Effects of Citrate on the Different Phases of Calcium Oxalate Crystallization

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EFFECTS OF CITRATE ON THE DIFFERENT PHASES OF CALCIUM OXALATE CRYSTALLIZATION

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Abstract

Urinary citrate appears to be an important factor in the crystallization process of calcium oxalate and calcium phosphate. The urinary excretion of citrate was found to be significantly lower in patients with calcium oxalate stone disease as compared with normal subjects, and about 30 per cent of the calcium stone formers can be considered as hypocitraturic. The lowest excretion of citrate was recorded in urine collected during the night. Citrate has significant effects on supersaturation with respect to both calcium oxalate and calcium phosphate, it also inhibits the growth of these crystals. In addition, citrate appears to be capable of inhibiting the aggregation of crystals composed of calcium oxalate, brushite, and hydroxyapatite. The heterogenous growth of calcium oxalate on calcium phosphate is also counteracted by citrate. As a consequence of the crucial role of citrate in these processes, stone prevention with alkaline citrate has become an attractive form of treatment in patients with recurrent stone formation. Single evening dose administration of sodium potassium citrate resulted in an increased excretion of citrate, reduced levels of the calcium/citrate ratio as well as supersaturation with respect to calcium oxalate and a decreased rate of stone formation. However, conflicting results of stone preventive treatment with alkaline citrate have been reported by different groups, and long-term follow-up of patients treated in a randomized way is necessary to definitely assess the efficacy of alkaline citrate.

Key words: Citrate, calcium oxalate, calcium phosphate, renal stone disease, inhibition, crystal growth, crystal aggregation, alkaline citrate, recurrence prevention.

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Introduction

Renal stone disease represents an everyday problem to urologists and nephrologists because of the high recurrence rate encountered in these patients. Calcium oxalate (CaOx) is the type of crystal most often found in renal calcium stones. These crystals occur either in monohydrate (COM) or dihydrate form or both, and frequently together with calcium phosphate. A detailed understanding of the crystallization process that results in the formation of a renal stone is necessary for design of a rational recurrence preventive treatment.

Intensive research activities in this field during the last 15-20 years have also definitely given us valuable insights in this apparently very complicated process. A urine, sufficiently supersaturated with calcium oxalate, is certainly the most important prerequisite for starting the crystallization. It needs to be emphasized however, that a primary crystallization of calcium phosphate (CaP) is capable of inducing heterogenous crystallization of CaOx. In this way crystallization of CaOx on nuclei of CaP can occur at lower supersaturation levels than when pure CaOx crystals are formed. The crystals thus formed will grow and aggregate to large crystals and crystal masses, that constitute the renal stone. It has also been demonstrated that the number and sizes of crystals are greater in patients with stone disease than in normal subjects (Robertson et al., 1971). In order to explain the subsequent formation of a renal stone it is also essential to consider a retention factor. The formation of a stone might thus start with a nucleation followed by growth and aggregation of fixed or slowly moving crystal masses in the nephron. A number of urine constituents has been shown to exert effects on these different phases of the crystallization process, mainly as inhibitors of crystal growth, crystal aggregation or both. Small molecules such as pyrophosphate, citrate, and trace metals as well as macromolecules such as glycosaminoglycans, nephrocalcin, Tamm Horsfall mucoprotein and uropontin might be contributive in this respect (Robertson, 1976; Finlayson, 1977; Fleisch, 1990; Meyer, 1990; Coe et al., 1991; Hoyer, 1992).

During recent years, evidence has accumulated indicating that citrate might have a crucial role as

regulator of the calcium salt crystallization. Citrate can thereby affect supersaturation with respect to both CaOx and CaP by complex formation with calcium ions and inhibit growth of these two crystal types at least in diluted urine (Fleisch, 1990; Tiselius and Fornander, 1981). There have also been some observations of an inhibition of citrate on CaOx crystal aggregation. In as much as CaOx and CaP often are found together in calcium stones (Herring, 1962; Leusmann *et al.*, 1990; Otnes, 1983; Öhman *et al.*, 1992), maintenance of an adequate urinary citrate level might, therefore, be of great importance in the treatment of patients with calcium stone disease.

Attributable to the these properties of citrate, in addition to the reduced risk of CaOx crystallization at a pH above 6.5 (Tiselius, 1984; Ahlstrand *et al.*, 1984), there has been a considerable interest for the use of alkaline citrate in prevention of recurrent calcium stone formation. Successful results with this form of therapy have been reported by several authors (Butz, 1982; Pak *et al.*, 1985; Preminger *et al.*, 1988; Schwille *et al.*, 1987; Berg *et al.*, 1992), but not by others (Hofbauer *et al*, 1992; Trinchieri *et al.*, 1992).

The aim of this review is to summarize some experimental and clinical support for the effects of citrate in this respect and to determine the value of citrate analysis in patients with calcium stone disease.

Urinary Excretion of Citrate

A low urinary excretion of citrate is a common finding in patients with calcium stone disease. The fraction of patients with hypocitraturia varies between different reports (Butz and Knispel, 1984; Menon and Mahle, 1983; Nicar *et al.*, 1983; Pak, 1991; Robertson *et al.*, 1978; Rudman *et al.*, 1982; Schwille *et al.*, 1979; Welshman and McGeown, 1976; Rudman *et al.*, 1982). This variation certainly reflects both different selection of patients and different normal levels.

