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Circadian Rhythm of Lithogenic Substances in the Urine

E. W. Vahlensieck, D. Bach, and A. Hesse

Department of Urology, University Medical School, Bonn, Federal Republic of Germany

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Summary. After determination of the lithogenic and inhibitory substances in serum and urine of 18 healthy control subjects and 20 patients with calcium oxalate urolithiasis on an uncontrolled diet, the alteration of the parameters while taking a standard diet was investigated. After attainment of a steady state, the investigations were performed over 48 h on 3-h aliquots of urine. Typical circadian rhythms were detected for all lithogenic parameters in the urine and normal ranges were established. This special investigation permits detection of "peaks" in the excretion of lithogenic substances which were masked in investigation of 24-h urine samples alone.

Key words: Urolithiasis, Lithogenic and inhibitory substances in urine, Circadian rhythms.

Introduction

The concentration of lithogenic and inhibitory substances in the urine plays a decisive role in the development of urolithiasis. The risk of nucleation is always present when oversaturation of lithogenic substances occurs and/or there is a deficiency of inhibitors of nucleation, crystallisation and crystal aggregation. In certain disorders (e.g. pHPT, renal leak hypercalciuria, primary hyperoxaluria, primary hyperuricosuria), a permanently raised excretion and concentration of lithogenic substances could be demonstrated in 24-h urine samples. However, these disorders are relatively rare and it must be assumed that brief excretion and concentration peaks are very much more frequently the cause of nucleation. Such peaks cannot be detected by investigations on 24-h urine samples, since they are masked by the resulting mean value. The 24-h urine also does not give any pointer to possible circadian fluctuations of the inhibitory substances. On the other hand, investigation of 3-h urine samples over 48 h gives a very much better picture of the circadian rhythm of the lithogenic and inhibitory substances in the urine [3–5, 8, 9]. Such investigations can be carried out while the patients remains on their usual diet. If peaks are seen, however, it cannot be decided whether these are attributable to variations in the food intake or whether they are of endogenous origin. On the other hand, if the patients are kept on a standard diet and standardised fluid intake, a steady state is obtained after around 5 days. After securing this status over 3 days, we then investigated 3-h urine portions on the 9th and 10th day with regard to the excretion and concentration of lithogenic and inhibitory substances in order to detect in this way the circadian rhythm independent of the imponderable dietary influences.

Materials and Methods

1. The investigations were carried out in two groups:

1.1. *Group I.* 20 patients of normal weight (12 men, 8 women) with recurrent calcium oxalate urolithiasis. The average age of the men was 40.3 years, and that of the women 37.4 years. All patients had been repeatedly investigated, but no cause of the recurrent lithogenesis had been found so far.

1.2. *Group II.* 18 healthy subjects of normal weight (8 men, 10 women) as control group. The average age of the men was 28.8 years, and of the women 29.3 years.

2. To establish the initial situation on an uncontrolled diet, the parameters shown in Table 1 were investigated in the serum and urine on the first day of inpatient admission, or on an out-patient basis.

3. During a 12-day period of inpatient residence, the urolithiasis patients and healthy test subjects received a standard diet with approximately the same proportions of protein, fat, carbohydrates, minerals and trace elements each day. The composition of the standard diet corresponded to the recommendations of the German Nutritional Society (DGE) for a "normal diet" for a patient of normal weight.

Table 2 shows the composition of the standard diet for the first day. Breakfast (8 a.m.), midmorning break (10 a.m.) and evening meal (5.30 p.m.) were identical on all 12 investigation days, whereas there were five variations with regard to the midday meal (12.30 p.m.), i.e. the composition was repeated on the 6th day and 11th day.

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Table 1. Lithogenic parameters investigated in serum and urine in healthy subjects and urolithiasis patients

	Serum	Urine	Method
Lithogenic substances	Ca	Ca	Flame photometry Atomic absorption spectrography
	Inorganic P	Inorganic P	Photometry[a]
	Uric acid	Uric acid	Enzym. photometry[b]
		Oxalic acid	Gas-chromatography
Inhibitory substances	Mg	Mg	Atomic absorption spectrography
		Citric acid	Enzym. photometry[c]
Miscellaneous		pH	Photometry
		specific gravity	Urimeter

a Testkombination "Anorganisches Phosphat" (Merck)
b Urica-quant (Boehringer, Mannheim)
c Citrat-Lyase (Boehringer, Mannheim)

Table 2. Composition of the standard diet on the first day of the study (calculated according to Geigy tables 1977 and Wirths 1976 in relation to 100 g edible substance)

	Protein (g)	Fat (g)	Carbo hydrate (g)	H_2O (ml)	Oxalic acid (mg)	Ca (mg)	P (mg)	Mg (mg)	Na (mg)	K (mg)	Cl (mg)	kcal
Breakfast	10	22	50	265	14	45	159	22	316	163	147	437
Morning break	9	6	39	280	1	237	242	83	95	805	93	238
Lunch	32	32	76	392	15	130	298	197	332	1,154	386	729
Dinner	20	30	82	463	24	376	409	81	662	679	778	648
Total	71	90	247	1,400	54	788	1,108	383	1,405	2,801	1,404	2,052
	Recommendations of the Deutsche Gesellschaft für Ernährung (DGE)											
	95	63–100	285–412			700–800	700–800	220–260				2,200–2,600

The fluid intake was likewise standardised at 1,400 ml/day. At breakfast there were 200 ml coffeine-free coffee, at the evening meal 200 ml rosehip tea, and 1,000 ml orange juice distributed over the day. Patients and healthy control subjects consumed exactly these amounts.

