SECTION 9

The patient with urinary stone disease

CHAPTER 199

Epidemiology of nephrolithiasis

Eric N. Taylor and Gary C. Curhan

Prevalence, incidence, and recurrence

Prevalence

The prevalence of nephrolithiasis, defined as a history of stone disease, varies by age, sex, race, and geography. The prevalence increases with age and the lifetime risk of stone formation in the United States exceeds 12% in men and 6% in women (Johnson et al., 1979; Stamatelou et al., 2003). The prevalence appeared to be increasing in the last quarter of the twentieth century for men and women, whether black or white (Stamatelou et al., 2003). A history of stone disease in the United States is most common among older white males (~12%) and lowest in younger black females (~1%) (Soucie et al., 1994; Stamatelou et al., 2003). Increased detection of asymptomatic stones resulting from the increasing use and sensitivity of radiologic studies may explain in part the rise in prevalence.

Few population-based studies of the prevalence of nephrolithiasis have been conducted outside of the United States. Prevalence of stone disease has increased in Japan (Yoshida and Okada, 1990) and Germany (Hesse et al., 2003).

A study of > 1 million individuals found geographic variability with a north–south and west–east gradient; the highest prevalence of self-reported nephrolithiasis was in the Southeastern United States (Soucie et al., 1996).

A decrease in the male to female ratio was suggested by a recent study of hospital discharges (Scales et al., 2007). Data from the Nationwide Inpatient Survey between 1997 and 2002 found a male to female ratio of 1.3:1, substantially lower than the commonly reported ratio of 2–3:1.

Incidence

The incidence of nephrolithiasis, defined as the first stone event, varies by age, sex, and race. White males have the highest incidence rates. In men, the incidence begins to rise after age 20, peaks between 40 and 60 years at approximately 3/1000/year and then declines (Johnson et al., 1979; Hiatt et al., 1982; Curhan et al., 1993). In women, the incidence is higher in the late 20s at 2.5/1000/year and then decreases to 1/1000/year by age 50, remaining at this rate for the next several decades (Johnson et al., 1979; Hiatt et al., 1982; Curhan et al., 1997b, 2004).

A recent study from Rochester, Minnesota, United States, raised the possibility that incidence rates may be decreasing. Using the same methodology as a study performed 30 years earlier, the recent study reported incidence rates since 1990 may be falling in men and have levelled off in women (Lieske et al., 2006). Because there were only 157 cases in men and 91 in women, additional larger studies are needed.

Recurrence rates

Case series suggest 30–40% of untreated individuals will form another stone within 5 years after the initial episode (Johnson et al., 1979). The risk of recurrence is influenced by stone type and urinary composition. Fortunately, randomized trials demonstrated that interventions can reduce the likelihood of recurrence by 50% or more (Ettinger et al., 1986, 1988, 1997; Borghi et al., 2002).

Non-dietary risk factors

Family history

Studies of twins and populations have demonstrated that the common forms of stone disease are heritable (Goldfarb et al., 2005). The risk of stone formation is twofold higher in individuals with a family history of stone disease (Curhan et al., 1997a). The increased risk is likely due to both genetic predisposition and similar environmental exposures. Genetic causes of rare forms of nephrolithiasis (e.g. cystinuria and Dent disease) have been identified, but information is still limited on genes that contribute to risk of the common forms of stone disease.

Race/ethnicity

In a cross-sectional Canadian study, individuals of Arabic, West Indian, west Asian, and Latin American descent were more likely to be stone formers than those of European descent (Mente et al., 1997). Overall, African Americans have a lower frequency of stones (Stamatelou et al., 2003).

Systemic disorders

There is substantial evidence that nephrolithiasis is a systemic disorder. Well-known conditions associated with calcium-containing stones include primary hyperparathyroidism, renal tubular acidosis, and Crohn disease.

Several other common conditions, including obesity, gout, diabetes mellitus, and gallstones, have recently been convincingly linked to nephrolithiasis. Increasing body size, assessed by weight, body mass index (BMI), or waistline, increases the risk of stone formation independent of other risk factors including diet (Taylor et al., 2005b); for unexplained reasons, the impact is greater in women than in men. For example, the risk of stone formation for individuals with a BMI ≥ 30 kg/m^2 compared to those with a BMI 21–23 was 30% higher among men but nearly twofold higher among women.

In a cross-sectional study, individuals with gout were 50% more likely to have a history of stones (Kramer and Curhan, 2002). When examined prospectively, individuals with a history of gout had a twofold higher risk of incident nephrolithiasis, independent of diet, weight, and medications (Kramer et al., 2003).

Diabetes mellitus has also been associated with an increased risk of stone formation, independent of diet and body size (Taylor et al., 2005a). Cross-sectionally, individuals with a history of diabetes were > 30% more likely also to have a history of nephrolithiasis. Prospectively, a history of DM increased the risk of stone formation by 30–50% in women but not in men. In support of these findings, a recent study based on the National Health and Nutrition Examination Survey (NHANES) III data found that the risk of being a stone former increased with an increasing number of metabolic syndrome trait (West et al., 2008).

Environmental factors

Occupations or settings with higher insensible fluid losses, such as a hot environment, increase risk of stone formation (Atan et al., 2005). The risk will also be higher when individuals have restricted access to water or bathroom facilities, leading to lower fluid intake and lower urine volume.

Diet and stone disease

Because most data on the relation between diet and stone disease come from observational and physiologic studies, care must be taken when interpreting studies of diet and risk. Retrospective studies may be biased because individuals who develop stones may subsequently change their diet. Results from studies that use change in urine composition as a surrogate for actual stone formation should be viewed with caution because the composition of the urine does not completely predict risk and not all the components that modify risk are included in the calculation of supersaturation (e.g. urine phytate). Thus, prospective studies that assess a variety of nutrients are best suited for examining the associations between dietary factors and risk of actual stone formation. Finally, associations between specific dietary factors and risk may vary by age, sex, and body size.

Dietary risk factors: calcium oxalate stones

More than 80% of kidney stones contain calcium, and the majority of calcium stones consist primarily of calcium oxalate (Coe et al., 1992). Because calcium oxalate is most common, the majority of studies have focused on risk factors for this stone type. Dietary factors associated with increased or decreased risk are listed in Table 199.1.

Table 199.1 Dietary factors that may increase or decrease the risk of calcium oxalate kidney stones

Dietary factor	Proposed mechanism(s)
Increase risk	
Oxalate	Increased urinary oxalate excretion
Sodium	Increased urinary calcium excretion
Animal protein	Increased urinary calcium and uric acid excretion; reduced urinary citrate excretion
Vitamin C	Increased oxalate generation and excretion
Carbohydrates	Increased urinary calcium excretion
Decrease risk	
Dietary calcium	Binding of dietary oxalate in gut
Potassium-rich foods	Increased urinary citrate excretion; reduced urinary calcium excretion
Phytate	Inhibition of calcium oxalate crystal formation
Magnesium	Reduced dietary oxalate absorption; inhibition of calcium oxalate crystal formation
Vitamin B_6	Vitamin B_6 deficiency may increase oxalate production and oxaluria

Calcium

In the past, higher calcium intake was believed to increase the risk of stone formation. However, there is now substantial evidence demonstrating that a higher calcium diet is associated with a *reduced* risk of stone formation. One potential mechanism to explain this apparent paradox is that the higher calcium intake will bind dietary oxalate in the gut, thereby reducing oxalate absorption and urinary excretion. It is also possible that dairy products (the major source of dietary calcium) may contain inhibitory factors.

Several large, prospective observational studies in men and women consistently support a reduced risk of stone formation with increasing dietary calcium intake. Compared to individuals in the lowest quintile of dietary calcium intake, those in the highest quintile had a > 30% lower risk of forming a stone (Curhan et al., 1993, 1997b, 2004). These results were adjusted for multiple factors, including age, BMI, total fluid intake, the use of thiazide diuretics, and the intake of nutrients such as animal protein, magnesium, phosphorous, sodium, and potassium. Calcium intake is an example of how the impact of a risk factor may vary by age: there was no association between dietary calcium and stone formation in men aged 60 years or older (Taylor et al., 2004).

The observational findings were subsequently confirmed by a 5-year randomized controlled clinical trial that compared stone recurrence in individuals with a history of calcium oxalate nephrolithiasis and idiopathic hypercalciuria assigned to a diet low in calcium (400 mg/day) or to a diet with 'normal' calcium content (1200 mg/day) and lower amounts of animal protein and sodium (Borghi et al., 2002). The risk of developing a recurrent stone on the higher calcium diet was 51% lower than for the low-calcium diet (Borghi et al., 2002). Because dietary sodium and animal protein may both contribute to the formation of calcium stones, this trial, although suggestive, did not directly address the independent role of dietary calcium in the pathogenesis of kidney stones.

