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H.-G. Tiselius

Estimated levels of supersaturation with calcium phosphate and calcium oxalate in the distal tubule

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Abstract Approximate estimates of the ion-acitivity products of calcium phosphate and calcium oxalate in distal tubular urine were derived from the 16-h urinary excretion of calcium, oxalate, citrate, magnesium and phosphate. Urine variables were obtained from 96 normal subjects and 277 calcium stone formers and the calculations were carried out with iterative approximation using the EQUIL2 program. With respect to other ions of importance for the ion-activity products, the urine was assumed to have a fixed composition with pH 6.45. Significantly higher ion-activity products of both calcium phosphate and calcium oxalate were recorded in stone formers. It was concluded that diurnal variations in urine composition and pH might result in peaks of calcium phosphate supersaturation in distal tubular urine whereby a crystallization can occur. In association with abnormalities in terms of promotion and inhibition of calcium salt crystallization, such a precipitation can be of importance for the subsequent formation of calcium renal stones.

Key words Calcium oxalate · Calcium phosphate · Ion-activity product · Renal tubule · Urine · Renal stone disease

The majority of patients with calcium renal stone disease form stones containing both calcium oxalate (CaOx) and calcium phosphate (CaP) [25, 31]. Under physiological conditions, however, the urine supersaturation with respect to CaOx is never sufficiently high to result in a homogeneous nucleation and a promoter is likely to contribute to the precipitation of this salt [30]. Accordingly, it has been suggested that crystals of CaP might constitute one necessary promoter for starting a crystallization of CaOx [3, 6, 11, 29, 32, 34, 47].

Both brushite and hydroxyapatite can induce heterogeneous nucleation of CaOx [4, 5, 14, 29, 33, 35]. Recent experiments have also shown that spherulites of CaP developed under physiological conditions have a close similarity with crystals found in the nucleus of mixed CaOx/CaP stones and that such spherulites are capable of inducing CaOx crystal growth [1].

It is reasonable to assume that the initial steps in most forms of calcium stone disease take place in the nephron. The physicochemical properties at nephron levels above the collecting duct undoubtedly favour the precipitation of CaP [8, 9, 21, 27]. It has thus been suggested that a precipitation of CaP might occur either in the thin segment of the loop of Henle [8, 9] or in the distal tubules [27]. Precipitates of CaP have also been demonstrated with scanning electron microscopy in both human [16] and animal [18] tubules .

From our previously published experiments with solutions given a composition approximately corresponding to that in different parts of the nephron [27], it was concluded that during periods with an increased excretion of calcium, urine in the distal part of the distal tubule might be at the greatest risk of crystallization with CaP, whereas in the lower part of the collecting ducts and in the final urine, the risk of CaOx crystallization prevails.

In view of these findings it can be hypothesized that a crystallization of CaP in the distal tubule might be of importance for initiating calcium stone formation. If this is so the biochemical risk evaluation in calcium stone formers should accordingly include information on the crystallization properties in the distal tubular urine. The present study reports approximate ion-activity products of CaOx and different CaP salts in distal tubular urine as derived from the amounts of calcium, oxalate, citrate, magnesium and phosphate excreted in urine from calcium stone formers and normal subjects.

H.-G. Tiselius (✉)
Department of Urology and the Clinical Research Centre, University Hospital, and the Faculty of Health Sciences, Linköping, Sweden

Materials and methods

Urine variables

The excretion of calcium, oxalate, citrate, magnesium and phosphate in 16-h urine samples was analysed on a routine basis and the variables used in the calculations. Urine had been collected between 0600 and 2200 hours in bottles containing 15 ml of 6 mol/l hydrochloric acid and the analysis was carried out with methods described in detail elsewhere [15, 24, 26, 44, 45]. A complete set of analytical data were available in urine from 96 normal subjects [51 men, NM, and 45 women, NW) and 277 calcium stone formers (179 men, SFM, and 98 women, SFW).