4. On days 1–8, the parameters listed in Table 1 were determined in the serum and in 24 h urine collections. The volume of the 3-h urine samples was measured on the 9th and 10th day and the pH value as well as the effective excretion and concentration of calcium, magnesium, phosphate, oxalic acid, uric acid and citric acid determined in each urine fraction, were also recorded.

5. *Statistical Methods*

5.1. *Significance Calculation.* The *t*-test according to Student with assumption of normal distribution and the Wilcoxon test for pair differences was applied for the basic collectives. In the test, a probability of error $\alpha = 0.05$ was used, as it is usual for medical investigations.

The *t*-test according to Student for equal and unequal variances tests the hypothesis: mean value 1 $\neq$ mean value 2 against the alternative mean value 1 = mean value 2. The equality and inequality of the variances was tested with the F-test. Like the *t*-test, the Wilcoxon test for pair differences tests the hypothesis: the basic collectives and thus the mean values and distribution function are the same.

5.2. *Determination of the Circadian Curve Courses by Polynomials.* A precondition for this kind of representation of biological processes is that the variations of the individual parameters are "constant curves" in the course of the day. Since every constant curve can be approximated as closely as desired by polynomials (set of Weierstrass), the possibility of representing the course of individual parameters by means of polynomials entails a solution of the problem of representing biological sequences. We have reported on details of this mathematical technique in an earlier publication [6]. The curves were calculated and plotted by machine on an IBM 370/168 in the Bonn University Computer Center.

6. Results

6.1. *Urinary Volume*

The maximum urinary volumes are at midday (11 a.m. – 2 p.m.) or in the early afternoon (2 p.m. – 5 p.m.) in

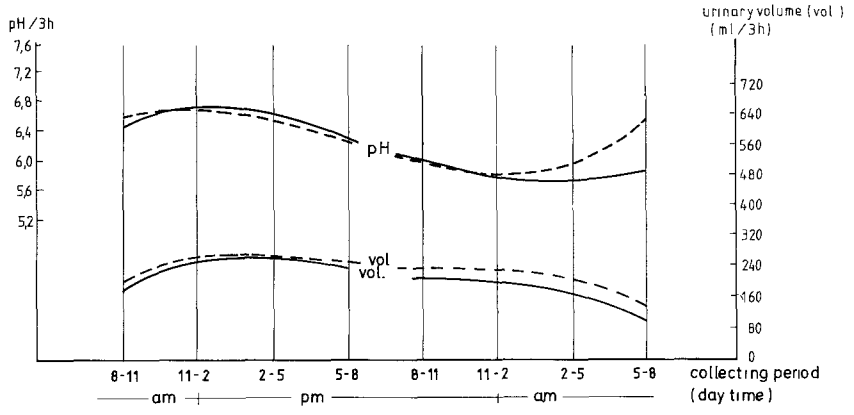


Fig. 1. Circadian rhythm of the urinary pH value in 18 healthy subjects (*N*) and 20 calcium oxalate urolithiasis patients (*SF*) on a standard diet

N = ——, SF = – – –

healthy subjects and in urolithiasis patients. The lowest volumes were at the same time (5 p.m. – 8 p.m.) in both groups. The circadian curves of the urinary volumes are drawn in the Figs. 1–7.

6.2. *pH Value (Fig. 1)*

The circadian course of the pH value is almost the same for healthy subjects and for urolithiasis patients: maximum between 11 a.m. and 2 p.m., and minimum between 2 a.m. and 5 a.m.

6.3. *Calcium (Figs. 2a and b)*

Two peaks are observed for the calcium concentration both in healthy subjects and in urolithiasis patients: the first peak is between 5 a.m. and 8 a.m. and the second between 8 p.m. and 11 p.m. Two troughs of calcium concentrations were observed in healthy subjects and urolithiasis patients at noon (11 a.m. –2 p.m.) and in the early morning (2 a.m. – 5 a.m.). The calcium excretion was maximal in both groups between 8 p.m. and 11 p.m. and at its lowest between 5 a.m. and 8 a.m. The values for calcium concentration and excretion are significantly higher in urolithiasis patients than in healthy subjects.

6.4. *Phosphate (Figs. 3a and b)*

The maximum phosphate concentration was in the early morning (2 a.m. – 5 a.m. or 5 a.m. – 8 a.m.) and the minimum in the morning (8 a.m. – 11 a.m.) in both groups. Phosphate excretion was maximal in the afternoon (2 p.m. –5 p.m.) or in the early evening (5 p.m. – 8 p.m.). The least phosphate was excreted in the morning (8 a.m. – 11 a.m.).

There were differences in the level of the values between healthy subjects and urolithiasis patients in only a few urine fractions. However, significant correlations could not be calculated.

6.5. *Uric Acid (Figs. 4a and b)*

The highest uric acid concentration was found in healthy subjects between 5 a.m. and 8 a.m. and the lowest uric acid concentration between 11 p.m. and 2 a.m. A circadian rhythm could not be detected in the urolithiasis patients. The maximum uric acid excretion was found between 11 a.m. and 5 p.m. (healthy subjects) and 8 p.m. and 11 p.m. (urolithiasis patients). Healthy subjects had a significantly higher uric acid concentration and excretion in all urine fractions.

