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Measurement of diurnal variations in urinary cystine saturation

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Abstract In an attempt to improve the diagnostic value of urine analysis in patients with homozygous cystinuria, we studied the diurnal variation in urine composition. A simplified estimate of the ion-activity product of cystine was used to increase the probability of identifying patients with a particular risk of stone formation. Eight 6-h urine samples were collected during two 24-h periods. The highest urinary excretion of cystine was recorded between 1200 and 1800 hours and the lowest between 0000 and 0600 hours, whereas the urinary cystine concentration was highest between 0000 and 0006 hours and lowest between 1200 and 1800 hours. The approximate ion-activity product of cystine had a maximal level between 0000 and 0600 hours but a minimal level between 0600 and 1200 hours. The differences between different periods were numerically more pronounced in terms of the ion-activity product of cystine than in terms of concentration. The peak concentrations of cystine in 6-h samples were about 90% higher than the corresponding concentrations in 24-h urine samples. It is concluded that the analysis of cystine in 6-h urine samples reveals transient episodes of cystine supersaturation that otherwise will remain undetected. Further studies are, however, needed to establish its usefulness in clinical practice.

Key words Homozygous cystinuria · Diurnal variation · Stone formation · Diagnosis

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In the management of cystinuria, urinary supersaturation of cystine can be counteracted by an increased fluid intake and alkalinization of the urine. This regimen was introduced by Dent and Senior in 1955 [9] and was found to be effective in 12 of their 18 patients [11]. A drawback is the inconvenient polyuria, which is a cause of noncompliance, and the fact that the regimen is sufficient only for patients with moderately active stone disease. With the introduction of D-penicillamine in the treatment of cystinuria, it became possible to reduce the urinary excretion of free cystine [7]. The use of D-penicillamine has, however, been limited by a high frequency of adverse effects [13]. Another effective SH compound, tiopronin (2-mercapto-propionylglycine), which has less pronounced side effects, has since come into clinical use [8, 15, 17, 20, 23, 25].

In a long-term follow-up of cystinuric patients treated with tiopronin, we recently found that individualization of stone-preventive treatment based on monitoring of urinary cystine excretion is necessary to achieve an adequate reduction of cystine concentration in the individual patient (in preparation). Dent and Senior [9] experimentally showed that the solubility of cystine in human urine is about 1200 μmol/l. The formation of cystine stones can be assumed to take place when the urinary concentration of cystine exceeds the level of saturation, and we have shown that the activity of renal stone formation in cystinuria is related to the urinary cystine concentration in 24-h urine samples. A well-defined level of cystine concentration above which the formation of cystine stones was sharply increased could not however, be demonstrated [22]. The cystine concentration can be expected to fluctuate because of variations in urine flow [1, 9, 26]. In addition, the solubility of cystine is influenced by the urinary pH [9], which varies during the day and night. Periods with a particularly high risk of stone formation may therefore be undetected when 24-h urine samples are analyzed. For similar

reasons, periods of urine collection shorter than 24-h have been used in the biochemical evaluation of patients with calcium oxalate stone disease [2].

The purpose of the present investigation was to study the diurnal variation in the urinary cystine excretion and concentration, and to obtain an estimate of the ion-activity product of cystine. We also wanted to decide whether such measurements offer any advantages over analysis of 24-h samples for the monitoring of anticystinuric therapy.

Patients and methods

Patients

Eight patients took part in the study. The clinical characteristics of the patients are shown in Table 1. The diagnosis of homozygous cystinuria had in all cases been established by determination of the urinary excretion of cystine and the dibasic amino acids [16]. Five of the patients were women and three were men. The mean age was 43 years (range 28–74 years). The mean age at the first symptoms of renal stone disease was 23 years (range 13–42 years). All of the patients had a history of active stone formation, and six of them had been subjected to renal stone surgery or extracorporeal shock wave lithotripsy (ESWL) within the last 10 years. The number of formed renal stones specified in Table 1 includes only those verified on radiograms and stone passages, and is probably an underestimate. Two of the patients (Nos. 2 and 5) were unilaterally nephrectomized. Two patients (Nos. 6 and 7) had nonobstructing unilateral renal stones on their latest radiographic examination, whereas the remaining six patients were free of renal stones. There were no signs or symptoms of the renal stone disease during the study.

