
Metabolic activity score	–	19 ±2	23 ±3	–	18 ±2	21 ±2
Fasting blood data						
Creatinine (mg/dl)	1.05 ± 0.02	1.16 ± 0.02**	1.15 ± 0.03**	0.93 ± 0.03	0.96 ± 0.04	0.84 ± 0.04
Total calcium (mg/dl)	9.43 ± 0.05	9.52 ± 0.08	9.72 ± 0.07**	9.12 ± 0.07	9.51 ± 0.06***	9.41 ± 0.05***
Fasting (2-h) and postprandial (3-h) urine data						
2 h cyclic AMP (μmol/g)[a]	3.00 ± 0.31	3.08 ± 0.88	3.06 ± 0.40	nd	4.12 ± 0.61	4.15 ± 0.93
3 h cyclic AMP, (μmol/g)[a]	2.81 ± 0.38	1.75 ± 0.38	2.34 ± 0.24	nd	3.09 ± 0.55	3.00 ± 0.32

Values are given as mean ± SEM or median and range (indicated by parentheses).
P values are all versus controls of the same sex
NC, Normocalciuria; IHC, idiopathic hypercalciuria; nd, not determined
* $P<0.05$; ** $P<0.01$; *** $P<0.001$
[a] Per gram creatinine in the same sample

carried out only under external conditions that more or less ideally reflect the in vivo situation.

A recently reported technique [37], based on examination of freshly voided bladder urine, appeared suitable for systematic and serial investigations, the latter because of the relative simplicity of the technique. Using this method differences in crystalluria were found between patients with calcium urolithiasis and normal individuals [38]. In our preliminary reports we showed a certain predominance of calcium phosphate and urate-containing phases [32] both in normal subjects and in patients with idiopathic recurrent calcium urolithiasis (RCU) and also an absence of calcium oxalate crystals in healthy females [31]. Additional uncertainties in the correct identification of optically recognizable phases (see below) prompted us to undertake the reevaluation reported in the present work.

Materials and methods

Study participants

Eighty patients with RCU (40 males, 40 females) and 40 healthy controls (20 males, 20 females) were examined, the patients being divided into normocalciuria (NC; 20 males, 20 females) and idiopathic hypercalciuria (IHC; 20 males, 20 females). Calciuria was classified on the basis of data from our standardized laboratory program [28], to which all participants were submitted. Criteria for NC or IHC were: calcium in 24-h urine collected at home, over 250 mg per 1.73 m^2 body surface (NC, IHC); calcium in 2-h fasting urine less than 0.12 (NC) or over 0.12 (IHC) mg/mg urinary creatinine; calcium in 3-h postprandial urine less than 0.27 (NC) or over 0.27 (IHC) mg/mg creatinine. The calcium levels in urine of controls were normal. Crystalluria was evaluated in all RCU groups and in controls. We had previously found that the average stone recurrence in IHC was higher than in NC, and the metabolic activity was therefore determined (Table 1) [5]. Also in Table 1 the age distribution and the anthropometric characteristics weight, height, body mass index and lean body mass [14] are shown. Patients with stones with conditions known to cause urolithiasis, such as urinary tract infection, anatomical abnormalities of the kidney and the urinary tract, renal tubular acidosis, primary hyperparathyroidism, overt gout, enteric hyperoxaluria, as well as patients with impaired kidney function (creatinine clearance <60 ml/min) were excluded from the study. None of the participants had taken drugs during the week preceeding the examination and all had been eating normally until the evening before attending the laboratory, in which each participant remained, on average, from 8:00 a.m. to 2:00 p.m.

Measurement of crystalluria

We searched for crystals in urine using the filter technique [37] (polycarbonate filter, code SN 110 606, diameter 25 mm, pore size 0.2 μm; Nuclepore, Pleasanton, USA). Bladder urine was directly voided into a beaker prewarmed to 37°C. Immediately, 2 ml were pipetted into a Millipore filtration device with the reservoir kept at 37°C; the vacuum-assisted filtration was completed within 2–3 min. The (surface of the) filter was rinsed with 500 μl distilled water to remove traces of solubilized salts; filtration was repeated and the filter air-dried at room temperature. With this procedure the stability of the crystalline and spheroidal phases (see below) was maintained over the long term, and the stored filter remained accessible to observer-independent evaluation.

The minerals were classified as isotropic or anisotropic by polarization microscopy. The nature of the crystal was identified on the basis of birefringence and light refraction, after immersing the surface of the filter in immersion oils of varying refraction index and applying Becke's light line.