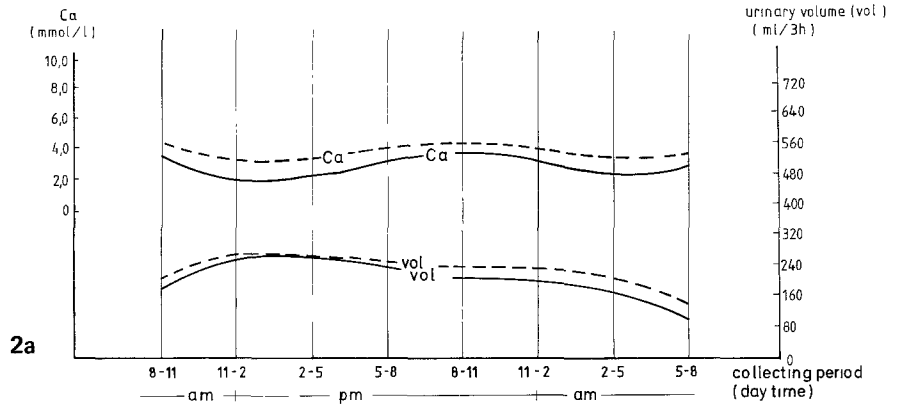
6.6. *Oxalic Acid (Figs. 5a and b)*

The highest concentrations were found in healthy subjects and urolithiasis patients in the early morning (5 a.m. – 8 a.m.) or in the morning (8 a.m. – 11 a.m.), and the lowest concentrations towards midnight (11 p.m. – 2 a.m.). The excretion was highest in healthy subjects and in urolithiasis patients in the afternoon (2 p.m. – 5 p.m.) and lowest in the early morning (2 a.m. – 8 a.m.). There were no significant differences in the levels of concentration and excretion between healthy subjects and urolithiasis patients.

6.7. *Magnesium (Figs. 6a and b)*

The highest concentration was found in healthy subjects in the early morning (5 a.m. – 8 a.m.), and in urolithiasis patients in the evening (8 p.m. – 11 p.m.). The lowest concentration was found in both groups towards midday (11 a.m. – 2 p.m.). The excretion curve had an identical course in both groups: maximum in the evening (8 p.m. – 11 p.m.) and minimum in the early morning (5 a.m. – 8 a.m.).

Significantly higher values were found for the magnesium concentration in urolithiasis patients than in healthy subjects; this does not apply to the excretion.



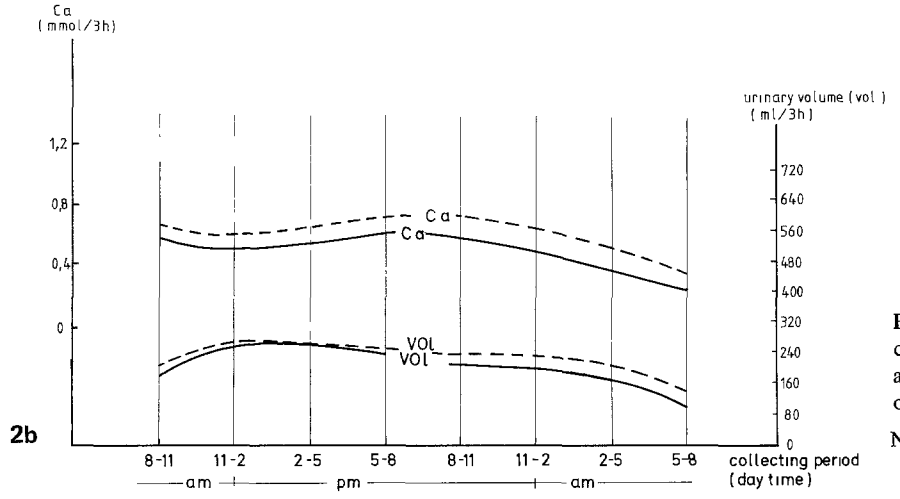
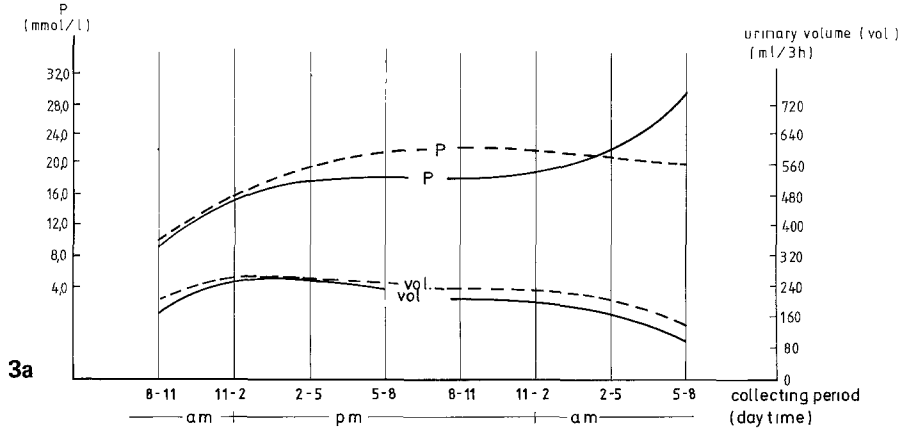


Fig. 2. Circadian rhythm of the calcium concentration (a) and excretion (b) in 18 healthy and 20 calcium oxalate urolithiasis patients on a standard diet