The impact of supplemental calcium on stone risk may be different from dietary calcium. In an observational study of older women, calcium supplement users were 20% more likely to form a stone than women who did not take supplements (Curhan et al., 1997). The Women's Health Initiative randomized trial also found an increased risk with calcium supplementation (1000 mg/day), though the supplements also contained 400 IU/day of vitamin D_3 (Jackson et al., 2006). In younger women and men, there was no association between calcium supplement use and risk of stone formation (Curhan et al., 1993, 2004). The discrepancy between the risks from dietary calcium and calcium supplements, at least in the observational study, may be due to the timing of calcium intake. Calcium supplements are not typically taken with meals, which would diminish binding of dietary oxalate.

Because the absolute risk of forming the first kidney stone by a supplement user is only slightly increased (1.2 cases/1000 women per year compared to 1.0/1000 per year), supplement use is not a major contributor to stone risk. However, for an individual who has had a stone, the impact of calcium supplementation on 24-hour urine composition should be evaluated.

Oxalate

Although urine oxalate is an important risk factor for calcium oxalate stone formation, the role of dietary oxalate in the pathogenesis of calcium oxalate nephrolithiasis remains unclear (Holmes and Assimos, 2004). First, the proportion of urinary oxalate derived from dietary oxalate is controversial; estimates range from 10% to 50% (Holmes and Assimos, 2004). Thus, a substantial proportion of urinary oxalate is derived from the endogenous production such as the metabolism of glycine, glycolate, and hydroxyproline. Second, other dietary factors influence urine oxalate. For example, vitamin C supplementation appears to be an important contributor (Taylor and Curhan, 2008a) because it can be metabolized to oxalate. Third, much of the oxalate in food may not be readily absorbed due to low bioavailability. Finally, significant variation can exist between individuals with respect to the gastrointestinal absorption of oxalate. For instance, up to one-third of patients with calcium oxalate nephrolithiasis may experience increased absorption of dietary oxalate. A recent study found individuals with a history of calcium oxalate nephrolithiasis were less likely to be colonized with *Oxalobacter formigenes*, an intestinal bacterium that degrades oxalate (Kaufman et al., 2008).

Older reports of the oxalate content in food may be unreliable due to measurement issues, related to the quality of the assay procedure as well as the variability in oxalate content of the same food items. Recently, however, reliable assays for the direct determination of the oxalate content of food, including ion chromatography and capillary electrophoresis, have been developed (a list of the oxalate content of several hundred food items can be found at <https://regepi.bwh.harvard.edu/health/Oxalate/files>), and large-scale prospective studies of the relation between dietary oxalate and kidney stone formation have been completed. Surprisingly, the impact of dietary oxalate, even when comparing substantial differences in intake, was minimal in men and older women and not associated with stone formation in younger women (Taylor and Curhan, 2007).

Sodium

Higher sodium intake results in decreased proximal sodium reabsorption with a subsequent reduction in calcium reabsorption. A recent randomized trial of dietary salt restriction in calcium-oxalate stone formers with idiopathic hypercalciuria demonstrated that for every 100 mmol decrease in urine sodium, urine calcium decreased by 64 mg/day (Nouvenne et al., 2010). Previous observational studies found a positive, independent association between sodium consumption and new kidney stone formation in women but not men (Curhan et al., 1993, 1997b).

Other nutrients

A variety of other nutrients have been implicated in stone formation. Higher animal protein intake may increase urinary calcium and decrease urinary citrate (Breslau et al., 1988), thereby increasing the risk of stone formation. However, when studied prospectively, animal protein was associated with an increased risk in men but not women (Curhan et al., 1993, 1997b, 2004). Further, the increased risk in men was only found among men with a BMI < 25 kg/m^2 (Taylor et al., 2004). Higher dietary potassium intake decreased risk in men and older women (Curhan et al., 1993, 1997b; Taylor et al., 2004) possibly by reducing urine calcium excretion (Lemann et al., 1991) or increasing urine citrate. Higher intake of sucrose (Lemann et al., 1969) increases urinary calcium excretion independent of calcium intake. In prospective studies, sucrose was associated with an increased risk in women and fructose increased risk in men and women (Curhan et al., 1997b, 2004; Taylor and Curhan, 2008b). Phytate, found in whole grains and beans, was observed to reduce risk of stone formation in younger women (Curhan et al., 2004), possibly by directly inhibiting calcium oxalate crystal formation.

Although magnesium may reduce dietary oxalate absorption, randomized trials of magnesium supplements did not find a protective effect on stone recurrence, though the dropout rates were high. In prospective observational studies, higher dietary magnesium was associated with a lower risk of stone formation in men (Taylor et al., 2004) but not women (Curhan et al., 1997b, 2004).

Vitamin C (ascorbic acid) can be metabolized to oxalate. Consumption of 1000 mg of supplemental vitamin C twice daily increased urinary oxalate excretion by 22% (Traxer et al., 2003). In a prospective observational study, men who consumed 1000 mg or more per day of vitamin C had a 40% higher risk of stone formation compared to men who consumed < 90 mg/day (the recommended dietary allowance) (Taylor et al., 2004). While these data do not support the restriction of *dietary* vitamin C (because foods high in vitamin C contain inhibitory factors such as potassium), many clinicians reasonably suggest that calcium oxalate stone formers avoid vitamin C supplements.

Although high-dose vitamin B_6 (pyridoxine) may reduce oxalate production in selected patients with type 1 primary hyperoxaluria, it is unclear if there would be benefit from the use of vitamin B_6 supplements to prevent common stone disease. In observational studies, higher intake of vitamin B_6 was associated with a reduced risk of kidney stone formation in women (Curhan et al., 1999) but not in men (Curhan et al., 1996).

Dietary patterns

Relatively few studies have examined the impact of overall diet or dietary patterns on risk. The Dietary Approaches to Stop Hypertension (DASH) diet, which is high in fruits and vegetables, moderate in low-fat dairy products, and low in animal protein represents a novel potential means of kidney stone prevention. In a recent large, prospective observational study of three distinct cohorts, a higher dietary DASH score was associated with 40–45% reduction in the risk of incident kidney stone formation (Taylor et al., 2009). This relation was independent of age, body size, hypertension, diabetes, thiazide use, and intakes of total calories, fluid, caffeine, and alcohol.

Dietary risk factors: other stone types

For the less common stone types, little data exist to support the role of specific dietary factors in kidney stone formation. It is possible to speculate about the impact of diet based on pathophysiology, but it should be remembered that the many of the older

recommendations for calcium oxalate stone prevention based on pathophysiologic considerations were later found to be unhelpful.

Uric acid stones

The two driving forces for uric acid crystal formation are the uric acid concentration and urine pH (the solubility of uric acid increases substantially as the urine pH increases from 5.0 to 6.5). Decreasing consumption of meat, chicken, and seafood will decrease purine intake and, therefore, uric acid production, and may also increase urinary pH. Higher intake of fruits and vegetables should raise the urine pH and reduce the risk of uric acid crystal formation.

Cystine stones

Restricting sodium intake may reduce the urinary excretion of cysteine (Lindell et al., 1995). The solubility of cystine increases as urinary pH rises (Nakagawa et al., 2000), thus higher fruit and vegetable consumption may be beneficial. There is little evidence to support the dietary restriction of proteins high in cystine, though reducing animal protein intake may be beneficial by increasing urine pH.

Calcium phosphate stones

Because patients with type 1 renal tubular acidosis and stone disease may benefit from alkali supplementation, they may also benefit from a diet high in fruits and vegetables. It should be noted, however, that an increase in urinary pH can increase the risk of calcium phosphate crystal formation. Dietary manoeuvres directed at decreasing urinary calcium excretion (such as sodium and animal protein restriction) would also be expected to decrease calcium phosphate stone recurrence.

Beverages and calcium stones

Total fluid

Nephrolithiasis is a disease driven by the urinary concentration of lithogenic factors. Thus, fluid intake, the main determinant of urine volume, plays a critical role in kidney stone formation. Observational studies (Curhan et al., 1993, 1997b, 2004) and a randomized controlled trial (goal urine output 2 L/day) (Borghi et al., 1996) demonstrated that higher fluid intake reduces the risk of stone formation.

Individual beverages

The associations of specific beverages, beyond just fluid intake, with kidney stone formation are presented in Table 199.2. Despite previous beliefs to the contrary, alcoholic beverages, coffee, and tea do not increase the risk of stone formation. In fact, observational studies have found that coffee, tea, beer, and wine are associated with a *reduced* risk of stone formation (Curhan et al., 1996b, 1998). The mechanisms for these protective associations may be related to inhibition of antidiuretic hormone action in the kidney by caffeine and inhibition of antidiuretic hormone secretion by alcohol. The role of tea deserves special mention. There is a widespread belief that tea is high in oxalate and should be avoided. A cup of tea contains 14 mg of oxalate. While this is not insignificant, the bioavailability does not appear to be high. A feeding study of tea demonstrated a negligible impact on urinary oxalate (Brinkley et al., 1990). Citrus juices, such as orange and grapefruit juice, theoretically could reduce the risk of stone formation by increasing urine citrate. However, the prospective studies found no association with orange juice, and grapefruit juice intake was associated with a 40% higher risk of stone formation (Curhan et al., 1996b, 1998). One feeding study found that grapefruit consumption did increase urine citrate but also substantially increased urine oxalate (Goldfarb and Asplin, 2001).