Amorphous calcium phosphate was the only isotropic phase. Anisotropic crystals were whewellite, weddellite and a further type of crystal tentatively designated uric, a term that could stand for uric

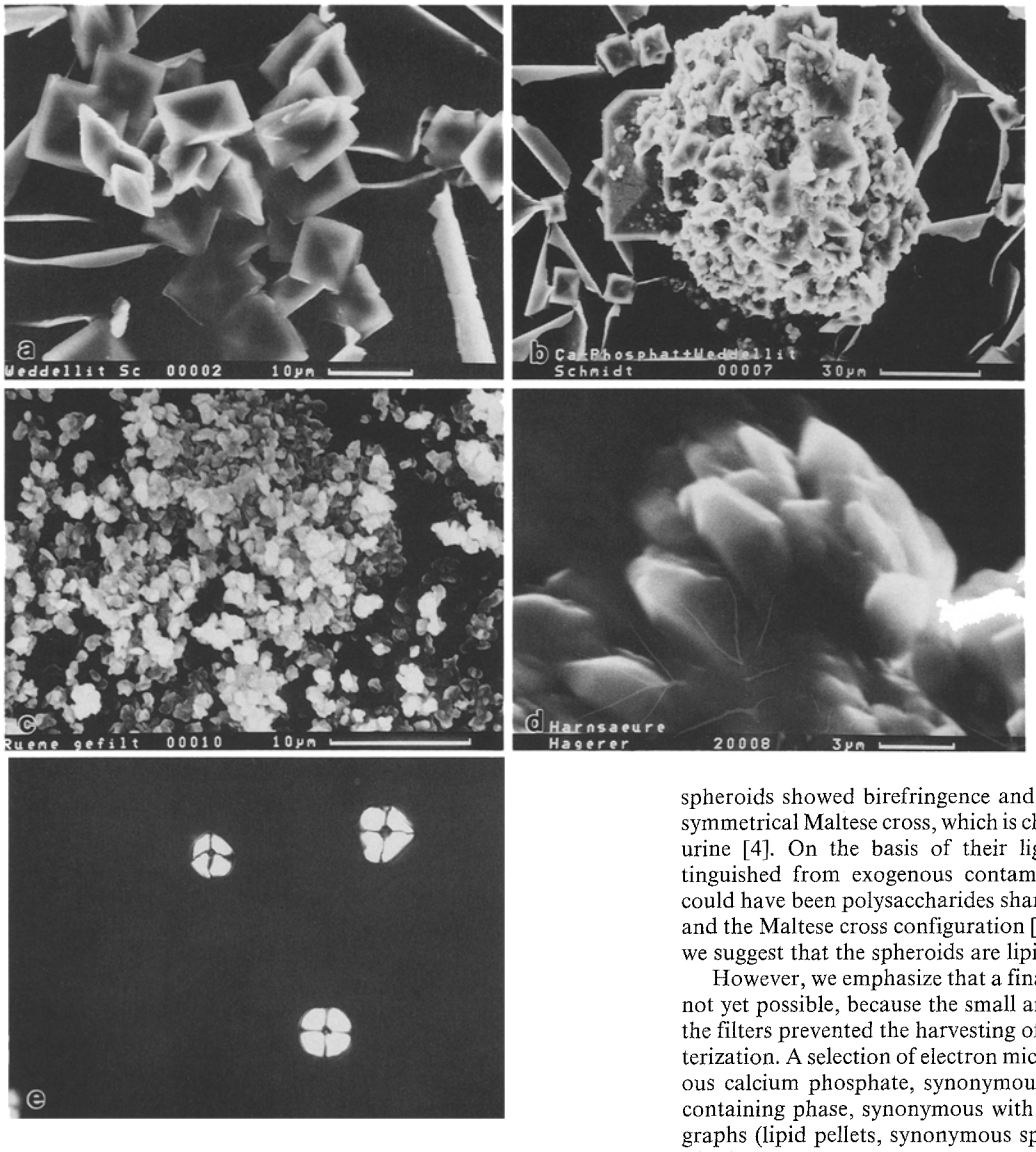

Fig. 1 a–e. Micrographs of crystals identified on filters. **a** weddellite, **b** weddellite and isotropic, **c** isotropic, **d** uric (all from scanning electron microscopy) and **e** spheroids, showing a Maltese cross configuration (from polarization microscopy). For details see "Methods"

acid, its dihydrate, or salts of uric acid (e.g., monosodium, monopotassium, monoammonium urate, or calcium urate). Since whewellite could easily be distinguished from weddellite by birefringence, we used only one refraction liquid (index n = 1.65, bromnaphtaline) for the routine assessment of crystalluria, which also permitted differentiation between whewellite and the uric phase. A score (see below) was used for the semiquantitative determination of crystalluria.