The 24 hour excretion of citrate in men and women in a large group of patients with recurrent stone disease is shown in Figure 1. During the 24 hour period 10 per cent of the normal men excreted less than 1.85 mmol and 10 per cent of the normal women less than 1.75 mmol.

In our investigation, approximately 27 per cent of both stone forming men and women had a citrate excretion below these levels. Pak (1991), who used a slightly lower normal citrate limit of 1.69 mmol per 24 hours, found that approximately 50 per cent of the investigated patients were hypocitraturic. None of these observations gave support to a sex difference in terms of citrate excretion, which previously has been observed (Hodgkinson, 1962; Marangella *et al.*, 1987). In our measurements the mean (standard deviation, SD) citrate excretion were in normal men and women 3.10 (1.08) and 3.10 (1.04) mmol, respectively; and in stone-formers 2.73 (1.70) and 2.78 (1.49) mmol. The values between stone formers and normal subjects differed statistically for men ($p < 0.05$), but not for women ($p > 0.05$).
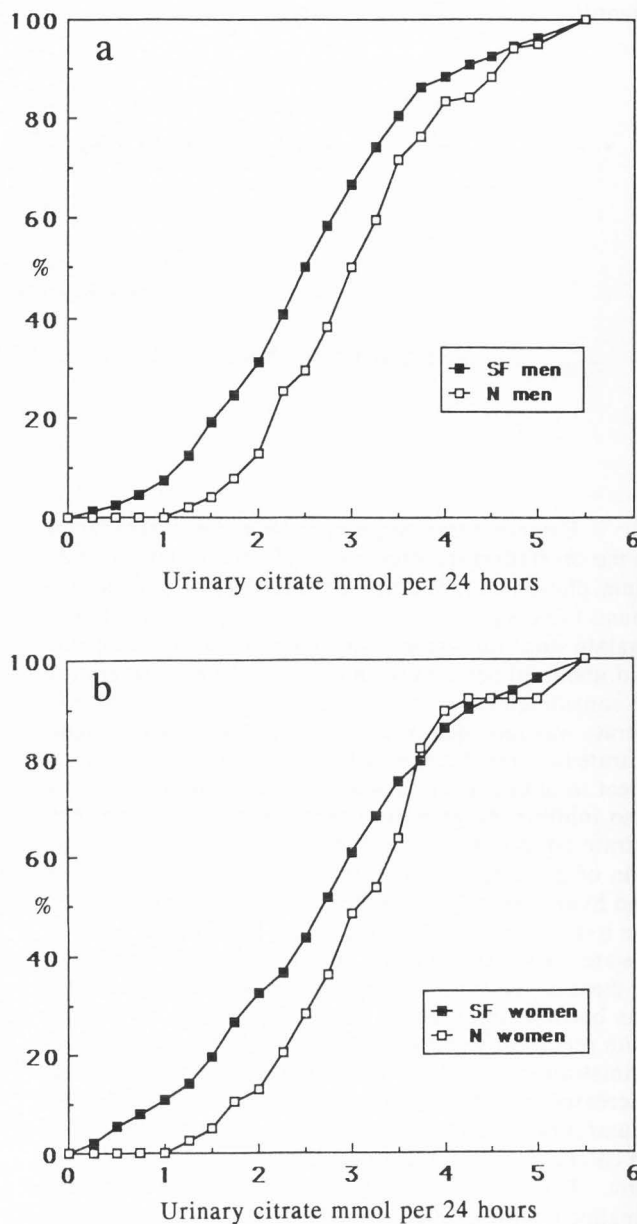

Urinary citrate mmol per 24 hours

Urinary citrate mmol per 24 hours

Figure 1. Cumulative frequency distribution curves of 24 hour urinary excretion of citrate in normal (N) men and stone-forming (SF) men (**a**) and normal women and stone-forming women (**b**).

In terms of creatinine related urinary excretion, 13 percent of the normal men had values below 125 mmol per mol of creatinine whereas 13 per cent of the normal women had values below 175. Corresponding fractions of stone forming men and women were 33 and 29 per cent, respectively. The mean (SD) values were 190 (68) and 287 (181) mmol per mol of creatinine for normal men and women, and 191 (95) and 271 (129) for stone forming men and women. There was no significant difference between stone formers and normal subjects in this respect. If we thus define the normal range
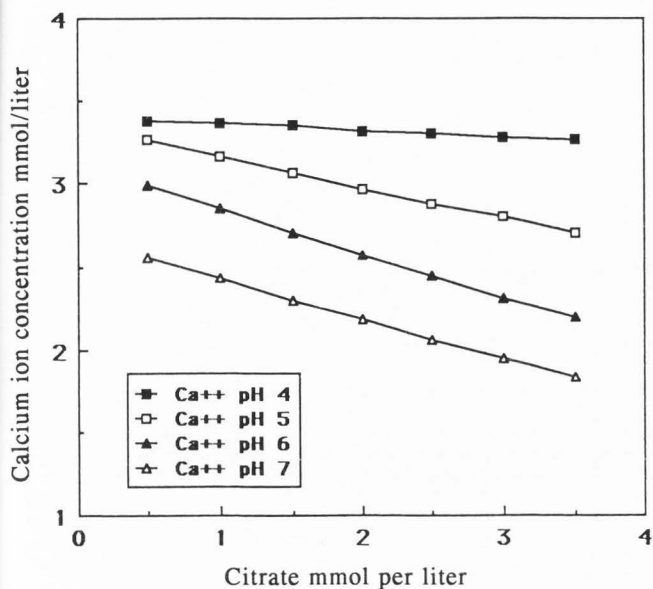
Figure 2. The relationship between pH and the free calcium ion concentration in a solution which contained: 200 mmol of sodium, 80 mmol of potassium, 5 mmol of calcium, 3 mmol of magnesium, 0.25 mmol of oxalate, 30 mmol of phosphate, 20 mmol of sulfate and 20 mmol of ammonium, per liter.