The relation between soda ('soft drink') consumption and stone risk is complicated. Dietary patterns associated with sweetened soda consumption were found to increase the risk of stone formation and sweetened sodas contain fructose, which increases the risk of stone formation.

Table 199.2 Select beverages, risk, and possible mechanism for calcium oxalate stone formation[a]

Beverage type	Risk	Proposed mechanism(s)
Coffee and tea	Decreased	Caffeine interferes with antidiuretic hormone action, leading to decreased urinary concentration
Alcohol	Decreased	Alcohol inhibits secretion of antidiuretic hormone, leading to decreased urinary concentration
Milk	Decreased	Binding of dietary oxalate in gut
Grapefruit juice	Increased	Possible increased oxalate production

[a]Orange juice and soda are discussed in the text.

Urinary factors

The 24-hour urine collection provides important prognostic information and guides preventive recommendations. Like many laboratory tests, urine results have traditionally been categorized into 'normal' and 'abnormal'. However, recent data has revealed this grouping is unsatisfactory (Curhan and Taylor, 2008). Urine values are continuous so the dichotomization into 'normal' and 'abnormal' is arbitrary and potentially misleading. Although terms of abnormal excretion, such as 'hypercalciuria' or 'hypocitraturia' are often used clinically and in the scientific literature, the limitations of these terms should be acknowledged.

Higher urine calcium

Hypercalciuria is commonly defined as urine calcium excretion ≥ 300 mg/day in men and ≥ 250 mg/day in women (Hodgkinson and Pyrah, 1958) on a 1000 mg/day calcium diet (but a variety of definitions are in use). Using these traditional definitions, approximately 20–40% of patients with calcium stone disease will have hypercalciuria. Although possibly reasonable from a calcium balance perspective, there is insufficient justification for different thresholds for males and females.

Higher urine oxalate

Hyperoxaluria is typically defined as urinary oxalate excretion > 45 mg/day, though here too a variety of thresholds are in use. Elevated urinary oxalate excretion is three to four times more common among men (~40%) than in women (~10%) (Curhan and Taylor,

2008). Mean urinary oxalate levels are only slightly higher in cases than in controls, but in multivariate models urine oxalate is clearly an important independent risk factor for stone formation (Curhan and Taylor, 2008). Of note, the risk begins to rise well below the 45 mg/day level.

Higher urine uric acid

The relation between uric acid excretion and calcium stone disease is unsettled. Some early cross-sectional studies reported that hyperuricosuria (typically defined as > 800 mg/day in men or 750 mg/day in women) is more frequent in patients who form calcium stones than controls (Coe, 1978). However a recent study of > 2200 stone formers and 1100 non-stone formers reported that a higher urine uric acid was associated with a lower likelihood of being a stone former in men, and there was no increase in risk for women (Curhan and Taylor, 2008). A double-blind trial of allopurinol successfully decreased recurrence rates of calcium stones in patients with hyperuricosuria suggesting that uric acid is important (Ettinger et al., 1986), but it is possible that the beneficial effect of allopurinol was through a mechanism unrelated to lowering of urine uric acid.

Lower urine citrate

Hypocitraturia, often defined as 24-hour excretion ≤ 320 mg/day, increases risk of stone formation (Pak, 1994) and is found in 5–11% of first-time stone formers (Curhan and Taylor, 2008). There is suggestive evidence that increasing urinary citrate into the high-normal range could provide additional protection (Curhan and Taylor, 2008).

Summary

Epidemiologic studies have greatly expanded our understanding of risk factors for stone disease. A variety of dietary, non-dietary, and urinary risk factors contribute to the risk of kidney stone formation and the importance of these varies by age, sex, and BMI. There is a paucity of randomized trials in the field, and more interventional studies are needed to examine the effect of dietary patterns and/or weight loss on the 'hard' outcome of kidney stone formation.

References

Atan, L., Andreoni, C., Ortiz, V., *et al.* (2005). High kidney stone risk in men working in steel industry at hot temperatures. *Urology*, 65(5), 858–61.

Borghi, L., Meschi, T., Amato, F., *et al.* (1996). Urinary volume, water and recurrences in idiopathic calcium nephrolithiasis: a 5-year randomized prospective study. *J Urol*, 155, 839–43.

Borghi, L., Schianchi, T., Meschi, T., *et al.* (2002). Comparison of two diets for the prevention of recurrent stones in idiopathic hypercalciuria. *N Engl J Med*, 346(2), 77–84.

Breslau, N., Brinkley, L., Hill, K. D., *et al.* (1988). Relationship of animal protein-rich diet to kidney stone formation and calcium metabolism. *J Clin Endocrinol Metab*, 66, 140–6.

Brinkley, L., Gregory, J., and Pak, C. (1990). A further study of oxalate bioavailability in foods. *J Urol*, 144, 94–6.

Coe, F. L. (1978). Hyperuricosuric calcium oxalate nephrolithiasis. *Kidney Int*, 13(5), 418–26.

Coe, F. L., Parks, J. H., and Asplin, J. R. (1992). The pathogenesis and treatment of kidney stones. *N Engl J Med*, 327(16), 1141–52.

Curhan, G. C. and Taylor, E. N. (2008). 24-h uric acid excretion and the risk of kidney stones. *Kidney Int*, 73(4), 489–96.

Curhan, G. C., Willett, W. C., Knight, E. L., *et al.* (2004). Dietary factors and the risk of incident kidney stones in younger women: Nurses' Health Study II. *Arch Intern Med*, 164(8), 885–91.

Curhan, G. C., Willett, W. C., Rimm, E. B., *et al.* (1993). A prospective study of dietary calcium and other nutrients and the risk of symptomatic kidney stones. *N Engl J Med*, 328, 833–8.

Curhan, G. C., Willett, W. C., Rimm, E. B., *et al.* (1996a). A prospective study of the intake of vitamins C and B6, and the risk of kidney stones in men. *J Urol*, 155(6), 1847–51.

Curhan, G. C., Willett, W. C., Rimm, E. B., *et al.* (1996b). Prospective study of beverage use and the risk of kidney stones. *Am J Epidemiol*, 143(3), 240–7.

Curhan, G. C, Willett, W. C., Rimm, E. B., *et al.* (1997a). Family history and risk of kidney stones. *J Am Soc Nephrol*, 8, 1568–73.

Curhan, G. C, Willett, W. C., Speizer, F. E., *et al.* (1997b). Comparison of dietary calcium with supplemental calcium and other nutrients as factors affecting the risk for kidney stones in women. *Ann Intern Med*, 126, 497–504.

Curhan, G. C., Willett, W. C., Speizer, F. E., *et al.* (1998). Beverage use and risk for kidney stones in women. *Ann Intern Med*, 128(7), 534–40.

Curhan, G. C., Willett, W. C., Speizer, F. E., *et al.* (1999). Intake of vitamins B6 and C and the risk of kidney stones in women. *J Am Soc Nephrol*, 10(4), 840–5.

Ettinger, B., Citron, J. T., Livermore, B., *et al.* (1988). Chlorthalidone reduces calcium oxalate calculous recurrence but magnesium hydroxide does not. *J Urol*, 139(4), 679–84.

Ettinger, B., Pak, C. Y., Citron, J. T., *et al.* (1997). Potassium-magnesium citrate is an effective prophylaxis against recurrent calcium oxalate nephrolithiasis. *J Urol*, 158(6), 2069–73.

Ettinger, B., Tang, A., Citron, J. T., *et al.* (1986). Randomized trial of allopurinol in the prevention of calcium oxalate calculi. *N Engl J Med*, 315(22), 1386–9.

Goldfarb, D. S. and Asplin, J. R. (2001). Effect of grapefruit juice on urinary lithogenicity. *J Urol*, 166(1), 263–7.

Goldfarb, D. S., Fischer, M. E., Keich, Y., *et al.* (2005). A twin study of genetic and dietary influences on nephrolithiasis: a report from the Vietnam Era Twin (VET) Registry. *Kidney Int*, 67(3), 1053–61.

Hesse, A., Brändle, E., Wilbert, D., *et al.* (2003). Study on the prevalence and incidence of urolithiasis in Germany comparing the years 1979 vs. 2000. *Eur Urol*, 44(6), 709–13.

Hiatt, R. A., Dales, L. G., Friedman, G. D., *et al.* (1982). Frequency of urolithiasis in a prepaid medical care program. *Am J Epidemiol*, 115, 255–65.

Hodgkinson, A. and Pyrah, L. N. (1958). The urinary excretion of calcium and inorganic phosphate in 344 patients with calcium stone of renal origin. *Br J Surg*, 46(195), 10–18.

Holmes, R. P. and Assimos, D. G. (2004). The impact of dietary oxalate on kidney stone formation. *Urol Res*, 2004. 32(5), 311–16.