Assumptions on the composition in the distal part of the distal tubule

The main theoretical basis and the considerations relating to the average ion composition of urine in the distal part of the distal tubule (DTd) have previously been presented in detail [27]. The concentrations of calcium, oxalate, citrate, magnesium and phosphate in DTd urine were calculated for a 16-h urine flow of 6000 ml through this part of the nephron. It was thereby assumed that the amounts of calcium and magnesium at this level were 1.48 and 1.10 times higher than those in final urine as a result of reabsorption in the collecting duct. Oxalate, citrate and phosphate were considered unaffected by the passage through the collecting ducts [22, 27]. Other important ions were assumed to have the following concentrations: sodium 96 mmol/l, potassium 58 mmol/l, sulphate 13.8 mmol/l and urate 0.42 mmol/l. The average pH was set to 6.45 [36].

Ion-activity products

The ion-activity products of calcium oxalate (AP_{CaOx}), hydroxy apatite (AP_{HAP}), octa calcium phosphate (AP_{OCP}), amorphous calcium phosphate (AP_{ACP}) and brushite (AP_{Bru}) were calculated by means of the EQUIL2 program [46]. The formulas used for calculation of the different ion-activity products were as follows [43]:

$$AP_{CaOx} = C_{Ca^{2+}} \cdot C_{Ox^{2-}} \cdot [f_2]^2$$

$$AP_{HAP} = [C_{Ca^{2+}}]^5 \cdot C_{OH^-} \cdot [C_{PO_4^{3-}}]^3 \cdot f_1 \cdot [f_2]^5 \cdot [f_3]^3$$

$$AP_{OCP} = [C_{Ca^{2+}}]^4 \cdot 10^{-pH} \cdot [C_{PO_4^{3-}}]^3 \cdot f_1 \cdot [f_2]^4 \cdot [f_3]^3$$

$$AP_{Bru} = C_{Ca^{2+}} \cdot C_{HPO_4^{2-}} \cdot [f_2]^2$$

in which f_1, f_2 and f_3 are the activity coefficients for ions with valances of one, two and three, respectively, and C represents the concentration of the free ion. For ACP we used the following formula [7]:

$$Ca(PO_4)_{0.74}H_{0.22}$$

and accordingly the ion-activity product:

$$AP_{ACP} = C_{Ca^{2+}} \cdot [10^{-pH}]^{0.22} \cdot [C_{PO_4^{3-}}]^{0.74} \cdot f_2 \cdot [f_1]^{0.22} \cdot [f_3]^{0.74}$$

The mathematical form of the ion-activity products explains their numerical levels and the differences recorded between the different salts.

In addition to the ion-activity products listed above a simplified expression of the ion-activity product of CaP was derived (AP_{CaP}):

$$AP_{CaP} = C_{Ca^{2+}} \cdot f_2 \cdot C_{PO_4^{3-}} \cdot f_3$$

Estimates of ion-activity products of CaOx and CaP in 16-h urine

The following formula was used for deriving an approximate estimate of the ion-activity product of CaOx in 16-h urine [39, 42]:

$$AP(CaOx)index = \frac{2.3 \cdot Calcium^{0.84} \cdot Oxalate}{Citrate^{0.22} \cdot Magnesium^{0.12} \cdot Volume^{1.03}}$$

The urine variables are expressed as mmol excreted during 16 h and the urine volume during this period in litres. The factor 2.3 is specific for a collection period of 16 h. A standardized index calculated for a 16-h urine volume of 1000 ml thereby had the following form [41, 42]:

$$AP(CaOx)index(s) = \frac{2.3 \cdot Calcium^{0.84} \cdot Oxalate}{Citrate^{0.22} \cdot Magnesium^{0.12}}$$

A standardized expression of the ion-activity product of CaP was obtained for a 16-h urine volume of 1000 ml and a pH of 7.0 as follows [40]:

$$AP(CaP)index(s) = \frac{3.0 \cdot 10^{-3} \cdot Calcium^{1.07} \cdot Phosphate^{0.70} \cdot (7.0 - 4.5)^{6.8}}{Citrate^{0.20}}$$

The factor $3.0 \cdot 10^{-3}$ is specific for a collection period of 16 h [43] and with a urine volume of 1 l the volume factor in the denominator is equal to 1.0.