All patients had been followed up 2–3 times a year as outpatients at the Department of Nephrology for an average of 5.8 years (range 1–9 years). Two patients (Nos. 4 and 5) had glomerular filtration rates below the age-related normal range [12]. As part of the prevention of cystine stone formation, one of the patients (No. 1) was treated with potassium-sodium citrate (Uralyt, Madaus, Cologne, FRG), and six were treated with sodium bicarbonate in doses of 1–15 g (mean 8.8 g). All the patients had previously been encouraged to maintain a high level of fluid intake. No patient was treated with any sulfhydryl compound at the time of the study. In two patients (Nos. 2 and 6) there was a history of adverse reactions to both D-penicillamine and tiopronin, and in the remaining six to tiopronin only [21]. One patient (No. 1) was medicated with 50 mg atenolol and 40 mg furosemide daily for mild hypertension, and one patient (No. 5) took 0.15 mg levothyroxine for hypothyroidism.

Table 1 Clinical characteristics of the eight cystinuric patients

Patient no.	Sex	Age at time of study (years)	Age at onset of symptoms (years)	GFR (ml/min ×1.73 m^2)	Renal stone formation[a] (number/years)
1	F	31	22	137[b]	5/9
2	F	35	13	93[c]	13/22
3	F	38	15	110[b]	4/23
4	F	44	42	75[c]	2/2
5	F	74	41	50[b]	11/33
6	M	28	16	101[b]	6/12
7	M	37	22	113[b]	4/15
8	M	57	14	134[c]	7/43

[a] Verified by radiographic examinations and stone passages
[b] ^{51}Cr-EDTA plasma clearance
[c] ^{99m}Tc-DTPA plasma clearance

Study design

The patients were admitted to the ward for 48 h. Prior to their admittance, they had collected one 24-h urine sample (Period 0). The participation of one patient (No. 3) was limited to 24 h. Eight 6-h urine samples were collected starting at 1800 hours and stored at + 4 °C. Immediately after each 6-h period the urine samples were analyzed with respect to pH, specific gravity and specific electrical conductivity. After 24 h (Period 1) and 48 h (Period 2), the volumes of the four 6-h urine samples were measured, and two 10-ml aliquots from each sample were frozen at −20 °C without addition of chemicals for later determination of free cystine. The medication with sodium bicarbonate was stopped 1 week prior to the study, and withheld until the collection of urine was completed. The patients were served a regular hospital diet with breakfast at 0730 hours, lunch at 1130 hours and dinner at 1700 hours. They were encouraged to maintain their ordinary drinking habits.

The average 24-h concentration of cystine in urine during Periods 1 and 2 was calculated by dividing the sum of the 6-h excretions of cystine by the sum of the 6-h urinary volumes. The ion-activity product of cystine was calculated by the formula described by Tiselius [27]:

$$\frac{(10^{-pH})^2 \times Conc_{cystine} \times 0.155}{\{1 + (0.39 \times 10^{10} \times 10^{-pH}) + [(10^{-pH})^2 \times 3.51 \times 10^{16}]\}}$$

Analytical methods

The urinary amino acids were determined by ion-exchange chromatography at the Department of Clinical Chemistry, University Hospital, Malmö [8, 16]. Prior to chromatography, internal standards of amino-ethylcysteine were added to the samples, and urinary proteins were precipitated with sulfosalicylic acid. Separation and detection of analyzed constituents were performed by an amino acid analyzer (LKB 4151 Alpha Plus). The ninhydrin complexes were detected spectrophotometrically at 570 nm, and concentrations were calculated by an automatic integrator (Shimadzu C-R4A).

Urinary pH was measured with a glass electrode (Extech, Boston, Mass., USA), and the specific gravity was measured by a floating hydrometer. The specific electrical conductivity was measured by a personal electronic device (Urimho, Gyrus Medical, UK). All the measurements were carried out at room temperature. The results were recorded as arbitrary units (0–5). Glomerular filtration rate was determined as plasma clearance of ^{51}Cr-EDTA [3] or ^{99m}Tc-DTPA [18].

Statistics

Student's *t*-test and Wilcoxon's signed-rank test were used for significance testing of differences between means.