N = ——, SF = – – –



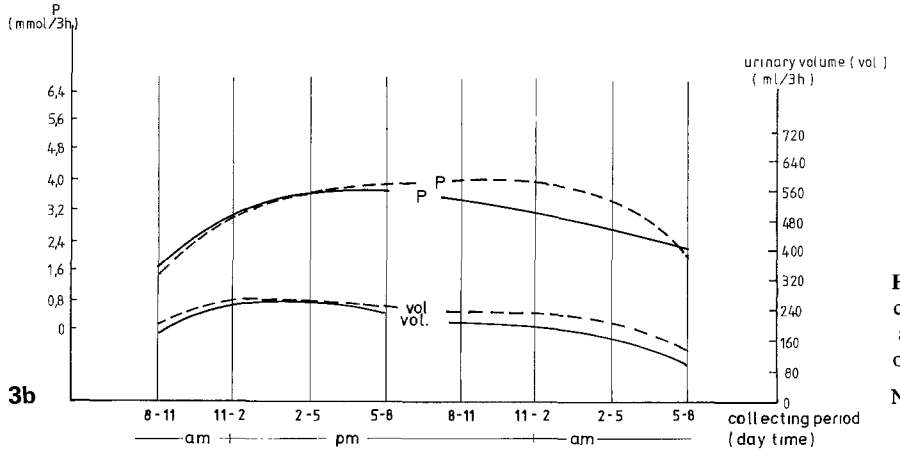


Fig. 3. Circadian rhythm of the phosphate concentration (a) and excretion (b) in 18 healthy and 20 calcium oxalate urolithiasis patients on a standard diet

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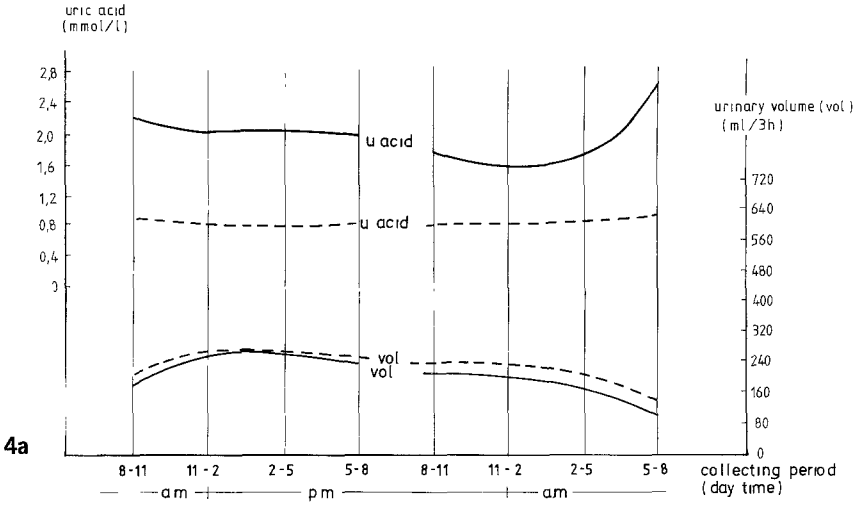
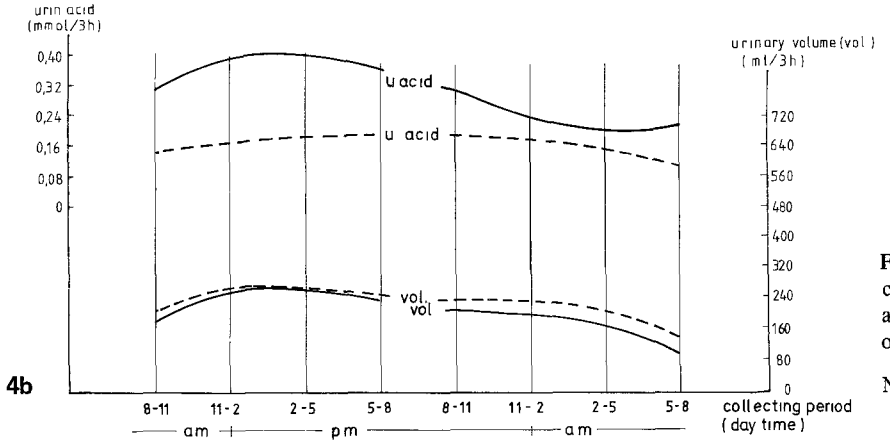