Spontaneous crystallization of calcium phosphate

The propensity of spontaneous CaP crystallization in samples with an ion composition similar to that in DTd was varied by increasing the pH and the concentration of calcium. The composition of those samples in which the first indication of crystallization was recorded, either by particle appearance in the size range above 3.5 μm in the Coulter counter or by consumption of ^{45}Ca, was used to calculate a rough estimate of AP_{CaP}. Aliquots for assessment of crystallization were drawn 1, 6, 12 and 24 h after preparation of the experimental solution. During this period the samples were continuously stirred.

Statistical analysis

The different variables and parameters were expressed as means and SD. Group comparison was carried out with Student's t-test and regression analysis with standard methods using Statistica software (StatSoft, Tulsa, Oklahoma, USA).

Results

The ion-activity products of different CaP salts and CaOx in DTd urine derived from 16-h urine variables are shown in Table 1. AP_{ACP}, AP_{OCP}, AP_{Bru}, AP_{CaP} as well as AP_{CaOx} were significantly higher in SFM than in NM. Although the mean AP_{HAP} was numerically higher in SFM than in NM, the difference was not statistically significant. In women significantly higher ion-activity products of ACP, CaP and CaOx were recorded in SFW, whereas AP_{OCP}, AP_{HAP} and AP_{Bru} did not differ significantly from the levels in NW. The AP_{CaP} levels recorded in NM and SFM are shown by the cumlative frequency distributions curves in Fig. 1 and those in NW and SFW in Fig. 2.

A comparison between NM and NW disclosed a slightly higher AP_{Bru} ($P = 0.031$), a higher AP_{CaOx} ($P =$

Table 1 Mean (SD) ion-activity products of calcium phosphates and calcium oxalate in distal tubular urine calculated by extrapolation from the composition of the 16 h urine in stone forming and normal subjects.

Variable	Normal men	Stone-forming men	Significance of difference	Normal women	Stone-forming women	Significance of difference
	n = 51	n = 179	–	n = 45	n = 98	–
$10^{12}\cdot AP_{ACP}$	1.36 (0.70)	1.85 (0.87)	p = 0.0003	1.18 (0.50)	1.63 (0.87)	p = 0.0013
$10^{43}\cdot AP_{OCP}$	9.96 (25.69)	27.94 (51.37)	p = 0.017	3.73 (5.96)	24.9 (108.3)	p = 0.193
$10^{50}\cdot AP_{HAP}$	35.1 (123.4)	105.9 (266.3)	p = 0.067	4.40 (9.72)	43.4 (194.5)	p = 0.181
$10^{8}\cdot AP_{Bru}$	5.62 (3.97)	7.32 (4.83)	p = 0.022	4.15 (2.26)	5.23 (4.42)	p = 0.124
$10^{14}\cdot AP_{CaP}$	0.88 (0.63)	1.13 (0.73)	p = 0.031	0.60 (0.35)	0.82 (0.70)	p = 0.049
$10^{9}\cdot AP_{CaOx}$	1.29 (0.80)	1.85 (0.96)	p = 0.0002	0.92 (0.46)	1.44 (0.81)	p = 0.0001