On account of the small quantity of crystals observed on the filters, only isotropic amorphous calcium phosphate could be analyzed by scanning electron microscopy, X-ray microanalysis, or X-ray diffractometry. In the diagram of the latter this substance showed lines like those of apatite, but the molar calcium to phosphate ratio was regularly less than 1:5, thereby contrasting with apatite (calcium to phosphate ratio over 1.5) [6]. However, true apatite is anisotropic, although it can present as isotropic in urinary stones after changing its symmetry. Bearing in mind the possibility that the two different procedures could lead to different assessments of crystals, we chose the term isotropic for the calcium phoshate observed on the filters.

Besides the mineralogical phases described we also found a spherulytic phase on some of the filters. In the present study this was tentatively termed spheroid phase, but in a previous work, the term hydroxyapatite was used [32, 31]. Repeated evaluation revealed that a crystal state could not be assigned to these particles, neither on electron microscopy nor on light microscopy. With the latter, spheroids showed birefringence and a configuration resembling a symmetrical Maltese cross, which is characteristic of lipid droplets in urine [4]. On the basis of their light refraction they were distinguished from exogenous contaminations which, for example, could have been polysaccharides sharing anisotropy with spheroids and the Maltese cross configuration [11]. On the basis of these facts we suggest that the spheroids are lipid pellets.

However, we emphasize that a final identification of spheroids is not yet possible, because the small amounts of material present on the filters prevented the harvesting of quantities for further characterization. A selection of electron micrographs (weddellite; amorphous calcium phosphate, synonymous with isotropic phase; urate-containing phase, synonymous with uric) and polarization micrographs (lipid pellets, synonymous spheroid phase) are depicted in Fig. 1.

Analyses

Clinical chemistry variables in blood and urine were obtained by established methods; calcium (complexometry), magnesium (AAS), phosphorus (colorimetry), creatinine (Autoanalyzer Technicon), urine pH (glass electrode), cyclic AMP (radioimmunoassay). Citrate was measured enzymatically, oxalate by ion chromatography [33].

Calculations and statistics

The semiquantitative crystalluria score was based on the number of observed particles on one half of the filter: 0 particles (score 0), 1–3 (0.25), 4–10 (0.5), 11–20 (1), 21–50 (1.5), 51–100 (2), 101–200 (2.5), over 200 (3). Diameter and shape of crystals were not taken into account. To quantitate the risk of calcium phosphate and calcium oxalate crystallization in urine we calculated the risk indices as described by Tiselius [35, 36], the former after slight modification:

$$\text{CaP risk index} = (Ca/Cr)^{1.07} \times (P/Cr)^{0.7} \times (pH - 4.5)^{6.8} \times (Cit/Cr)^{-0.20}$$

Table 2. Crystalluria and risk index for crystallization of calcium phosphate and calcium oxalate in 2-h morning (fasting) and 3-h postprandial urine for the male study participants

	Males								
	Controls ($n = 20$)[a]			NC ($n = 20$)			IHC ($n = 20$)		
	P/A	$\bar{X}$	Range	P/A	$\bar{X}$	Range	P/A	$\bar{X}$	Range
Fasting urine									
Isotropic	5/14	0.211	(0–2)	11/9	0.700	(0–3)	9/11	0.613	(0–3)
Uric	13/6	0.171	(0–0.25)	11/9	0.163	(0–0.5)	14/6	0.175	(0–0.25)
Spheroids	12/7	0.224	(0–0.5)	12/8	0.213	(0–0.5)	10/10	0.163	(0–0.5)
Whewellite			0.25; 0.25	cnd					0.25; 0.25; 1; 1.5
CaP risk index $\times 10^{-6}$		5.30	(0.001–33.40)		2.19	(0.001–11.19)		7.19	(0.16–34.66)*
CaOx risk index		202	(48–573)		213	(45–450)		345	(40–628)***
Postprandial urine									
Isotropic	5/12	0.250	(0–1.5)	12/8	0.613	(0–3)	10/10	0.375	(0–3)
Uric	12/5	0.206	(0–0.5)	12/8	0.188	(0–0.5)	15/5	0.188	(0–0.25)
Spheroids	6/11	0.118	(0–0.5)	6/14	0.100	(0–0.5)	7/13	0.125	(0–0.5)
Whewellite			0.25	cnd					0.25; 0.25
CaP risk index $\times 10^{-6}$		0.45	(0.002–4.27)		3.51	(0.001–44.72)		1.90	(0.07–12.96)*
CaO risk index		365	(163–738)		394	(31–1136)		544	(73–1067)**

Crystalluria was evaluated in accordance with the presence or absence of the various types of crystal on the filters, and by score. For further details and group symbols see "Methods". Except for the P/A ratio the data are mean values and range; for whewellite individual values are given. *P* values are all versus controls