Jackson, R. D., LaCroix, A. Z., Gass, M., *et al.* (2006). Calcium plus vitamin D supplementation and the risk of fractures. *N Engl J Med*, 354(7), 669–83.

Johnson, C. M., Wilson, D. M., O'Fallon, W. M., *et al.* (1979). Renal stone epidemiology: a 25-year study in Rochester, Minnesota. *Kidney Int*, 16(5), 624–31.

Kaufman, D. W., Kelly, J. P., Curhan, G. C., *et al.* (2008). Oxalobacter formigenes may reduce the risk of calcium oxalate kidney stones. *J Am Soc Nephrol*, 19(6), 1197–203.

Kramer, H. J., Choi, H. K, Atkinson, K., *et al.* (2003). The association between gout and nephrolithiasis in men: The Health Professionals' Follow-Up Study. *Kidney Int*, 64(3), 1022–6.

Kramer, H. M. and Curhan, G. (2002). The association between gout and nephrolithiasis: the National Health and Nutrition Examination Survey III, 1988–1994. *Am J Kidney Dis*, 40(1), 37–42.

Lemann, J., Jr., Piering, W. F., and Lennon, E. J. (1969). Possible role of carbohydrate-induced calciuria in calcium oxalate kidney-stone formation. *N Engl J Med*, 280(5), 232–7.

Lemann, J., Jr., Pleuss, J. A., Gray, R. W., *et al.* (1991). Potassium administration reduces and potassium deprivation increases urinary calcium excretion in healthy adults [corrected]. *Kidney Int*, 39(5), 973–83.

Lieske, J. C., Peña de la Vega, L. S., Slezak, J. M., *et al.* (2006). Renal stone epidemiology in Rochester, Minnesota: an update. *Kidney Int*, 69(4), 760–4.

Lindell, A., Denneberg, T., Edholm, E., *et al.* (1995). The effect of sodium intake on cystinuria with and without tiopronin treatment. *Nephron*, 71(4), 407–15.

Mente, A., Honey, R. J., McLaughlin, J. R., *et al.* (2007). Ethnic differences in relative risk of idiopathic calcium nephrolithiasis in North America. *J Urol*, 178(5), 1992–7.

Nakagawa, Y., Asplin, J. R., Goldfarb, D. S., *et al.* (2000). Clinical use of cystine supersaturation measurements. *J Urol*, 164(5), 1481–5.

Nouvenne, A., Meschi, T., Prati, B., *et al.* (2010). Effects of a low-salt diet on idiopathic hypercalciuria in calcium-oxalate stone formers: a 3-mo randomized controlled trial. *Am J Clin Nutr*, 91(3), 565–70.

Pak, C. Y. (1994). Citrate and renal calculi: an update. *Miner Electrolyte Metab*, 20(6), 371–7.

Scales, C. D., Jr., Curtis, L. H., Norris, R. D., *et al.* (2007). Changing gender prevalence of stone disease. *J Urol*, 177(3), 979–82.

Soucie, J., Coates, R. J., McClellan, W., *et al.* (1996). Relation between geographic variability in kidney stones prevalence and risk factors for stones. *Am J Epidemiol*, 143, 487–95.

Soucie, J. M., Thun, M. J., Coates, R. J., *et al.* (1994). Demographic and geographic variability of kidney stones in the United States. *Kidney Int*, 46(3), 893–9.

Stamatelou, K. K., Francis, M. E., Jones, C. A., *et al.* (2003). Time trends in reported prevalence of kidney stones in the United States: 1976–1994. *Kidney Int*, 63(5), 1817–23.

Taylor, E. N. and Curhan, G. C. (2007). Differences in 24-hour urine composition between black and white women. *J Am Soc Nephrol*, 18(2), 654–9.

Taylor, E. N. and Curhan, G. C. (2008a). Determinants of 24-hour urinary oxalate excretion. *Clin J Am Soc Nephrol*, 3(5), 1453–60.

Taylor, E. N. and Curhan, C. G. (2008b). Fructose consumption and the risk of kidney stones. *Kidney Int*, 73(2), 207–12.

Taylor, E. N., Fung, T. T., and Curhan, G. C. (2009). DASH-style diet associates with reduced risk for kidney stones. *J Am Soc Nephrol*, 20(10), 2253–9.

Taylor, E. N., Stampfer, M. J., and Curhan, G. C. (2004). Dietary factors and the risk of incident kidney stones in men: new insights after 14 years of follow-up. *J Am Soc Nephrol*, 15(12), 3225–32.

Taylor, E. N., Stampfer, M. J., and Curhan, G. C. (2005a). Diabetes mellitus and the risk of nephrolithiasis. *Kidney Int*, 68(3), 1230–5.

Taylor, E. N., Stampfer, M. J., and Curhan, G. C. (2005b). Obesity, weight gain, and the risk of kidney stones. *JAMA*, 293(4), 455–62.

Traxer, O., Huet, B., Poindexter, J., *et al.* (2003). Effect of ascorbic acid consumption on urinary stone risk factors. *J Urol*, 170(2 Pt 1), 397–401.

West, B., Luke, A., Durazo-Arvizu, R. A., *et al.* (2008). Metabolic syndrome and self-reported history of kidney stones: the National Health and Nutrition Examination Survey (NHANES III) 1988–1994. *Am J Kidney Dis*, 51(5), 741–7.

Yoshida, O. and Okada, Y. (1990). Epidemiology of urolithiasis in Japan: a chronological and geographical study. *Urol Int*, 45, 104–11.

CHAPTER 200

Approach to the patient with kidney stones

Bhavna Chopra and Stanley Goldfarb

Clinical presentation

Table 200.1 gives an overview of stone types and features.

History specific for stone evaluation

It is helpful to narrow down the potential causes of kidney stone formation by taking a detailed history. It is important to know the age and family history of the patient to identify the possibility for inherited risk factors. A history of known renal structural abnormalities should be sought. Occupation can be important as conditions that lead to dehydration and volume depletion from low fluid intake or excessive heat-related fluid losses could predispose to kidney stones. A careful dietary history is important to identify the calcium, oxalate, sodium, protein, potassium, and purine content in the subject's diet. Previous history of kidney stones increases the risk of recurrent stone to about 50% in the subsequent decade after stone passages (Trinchieri et al., 1999). It is also important to identify the duration of stone disease, a history of any stone-related procedures, and to determine if stone analysis was done in the past.

Other important information includes history of gastrointestinal diseases such as chronic diarrhoea, ileal resection, jejunoileal bypass, or bariatric surgery. Previous conditions that can lead to hypercalcaemia and hypercalciuria such as primary hyperparathyroidism, multiple myeloma, immobilization, or sarcoidosis need to be noted. Distal renal tubular acidosis is very commonly associated with kidney stones and a history of nephrocalcinosis would suggest that diagnosis. A history of recurrent urinary tract infections especially with urease-positive organisms should be sought as it can cause stag horn calculi. Gout, diabetes, obesity, and the metabolic syndrome are risk factors for uric acid stones.

Medication history

- Corticosteroids, calcium carbonate supplements and vitamin D supplements can cause hypercalciuria.
- Vitamin B_6 and vitamin C can cause hyperoxaluria.
- Carbonic anhydrase inhibitors cause hypocitraturia, hypercalciuria, and high urine pH resulting in increased risk for calcium phosphate stones.
- Indinavir and triamterene cause insoluble stones.

Dietary history

Patients who do not drink enough fluids and live or work in conditions where there is a large amount of fluid loss due to the environment, such as miners, foundry workers, or those who live in warm dry climates, have an increased risk of forming stones (Atan et al., 2005). A diet rich in protein and salt will increase calcium excretion in the urine and the chances of stone formation. Low calcium intake can lead to increased oxalate absorption in the gut leading to increased oxaluria and hence raise the risk of calcium oxalate stones (Lemann, 1993).While excessive intakes of calcium and vitamin D supplements can result in calcium phosphate stones, a moderate amount of calcium in the diet is associated with a reduced risk of stone formation through its action to reduce gastrointestinal oxalate absorption. Diets rich in animal protein, protein supplement powders, and sugar loads, especially fructose, can also increase the risk of calcium-containing stones.

Genetic disorders

Hereditary and genetic factors such as cystinuria (Chapter 200), where there is decreased renal reabsorption of cystine, can play a role in stone formation. Dent disease and primary hyperoxaluria can cause calcium oxalate stones (Chapters 201 and 205). Kidney stones can also follow a polygenic inheritance (Vezzoli et al., 2008) such that there is an increased incidence of kidney stones in families. There is a high concordance found in twin studies.

Signs and symptoms of renal colic

Active passage of stone is associated with often unbearable pain, haematuria, nausea, and vomiting. Pain typically occurs in the flank area and usually begins suddenly and then plateaus at 30 minutes to 2 hours. The pain does not improve with movement or posture. There may be intense nausea with or without vomiting.