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so that it includes 90 per cent of the normal subjects, approximately 30 per cent of stone formers can be considered as hypocitraturic. This figure is in good agreement with results reported by others.

Citrate forms stable and soluble complexes with calcium ions and is therefore efficient in modifying the free calcium concentration, a factor of great importance for the ion-activity products of both CaOx and CaP. The effect of citrate concentrations on the free calcium ion concentration at different pH-levels is demonstrated in Figure 2. The complex formation is greatest at alkaline pH, as a result of the increased dissociation of citrate. At pH 6 an increment of citrate from 1.5 to 3.0 mmol/l resulted in a 15 per cent reduction of the Ca^{++} concentration. If pH simultaneously is increased to 7, the reduction will be 28 per cent. The most frequently encountered urine abnormality in calcium stone formers is hypercalciuria. For this reason, and in view of the important complex formation between calcium and citrate, it might be of interest to consider the urinary calcium/citrate (Ca/Cit) ratio in urine (Butz, Knispel, 1984; Hodgkinson, 1962).

The cumulative frequency distribution curves of Ca/Cit-ratios (Figure 3) clearly show high values in stone formers. Whereas only 6 per cent of normal subjects had a ratio above 3.0, as many as 35 per cent of the stone formers had greater values.

The mean (SD) Ca/Cit ratios were in stone-forming men and women 3.33 (3.09) and 3.16 (4.76), respectively. The corresponding figures for normal subjects
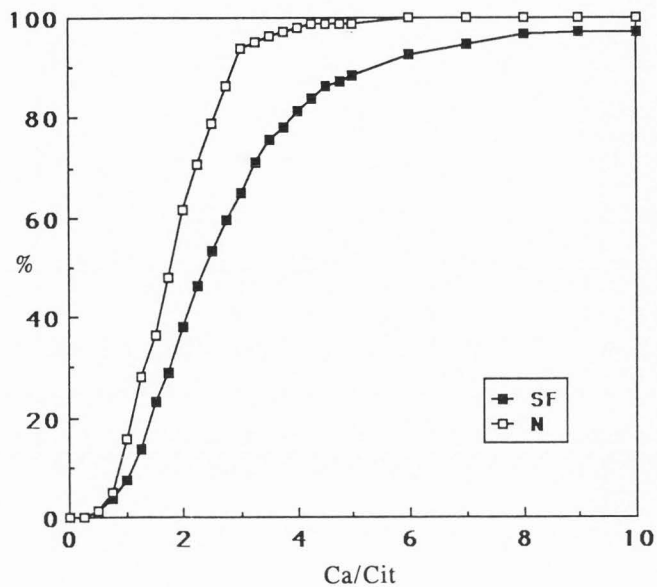

Figure 3. Cumulative frequency distribution curves of calcium/citrate-ratios in normal subjects and stone-formers (1389 urine samples).

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were 1.96 (0.91) and 1.66 (0.77). The ratios were significantly higher in both stone-forming men (p < 0.001) and women (p < 0.05).

Parks and Coe (1986) found that analysis of urinary calcium and citrate offered the best possibilities to distinguish between normal subjects and stone formers. Based on discriminant analysis they formulated a calcium-citrate index by means of which a better separation between the groups was obtained, than with any other measurement including direct measurement of the supersaturation. The distribution pattern of calcium-citrate discriminant score was very similar to that obtained with the Ca/Cit-ratio determinations in our study.

Diurnal Excretion Pattern of Citrate

The citrate excretion during the day is only subject to minor variations and a comparison between the excretion of citrate per mol of creatinine in 24 hour urine correlated very well with that recorded in a 4 hour urine sample collected between 0600 and 1000 hours (Berg, et al., 1987). This might be clinically important by allowing for appropriate determination of the citrate level even in short-term urine collections. Higher citrate levels were observed during daytime (Figure 4) and are certainly explained by the increased pH after meals. A low level was found during the night hours (Tiselius, 1981a; Ahlstrand et al., 1984).