P, presence; A, absence; cnd, crystals not detectable; CaP, calcium phosphate; CaOx, calcium oxalate

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

[a] except number of filters evaluated

Table 3. Crystalluria and risk index for crystallization of calcium phosphate and calcium oxalate in 2-h morning (fasting) and 3-h postprandial urine for the female study participants

	Females								
	Controls ($n = 20$)			NC ($n = 20$)			IHC ($n = 20$)		
	P/A	$\bar{X}$	Range	P/A	$\bar{X}$	Range	P/A	$\bar{X}$	Range
Fasting urine									
Isotropic	11/9	0.950	(0–3)	6/14	0.300	(0–3)	9/11	0.388	(0–2.5)
Uric	18/2	0.238	(0–0.5)	13/7	0.213	(0–0.5)	17/3	0.275	(0–1)
Spheroids	6/14	0.088	(0–0.05)	11/9	0.213	(0–0.5)	12/8	0.200	(0–1)
Whewellite	cnd					0.25; 0.25; 0.25	cnd		
Cap risk index $\times 10^{-6}$		1.70	(0.002–5.77)		5.77	(0.001–44.35)		19.22	(0.02–59.24)
CaOx risk index		148	(42–393)		287	(107–509)***		489	(157–958)***
Postprandial urine									
Isotropic	12/8	0.925	(0–2.5)	10/10	0.700	(0–2.5)	16/4	1.213	(0–3)
Uric	16/4	0.300	(0–1.5)	12/8	0.163	(0–0.5)	16/4	0.213	(0–0.5)
Spheroids	5/15	0.063	(0–0.25)	10/10	0.175	(0–1)	16/4	0.238	(0–0.5)
Whewellite	cnd			cnd			cnd		
CaP risk index $\times 10^{-6}$		1.52	(0.005–5.69)		1.99	(0.001–13.89)		8.19	(0.003–68.50)
CaOx risk index		277	(69–492)		517	(71–1372)**		740	(356–1565)***

Crystalluria was evaluated in accordance with the presence or absence of the various types of crystal on the filters, and by score. For further details and group symbols see "Methods". Except for the P/A ratio the data are mean values and range; for whewellite individual values are given. *P* values are all versus controls

P, presence; A, absence; cnd, crystals not detectable; CaP, calcium phosphate; CaOx, calcium oxalate

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

$$\text{CaOx risk index} = (Ca/Cr)^{0.71} \times (Ox/Cr) \times (Mg/Cr)^{-0.14} \times (Cit/Cr)^{-0.10}$$

Results were given either as mean values ± 1 SEM or as median and range of individual values. The significance of observed differences was examined by the *t*-test for paired and unpaired data (Gaussian distribution) and the U-test (non-Gaussian distribution), as appropriate.

Results

Individual data and clinical chemistry in fasting blood and urine (Table 1)

With respect to age, the groups NC, IHC and controls were comparable. The body weight was elevated in female NC, but height was decreased (female NC and IHC). The body mass index was elevated in both female patient groups, but lean body mass was decreased only in IHC. Based on these two parameters female, not male, patients evidently were obese.

When related to serum creatinine, the kidney function in male patients was slightly reduced, but still remained within the normal range, whereas females seemed normal. Total calcium in the serum was generally higher in RCU than in controls, although all the individual values were below the upper limit of normalcy (10.2 mg/dl); the differences were significant for NC (females) and IHC (males, females). Since the mean serum parathyroid hormone concentrations (data not shown) and the mean cyclic AMP excretion in fasting and postprandial urine were unremarkable, the parathyroid gland function was considered normal.

Crystalluria

Overview of the frequency and score of the observed phases (Tables 2, 3). The data obtained in fasting and postprandial urine showed the following: of the three phases, the uric phase was the most common, being seen on all the filters investigated (see presence to absence values for males and females); this was followed by the isotropic and spheroid phases. With respect to the score assessed on each individual filter, that of the isotropic phase was at least equal to those of the uric and spheroid phases, but was usually even higher. The mean scores of uric and spheroid phases were roughly equal. Only exceptionally were calcium oxalate crystals (whewellite) observed (on 14 of the 236 filters), as indicated in the data of the Tables 2 and 3. Between the subgroups NC and IHC no statistical differences were detectable. However, in terms of quantity and quality of crystalluria there were substantial differences between males and females.

Influence of stone disease and urine collection period in males (Table 2). In NC and IHC the mean isotropic score was about three times as high as that in controls. Compared with the latter, isotropic of NC was significantly higher in 2-h urine, while the higher mean value in 3-h urine was of borderline significance. In contrast, the mean scores of the uric and spheroid phases were roughly equal in all groups (2-h, 3-h urine). Calcium oxalate crystals were observed only sporadically (controls, IHC; 2-h, 3-h urine).