Results

Figure 1a–c shows the 24-h excretion of cystine, the 24-h urine volume and the urinary cystine concentration during Period 0 and during the 2 days of 6-h urine collection (Periods 1 and 2). There were no differences between Period 1 and Period 2. The 24-h cystine

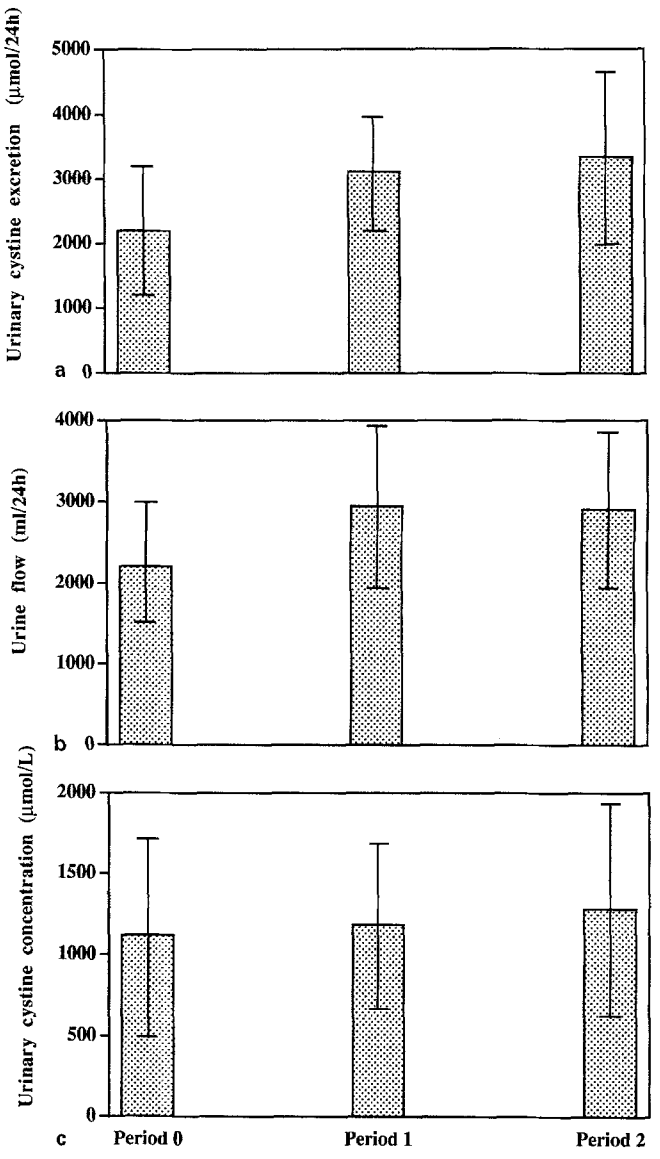

Fig. 1a–c The 24-h urinary cystine excretion, urine volume and cystine concentration during periods 0, 1 and 2. *Bars* 1 SD

excretion and urine volume was slightly lower during Period 0 ($P < 0.05$), but the urinary cystine concentration was similar during the three 24-h periods. There were no significant differences in urinary pH between Periods 1 and 2.

Figure 2a–e demonstrates the mean urinary volume, cystine concentration, cystine excretion, pH and ion activity product of cystine in the 6-h samples. The values were calculated as the means in corresponding 6-h periods from the two 24-h periods observed. The urine flow was lowest between 0000 and 0600 hours (mean 490 ml, range 195–680 ml) and highest between 1200 and 1800 hours (mean 973 ml, range 220–1555 ml, $P < 0.001$). The concentration of cystine was highest between 0000 and 0600 hours (mean 1761 μmol/l, range 638–7074 μmol/l) and lowest between 1200 and 1800 hours (mean 1288 μmol/l, range 449–5919 μmol/l, $P < 0.05$). The excretion of cystine was lowest between 0000 and 0006 hours (mean 673 μmol/6 h, range 408–1379 μmol/6 h) and highest between 1200 and 1800 hours (mean 960 μmol/6 h, range 501–2224 μmol/6 h, $P < 0.01$). The urinary pH was lowest between 0000 and 0600 hours (mean 6.1, range 5.4–6.7) and highest between 0600 and 1200 hours (mean 6.8, range 6.1–7.5, $P < 0.001$). The ion-activity product was highest between 0000 and 0600 hours (mean 6.9×10^{-21} M^2, range 2.0–30.0 $\times 10^{-21}$ M^2) and lowest between 0600 and 1200 hours (mean 3.1×10^{-21} M^2, range 0.9–7.9 $\times 10^{-21}$ M^2, $P < 0.01$).