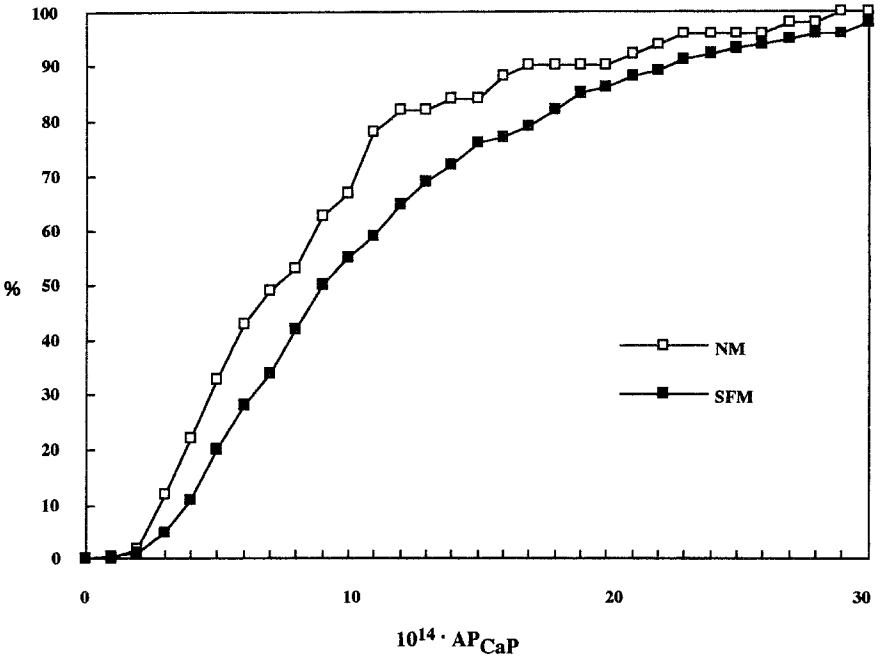

Fig. 1 Cumulative frequency distribution curves of $10^{14}\cdot AP_{CaP}$ derived from the 16-h excretion of calcium, oxalate, citrate, magnesium and phosphate in 51 NM and 179 SFM. For detailed explanation see "Materials and methods"

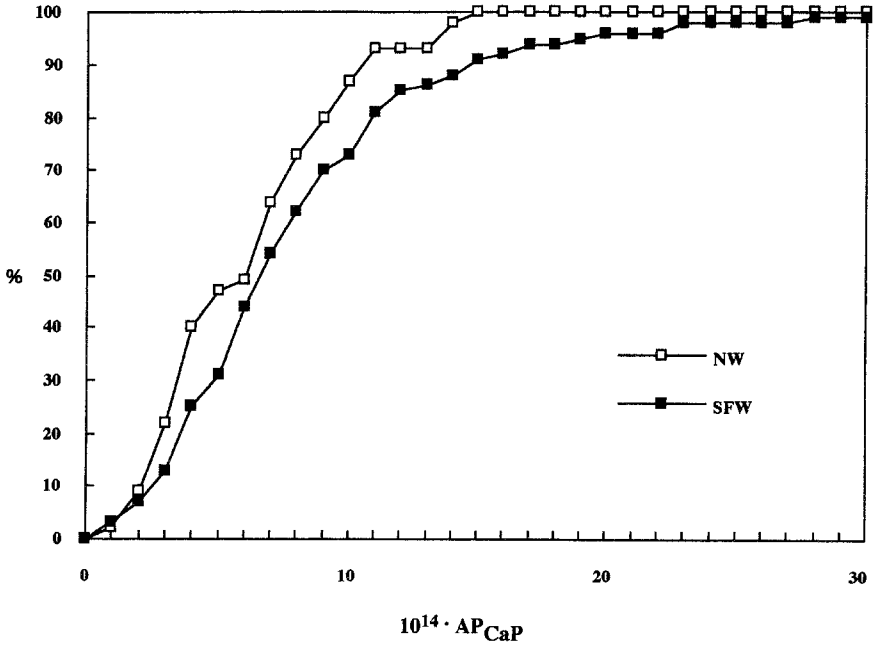

Fig. 2 Cumulative frequency distribution curves of $10^{14}\cdot AP_{CaP}$ derived from the 16-h excretion of calcium, oxalate, citrate, magnesium and phosphate in 45 NW and 98 SFW. For detailed explanation see "Materials and methods"

0.009) and a higher AP_{CaP} ($P = 0.009$) in NM, but there were no differences in terms of AP_{ACP} ($P = 0.149$), AP_{OCP} ($P = 0.116$) or AP_{HAP} ($P = 0.099$). In contrast SFM had significantly higher levels of AP_{ACP} ($P = 0.05$), AP_{Bru} ($P = 0.0004$), AP_{HAP} ($P = 0.04$), AP_{CaP} ($P = 0.009$) and AP_{CaOx} ($P = 0.0004$) than SFW.