In 3-h urine, i.e., after ingestion of the calcium-rich test meal, only the mean scores of the isotropic and spheroid phases decreased – except in controls (isotropic) – while the score of the uric phase increased (differences between 2-h and 3-h urine not tested).

Influence of stone disease and urine collection period in females (Table 3). In 2-h urine of female controls the mean isotropic score was higher than in that of NC or IHC by a factor of 3, but in 3-h urine the score of the latter was of the same order of magnitude as that of controls. The uric phase score was comparable in NC, IHC, and controls. Spheroids were regularly observed in NC and IHC, but only rarely in controls; the score between NC, IHC and controls differed slightly but significantly (2-h, 3-h urine). Calcium oxalate crystals were found only in 2-h urine of NC.

The response of the uric and spheroid phases to the test meal was not uniform (score in 3-h urine) – in contrast to the response of the isotropic phase (see above).

Risk indices of calcium phosphate and calcium oxalate (Tables 2, 3). For calcium phosphate the scatter of individual values was considerable, with mean values ranging from 0.45×10^6 (male controls; 3-h urine) to 19.22×10^6 (female IHC; 2-h urine). Compared with controls, the risk index was significantly elevated but statistically unchanged in male NC (2-h, 3-h urine) and in male IHC (2-h, 3-h urine). In female IHC the risk index was significantly elevated in 2-h urine but only insignificantly higher in 3-h urine as compared with controls (2-h, 3-h urine).

For calcium oxalate the scatter range of the individual values was from 31 (male NC; 3-h urine) to 1565 (female IHC; 3-h urine). Irrespective of gender and urine collection period the risk index was lowest in controls, higher in NC and highest in IHC. In males the differences between IHC and controls were significant (2-h, 3-h urine); in females NC and IHC differed from those in controls.

After ingestion of the calcium-rich test meal the calcium phosphate risk index of all groups was lower in the 3-h than in the 2-h urine, mainly owing to the more acidic pH in 3-h urine. This variable is of importance for the calcium phosphate supersaturation product (see "The pH in urine"), and hence for the risk index as well. In contrast, the calcium oxalate risk index was higher in 3-h urine, presumably as a result of the increase in urinary calcium following the calcium-rich test meal [28, 30].

In RCU, the generally higher calcium oxalate risk index contrasts with the only rare occurrence of calcium oxalate crystals.

The pH in urine (Fig. 2)

Besides the molar concentrations of calcium and phosphate, urinary pH is an important determinant of the degree of urinary saturation with calcium phosphate; in

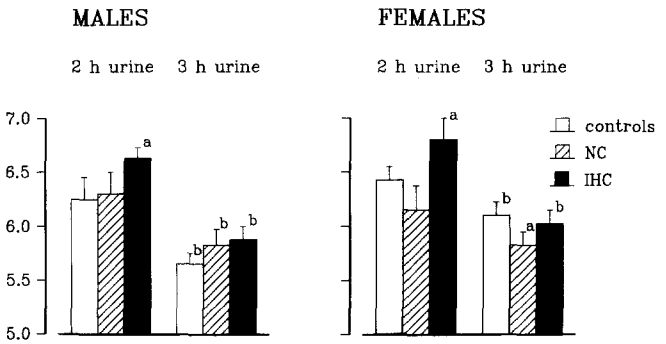

Fig. 2. Urinary pH in male and female participants in the study. Data are depicted for 2-h fasting and 3-h postprandial urine of controls, NC and IHC stone patients. For details see "Methods". Mean values ± SEM. $a = P < 0.05$ or smaller, versus controls in the same urine (*t*-test; unpaired data); $b = P < 0.05$ or smaller, versus the same group in 2-h urine (*t*-test; paired data)

hydroxyapatite a less acidic, or even alkaline, pH is the leading factor (P.O. Schwille, unpublished data). The pH was increased in IHC (males and females), as compared with controls, while in NC it was not. The calcium-rich test meal effected a decrease in pH in all groups, the greatest decrease being seen in IHC (males, females). Compared with controls there was a tendency towards higher pH in 3-h urine of NC and IHC, while in females the reverse was the case.

Correlations

Significant correlations were observed only in 3-h urine. The calcium phosphate risk index correlated with the isotropic phase in IHC (males: $n = 20$, $r = 0.75$, $P < 0.0003$; females: $n = 20$, $r = 0.45$, $P < 0.05$), but not in NC (males: $n = 20$, $r = -0.05$, $P < 0.85$; females: $n = 20$, $r = -0.21$, $P < 0.36$) and controls (males: $n = 20$, $r = -0.19$, $P < 0.47$; females: $n = 20$, $r = 0.08$, $P < 0.73$).