The location of the stone further characterizes the pain. Stones obstructing the ureteropelvic junction may present with mild-to-severe deep flank pain without radiation to the groin. The pain is due to distension of the renal capsule from the hydrostatic pressure caused by ureteral obstruction. Stones impacted within the ureter cause abrupt, severe, colicky pain in the flank and ipsilateral lower abdomen with radiation to the groin area. Stones lodged at the ureterovesical junction also may cause irritating voiding symptoms, such as urinary frequency and dysuria. Calculi that have entered the bladder are usually asymptomatic and are passed relatively easily during urination. Rarely, a patient may report positional urinary retention (obstruction precipitated by standing, relieved by recumbency), which is due to the ball-valve effect of a large stone located at the bladder outlet. Macroscopic or microscopic haematuria is commonly seen in stone disease but it is neither specific nor highly sensitive as a marker of stone passage. For example, macroscopic/gross haematuria occurs with renal colic pain due to large calculi

Table 200.1 Clinical characteristics of common types of kidney stones

	Calcium oxalate (Worcester and Coe, 2010)	**Calcium phosphate**	**Cystine (Chillaron et al., 2010)**	**Struvite (Koga et al., 1991; Teichman et al., 1995)**	**Uric acid (Cameron and Sakhaee, 2007)**
History and risk factors	Genetic disorders: primary hyperoxaluria, disorders of glyoxylate metabolism (type I/II) Secondary hyperoxaluria, due to fat malabsorption syndromes, such as gastric bypass surgery, inflammatory bowel disease, chronic pancreatitis, ethylene glycol poisoning, diet rich in oxalate, and vitamin C	*Medical conditions* that increase risk are: sarcoidosis, renal tubular acidosis, bone disease or fracture, immobilization, milk-alkali syndrome, medullary sponge kidney, primary hyperparathyroidism *Medications associated*: topiramate, calcium supplements, carbonic anhydrase inhibitor, and vitamin D	Rare genetic cause of kidney stones. Usually 1–2% of stone former have cystine stone. Genetic defect in the transport of cystine → ↓ proximal tubular reabsorption of filtered cystine → ↑urinary excretion and cystine nephrolithiasis. The gene for cystinuria (*SLC3A1*) has been localized to chromosome 2p21 by fluorescence *in situ* hybridization	Recurrent urinary tract infection (UTI). Patients with neurogenic bladder, or frequent instrumentation are at risk for UTI. They form in urine infected by urea-splitting bacteria. Struvite stones grow rapidly to progress to staghorn stones	Primary gout, increase purine intake in diet, glycogen storage disorders; G6PD deficiency, increased phospho-ribosyl pyro-phosphate synthetase activity, hypoxanthine-guanine phosphoribosyltransferase deficiency (Lesh–Nyhan syndrome), neoplastic diseases, polycythemia, haemoglobinopathy and psoriasis. Small bowel disease leading to ileostomy
Clinical features	Stones could be asymptomatic or associated with renal colic pain and haematuria. Some of the hyperoxaluria syndromes can lead to calcium oxalate precipitation in other tissues besides the kidney	Can cause nephrocalcinosis, when associated with renal distal tubular acidosis, or medullary sponge kidney	Genetic disorder, strong family history	Patients can be asymptomatic or may have obstruction in collecting system. Some patients might have vague abdominal/flank pain or gross haematuria	Acidic urine promotes uric acid stones. Risk increases in chronic diarrhoeal states associated with bicarbonate loss + volume depletion. Also seen in other metabolic defects such as gout, diabetes, insulin resistance and obesity
Biochemical	Calcium oxalate crystals in urine, oxalate excretion > 44 mg/day, Plasma oxalate levels high	High urine pH. Hypercalciuria, brushite (calcium monohydrate stones)	Pathognomonic hexagonal cystine crystals seen in urine	Persistently alkaline urine, pH > 7.0; urine has magnesium ammonium phosphate crystals	Persistently acidic urine. 24-hour urine may not have hyper-uricosuria
Radiology	Helical CT is the gold standard, but they are radio-opaque and if large enough can be seen on KUB	Radio-opaque stones. Nephrocalcinosis can be seen on plain X-ray. Dual energy CT scan, can identify	They are visible on abdominal X-rays, because of the dense sulfa and cystine molecule	They can usually be seen on plain abdominal X-ray films; however CT scan has been thought to be the most sensitive tests to diagnose	Helical CT. Radiolucent stones, not visualized in plain X-ray. Dual-energy CT can identify the composition
Treatment	Calcium oxalate stones from primary hyperoxaluria can often cause nephrocalcinosis and end-stage renal disease. Simultaneous liver kidney transplant is needed to correct the enzymatic defect. In secondary hyperoxaluria, calcium supplements, high fluid, and low oxalate diet is recommended	Treatment of distal renal tubular acidosis with alkali supplements which increases citrate, inhibiting precipitation of CaP. Thiazide can decrease hypercalciuria	Treatment is to reduce excretion and increase solubility of cystine, this can be achieved by reducing methionine intake. There is a very high risk of recurrence of these stones even after surgical removal of the stone	Natural history of these stones is associated with high morbidity. Conservative management is associated with a high 10-year mortality rate of ~28%, whereas surgical management can decrease the mortality rate to ~7.8%. Conservative treatment was also shown to be associated with a much higher, 36%, rate of chronic kidney disease	Alkalinization of urine increases the pH and increases the solubility of uric acid stones. Potassium bicarbonate/potassium citrate. Uric acid synthesis can be decreased by xanthine oxidase inhibitors such as allopurinol

but also is seen in patients with urinary tract infection or in males with prostatitis. Microscopic haematuria may be found in patients with hypercalciuria without stone formation.

Biochemical features

Evaluation of a patient with a single stone episode

A focused history targeting risk factors of kidney stones as described above along with basic laboratory investigation and radiology imaging should be obtained in all patients.

There is controversy over whether a complete metabolic evaluation is required after a single stone episode (Pak, 1982). Some limited data suggests that the same physiological and environmental disturbances characterize stone formation in patients with a single stone episode as with those with recurrent stone formation indicating the need for diagnostic evaluation even after a single stone episode (Strauss et al., 1982). The likelihood of recurrence of a second kidney stone episode after an untreated first stone in males is approximately 15% in 1 year, 35–40% in 5 years, and 50% in 10 years (Uribarri, 1989). This rate is slightly lower in females.

There are three ways to approach a patient with a first episode of kidney stone. In all settings, stone analysis is crucial as uric acid stones, cystine stones, and calcium phosphate stones require specific interventions:

1. *Limited evaluation*: since the recurrence rate of a second kidney stone event is so variable (Coe et al., 1977), a limited approach has been acceptable. This should include basic serum chemistries including calcium, phosphorus, and bicarbonate levels. Hyperparathyroidism is a common treatable cause of kidney stones, and hence should be ruled out (Parks et al., 1980). Similarly, distal renal tubular acidosis or metabolic acidosis due to chronic diarrhoea should also be ruled out. All patients should have imaging to evaluate the stone burden.
2. *Targeted evaluation*: a targeted approach of evaluation is based on the risk stratification for new stone formation. Patients with a low risk of stone recurrence should undergo a limited evaluation, whereas those with a high risk of recurrence should have a complete metabolic evaluation. The high-risk groups include white males with a family history of stones, and patients with malabsorption syndromes, metabolic syndrome, gout, urinary tract infection, pathological fractures, and osteoporosis. A patient with a known cystic, struvite, calcium phosphate, or uric acid stone has higher risk of recurrence and warrants a complete metabolic evaluation.
3. *Comprehensive evaluation*: since there is a potentially higher rate of recurrence of kidney stones after the first stone episode, another school of thought suggests that prophylactic therapy can lower the morbidity associated with stone disease. A retrospective analysis has shown that male calcium stone patients have a higher risk of relapse on medical therapy, and this is directly related to the number of pre-treatment kidney stones (Parks and Coe, 1994). Hence, patients who are willing to accept the therapeutic interventions should undergo a comprehensive evaluation. On the other hand, there are data supporting the notion that a comprehensive medical evaluation and prophylactic medical treatment in patients with history of single kidney stone is not cost-effective (Chandhoke, 2002). This comprehensive metabolic evaluation consists of blood tests, urine analysis, and two 24-hour urine collections.

Blood tests

This includes a calcium and phosphorus level. If the calcium level is in the high or the high-normal range; then intact parathyroid hormone (iPth) level should be checked. Primary hyperparathyroidism is associated with high/or high-normal calcium, low phosphorus, and high or inappropriately normal parathyroid hormone level and is more common in women. In a study of 48 patients with proven hyperparathyroidism and stone formation, serum calcium levels were in the normal range of 10.15–10.95 mg/dL (2.55–2.75 mmol/L) in 63% of the patients (Parks et al., 1980).