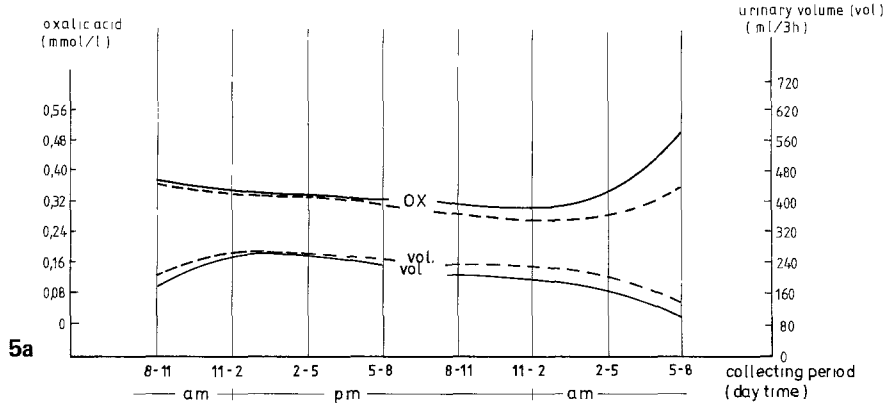
4a



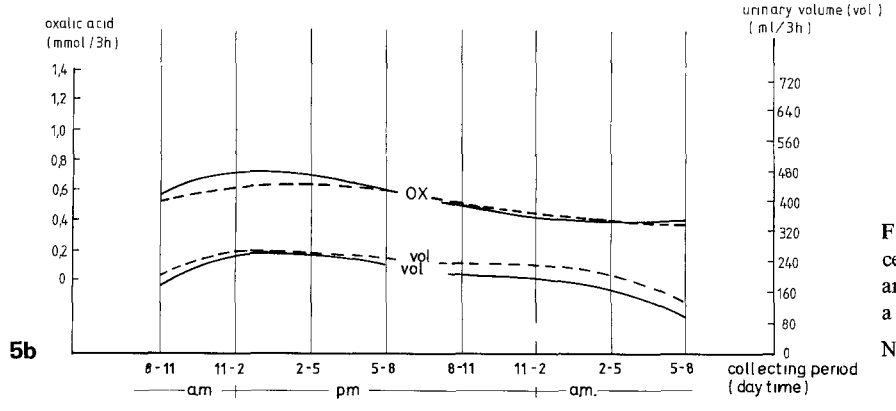
4b

Fig. 4. Circadian rhythm of the uric acid concentration **(a)** and excretion **(b)** in 18 healthy and 20 calcium oxalate urolithiasis patients on a standard diet

N = ——, SF = – – –



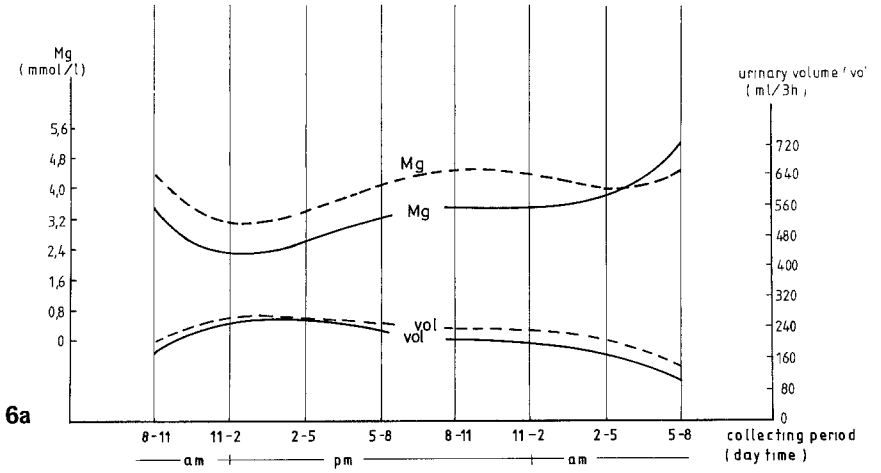
5a



5b

Fig. 5. Circadian rhythm of the oxalic acid concentration **(a)** and excretion **(b)** in 18 healthy and 20 calcium oxalate urolithiasis patients on a standard diet

N = ——, SF = – – –



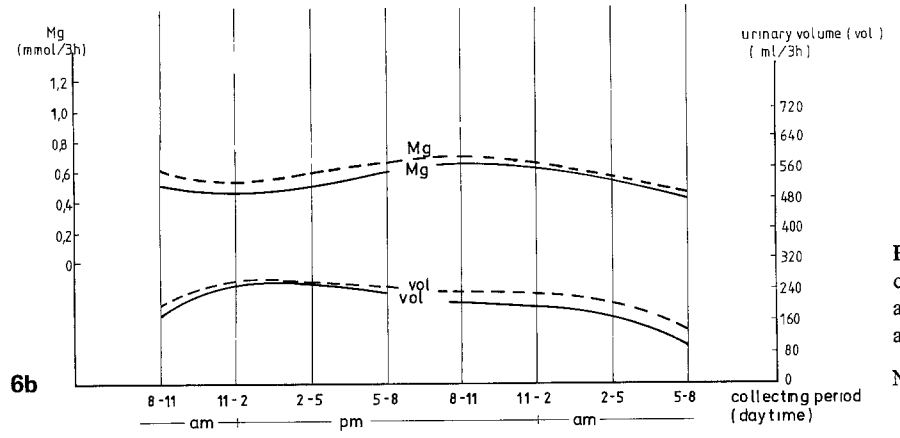


Fig. 6. Circadian rhythm of the magnesium concentration **(a)** and excretion **(b)** in 18 healthy and 20 calcium oxalate urolithiasis patients on a standard diet

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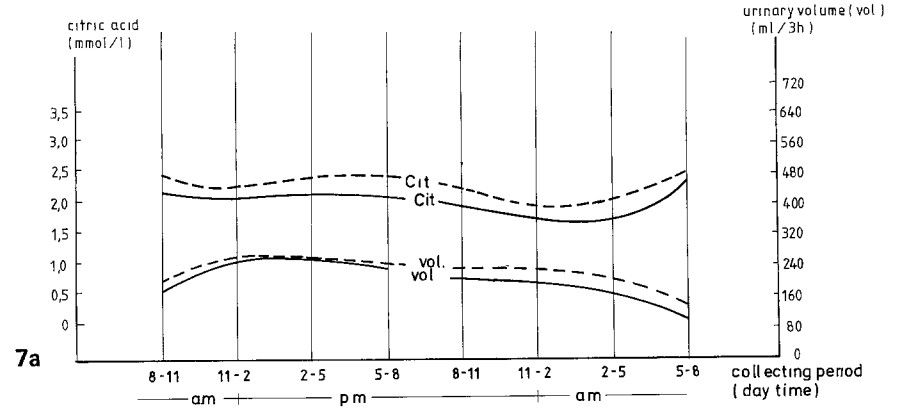