Since female patients were obese (Table 1), and also had more spheroids on the filters than controls (Tables 2, 3), we assumed a relationship between spheroids - the phase conceived as lipids - and lean body mass. Significant correlations were, however, restricted to the pooled group of females (controls plus RCU patients: $n = 60$, $r = -0.31$, $P < 0.02$) and to male controls ($n = 20$, $r = -0.53$, $P < 0.03$).

Discussion

Assuming that the composition of bladder urine and that of the upper urinary tract is identical, the data of the present study may reflect virtually natural conditions. However, it is still unclear whether crystalluria is of relevance in the pathophysiology and diagnosis of RCU, and our results deviate from those reported, especially with respect to calcium oxalate, although they are in agreement with those of other reports with respect to calcium phosphate [38, 39].

Criticism of the crystalluria technique

We consider it necessary that the temperature be kept constant during filtration, thus precluding the possibility that observed changes in crystalluria are achieved inadvertently. Immediate covering of the dried filter with refraction liquid, as proposed by the original authors [37], would have helped preserve the crystalline phases, but would also have rendered the spheroid phase unstable (see "Material and methods"); moreover, retaining the filters for later evaluation by independent observers as well as long-term storage would have been made impossible. As a rule, we observed only a few crystals on each filter (Tables 2, 3), which is in contrast with results from other investigators, who found up to 10 times as many crystals [38]. Procedures deviating substantially from that used in our study, such as collection and treatment of urine after prior addition of chemicals, or uncontrolled standing at room temperature and below, could be responsible.

In our preliminary studies the low yield of crystals, which is characteristic of the technique, may have led to a misinterpretation of specific phases, especially the false identification of spheroids as apatite [32, 31]. Since there is a likelihood that spheroids are not minerals, but lipids (see "Material and methods"), these particles were validated as a separate entity. Their nature and chemistry need further characterization though, above all to avoid confusion with urinary bodies displaying a Maltese cross configuration [13]. Techniques permitting enrichment of urine with spheroids, their isolation and their staining with specific dyes may be helpful.

Calcium metabolism, urinary pH and associated crystalluria

Earlier studies by our laboratory disclosed some misregulation of serum calcium in RCU, which may be unrelated to parathyroid gland function [30, 29]. In the present study involving other subjects we again found significantly higher serum total calcium in patients with stones than in healthy controls. The nature of this anomaly is still unknown; increased bone resorption [1] or altered bone turnover [16] may play a role and may also have contributed to the well-known high urine calcium in IHC. It may be surprising that crystalluria in IHC was not more pronounced than in NC. In hypercalciuria it was found that inhibitors and promoters of precipitation were modulated in opposite senses [40]. Thus, the occurrence of crystalluria and the types of crystals evolving during hypercalciuria may depend on which action is predominant.

The higher urinary pH in RCU, especially in IHC, was unexpected, since the generation of protons by the distal renal tubules of RCU was found to be normal in our laboratory (P.O. Schwille; unpublished data). High oral calcium load decreases parathyroid gland activity, when measured in terms of the serum concentration of bioactive parathyroid hormone or fragments of it or cyclic AMP excretion in the urine [30]. As parathyroid hormone normally reduces the reabsorption of bicarbonate ions in

the proximal tubules [21], the higher urine pH in RCU could reflect some degree of nonsuppression of parathyroid hormone-induced bicarbonate excretion in the urine; alternatively, a tubular leak for bicarbonate may be considered. However, in the present work the mean cyclic AMP was normal in all groups and decreased markedly after consumption of the calcium-rich test meal, indicating that parathyroid gland suppression by calcium ions was sufficient. In RCU, the existence of a proximal-tubular defect of unknown etiology has been proposed [15, 34], and the data (Fig. 2) permit the speculation that the defect, if present, includes (the development of) a more alkaline urinary pH, especially in IHC males. Irrespective of the underlying cause, reduced urinary acidification favors precipitation of calcium phosphate [20].

The isotropic phase was less frequent in male controls than in male RCU. On the other hand, spheroids were only rarely observed in female controls, but were regularly present in female patients with stones. Assuming that crystalluria is consistent with microurolithiasis [7, 2], this situation may signal that the pathogenesis of RCU differs, depending on sex.