The diurnal excretion of citrate was also studied by Bach et al. (1980), who observed a high citrate excretion during the day and a low excretion during the night. Similar to the results in Figure 4, they recorded peak citrate concentrations in the morning and the lowest levels during the late night hours.
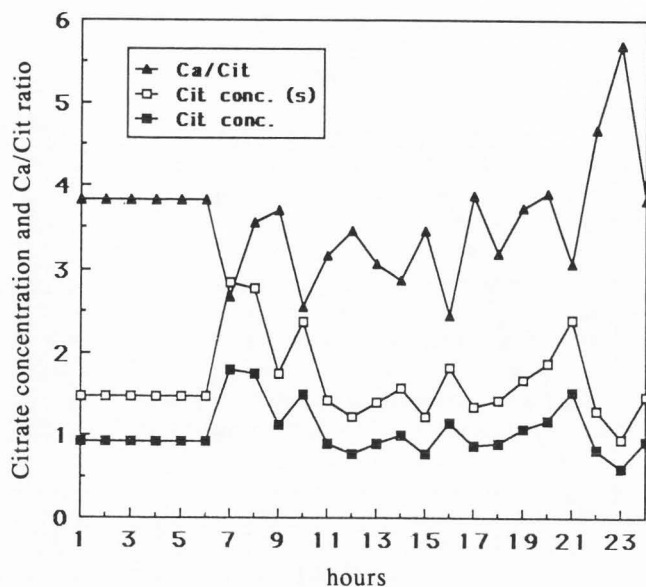
Figure 4. Diurnal variation in the urinary excretion of citrate and calcium/citrate-ratios. The citrate concentrations were calculated for a 24 hour urine volume of 1500 ml.

### Effects of Citrate on CaOx Supersaturation

Variations in citrate concentrations have significant effects on the ion-activity product of CaOx (AP_{CaOx}). A relationship between changes in citrate excretion and AP_{CaOx} was calculated by means of the EQUIL 2 program (Werness *et al.*, 1985) in a solution which at pH 6.0 and in a volume of 1500 ml contained 6.5 mmol of calcium, 4.5 mmol of magnesium, 0.3 mmol of oxalate, 200 mmol of sodium, 70 mmol of potassium, 25 mmol of phosphate, 30 mmol of sulfate, and 35 mmol of ammonium. With this urine composition, an increment of 24 hour urinary citrate from 2 to 4 mmol resulted in a AP_{CaOx} reduction from 1.37×10^{-8} to 1.26×10^{-8} $(mmol/l)^2$.

Citrate was more efficient than magnesium in changing AP_{CaOx}. The exponent for citrate was -0.22 and that for magnesium -0.12 (Tiselius, 1991).

Citrate affects AP_{CaOx} by complex formation with calcium, and the citrate response is therefore more pronounced in alkaline solutions. This is clearly shown in Figure 5. In a similar way, citrate influences the ion-activity product of brushite, a salt that might be of importance particularly for formation of stones containing both CaOx and CaP (Pak and Holt, 1976) (Figure 6).

Effects of Citrate CaOx Crystal Growth Inhibition

It has been reported by several authors that citrate in low concentrations is capable of inhibiting CaOx crystal growth in metastably supersaturated salt solutions (Meyer and Smith, 1975; Ryall *et al.*, 1981a; Tiselius *et al.*, 1981). The question can be raised, however, to what extent does citrate contribute to the crystal growth
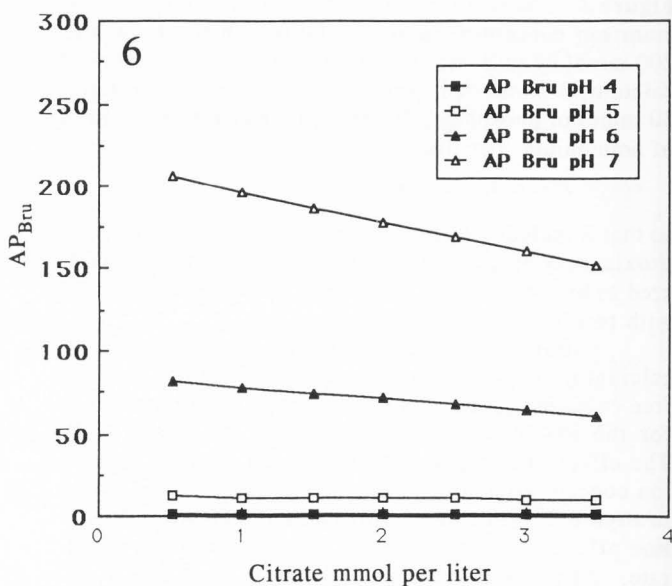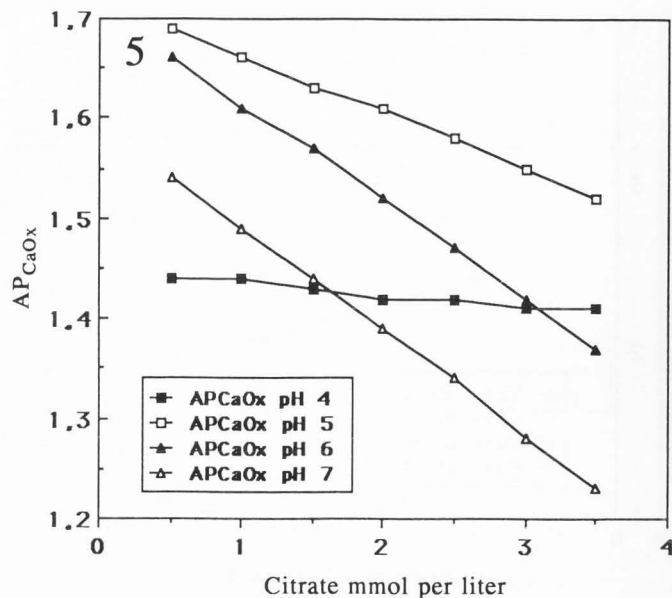
Figures 5 and 6. The effects of citrate on the ion-activity product of CaOx (AP_{CaOx}) (**Figure 5**) and brushite (AP_{Bru}) (**Figure 6**) at pH 4, 5, 6, and 7.