Significantly higher levels of $AP(CaOx)$ index, $AP(CaOx)$ index(s) and $AP(CaP)$ index(s) were recorded in 16-h urine from both SFM and SFW in comparison with NM and NW (Table 2). $AP(CaOx)$ index was also higher in NM than in NW ($P = 0.0015$) and in SFM compared with SFW ($P = 0.034$). Higher $AP(CaOx)$ index(s) values were recorded in NM and SFM in comparison with NW and SFW ($P = 0.003$ and $P = 0.008$, respectively). Similarly, $AP(CaP)$ index(s) was higher in NM than in NW ($P = 0.02$) and in SFM compared with SFW ($P = 0.0004$).

As could be expected, AP_{CaP} in DTd urine was positively correlated with AP_{ACP} ($r = 0.87$; $P < 0.00001$), AP_{OCP} ($r = 0.68$; $P < 0.00001$), AP_{HAP} ($r = 0.80$; $P < 0.00001$) and AP_{CaOx} ($r = 0.75$; $P < 0.00001$). These good correlations are explained by the strong relationship between the derived calcium ion concentration and AP_{CaP} ($r = 0.87$; $P < 0.00001$). Although also significantly correlated ($P < 0.00001$), the coefficient of correlation was only 0.44 between the oxalate ion concentration and AP_{CaOx}, whereas the correlation between the calcium ion concentration and AP_{CaOx} was better ($r = 0.82$; $P < 0.00001$).

A close correlation between AP_{CaP} and the ion-activity products of *ACP*, *OCP*, *HAP*, and *Bru* obviously renders the former expression suitable for expressing approximately the supersaturation with CaP salts in DTd urine. In this respect, it is noteworthy that the standardized estimate of the ion-activity product of CaP [$AP(CaP)$ index(s)] in 16-h urine correlated well with AP_{CaP} calculated for DTd ($r = 0.98$; $P < 0.00001$). The equation for the regression line for this relationship had the following form:

$$10^{14} \cdot AP_{CaP} = -0.96 + 0.1996 \cdot AP(CaP) \text{ index(s)}$$

In accordance with the close relationship between AP_{CaP} and the ion-activity products of the different CaP salts and the good correlation between AP_{CaP} in DTd and $AP(CaP)$ index(s) in 16-h urine, good correlations were also recorded between the ion-activity products of the various CaP phases calculated in DTd urine and the 16-h $AP(CaP)$ index(s) in final urine. The following regression equations were obtained:

$$10^{12} \cdot AP_{ACP} = 0.47 + 0.022 \cdot AP(CaP) \text{ index(s)} \quad (r = 0.93)$$

$$10^{8} \cdot AP_{Bru} = -0.52 + 0.13 \cdot AP(CaP) \text{ index(s)} \quad (r = 0.98)$$

$$10^{43} \cdot AP_{OCP} = -48.4 + 1.33 \cdot AP(CaP) \text{ index(s)} \quad (r = 0.70)$$

$$10^{50} \cdot AP_{HAP} = -182.2 + 4.74 \cdot AP(CaP) \text{ index(s)} \quad (r = 0.77)$$

The less perfect correlation between $AP(CaP)$ index(s) and AP_{OCP} and AP_{HAP} was explained by the fact that these two relations were non-linear. No major differences were recorded when the regression analysis was carried out separately for SF and N .

The ion-activity products in DTd were all calculated for a pH of 6.45. Due to the pronounced influence of pH on the supersaturation with CaP salts, pH deviations from 6.45 will strongly influence the crystallization properties. Figures 3 and 4 show the effect of variations in pH on AP_{CaP} in DTd as derived from the mean urine excretion of calcium, oxalate, citrate, magnesium and phosphate in 16-h urine from NM and SFM as well as from NW and SFW.