Various other conditions that could lead to hypercalcaemia and increased stone risk should be evaluated. Sarcoidosis and other granulomatous diseases are associated with high serum calcium due to high circulating levels of 1,25(OH) vitamin D_3 (calcitriol), released from resident macrophages in granulomatous lesions. In these cells, 25(OH) vitamin D_3 1-hydroxylase enzyme is not responsive to the iPth level. Patients with hypoparathyroidism on vitamin D supplementation also tend to have hypercalcaemia. Hyperthyroidism may also cause hypercalcaemia, thought to be due to thyroxine-mediated increased bone turnover (Chandhoke, 2002). Parathyroid hormone-related peptide can also cause hypercalcaemia in various malignancies although stone formation is rare in that setting. Lithium causes hypercalcaemia, by decreasing parathyroid gland sensitivity to calcium (Brown, 1981).

Plasma oxalate should be measured in patients with calcium oxalate stones especially in conditions associated with primary or secondary hyperoxaluria. The normal plasma oxalate level ranges from 1.3 to 3.1 μmol/L, and a linear correlation was found between plasma oxalate and plasma creatinine (Kasidas and Rose, 1986). High levels are associated with increased risk of calcium oxalate precipitation.

Urine analysis

Microscopic examination of freshly voided urine specimen may show crystals in urine (Chapter 6) which could give an idea about the type of stone (Fig. 200.1). For example:

- Calcium oxalate: dumb-bell-shaped and double pyramids
- Calcium monohydrogen phosphate (brushite): amorphous crystals
- Struvite crystals: coffin lids
- Uric acid crystals: pears and diamond
- Cystine crystals: hexagons
- Indinavir crystals: needle-shaped crystals.

Hypercalciuria can be associated with haematuria as well. A urine pH of > 7.0 is strongly associated with calcium phosphate or struvite stone. Uric acid and calcium oxalate crystals can be present in urine even in the absence of kidney stones. Indinavir crystals are present in patients with HIV being treated with indinavir.

Twenty-four-hour urine collection

At least two 24-hour urine collections should be obtained while the patients are on their usual diet, fluid intake, and activity level. In case there is great variability in the two urine collections, a third 24-hour collection may be required. The urine volume, pH, and excretion of calcium, uric acid, citrate, oxalate, and sodium are measured. In order to make sure that it is an adequate collection the creatinine excretion is also measured.

Fig. 200.1 Urinary crystals commonly seen in nephropathies. Calcium oxalate dihydrate crystals are shown in Panel A. Dumbbell-shaped calcium oxalate monohydrate crystals, which are the size of erythrocytes, are shown to the left of the pyramidal dihydrate crystals in Panel B, elongated, lath-shaped (like a plank of wood) brushite crystals can be seen in Panel C, rhomboidal uric acid crystals in panel D, uric acid microcrystallites in Panel E, coffin-lid-shaped struvite crystals in Panel F, and cystine crystals in Panel G.
Reproduced with permission from Coe, F.L., Parks, J. H., and Asplin, J. R. (1992). The pathogenesis and treatment of kidney stones. *N Engl J Med*, 327(16), 1141–52.

Normal ranges of the urine chemistries have varied at different labs, and are given as follows:

- Volume > 1.5 L/day
- pH: 5.8–6.2
- Calcium: < 250 mg/dL, 62.5 mmol/L (women); < 300 mg/dL, 75 mmol/L (men)
- Oxalate: < 45 mg/day
- Citrate: > 550 mg/dL (women), > 450 mg/dL (men)
- Uric acid: < 750 mg/dL (women), < 800 mg/dL (men)
- Phosphate: 500–1500 mg/day.

There have been various studies to determine the adequate number of urine collections in order to assess stone risk. In a study by Hess et al. (1997), the relative diagnostic utility of one, two, or three 24-hour urine collections was compared in 75 recurrent, idiopathic calcium stone formers. Three urine collections were associated with finding a significant metabolic abnormality when compared to one or two 24-hour urine collections. Similar results were found in a study performed in 1132 patients (Parks et al., 2002), where more than one urine collection was recommended.

The timing of the urine collections is also important. In the study by Hess et al. (1997), it was noted that the diagnostic risk factor assessment becomes more accurate with an increase in time from the first stone event. It was also noted that urine collected during the weekend had more volume than the one collected during the week. It is important to inform patients to stay on their usual diet and activity level during the time of the fluid collection. It is suggested to wait at least 1–2 months after the patient has recovered from any surgical intervention or stone event. The urine should not be infected, and the patient should maintain their usual diet and not a temporarily modified diet soon after stone event.

Table 200.2 Kidney stones, urinary risk factors, and chemical effects

Type of stone	Urinary risk factor	Chemical effect
Cystine	↑ cystine	↑ SS for cystine
Uric acid stones	↓ pH, ↑ uric acid, ↓ volume	↑ SS for uric acid
Calcium stones	↓ volume, ↑oxalate, ↑ Ca, ↑ pH, ↑ promoters, ↓ inhibitors, ↓ uric acid	↑ CaOx and ↑ Ca PO_4 stones
Infection	↑↑ pH, ↑ NH_4+	↑ Mg ammonium PO_4 + Ca $3(PO_4)_2$ SS

SS = supersaturation.

The 24-hour urine measurements should be accompanied by an estimate of the relative super saturation of stone-forming constituents of the urine. This measurement would help to monitor the utility and subsequently, the potential benefits of various therapeutic interventions. In situations where the stone composition is not known, management and risk assessment of these patients is dependent on the results of the 24-hour urine supersaturation profile (Table 200.2). A urine volume of > 2L is preferred to avoid urine concentrations of stone-forming constituents that are above the supersaturation threshold that could lead to precipitation. A low salt, adequate calcium diet, and low-protein diet is preferred for all patients with stones.

Chemical structure of urine stones

See Table 200.3.

Inhibitors in urine that prevent stone formation

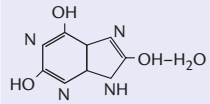
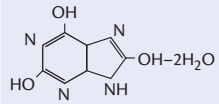
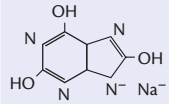
Nephrocalcin, a putative stone inhibitor, contains a gamma-carboxyglutamic acid (Gla) residue, which tends to aggregate to form a series of high-molecular-mass polymers. In some stone formers, nephrocalcin lacks the Gla residue, which decreases the ability to inhibit crystal growth (Nakagawa et al., 1983). An X-linked recessive nephrolithiasis disease has been reported where the affected males have a defect in nephrocalcin, leading to increased crystal growth.

Osteopontin, another putative stone inhibitor, is isolated from bone, kidney, inner ear, decidual glands, smooth muscle, and breast milk (Brown et al., 1992). It is expressed in response to growth factors and tissue injury. It is thought to be protective against calcium oxalate crystal aggregation when phosphorylated.

Tamm–Horsfall protein (uromodulin) is the major urinary glycoprotein of normal urine and in stone formers it may be modified so that it is less active in preventing crystal aggregation, especially in low pH and in high calcium concentrations (Hess, 1991). Tamm–Horsfall protein can act as a promoter or inhibitor of stone formation depending on the molecular size, state of aggregation, and concentration of citrate in urine. Similarly, citrate concentration has a linear relation with calcium oxalate crystal aggregation inhibition (Erwin et al., 1994).

Uromucoids are substances that promote calcium phosphate crystallization and it has also been shown that the presence of urate can enhance the precipitation of calcium oxalate.

Table 200.3 Chemical structure of kidney stones

	Chemical structure
Calcium oxalate monohydrate	$CaC_2O_4 \cdot H_2O$
Calcium oxalate dehydrate	$CaC_2O_4 \cdot 2H_2O$
Calcium phosphate/hydroxyapatite	$Ca_{10}(PO_4)_6(OH)_2$
Calcium hydrogen phosphate/brushite	$CaHPO_4 \cdot 2H_2O$
Calcium pyrophosphate	$Ca_9(Mg,Fe^{2+})(PO_4)_6(PO_3OH)$
Magnesium ammonium phosphate hexahydrate/struvite	$MgNH_4PO_4 \cdot 6H_2O$
Magnesium acid phosphate trihydrate	$MgHPO_4 \cdot 3H_2O$
Struvite stone	$(MgNH_4PO_4 \cdot 6H_2O)$ + $(Ca_{10}(PO_4)6 \cdot CO_3)$
Uric acid monohydrate	
Uric acid dihydrate	
Monosodium urate (exists only in conjunction with calcium stones)	

From Brenner and Rector.

Diagnosis of kidney stones

Radiological studies

- Non-contrast, helical computed tomography (CT scan; see Chapter 14) has become the gold standard to diagnose kidney stones and ureteral obstruction. CT scan cuts are 3–5 mm in thickness. The location and type of radiolucency might help determine the composition of the stone. Cystine stones are radio-opaque and not as dense as calcium oxalate stones. Calcium phosphate stones are usually bilateral and associated with nephrocalcinosis, typically also seen in medullary sponge kidney. Staghorn calculi are large infection-related stones which grow in all directions. Occasionally, one might miss very small non-obstructing ureteral phleboliths that course through the ureter and might be visualized as a small rim (Colistro et al., 2002).