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inhibition in the presence of the high concentrations of powerful macromolecules in urine (Tiselius *et al.*, 1987; Bek-Jensen and Tiselius, 1991). To address this question, direct determination of the crystallization was carried out in whole urine samples after addition of different amounts of citrate. As could be expected, increased concentrations of citrate resulted in a reduced crystallization risk, when determined by the oxalate addition required for formation of 100 crystals in the size-range of 3.5-5 μm (Tiselius, 1985).
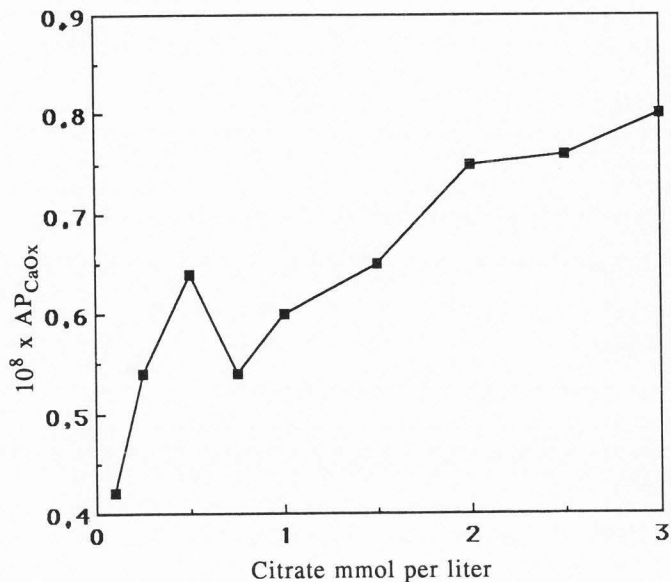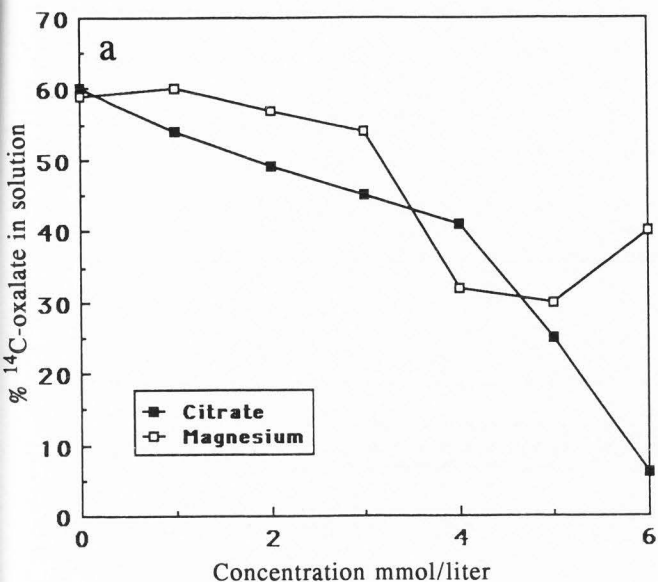
Figure 8. The ion-activity product of CaOx for the same precipitation of calcium oxalate in relation to the citrate concentration. Crystal growth was determined 30 minutes after addition of seed crystals by measuring the fraction of ^{45}Ca remaining in solution.
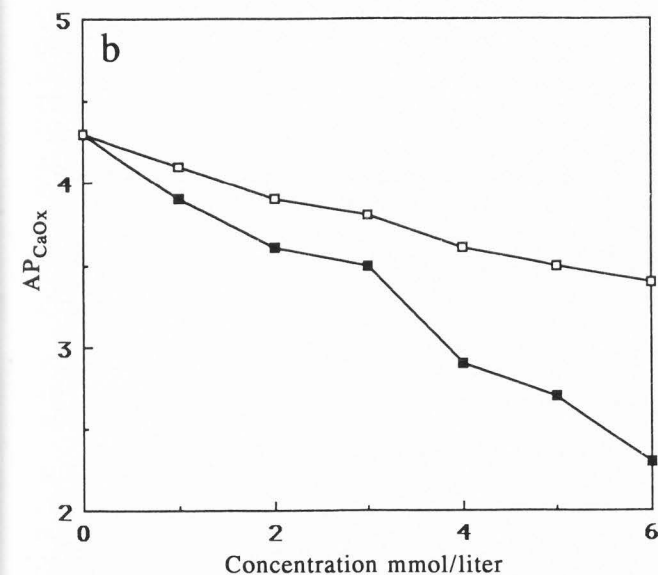

Figure 7. Effects of different concentrations of citrate and magnesium on the crystallization of calcium oxalate in a salt solution as determined from the fraction of ^{14}C-oxalate remaining in solution 60 minutes after supersaturation (**a**) and on the ion-activity product (**b**).