Table 3 summarizes the solubility products and formation products of ACP, OCP, HAP, Bru and CaOx reported in the literature [7, 10, 23, 37] and the median and range of the ion-activity products recorded in SF and N at pH 6.45. The ion-activity products for OCP and HAP were clearly above the solubility products and in the case of OCP the ion-activity product also exceeded the reported formation product. The AP_{ACP} levels were slightly below the apparent solubility product [7], but

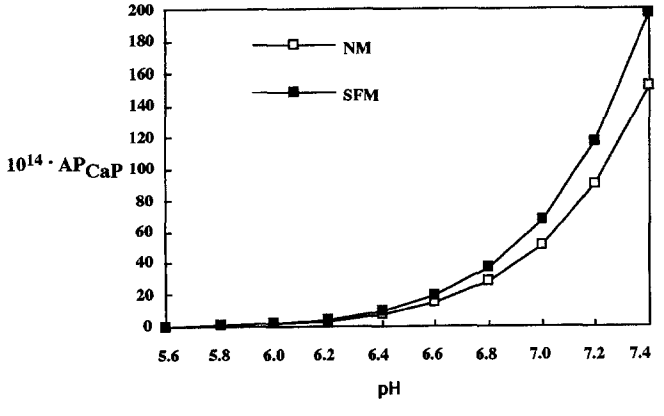

Fig. 3 Relationship between pH and $10^{14} \cdot AP_{CaP}$ in DTd urine. The AP_{CaP} values were derived from the mean 16-h excretion of calcium, oxalate, citrate, magnesium and phosphate in 51 NM and 179 SFM

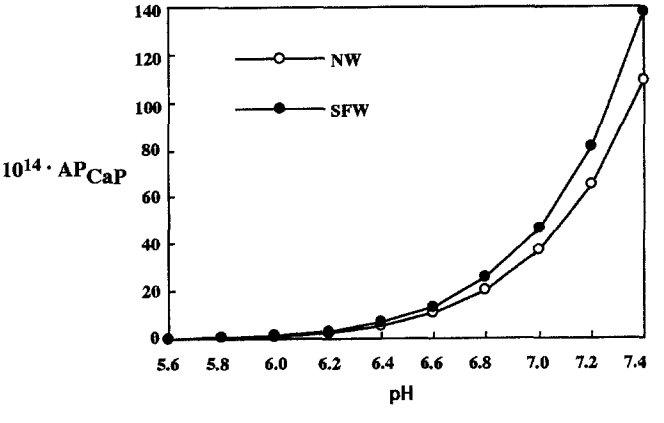

Fig. 4 Relationship between pH and $10^{14} \cdot AP_{CaP}$ in DTd urine. The AP_{CaP} values were derived from the mean 16-h excretion of calcium, oxalate, citrate, magnesium and phosphate in 45 NW and 98 SFW

Table 2 Mean (SD) AP(CaOx) index, AP(CaOx) index(s) and AP(CaP) index(s) in 16 h urine

Index	Normal men	Stone-forming men	Significance of difference	Normal women	Stone-forming women	Significance of difference
AP(CaOx) index	1.27 (0.59)	1.97 (1.38)	$p = 0.0005$	0.91 (0.49)	1.65 (0.85)	$p < 0.00001$
AP(CaOx) index(s)	1.31 (0.72)	2.00 (0.94)	$p < 0.00001$	0.94 (0.41)	1.55 (0.79)	$p < 0.00001$
AP(CaP) index(s)	46.2 (29.7)	62.8 (37.5)	$p = 0.0039$	34.2 (17.9)	46.6 (34.1)	$p = 0.0228$

Table 3 Solubility (SP) and formation products (FP) of different calcium salts and the median and range of AP-levels in DTd at pH 6.45 derived by extrapolation from the excretion of calcium, oxalate, citrate, magnesium, and phosphate 16 h urine (7, 10, 23, 37)

Crystal phase	SP	FP	Normal subjects Median	Normal subjects Range	Stone formers Median	Stone formers Range
ACP $(mol/l)^2$	$2.29 \cdot 10^{-11}$	–	$1.18 \cdot 10^{-12}$	$0.23–3.62 \cdot 10^{-12}$	$1.63 \cdot 10^{-12}$	$0.17–5.78 \cdot 10^{-12}$
OCP $(mol/l)^8$	$8.30 \cdot 10^{-48}$	$2.5 \cdot 10^{-45}$	$1.56 \cdot 10^{-43}$	$0–156 \cdot 10^{-43}$	$5.98 \cdot 10^{-43}$	$0–1039 \cdot 10^{-43}$
HAP $(mol/l)^9$	$2.35 \cdot 10^{-59}$	–	$1.01 \cdot 10^{-50}$	$0–839 \cdot 10^{-50}$	$3.94 \cdot 10^{-50}$	$0–1938 \cdot 10^{-50}$
Brushite $(mol/l)^2$	$1.87 \cdot 10^{-7}$	$2.0 \cdot 10^{-6}$	$4.22 \cdot 10^{-8}$	$0.48–18.0 \cdot 10^{-8}$	$5.27 \cdot 10^{-8}$	$0.38–35.6 \cdot 10^{-8}$
CaOx $(mol/l)^2$	$0.23 \cdot 10^{-8}$	$2.0 \cdot 10^{-8}$	$0.09 \cdot 10^{-8}$	$0.02–0.40 \cdot 10^{-8}$	$0.15 \cdot 10^{-8}$	$0.01–0.58 \cdot 10^{-8}$