- Dose of radiation (Chapter 10): radiation dose has been a concern for patients with a large stone burden who may undergo repeated studies. Low-dose CT scan may serve as an effective tool in evaluating patients with urolithiasis for follow-up studies by minimizing the dose of radiation. The sensitivity and specificity is similar to standard CT and can readily diagnose obstruction. It may be problematic in diagnosing small stones, < 2 mm (Zilberman et al., 2011). Ultra-low-dose CT scan protocols detect distal ureteral calculi in a fashion similar to that of conventional CT protocols in a cadaveric model. These protocols may decrease the radiation dose up to 95% (Jellison et al., 2009).

- Intravenous urography (Chapter 11): intravenous urography is easy to perform, inexpensive, has moderate radiation exposure, and was once considered the gold standard test. It is not a preferred mode of diagnosis anymore because of the contrast agent exposure and lower sensitivity than CT exams. It is still occasionally used in planning therapy and confirming diagnosis.
- Renal ultrasound (Chapter 13): this is a procedure of choice in order to avoid radiation exposure, especially in pregnant women, but is less sensitive than a CT scan. It is helpful in diagnosing in hydronephrosis or obstruction. Its sensitivity can improve by using colour Doppler technology to assess urine flow rates and the presence of a post-obstructive blush. The disadvantages are that smaller stones cannot be easily visualized. In pregnancy it is reported to have 60% sensitivity. Transvaginal ultrasounds can detect distal ureteral stones (Butler et al., 2000). In pregnancy it can be difficult to distinguish between an obstructing stone and the physiological changes to the ureter seen during pregnancy (Laing et al., 1994).
- Abdominal X-ray: only radio-opaque stones can be visualized by a plain film. These include calcium, struvite, and cystine stones. Uric acid stones cannot be visualized as they are radiolucent. Large radio-opaque stones in the pelvis are most likely struvite stones. Bilateral diffuse calcification in the cortico-medullary junction is most likely calcium phosphate stones, typically seen in nephrocalcinosis due to renal tubular acidosis or medullary sponge kidney. Advantages of this modality are that it is inexpensive and there is minimal radiation exposure but the disadvantage is that it has very limited sensitivity and specificity. A combination of ultrasonography and abdominal plain film may be a good compromise for following patients at risk of stone recurrence while minimizing radiation exposure.
- Dual-energy CT scan (Zilberman et al., 2010): this is an enhanced CT scan modality which utilizes two energy sources. Calcium phosphate and various types of calcium oxalate stones can be distinguished using this technology. The heterogeneity of the stone composition can be used to assess fragility of the stone and can therefore predict the efficacy of shock wave lithotripsy (Williams et al., 2002).
- Magnetic resonance imaging (MRI; Chapter 15): MRI is a rarely used modality for stone imaging since it is expensive and the image resolution is similar to a CT scan. MRI is used for stone detection primarily in pregnant patients in order to minimize exposure to ionized radiation. There are no reported harmful effects to the pregnant mother or fetus from MRI. However, since there is limited data on safety of MRI during organogenesis, the National Radiology Protection Board advises to avoid MRI in the first trimester (Duncan, 1996).
- Imaging of stones due to indinavir: indinavir, an HIV protease inhibitor, may form stones that are not radiopaque and signs of obstruction may be minimal or absent; thus, the diagnosis may be missed with intravenous urogram, ultrasonography, and non-contrast CT scans. In such patients, contrast-enhanced CT scanning may be required to establish the diagnosis (Schwartz et al., 1999).

Stone analysis

Patients should be strongly encouraged to retrieve any passed stones so that analysis is possible. Compositional stone analysis should be an integral part of the metabolic evaluation of patients with nephrolithiasis, as stone analysis alone may provide guidance for therapeutic treatment and may obviate a formal metabolic evaluation (Kourambas et al., 2001).

Each time a patient passes a urinary stone, it should be analysed by sensitive imaging techniques such as infrared spectroscopy or by X-ray diffraction to determine the type of stone. Passage of recurrent stones should also be analysed as the type of stone can change with time and treatment. The different methods used for stone analysis include:

- Quantitative wet chemistry (Hesse et al., 2005): this technique detects the individual ions in the stone. This usually provides a simple qualitative and semi-quantitative analysis of the stone and provides approximate information of the stone composition. These methods are now considered obsolete.
- X-ray diffraction photography: X-ray diffraction identifies the constituents of a calculus by its unique diffraction patterns or 'fingerprints' produced by monochromatic X-ray bombardment of crystalline material. The X-rays, when travelling intramolecular distances, are diffracted in particular patterns that identify the structure of the crystals. The diffractogram formed by the reflected X-rays allows definite identification of an unknown crystalline substance (Ansari et al., 2005). The main disadvantage of X-ray diffraction is its poor ability to identify some amorphous materials and constituents present in trace amounts.
- Infrared spectroscopy:
  - This is a physical analytic technique that reveals more detail of the fine structure of the stone and provides the capability for *in vivo* stone analysis. It employs a spectrophotometer which exposes stone molecules to infrared light. Most organic and inorganic solids have absorption patterns at different wavelengths that are characteristic of the particular make-up of the molecule. Correlation of specific observed patterns with the known reference spectra helps in identification of the stone molecules. Infrared spectroscopy is useful for the identification of non-crystalline materials, including amorphous and fatty substances. This gives it an advantage over X-ray diffraction, which is useful primarily for analysing crystalline compounds (Rebentisch et al., 1988). Such compounds as carbonate apatite and hydroxyl apatite may give weak, diffuse lines on an X-ray diffraction pattern but may be identified and measured by infrared spectroscopy. It is also able to identify the presence of drugs in the calculi, or artefacts.
  - Some newer techniques that have been developed include Fourier transform infrared spectrophotometry (FTIR), which allows identification by comparisons made to libraries of reference spectra (Sabot et al., 1999).
- Crystallography: the crystallographic techniques of polarization microscopy are useful to study the crystalline structure, order of deposition of components, and the nucleus. This needs a high degree of skill, and hence is performed at select laboratories. Electron microscopy helps in identifying the organic matrix along with the crystalline structure (Saint and Dyson, 1990; Daudon and Jungers, 1991).
- Thermogravimetric analysis: this is used to characterize the two hydrates of the calcium oxalate stones, the mono-and dihydrate.

Table 200.4 Differential diagnosis of uric acid stones

	Radio-opaque	Urine uric acid	Urine pH	Urine calcium	Serum uric acid	Response alkali Rx
Uric acid	No	Normal	↓	Normal	↑	Yes
Xanthine	No	Normal/↑	Normal	Normal	↓	No
2,8-Dihydroxyadenine	No	Normal	Normal	Normal	Normal	No
Mixed uric acid-calcium	Yes	Normal/↑	Normal/↓	Normal/↑	Normal/↑	Yes
Hyperuricosuric Ca	Yes	↑	Normal	Normal	Normal	Yes

From Cameron and Sakhaee (2007).

It is believed that this method may be able to inform us about the age of the stone and the activity of the disease (Kaloustian et al., 2003).

- Micro CT has been shown to provide exceptionally high quality imaging of the fine structural detail within urinary calculi and has also been used to identify the mineral composition of urinary stones non-destructively. Micro CT can analyse intact stones with excellent resolution of the structural detail and can discriminate multiple mineral types within the stones (Zarse et al., 2004).
- Various newer techniques have been developed in recognizing the atomic structure and trace elements in the stones. These include:
  - Atomic absorption spectrophotometry (Durak et al., 1998)
  - Inductivity coupled plasma atomic emission spectroscopy (Hofbauer et al., 1991)
  - Instrumental neutron activation analysis (INAA) (Lin et al., 1987)
  - Proton-induced characteristic X-ray emission spectroscopy (PIXE) (Saint and Dyson, 1990)
  - Nuclear reaction analysis (NRA) (Saint and Dyson, 1990).

Melamine crystalluria

Melamine is a synthetic product also called cyanuramide. It is not made for human or animal consumption, but if it is used as a contaminant, it leads to crystalluria and urinary tract obstruction. The 'melamine milk crisis' in 2008 was truly a disaster in China. There were six deaths and 294,000 children were affected, > 5000 of them hospitalized with urinary problems, including intratubular obstruction and kidney stones related to the consumption of melamine-contaminated infant formula (Langman et al., 2009; Hu et al., 2010).

Differential diagnosis

Clinical presentation of a patient with kidney stones includes colic pain and haematuria but other conditions could mimic these symptoms. Haematuria and renal colic pain may result from renal cell cancer. Haematuria from glomerular bleeding is usually not associated with pain, and is usually microscopic although occasionally gross. Renal colic pain may mimic pain from aortic aneurysm dissection, ectopic pregnancy, appendicitis, and intestinal obstruction, although these conditions would not cause haematuria.

Loin pain haematuria syndrome (Chapter 47) is a poorly defined condition in which pain may resemble renal colic pain with haematuria although there are no kidney stones or other cause of obstruction or pain identified (Cameron and Sakhaee, 2007).