Both the origin and its importance for stone formation of the isotropic phase are unknown. The development of the phase obviously depends on pH, as shown by the calcium phosphate risk index and the correlation between the index and the isotropic phase score. Since we were able to show apatite-like properties of the isotropic phase, we suggest a role similar to that of hydroxyapatite; the ability of the latter to induce heterogeneous nucleation of calcium oxalate monohydrate in vitro has been documented [17]. It could be argued that since calcium oxalate crystals were only exceptionally observed, this discrepancy might cast doubt on an initiatory role of the isotropic phase in the above sense. However, the absence of calcium oxalate crystals would be commensurable with crystal adhesion to upstream epithelial structures. A similar situation has been assumed for the kidney in experimental animals and humans [3], but final evidence is still lacking.

Spheroids are also still unclear. Because this phase was detected more frequently in the urine of RCU females otherwise characterized as being obese – a characteristic also reflected in the negative correlation between spheroids and lean body mass – some sort of functional link between these variables cannot be excluded. Diverse nutritional factors have been discussed as driving forces in RCU and its higher incidence was found to be associated with an abundant diet, especially in the industrialized countries of the Western hemisphere [25]; in this female RCU patients differ from healthy controls with regard to nutrition [10]. Further work may show whether this abnormal behavior leads not only to obesity, but also to spheroid crystalluria and urolithiasis.

Acknowledgements. We are grateful to G. Hofmann, B. Schreiber, and K. Schwille for technical assistance and I. Goldberg for additional secretarial work. The work was supported by the Deutsche Forschungsgemeinschaft, Bonn, FRG, Grant 210/4-2, and by the University of Erlangen Hospital Research Fund.

References

1. Barkin J, Wilson DR, Manuel MA, Baley A, Murray T, Harrison J (1985) Bone mineral content in idiopathic calcium nephrolithiasis. Min Electr Metab 11:19
2. Baumann JM (1985) Can the formation of calcium oxalate stones be explained by crystallization processes in urine. Urol Res 13:267
3. Burns JR, Finlayson B, Gauthier J (1984) Calcium oxalate retention in subjects with crystalluria. In: Ryall RL, Brockis JG, Marshall VR, Finlayson B (eds) Urinary stone. Proceedings of the Second International Urinary Stone Conference, Singapore, 1983. Churchill Livingstone, Melbourne, p 253
4. Comings DE (1963) Anisotropic lipids and urinary cholesterol excretion. JAMA 183:126
5. Dahlberg CJ, Kurzt SB, Wilson DM, Smith LH (1977) Clinical features and management of cystinuria. Mayo Clin Proc 52:533
6. Eanes ED, Meyer JL (1977) The maturation of crystalline calcium phosphates in aqueous suspensions at physiologic pH. Calcif Tiss Res 23:259
7. Finlayson B (1974) Renal lithiasis in review. Urol Clin North Am 1:181
8. Finlayson B (1978) Physicochemical aspects of urolithiasis. Kidney Int 13:344
9. Finlayson B, Khan SR, Hackett RK (1984) Mechanism of stone formation – an overview. Scanning Electron Microsc 3:1419
10. Griffith HM, O'Shea B, Keogh B, Kerancy JP (1986) A case-control study of dietary intake of renal stone patients. I. Preliminary analysis. Urol Res 14:67
11. Haber MH (1983) Farbatlas mikroskopischer Harnanalytik. Urban und Schwarzenberg, München, p 94
12. Hallson PC, Rose AG, Samuell CT (1981) Inhibitors of calcium oxalate and calcium phosphate crystal formation in urine – a critic and reappraisal. In: Smith LH, Robertson WG, Finlayson B (eds) Urolithiasis, clinical and basic research. Plenum, New York, p 589
13. Hudson JB, Dennis AJ, Gerhardt RE (1978) Urinary lipid and the Maltese cross. N Engl J Med 299:586
14. Hume R, Weyers E (1971) Relationship between total body water and surface area in normal and obese subjects. J Clin Path 24:234
15. Lau YK, Wasserstein A, Westby GR, Bosanac P, Grabie M, Mitnick P, Slatopolsky E, Goldfarb S, Agus ZS (1982) Proximal tubular defect in idiopathic hypercalciuria: Resistance to phosphate administration. Min Electr Metab 7:237
16. Liberman UA, Sperling O, Atsmon A, Frank M, Mordan M, DeVries A (1968) Metabolic and calcium kinetic studies in idiopathic hypercalciuria. J Clin Invest 47:2580
17. Meyer JL, Bergert JH, Smith LH (1975) Epitaxial relationship in urolithiasis: the calcium oxalate monohydrate-hydroxyapatite system. Clin Sci Mol Med 49:369
18. Deleted in proof
19. Nancollas GH (1976) The kinetics of crystal growth and renal stone-formation. In: Fleisch H, Robertson WG, Smith LH, Vahlensieck W (eds) Urolithiasis research. Plenum, New York, p 5
20. Nancollas GH, Salim MH, de Rooij JF (1984) Aspects of calcium phosphate crystallization under urinary conditions. Fortschr Urol Nephrol 22:198
21. Rasmussen H, Bordier P (1974) The physiological and cellular basis of metabolic bone disease. Williams & Williams, Baltimore, p 180
22. Robertson WG, Peacock M (1972) Calcium oxalate crystalluria and inhibitors of crystallization in recurrent renal stone-formers. Clin Sci 43:499
23. Robertson WG, Peacock M, Nordin BEC (1969) Calcium crystalluria in recurrent renal stone-formers. Lancet II:21
24. Robertson WG, Peacock M, Nordin BEC (1972) Measurement of the activity product in urine from stone-formers and normal subjects. In: Finlayson B, Hench LL, Smith LH (eds) Urolithiasis – physical aspects. National Academy of Science, Washington, p 79