The effect of citrate, as well as that of magnesium, was also determined in salt solutions brought to crystallization by supersaturation to an ion-activity product of 4.25×10^{-8} $(mol/l)^2$ with addition of sodium oxalate. As shown in Figure 7, there appeared to be a close relationship to the effects these compounds had on the supersaturation level in the sample. In an attempt to study this problem further, the crystal growth in samples containing 90 per cent of dialysed urine and CaOx seed crystals, was followed in the presence of various citrate

concentrations at different levels of supersaturation accomplished by the addition of oxalate (Tiselius *et al.*, 1987). The growth of CaOx was determined by measuring the amount of (^{45}Ca) remaining in solution in the samples 30 minutes after addition of the seed. A reduced rate of calcium oxalate precipitation was recorded with increased citrate concentrations. In order to determine whether this effect on crystallization reflected a direct inhibitory action of citrate or only was the result of the altered supersaturation, a comparison of the AP_{CaOx} at corresponding degrees of calcium precipitation was calculated. The AP_{CaOx} was calculated with the EQUIL 2 program. As is evident from Figure 8, the same CaOx precipitation was observed at increasing levels of AP_{CaOx} as the citrate concentration was increased. This indicates that citrate, even in the presence of whole urine concentrations of macromolecules, has the capacity to inhibit the growth of CaOx crystals (Tiselius, unpublished observation).

It is reasonable to assume that the effect of an increased pH on crystal growth, that has been demonstrated in diluted urine (Tiselius, 1981b), is also valid for whole urine.

Inhibition of Crystal Aggregation

It can be assumed that the process of renal stone formation includes a rapid formation of large crystal masses. This is accomplished by aggregation of crystals in a way that enables their entrapment in the distal part

of the collecting system. In this way, the initial crystal formation might result in a stone nidus, that can increase in size to a clinically significant stone by further growth and deposition of crystals. It has also been shown that the size of crystals and crystal aggregates is greater in stone formers than in normal subjects (Robertson *et al.*, 1971). Several authors have also suggested that the inhibition of crystal aggregation can be the most important discriminating risk factor between normal subjects and patients with calcium stone disease (Azoury *et al.*, 1987; Coe *et al.*, 1991; Kok *et al.*, 1986; Springmann *et al.*, 1986).

The assessment of crystal aggregation is not easy because of the simultaneous growth and aggregation that occurs in supersaturated solutions. Different methods can, however, give important information in this respect. A detailed mathematical analysis of the change in crystal size distribution over time was used by Ryall *et al.* (1981), whereas the conclusions drawn by Kok *et al.* (1986, 1991) were based on kinetic analyses of the course of CaOx crystallization. In a nice experimental system, Hess *et al.* (1989) demonstrated the inhibitory properties of urinary macromolecules on COM aggregation by measuring the rate of crystal sedimentation after a period of slow stirring.

We used a system similar to that of Hess *et al.* (1989), in which 0.5 ml of citrate solutions and dialysed urine were added to 14.5 ml of suspensions containing 3 mg per liter of calcium oxalate monohydrate in 90 mmol per liter sodium chloride and 10 mmol per liter Tris-buffer at pH 7.2. Following a 3 minute period of stirring, the sedimentation rate was followed by measuring the absorption at 690 nm in a Perkin-Elmer Lambda 2 spectrophotometer during the following 5 minutes. In this system, we were able to confirm a reduced rate of crystal sedimentation in the presence of urinary macromolecules by adding dialysed urine in different concentrations (Tiselius *et al.*, unpublished observations). The reduced rate of crystal sedimentation is assumed to reflect the state of aggregation because small crystals were found to sediment slower than large crystals (Hess *et al.*, 1989).

In contrast to the findings by Hess *et al.* (1989), however, we observed a pronounced reduction of crystal sedimentation when the crystal suspensions were preincubated with citrate in concentrations corresponding to those in whole urine. The effects of citrate in concentrations between 0.8 and 3.3 mmol/l at a pH of 7.2 is shown in Figure 9. The addition of citrate in the presence of dialysed urine resulted in a reduction of the sedimentation rate that was higher than that observed for citrate, but lower than that with only dialysed urine. Preliminary results indicated that an interaction between citrate and urinary macromolecules on the crystal surface might explain this phenomenon, but further studies in this respect are necessary. These results indicate that studies on isolated macromolecules (Felix *et al.*, 1977; Koide *et al.*, 1981), might give results different from those met under physiological conditions.
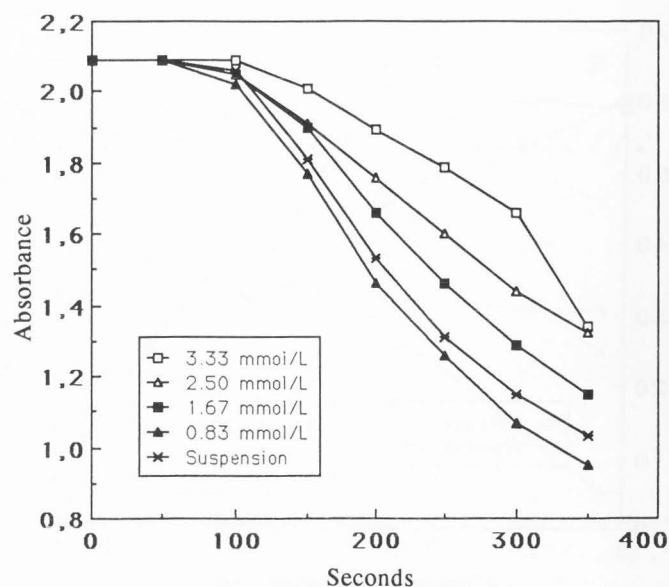

Figure 9. The sedimentation of calcium oxalate monohydrate crystals at pH 7.2 in solutions containing citrate in the concentration range of 0.8 to 3.3 mmol/l.