Figure 3 shows the urinary cystine concentration during Period 0, the calculated cystine concentration during Periods 1 and 2, and the highest concentration of cystine during each 24-h period as analyzed in the 6-h samples. The mean (SD, range) cystine concentration of the 15 24-h periods was 1230 μmol/l (566, 625–2185 μmol/l). The mean recorded peak concentration for the same 24-h periods was 2535 μmol/l (1863, 861–7074 μmol/l). The peak concentrations were on average 91% higher than the corresponding 24-h concentrations (SD 57%, range 15–224%, $P < 0.01$). The concentration of cystine reached its highest level between 0000 and 0600 hours in 6 out of the 15 collections, between 1800 and 2400 hours in 5 and between 0600 and 1200 hours in 2.

There was no apparent relationship between the excretion, concentration and ion-activity product of cystine on one hand, and the specific gravity and electrical conductivity on the other.

Discussion

Our results show that the urinary excretion of cystine was highest between 1200 and 1800 hours (Fig. 2a–e). The changes in cystine excretion of the present study were probably due to nutritional factors, since the ingestion of methionine, a component of animal protein, may cause an increase in cystine excretion within 2 h in cystinuric patients [10]. As expected, the urine flow was lowest between 1800 and 0600 hours. This resulted in a higher cystine concentration during the evening and night (1800–0600 hours) than during daytime (0600–1800 hours). This is in accordance with previous observations by Dent and Senior, who found a twofold increase in urinary cystine concentration during the night in one patient [9]. Similarly, Purkiss and Watts found the highest cystine concentrations in 6-h urine samples collected between 0200 and 0800 hours in three patients [26]. Backer et al. reported the highest concentrations between 0400 and 0800 hours in one patient [1].

The formation of cystine stones is counteracted by a reduced urinary supersaturation with respect to cystine. In an experimental study, Dent and Senior found that the solubility of cystine in urine was about