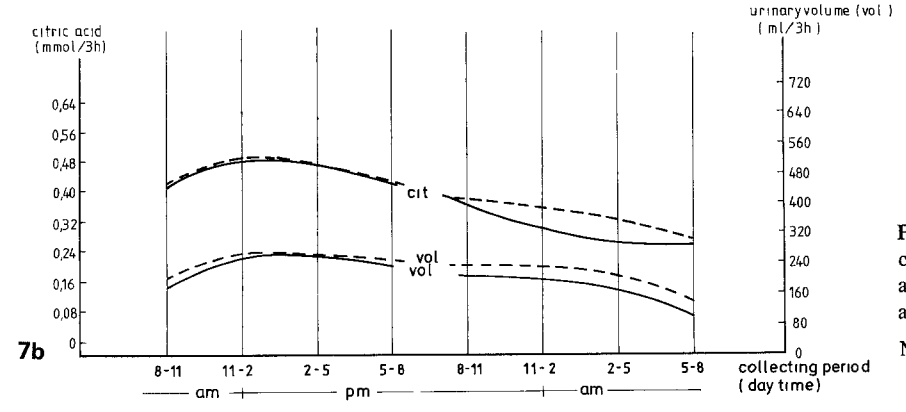

Fig. 7. Circadian rhythm of the citric acid concentration **(a)** excrection **(b)** in 18 healthy and 20 calcium oxalate urolithiasis patient on a standard diet

N = ——, SF = – – –

Table 3. Normal values (mean ± 2 SEM) of the concentration (mmol/l) of lithogenic parameters in the circadian rhythm in 18 healthy subjects on the 9th and 10th day under standard diet

	Collecting period							
	1	2	3	4	5	6	7	8
Ca	3.44 ± 0.68	2.14 ± 0.40	2.45 ± 0.52	2.59 ± 0.54	3.78 ± 0.88	2.55 ± 0.70	2.50 ± 0.60	3.00 ± 0.72
Mg	3.90 ± 0.60	2.20 ± 0.40	2.50 ± 0.40	3.10 ± 0.60	3.90 ± 0.80	3.0 ± 0.60	4.0 ± 0.60	4.70 ± 0.80
P	9.90 ± 1.32	13.78 ± 2.22	15.38 ± 2.46	19.66 ± 2.60	18.26 ± 2.60	16.70 ± 2.80	24.40 ± 2.60	28.60 ± 3.0
Uric acid	2.22 ± 0.46	1.93 ± 0.38	2.12 ± 0.48	1.92 ± 0.46	1.88 ± 0.38	1.30 ± 0.32	1.85 ± 0.38	2.49 ± 0.44
Oxalic acid	0.350 ± 0.044	0.319 ± 0.07	0.386 ± 0.108	0.336 ± 0.066	0.309 ± 0.052	0.269 ± 0.09	0.398 ± 0.092	0.487 ± 0.08
Citric acid	2.16 ± 0.22	2.05 ± 0.26	2.07 ± 0.26	2.1 ± 0.26	1.99 ± 0.28	1.5 ± 0.26	1.83 ± 0.24	2.05 ± 0.28

6.8. *Critric acid (Figs. 7a and b)*

The highest concentrations were found in healthy subjects in the morning (5 a.m. – 11a.m.) and in urolithiasis patients in the early morning (5 a.m. – 8 a.m.). The lowest concentrations were present in both groups around midnight (11 p.m. – 2 a.m.).

The excretion was highest at midday (11 a.m. – 2 p.m.) and lowest in the early morning (5 a.m. – 8 a.m.) in both groups. A higher concentration or excretion of citrate in the healthy subjects than in urolithiasis patients could only be detected for a few fractions. The differences were not significant.

Discussion

The circadian rhythm of biological processes in man is characterised in most cases (as in animals and plants) by a sinusoidal course with inflections in the early morning and afternoon hours [10]. The diurnal biological rhythm of body temperature and kidney function [1, 2] were already known in the last century.

Our knowledge of circadian fluctuations of urinary volume and the composition of the urine has been extended with increased sophistication of the investigation techniques. In general, the excretion of water and electrolytes is lowest towards the end of the period of night sleep and reaches a maximum during the normal waking period [7].

Endogenous factors are assumed to be the "regulators" causing the circadian rhythmicity [1, 2], even if exogenous factors can have an influence. The presence of such regulators has been inferred from the persistence of the circadian rhythm when the experience of day/night and time are artificially eliminated.

Our investigations also show an unequivocal circadian rhythm of the lithogenic parameters in the urine.

The maximal concentration of lithogenic and inhibitory substances are found in healthy subjects mainly in the early morning and morning hours, when the urinary volume is lowest. The lowest concentrations are not (as expected) at midday, when the urinary volume has its highest value: the lowest concentrations of uric acid, oxalic acid and citric acid are only detected around midnight. This phenomenon might be explained by the flat course of the circadian curve of the urine volume, as a result of which relatively high mean values of the urine volume can be registered even at midnight. Two peaks (5 a.m. – 18 a.m. and 8 p.m. – 11 p.m.) and two troughs (11 a.m. – 2 p.m. and 2 a.m. and 5 a.m.) are detected for calcium. The maximal effective excretion of lithogenic substances is found at midday and in the early afternoon, when the urinary volumes are also highest. The lowest are observed mainly in the early morning, when the urinary values are also lowest. The excretion maximum is in the early evening and the minimum in the early morning only for calcium and magnesium.