The demonstrated effect of citrate on crystal aggregation gives direct support to the findings by Kok *et al.* (1986; 1991) that citrate is a very potent inhibitor of COM crystal aggregation. The reason for the contrasting results, in comparison with those reported by Hess *et al.* (1989), has not been analyzed in detail, but differences in citrate concentrations, length of preincubation period, amount of crystals, and rate of stirring might have affected the result.

The Effects of Citrate on CaP Crystallization

A majority of calcium stones contain CaP in addition to CaOx (Herring, 1962; Otnes, 1983; Leusmann *et al.*, 1990; Öhman *et al.*, 1992) and there are certain indications that patients with recurrent stone disease more frequently have a high content of CaP stones than those who only have formed one stone (Tiselius and Larsson, unpublished observation). The crystallization of CaP might be an obvious risk-factor in alkaline urine, a fact that cannot be ignored in association with prophylactic treatment with alkaline citrate.

Studies on the crystal formation at different pH levels disclosed, that although there was a pronounced increment in CaP crystallization at high pH levels, the crystals were usually small (Tiselius, 1984). It is well known that citrate is an efficient inhibitor of CaP crystal growth (Bisaz *et al.*, 1978; Fleisch, 1980) and several experiments have shown that citrate is capable of efficiently inhibiting the heterogenous crystallization of CaOx on a hydroxyapatite surface (Berg and Tiselius, 1989; Grases *et al.*, 1989).

There is, however, only limited information available concerning the effects of citrate on CaP crystal aggregation. For this reason, we measured the effects of citrate on the sedimentation rate of brushite and hydroxyapatite crystals in a way similar to that described above for CaOx. The results showed that citrate also efficiently reduced the sedimentation rate of these crystals (Tiselius et al., unpublished observation). This information is encouraging for the further use of alkaline citrate in the preventive treatment of patients with recurrent calcium stone disease.

Treatment of Patients with Recurrent Calcium Stone Disease with Alkaline Citrate

Several authors have successfully used alkaline citrate preparations in prevention of recurrent calcium oxalate stone formation. Different preparations were administered, such as sodium potassium citrate, potassium citrate, and magnesium citrate (Butz, 1982; Pak et al., 1985; Preminger et al., 1988; Schwille et al., 1987). A 24 hour dose of 10-43 mmol given on two or three occasions appeared to be an efficient pharmacological therapy with pronounced effects on stone formation. The rational for this therapy is the increment in urinary citrate that is achieved with these preparations. In view of the risk situation that occurs during the late night and morning hours, with a low citrate concentration, a low pH, a low urine volume and a high Ca/Cit ratio, we found it reasonable to administer alkaline citrate in a single evening dose (Berg et al., 1992). That type of administration would accordingly counteract crystallization and stone formation during this particular risk period. Single dose administration might also result in a better patients' compliance than when the drug has to be administered several times a day.

A group of 39 men and 16 women with recurrent calcium oxalate stone disease were treated with 5.0 g (18 mmol) and 3.8 g (14 mmol) of sodium potassium citrate respectively, as a single evening dose. Their mean (SD) pretreatment 24 hour urinary excretion of citrate was 1.7 (1.2) mmol. In these patients mean (SD) urinary citrate increased from 138 (98) to 253 (127) mmol per mol of creatinine (p < 0.001), the Ca/Cit ratio decreased from 5.19 (3.88) to 2.07 (1.05) (p < 0.001) and the AP_{CaOx}-index, which is an estimate of the supersaturation, was reduced from 2.32 (0.83) to 1.45 (0.45) (p < 0.001). The clinical course of the disease was favorably affected by alkaline citrate and the number of stones formed during the treatment period up to 6 years [mean (SD) 3.5 (1.7)], was significantly lower than that recorded during a period of the same length following the first observation of a renal stone. The tolerance of the treatment was very good, certainly a result of the absence of side effects and the simplicity of drug-administration.

As could be expected, the mean (SD) AP(CaP) index, used as an estimate of CaP supersaturation, increased in morning samples from 4.1 (7.5) to 14.4 (16.9), a reflection of the increased pH. A reliable estimate of this index could be obtained only in urine collected between 0600 and 1000 hours. There was, however, a pronounced variation in the level of AP(CaP) index, and a significant increment was recorded only in a group of patients given an evening dose of 7.5 g.

Further long-term follow-up studies with single dose administration of alkaline citrate are in progress. From these studies, it is particularly important to determine whether the effect on urinary pH, with this regimen, also is transient, as recently observed by Schwille et al. (1992), who gave 6 g of potassium citrate three times a day. Such an effect can theoretically reduce the therapeutic effect of alkaline citrate despite a maintained increment in the citrate excretion.