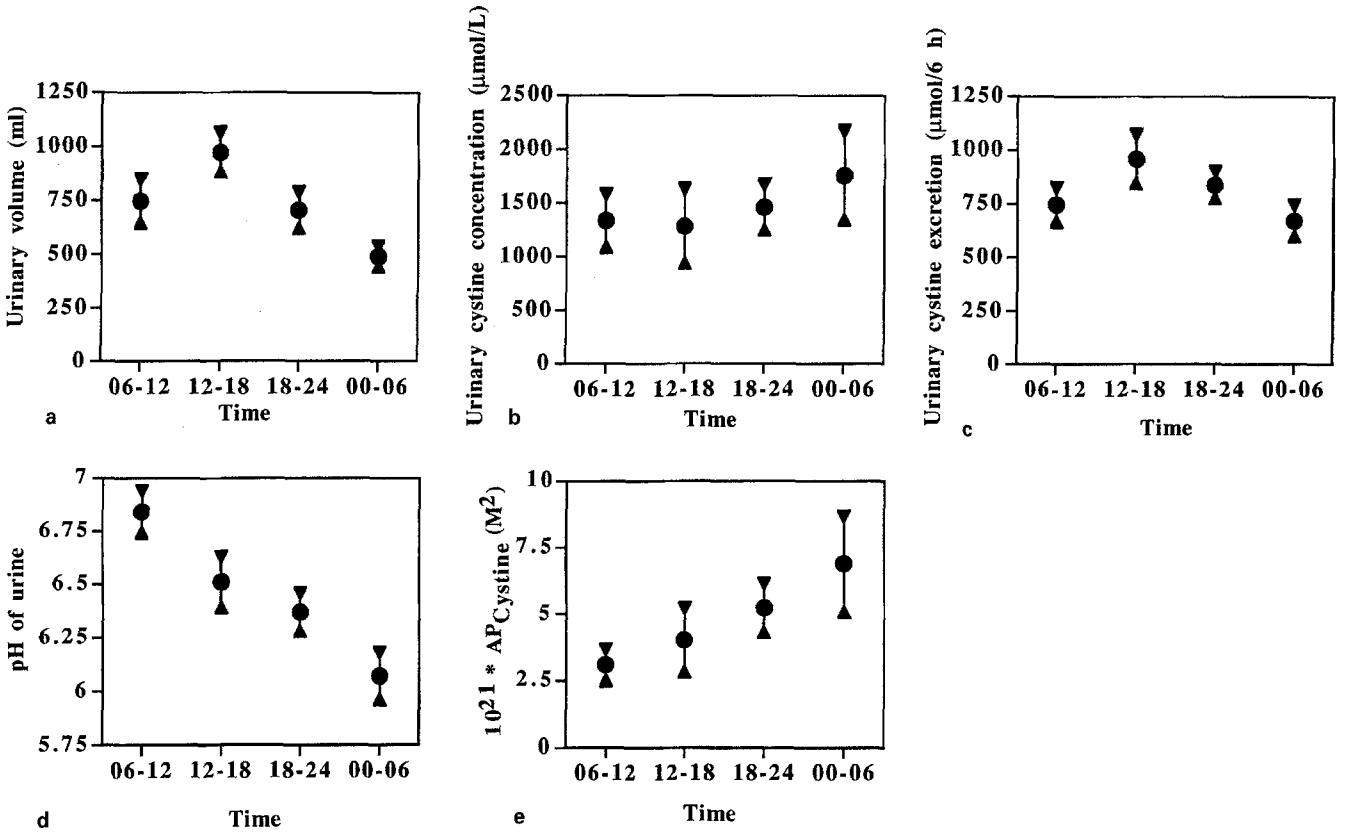

Fig. 2a–e Variations in urinary volume, cystine concentration, cystine excretion, pH and ion-activity product of cystine. The values are means and SE based on analysis of 15 6-h urine collections

1200 µmol/l [9]. The risk of stone formation should rise sharply when the cystine saturation exceeds the solubility product. The peak concentrations of 6-h urine samples recorded in this study were often substantially higher than the corresponding average 24-h concentration (Fig. 3). In a previous long-term study, we found that the rate of renal stone formation was satisfactorily controlled in two-thirds of the patients by tiopronin given at bedtime or twice daily [22]. The doses were governed by regular measurements of free cystine in 24-h urine samples. One-third of the patients continued to form stones in spite of urinary cystine concentrations below the assumed risk level of 1200 µmol/l. In evaluating the relation between the urine content of cystine and the formation of renal stones, we found that the rate of stone formation increased with increasing cystine concentrations already in the concentration range of 500–1100 µmol/l. It was not possible to define a level of cystine concentration above which the formation of cystine stones was sharply increased. It is reasonable to assume that the periods with the highest cystine concentration entail a risk of cystine crystallization even though the concentration on a 24-h basis is undersaturated. This is one probable explanation for the absence of a distinct risk level of cystine concentration in 24-h samples.

The pharmacokinetics of tiopronin have recently been elucidated, and it was shown that the effect on the excretion of free cystine lasted 6–12 h [4–6]. From a pharmacological point of view, the drug should thus be given at least twice daily when it is used to reduce the urinary cystine excretion. Similar conclusions can be drawn from the results of other investigators [1, 14, 19]. Consequently, the time of tiopronin administration is important since the urinary cystine concentration varies during day and night. In patients with active stone formation, despite an acceptable reduction of the 24-h cystine concentration, analysis of urine fractions may reveal periods of supersaturation, prompting a change in tiopronin therapy.

We have shown that the urinary cystine during the evening and night was critical in order to detect peak concentrations. This gives theoretical support for the reason for administration of tiopronin at bedtime. The results of other investigators suggest that the highest cystine concentrations occurred during the late night or early morning hours [1, 26], but we found a maximal cystine concentration between 1800 and 2400 hours in 5 of the 15 24-h periods. Because of the normal absence of fluid intake and the physiological increase in the secretion of antidiuretic hormone during the night, a collection period corresponding to the hours of sleep may be the most appropriate and at the same time convenient for the patient.