25. Robertson WG, Peacock M, Hodgkinson A (1979) Dietary changes and the incidence of the urinary calculi in the U.K. between 1958 and 1976 J Chron Dis 32:469
26. Rodgers AL, Wandt MAE (1985) Crystallization characteristics of synthetic urine in a fast evaporator. In: Schwille PO, Smith LH, Robertson WG, Vahlensieck W (eds) Urolithiasis and related clinical research. Plenum, New York, p 765
27. Ryall RL, Grover PK, Doyle IR, Harnett RM, Hibbered CM, Edyvane KA, Marshall VR (1988) The effect of macromolecules of the crystallization in urine. In: Martelli A, Buli P, Marchesini B (eds) Inhibitors of crystallization in renal lithiasis and their clinical application. Acta Medica, Rome, p 51
28. Scholz D, Schwille PO (1981) Klinische Laboratoriumsdiagnostik der Urolithiasis. Dtsch Med Wschr 106:99
29. Schwille PO, Scholz D, Schwille K, Engelhardt W, Schreiber B, Goldberg I, Sigel A (1982) Parathyroid gland function in subgroups of metabolically mediated urolithiasis as evaluated by serum parathyroid hormone, and urinary and nephrogenous cyclic nucleotides. Klin Wochen 69:229
30. Schwille PO, Rümenapf G, Schmidtler J, Köhler R (1987) Fasting and post-calcium load serum calcium, parathyroid hormone, calcitonin, in male idiopathic calcium urolithiasis – evidence for a basic disturbance in calcium metabolism. Exp Clin Endocrinol 90:71
31. Schwille PO, Kuch P, Berens H (1988) Crystalluria in health and idiopathic calcium stone disease. Urol Res 16:235
32. Schwille PO, Kuch P, Berger I, Berens H (1989) On the occurrence and nature of crystals in human urine. In: Walker VR, Sutton RAL, Cameron ECB, Pak CYC, Robertson WG (eds) Urolithiasis. Plenum, New York, p 31
33. Schwille PO, Manoharan M, Rümenapf G, Wölfel G, Berens H (1989) Oxalate measurement in the picomol range by ion chromatography: values in fasting blood and urine of controls and patients with idiopathic calcium urolithiasis. J Clin Chem Clin Biochem 27:87
34. Schwille PO, Rümenapf G, Köhler R (1989) Blood levels of glucometabolic hormones and urinary saturation with stone forming phases after an oral test meal in male patients with recurrent idiopathic calcium urolithiasis and in healthy controls. J Am Coll Nutr 8:557
35. Tiselius HG (1986) Measurement of the risk of calcium phosphate crystallization in urine. Urol Res 15:79
36. Tiselius HG (1985) Measurement of the risk of calcium oxalate crystallization in urine. Urol Res 13:297
37. Van den Berg, Cahill TM, Smith LH (1976) Crystalluria. In: Fleisch H, Robertson WG, Smith LH, Vahlensieck W (eds) Urolithiasis. Plenum, New York, p 365
38. Werness PG, Bergert JH, Smith LH (1981) Crystalluria. J Cryst Growth 53:166
39. Werness PG, Wilson JWL, Smith LH (1983) Hydroxyapatite and its role in calcium urolithiasis. In: Ryall RL, Brockis JG, Marshall VR, Finlayson B (eds) Urinary stone. Churchill Livingstone, Melbourne, p 273
40. Zerwekh JE, Hwang TIS, Poindexter J, Hill K, Wendell G, Pak CYC (1988) Modulation by calcium of the inhibitor activity of naturally occurring urinary inhibitors. Kidney Int 33:1005

Prof. Paul O. Schwille
Chirurgische Klinik
Universitätskrankenhaus
Maximiliansplatz 1
W-8520 Erlangen
Federal Republic of Germany