this observation should be considered in view of the difficulty in determining the SP_{ACP}. Both AP_{Bru} and AP_{CaOx} had values in the region of the solubility products, but in all cases below the formation products.

The AP_{CaP} derived from the urine composition corresponding to the first indication of crystal formation gave a variable range of values in the interval $225–435 \cdot 10^{-14}$ M^2 . As in all experiments based on spontaneous crystallization, the results were subject to considerable variation.

Discussion

Although the exact composition of urine in the distal tubules is unknown, it is reasonable to assume that the average composition corresponds approximately to that used in the calculations presented above. It needs to be emphasized, however, that all calculations were based on the 16-h excretion in urine, whereby physiological and dietary induced variations during that period of time were ignored. Nevertheless it stands to reason that a considerable variation in urine composition occurs during the day and that peak values particularly of calcium, phosphate and pH are extremely important determinants for the crystallization propensity.

Ideally calculations of this kind should be based on direct analysis of urine composition in the distal tubule, but this was not feasible. Several simplifications were therefore necessary in order to obtain estimates of the ion activities of calcium and phosphate. It was assumed that the amount of calcium passing DTd during the 16-h period was 48% higher and that of magnesium 10% higher than the amount recovered in urine [22]. Citrate, phosphate and oxalate were considered unaffected by the passage of urine through the collecting ducts. Inasmuch as sodium, potassium and sulphate had not been analysed in the 16-h urine, these variables were considered to have the same concentration in DTd urine from all subjects. On the other hand, the concentrations of ammonium, pyrophosphate and carbon dioxide were set to zero and although such an artificial state might be an oversimplification, the effects of this on the ion activities of calcium, oxalate and phosphate were negligible. The complexation between ions and macromolecules [38] was not accounted for, but inasmuch as the macromolecular concentration at this level of the nephron might only be between 15% and 20% of that in whole urine, this simplification was probably also without major importance for the conclusions . This concentration of macromolecules was deduced from the volume reduction that normally takes place in the collecting duct, giving an average concentration of approximately one-sixth of that in final urine. Although certain macromolecules are probably secreted in the urine-collecting system below the distal tubule, macromolecules known to be modifiers of the crystallization process are all secreted at higher levels.

The results show that the risk of forming a DTd urine critically supersaturated with CaP was higher in SF than in N. Although levels of the ion-activity products of the different calcium salts were commonly above the solubility products, at pH 6.45 they were in most cases lower than the formation products reported in the literature (Table 3).

It is evident that increased concentrations of calcium and phosphate during periods with a high pH can dramatically increase the ion-activity products of CaP salts. This increases the risk of CaP precipitation [2, 13], which has also been demonstrated during titration with calcium chloride [27]. It is not known which crystal phase most easily forms under these conditions, but it has been suggested that ACP might be the first crystal phase subsequently transformed to OCP [7].

Attributable to the complex structure of several CaP crystal phases, alterations in one or several urine vari-

ables will result in effects of the ion-activity products that differ considerably between the salts mainly as a result of their mathematical forms. A simple expression of the ion-activity product of CaP (AP_{CaP}) was therefore chosen to describe the risk situation in DTd irrespective of which CaP salt that is formed. The reasonably good correlation between AP_{CaP} and the ion-activities of the other CaP crystal phases seems to justify the use of this simple expression. It is important, however, to be aware of the fact that differences in the ratio between calcium and phosphate as well as various pH levels and probably also urinary macromolecules might have important influences on the crystal phase that forms. Despite this it seems reasonable to assume that AP_{CaP} can be used as a rough estimate of the risk of CaP nucleation.