The level of cystine saturation in urine is influenced by factors other than the concentration. Most

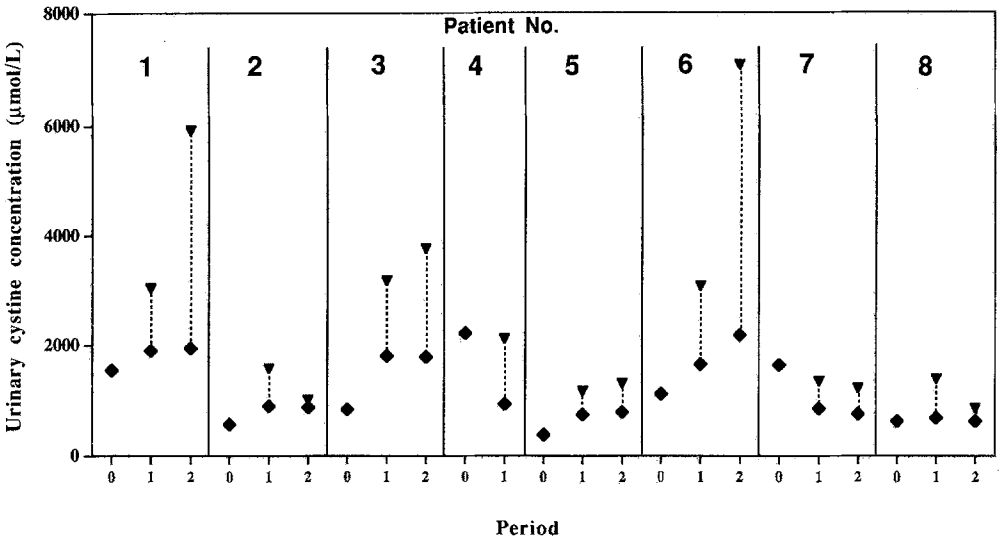

Fig. 3 Peak concentration of cystine (▼) recorded in 6-h urine samples compared with the average cystine concentration (◆) during the corresponding 24-h periods. Period 0 represents the 24 h preceding admission, during which no fractionated urine collection had been carried out

important is the strong pH dependence of cystine solubility [9], and reliable conclusions on saturation cannot be drawn only from the measurement of urinary cystine. Furthermore the solubility of cystine is influenced by the ionic strength and the concentration of macromolecules [24]. The estimate of the ion-activity product of cystine that we have used better reflects the degree of saturation despite the necessary approximations. In our previous study, we found that in 24-h urine samples the ion-activity product of cystine did not correlate with stone formation better than the cystine concentration [22]. A drawback with the pH measurement of a 24-h period is that it gives no information on diurnal variation. The ion-activity product from shorter collection periods may better reflect the degree of cystine saturation. The diurnal changes in the ion-activity product of cystine showed a different pattern than the urinary cystine concentration (Fig. 2a–e). The differences between the ion-activity products for the 6-h collection periods were also numerically more pronounced than for the concentrations. The reason for this is mainly that the urinary pH was lowest between 0000 and 0600 hours and between 0600 and 1200 hours. The ion-activity product in 6-h samples is probably a good indicator of the risk of cystine crystallization. Conclusions on the ability of the ion-activity product derived from analysis of 6-h urine samples to predict cystine stone formation, require however, a long-term follow-up of cystinuric patients.

In order to decide whether more simple laboratory analyses could replace cystine analyses to some extent, we assessed the specific gravity and electrical conductivity of each sample of urine. The correlation with the cystine excretion, the concentration and the ion-activity product was, however, very poor. The reason may be that the specific gravity and the electrical conductivity mainly reflect the sodium content of the urine.

It is concluded that the analysis of cystine in 6-h urine samples provides important additional information compared with the analysis of 24-h samples. Short-term collection periods may reveal transient episodes of supersaturation and enable the therapeutic design to be improved. This might be beneficial particularly in patients who have active stone disease despite an acceptable 24-h concentration of urinary cystine. In view of the 6- to 12-h duration of the effect of tiopronin, our observation of a high supersaturation with cystine during the night supports the idea of administering the drug at bedtime. The use of an estimate of the ion-activity product of cystine seems to be superior to that of cystine concentration only, but further studies are necessary to finally determine the clinical usefulness of this simplified estimate.

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