Mean values and SEM (standard error for the mean) were calculated from all values for concentration and effective excretion of the lithogenic parameters on the 9th and 10th day under standard diet in all 18 healthy control subjects (Tables 3 and 4). For more precise delimitation of the mean values, the two SEM range was used. This is a variation of the calculated mean value of the random sample from the true mean value of the total collective. Use of the two SEM values is possible because a random sample collective of more than 30 was used, so that only 5% of the "normal" values were outside the distribution curve for the mean value.

The maximal concentrations of lithogenic parameters in urolithiasis patients were observed at similar times of day and

Table 4. Normal values (mean ± 2 SEM) of the effective excretion (mmol/3 h) of lithogenic parameters in the circadian rhythm in 18 healthy test subjects on the 9th and 10th day under standard diet

	Collecting period							
	1	2	3	4	5	6	7	8
Ca	0.58 ± 0.12	0.59 ± 0.18	0.53 ± 0.12	0.58 ± 0.16	0.65 ± 0.12	0.55 ± 0.16	0.36 ± 0.14	0.3 ± 0.1
Mg	0,52 ± 0.06	0.45 ± 0.06	0.56 ± 0.1	0.59 ± 0.14	0.75 ± 0.22	0.57 ± 0.1	0.5 ± 0.08	0.42 ± 0.08
P	1.8 ± 0.4	2.8 ± 0.4	3.9 ± 0.4	3.5 ± 0.6	3.3 ± 0.4	3.1 ± 0.4	2.8 ± 0.6	2.1 ± 0.4
Uric acid	0.3 ± 0.04	0.38 ± 0.08	0.41 ± 0.08	0.32 ± 0.06	0.32 ± 0.04	0.23 ± 0.04	0.2 ± 0.04	0.21 ± 0.02
Oxalic acid	0.62 ± 0.14	0.68 ± 0.14	0.74 ± 0.12	0.56 ± 0.08	0.51 ± 0.06	0.5 ± 0.18	0.42 ± 0.08	0.4 ± 0.08
Citric acid	0.37 ± 0.06	0.49 ± 0.08	0.47 ± 0.08	0.4 ± 0.06	0.37 ± 0.06	0.3 ± 0.06	0.26 ± 0.06	0.25 ± 0.06

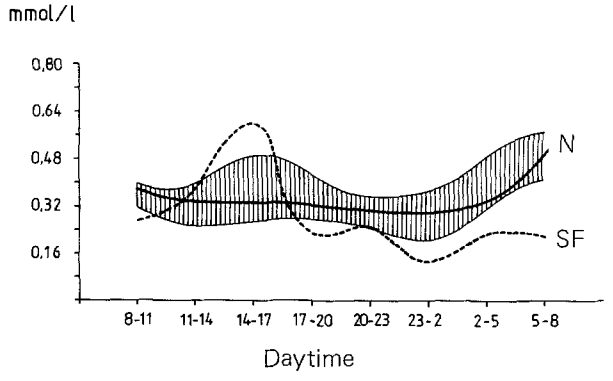

Fig. 8. Circadian rhythm of oxalic acid concentration (mmol/l) in urine in one calcium oxalate urolithiasis patient (*SF*) and 18 healthy subjects (*N*) (*hatched area* = normal range). *SF*: oxalic acid concentration in 24-h urine: 0.250 (normal range 0.237 ± 0.067) mmol/l

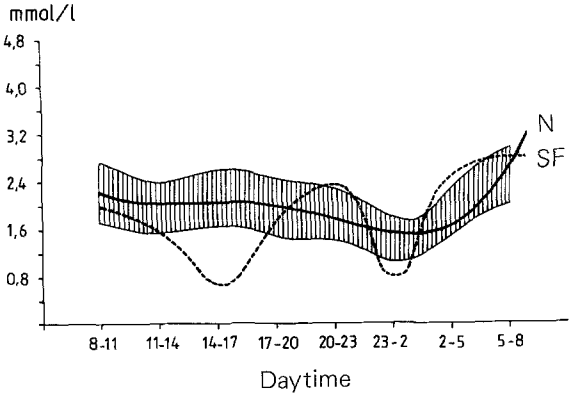

Fig. 10. Circadian rhythm of the uric acid concentration (mmol/l) in urine in one calcium oxalate urolithiasis patient (*SF*) and 18 healthy subjects (*N*) (*hatched area* = normal range). *SF*: uric acid concentration in 24-h urine: 1.26 (normal range 1.75 ± 0.36) mmol/l

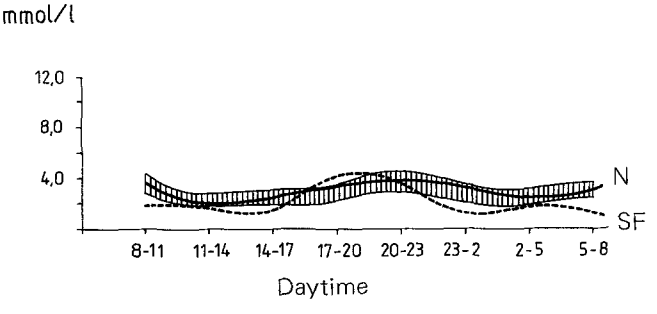

Fig. 9. Circadian rhythm of the calcium concentration (mmol/l) in urine in one calcium oxalate urolithiasis patient (*SF*) and 18 healthy subjects (*N*) (*hatched area* = normal range). *SF*: calcium concentration in 24-h urine: 1.54 (normal range 2.3 ± 1.05) mmol/l

night. No fixed rules can be drawn up for the timing of minimal concentrations.