A lack of stone prevention was reported by Trinchieri et al. (1992) who followed 19 patients treated with 5-10 g of potassium citrate during 2 years. They assumed that this might be attributable to an unaffected high calcium excretion. In a randomized study by Hofbauer et al. (1992), 25 patients treated with three daily doses of alkaline citrate to get a pH around 7, were compared with 25 patients only given metaphylactic instructions. The citrate was significantly higher in the group with alkaline citrate therapy, but there were no differences in the course of stone formation between the two groups.

Conclusions

Urinary citrate affects CaOx as well as CaP-crystallization by reduction of the ion-activity products of these salts. The CaOx crystal growth is also inhibited by urinary citrate in a way that adds inhibitory power to that accomplished by urinary macromolecules. The heterogenous crystallization of CaOx on CaP is significantly inhibited by citrate. Increments in citrate concentrations result in a reduction of the aggregation of CaOx, brushite and hydroxyapatite crystals. Although the high pH associated with alkaline citrate treatment results in CaP crystallization, the concomitant citrate increment seems to be one of the most important factors in reducing the risk of this precipitation. Citrate thereby apparently both maintains the crystals small and prevents the heterogenous growth of CaOx on CaP. The different experimental findings are supported by the successful effects of alkaline citrate on the course of calcium stone disease. The optimal dose and best way of administration however, has to await further long term studies.

Acknowledgement

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Discussion with Reviewers

W.G. Robertson: The difference in the citrate excretion of stone-formers and normals in the authors' own study is very small and would be predicted to have a trivial effect on the crystallization parameters quoted. How do such small differences explain stone-formation in the authors' patients?

Authors: It is true that the difference between stone formers and normal subjects in terms of mean 24 hour citrate excretion is relatively small. Citrate excretion levels below 1.0 mmol per 24 hours were, however, only found in stone formers and these patients might

constitute a special risk group. There is no proof that a low citrate excretion alone can explain why a person becomes a stone-former. Probably, in many patients, a low urinary citrate is rather a contributing factor to an already established risk situation, whether caused by increased concentrations of oxalate or calcium, or decreased inhibitory activities. In addition, we know that a high supersaturation occurs during the night, at which time the pH also is low. The low pH results in a further reduction of citrate excretion together with a decreased dissociation. Such mechanisms might result in much reduced activity of citrate during a period when the crystallization is unusually high.

A Hesse: Is there any evidence that citrate therapy is effective in different ways in patients forming whewellite and weddelite stones, respectively?
Authors: We have so far not studied differences in crystallization properties between these two crystal phases. All our measurements have been carried out with calcium oxalate monohydrate, but we are currently exploring the possibilities to measure at least aggregation in suspensions of calcium oxalate dihydrate. A comparison between stone composition (determined with x-ray diffraction technique) and citrate excretion indicated that male patients with less than 1.5 mmol of citrate excreted during 16 hours (between 0600 and 2200 hours) had a higher content of calcium oxalate dihydrate in their stones. According to these preliminary results, 16 per cent of the patients with a low citrate excretion had more than 70 per cent of dihydrate in their stones, compared with 8 per cent in patients with a higher citrate excretion.

A Hesse: Does citrate therapy favor stone passage after extracorporeal shock-wave (ESWL)-treatment?
Authors: This is a very interesting and most important question because of the problems associated with residual fragments after shock wave lithotripsy. There are a few observations in the literature which indicate that this might be the case. We have no personal experience to support this assumption, but we have recently started a randomized study to address this question.

A Hesse: High citrate concentrations in urine inhibit crystal aggregation or aggregation of disintegrates after ESWL-treatment. What kind of reaction may be responsible for this observation?
Authors: Citrate might either bind to the crystal surface or in any other way interact with the electrical double layer surrounding the crystals and/or disintegrates. There is also a possibility that citrate changes the physicochemical properties of aggregation promoting substances in urine (e.g., Tamm Horsfall mucoprotein). Furthermore, can the growth inhibition reduce the risk of crystal bridge formation between adjacent fragments.

B. Hess: The apparent contradiction between the authors' studies and our results (Hess *et al.*, 1989) with respect to citrate as an inhibitor of calcium oxalate crystal aggregation, tested at almost identical conditions, might not really exist, if one considers that we never studied citrate at more than 1 mmol/l. At this concentration, citrate inhibited by 7 + 3 per cent (mean + SD) in our system; in the authors' study, citrate at 0.83 mmol/l marginally promoted, whereas it slightly inhibited crystal aggregation at 1.67 mmol/l. These findings seem fully compatible with our own results and demonstrate that only high physiologic concentrations of citrate inhibit calcium oxalate crystal aggregation under saturated conditions at pH 7.2.
Authors: We agree completely with this comment. It is reasonable to assume that citrate concentrations above 1 mmol/l are necessary to get measurable effects on the crystal aggregation in this experimental setting. For appropriate evaluation of the definite role of citrate in terms of aggregation inhibition under physiological conditions, it is certainly necessary to improve the method by decreasing the concentration of crystals in the suspension and to carry out the measurements at pH levels representative for that of urine in different parts of the collecting system. Such work is in progress.