It is noteworthy that AP_{CaP} calculated for the DTd solution with its low concentrations is reflected so well in the $AP(CaP)$ index(s) derived from data in the 16-h urine. For clinical routine work it therefore appears sufficient to measure the excretion of calcium, phosphate and citrate in 16-h urine, calculate the $AP(CaP)$ index(s) and extrapolate this information to an AP_{CaP} value for DTd. Although the data in this study were all obtained from a 16-h urine collection, a 24-h urine sample would probably give similar results. It might, however, be even more informative to collect urine during shorter periods of time representing particular risk periods.

One prerequisite for a valid extrapolation of urine data to DTd properties is that the variables not specifically analysed or included in the calculations do not differ too much from the average figures used. It thus is important to be careful in interpreting the computed expressions when more pronounced abnormalities can be expected such as in patients with RTA and other tubular diseases.

The formation product of CaP is not easily assessed, but the preliminary experiments carried out in our laboratory indicate that crystals might form spontaneously at an AP_{CaP} level between 225 and $435 \cdot 10^{-14}$. This should be compared with an AP_{CaP} of $123–131 \cdot 10^{-14}$ computed from observations in DTd samples at the first appearance of crystals following stepwise addition of calcium [27]. It needs to be emphasized, however, that AP levels representing nucleation are difficult to determine with both Coulter counter and isotope techniques and the values presented above can only be used as a rough guide. Further conclusions in this respect require detailed analysis with more sensitive methods and it is also likely that the crystallization possible to achieve in vitro differs from that in the nephron.

As expected a marked increase in AP_{CaP} was recorded as a result of an increased pH, but with the mean values for SF and N the AP_{CaP} required for nucleation was not exceeded despite a pH above 7. Using the minimum and maximum values of calcium and phosphate, however, AP_{CaP} in DTd at a pH of 7.0 ranged between 6 and 256 in NM, 4 and 348 in SFM, 4 and 134 in NW and 2 and 400 in SFW. If the pH in DTd is considered to be affected only by the dilution, a pH of 7.0 in final urine would correspond to a pH of 7.8 in DTd, whereas at a urine pH of 6.5 the pH in DTd would be 7.3. With the latter pH AP_{CaP} in DTd calculated from the mean urine composition in SFM would be $154 \cdot 10^{-14}$, and with a urine pH of 7.0 a AP_{CaP} value in DTd of $530 \cdot 10^{-14}$ is obtained. These data suggest that there might be a pronounced risk of CaP precipitation in DTd under certain physiological conditions and that this risk is obviously higher in SF than in N. In addition macromolecules or other intratubular material might act as promoters for this crystallization, thus initiating a precipitation at lower supersaturation levels [12, 19, 20, 28].

In agreement with our previous results, the AP_{CaOx} in DTd was at a level where spontaneous crystal formation is extremely unlikely [27] and in a recent study crystals of CaOx were surprisingly uncommon in urine from calcium stone formers [17].

The lower grade of correlation between the DTd concentration of oxalate and AP_{CaOx} than between the concentration of calcium and AP_{CaOx} has not been clarified but is most likely a result of the high ratio between calcium and oxalate and the relatively low oxalate concentration in DTd urine.

Although further proof is necessary to support the theoretical considerations put forward in this paper, the results suggest that calcium stone formation might start as a crystallization of CaP in the distal tubule and that this risk is reflected with reasonable accuracy in terms of $AP(CaP)$ index(s), an index that is easily derived from analysis of calcium, phosphate and citrate in urine [40].

The pathogenetic importance of the formation of CaP crystals in the nephron remains to be shown. It is possible, however, that larger CaP particles formed as a result of a higher supersaturation with CaP and a greater tendency to aggregation at a level in the nephron, where the risk of CaOx crystal formation increases, play an important part in the development of a calcium stone. These aspects are currently subject to further studies.

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