Since the circadian curve of the urine volume has as flat a course in urolithiasis patients as in healthy subjects, very pronounced differences in the daily and nightly course (peaks) are not to be expected. Two special features are to be emphasised: on the one hand, the calcium concentration had two peaks and troughs in the urolithiasis patients. On the other hand, no circadian rhythm can be detected for the uric acid concentration. For the effective excretion of lithogenic parameters, unequivocal correlations with the level of the respective urinary volume can be demonstrated. The effective excretion is highest when the urinary volume has reached its highest level and vice versa.

The example of a recurrent urolithiasis patient is intended to demonstrate that a situation of danger can be found very much more readily by determination of the circadian rhythm

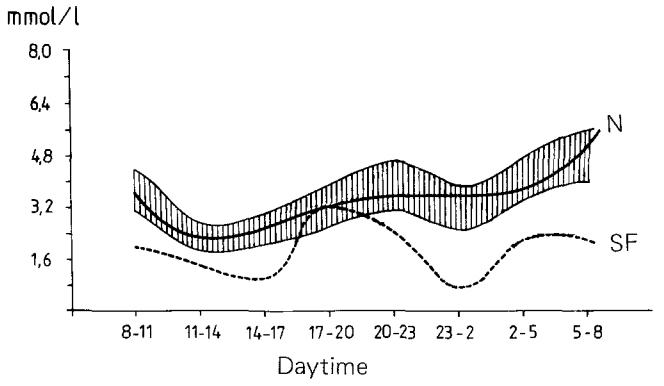

Fig. 11. Circadian rhythm of the magnesium concentration (mmol/l) in urine in one calcium oxalate urolithiasis patient (*SF*) and 18 healthy subjects (*N*) (*hatched area* = normal range). *SF*: magnesium acid concentration in 24-h urine: 1.42 (normal range 2.83 ± 0.7) mmol/l

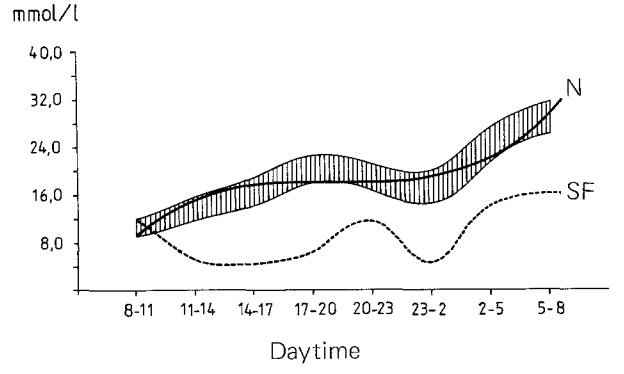

Fig. 12. Circadian rhythm of the phosphate concentration (mmol/l) in urine in 1 Ca oxalate urolithiasis patient (*SF*) and 18 healthy subjects (*N*) (*hatched area* = normal range). *SF*: phosphate concentration in 24-h urine: 6.57 (normal range 15.34 ± 2.26) mmol/l

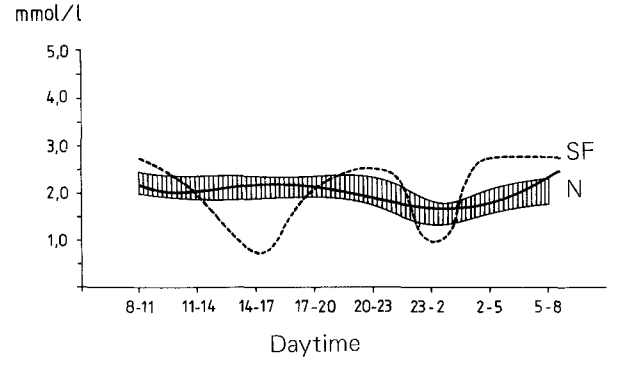

Fig. 13. Circadian rhythm of the citric acid concentration (mmol/l) in urine in 1 Ca oxalate urolithiasis patient (*SF*) and 18 healthy subjects (*N*) (*hatched area* = normal range). *SF*: citric acid concentration in 24-h urine: 1.47 (normal range 1.97 ± 0.39) mmol/l

of lithogenic substances than with the 24-h urine investigation. The 23 year old female patient had collected her urine over 24 h in a 3-h rhythm while on her normal diet. By the addition of the effective excretion values of the urolithiasis parameters and the concentration of lithogenic parameters in the 24-h collected urine calculated from this, the following values resulted: the concentrations of calcium and oxalic acid were in the normal range in the 24-h collected urine, whereas the values of magnesium, phosphate, uric acid and citrate were lowered to values below normal. In evaluation of the circadian concentrations of these parameters, which were related to the normal circadian curve, however, it was noticed that in the collection periods 2 and 3 the oxalic acid concentration (Fig. 8) was raised to a highly pathological value, whereas it was normal or subnormal in the remaining fractions. Thus a situation of danger around midday and in the afternoon was detected in the patient; this may possibly have been due to alimentary factors.

In addition, a situation of danger was also present for the calcium concentration (Fig. 9), as shown in the collection period 4, and likewise for the uric acid concentration (Fig. 10), for which pathological peaks were shown in the collection periods 5 and 7.

Furthermore, hypomagnesiuria (Fig. 11) and hypophosphaturia (Fig. 12) was found in almost all fractions as well as a citric acid concentration raised above normal in the morning (Fig. 13).

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Professor W. Vahlensieck, MD
Urologische Universitäts-Klinik
Sigmund-Freud-Str. 25
D-5300 Bonn