Differential diagnosis of calcium stones

- Hyperuricosuric calcium nephrolithiasis: especially with gouty diathesis, in patients with urine uric acid > 700 mg/day and urine pH < 5.5.
- Hyperoxaluric calcium nephrolithiasis (hyperoxaluria > 44mg/day):
  - Enteric hyperoxaluria in conditions such as inflammatory bowel disease, malabsorption syndrome, jejunoileal bypass, and ileal resection. Usually it is associated with hypocitraturia and low urine volume which might predispose to stone formation
  - Dietary hyperoxaluria
  - Primary hyperoxaluria: marked hyperoxaluria (> 80 mg/day) due to a genetic defect
- Hypocitraturic calcium nephrolithiasis (citrate in urine < 320 mg/day):
  - Distal renal tubular acidosis: non-gap metabolic acidosis due to impairment in acidification of urine, urine pH > 6.5 and is associated with hypokalaemia
  - Chronic diarrhoea leads to excessive alkali loss via gastrointestinal tract
  - Idiopathic hypocitraturia
- Hypomagnesiuric calcium nephrolithiasis (urinary Mg < 50 mg/day):

Differential diagnosis of uric acid stones

Table 200.4 shows the differential diagnosis of uric acid stones.

References

Ansari, M. S., Gupta, N. P., Hemal, A. K., *et al.* (2005). Spectrum of stone composition: structural analysis of 1050 upper urinary tract calculi from northern India. *Int J Urol*, 12(1), 12–16.

Atan, L., Andreoni, C., Ortiz, V., *et al.* (2005). High kidney stone risk in men working in steel industry at hot temperatures. *Urology*, 65(5), 858–61.

Brown, E. M. (1981). Lithium induces abnormal calcium-regulated pth release in dispersed bovine parathyroid cells. *J Clin Endocrinol Metab*, 52(5), 1046–8.

Brown, L. F., Berse, B., Van de Water, L., *et al.* (1992). Expression and distribution of osteopontin in human tissues: widespread association with luminal epithelial surfaces. *Mol Biol Cell*, 3(10), 1169–80.

Butler, E. L., Cox, S. M., Eberts, E. G., *et al.* (2000). Symptomatic nephrolithiasis complicating pregnancy. *Obstet Gynecol*, 96(5 Pt 1), 753–6.

Cameron, M. A. and Sakhaee, K. (2007). Uric acid nephrolithiasis. *Urol Clin North Am*, 34(3), 335–46.

Chandhoke, P. S. (2002). When is medical prophylaxis cost-effective for recurrent calcium stones? *J Urol*, 168(3), 937–40.

Chillaron, J., Font-Llitjós, M., Fort, J., *et al.* (2010). Pathophysiology and treatment of cystinuria. *Nat Rev Nephrol*, 6(7), 424–34.

Coe, F. L., Keck, J., and Norton, E. R. (1977). The natural history of calcium urolithiasis. *JAMA*, 238(14), 1519–23.

Coe, F. L., Parks, J. H., and Asplin, J. R. (1992). The pathogenesis and treatment of kidney stones. *N Engl J Med*, 327(16), 1141–52.

Colistro, R., Torreggiani, W. C., Lyburn, I. D., *et al.* (2002). Unenhanced helical CT in the investigation of acute flank pain. *Clin Radiol*, 57(6), 435–41.

Daudon, M. and Jungers, P. (1991). [Analytical methods of calculi and urinary crystals.] *Rev Prat*, 41(21), 2017–22.

Duncan, K. R. (1996). The development of magnetic resonance imaging in obstetrics. *Br J Hosp Med*, 55(4), 178–81.

Durak, I., Yasar, A., Yurtarslani, Z., *et al.* (1998). Analysis of magnesium and trace elements in urinary calculi by atomic absorption spectrophotometry. *Br J Urol*, 62(3), 203–5.

Erwin, D. T., Kok, D. J., Alam, J., *et al.* (1994). Calcium oxalate stone agglomeration reflects stone-forming activity: citrate inhibition depends on macromolecules larger than 30 kilodalton. *Am J Kidney Dis,* 24(6), 893–900.

Hess, B. (1991). The role of Tamm-Horsfall glycoprotein and nephrocalcin in calcium oxalate monohydrate crystallization processes. *Scanning Microsc*, 5, 689–95.

Hess, B., Hasler-Strub, U., Ackermann, D., *et al.* (1997). Metabolic evaluation of patients with recurrent idiopathic calcium nephrolithiasis. *Nephrol Dial Transplant*, 12(7), 1362–8.

Hesse, A., Kruse, R., Geilenkeuser, W. J., *et al.* (2005). Quality control in urinary stone analysis: results of 44 ring trials (1980-2001). *Clin Chem Lab Med,* 43(3), 298–303.

Hofbauer, J., Steffan I, Höbarth K., *et al.* (1991). Trace elements and urinary stone formation: new aspects of the pathological mechanism of urinary stone formation. [Erratum appears in *J Urol* 1992 Sep; 148(3): 898]. *J Urol*, 145(1), 93–6.

Hu, P., Lu, L., Hu, B., *et al.* (2010). The size of melamine-induced stones is dependent on the melamine content of the formula fed, but not on duration of exposure. *Pediatr Nephrol*, 25(3), 565–6.

Jellison, F. C., Smith, J. C., Heldt, J. P., *et al.* (2009). Effect of low dose radiation computerized tomography protocols on distal ureteral calculus detection. *J Urol*, 182(6), 2762–7.

Kaloustian, J., El-Moselhy, T. F., and Portugal, H. (2003). Determination of calcium oxalate (mono- and dihydrate) in mixtures with magnesium ammonium phosphate or uric acid: the use of simultaneous thermal analysis in urinary calculi. *Clin Chim Acta*, 334(1–2), 117–29.

Kasidas, G. P. and Rose, G. A. (1986). Measurement of plasma oxalate in healthy subjects and in patients with chronic renal failure using immobilised oxalate oxidase. *Clin Chim Acta*, 154(1), 49–58.

Koga, S., Arakaki, Y., Matsuoka, M., *et al.* (1991). Staghorn calculi—long-term results of management. *Br J Urol*, 68(2), 122–4.

Kourambas, J., Aslan, P., The, C. L., *et al.* (2001). Role of stone analysis in metabolic evaluation and medical treatment of nephrolithiasis. *J Endourol,* 15(2), 181–6.

Laing, F. C., Benson, C. B., DiSalvo, D. N., *et al.* (1994). Distal ureteral calculi: detection with vaginal US. *Radiology*, 192(2), 545–8.

Langman, C. B., Alon, U., Ingelfinger, J., *et al.* (2009). A position statement on kidney disease from powdered infant formula-based melamine exposure in Chinese infants. *Pediatr Nephrol*, 24(7), 1263–6.

Lemann, J. (1993). Composition of the diet and calcium kidney stones. *N Engl J Med*, 328(12), 880–2.

Lin, S. M., Tseng, C. L., and Yang, M. H. (1987). Determination of major, minor and trace elements in urinary stones by neutron activation analysis. *Int J Rad Appl Instrum A*, 38(8), 635–9.

Nakagawa, Y., Abram, V., Kézdy, F. J., *et al.* (1983). Purification and characterization of the principal inhibitor of calcium oxalate monohydrate crystal growth in human urine. *J Biol Chem*, 258(20), 12594–600.

Pak, C. Y. (1982). Should patients with single renal stone occurrence undergo diagnostic evaluation? *J Urol*, 127(5), 855–8.

Parks, J. H. and Coe, F. L. (1994). An increasing number of calcium oxalate stone events worsens treatment outcome. *Kidney Int*, 45(6), 1722–30.

Parks, J., Coe, F., and Favus, M. (1980). Hyperparathyroidism in nephrolithiasis. *Arch Intern Med*, 140(11), 1479–81.

Parks, J. H., Goldfisher, E., Asplin, J. R., *et al.* (2002). A single 24-hour urine collection is inadequate for the medical evaluation of nephrolithiasis. *J Urol*, 167(4), 1607–12.

Rebentisch, G., Berg, W., Pirlich, W., *et al.* (1988). Assessment and maintenance of the quality of urolith analyses in a comparison of methods. 4th International Ring Test to check quality. *Int Urol Nephrol*, 20(1), 35–45.

Sabot, J. -F., Bornet, C. E., Favre, S., *et al.* (1999). The analysis of peculiar urinary (and other) calculi: an endless source of challenge. *Clin Chim Acta*, 283(1–2), 151–8.

Saint, A. and Dyson, N. A. (1990). Nuclear techniques for the analysis of urinary calculi. *Br J Urol*, 66(3), 232–9.

Schwartz, B. F., Schenkman, N., Armenakas, N. A., *et al.* (1999). Imaging characteristics of indinavir calculi. *J Urol*, 161(4), 1085–7.

Spetie, D. N., Nadasdy, T., Nadasdy, G., *et al.* (2006). Proposed pathogenesis of idiopathic loin pain-hematuria syndrome. *Am J Kidney Dis*, 47(3), 419–27.

Strauss, A. L., Coe, F. L., and Parks, J. H. (1982). Formation of a single calcium stone of renal origin: clinical and laboratory characteristics of patients. *Arch Intern Med*, 142(3), 504–7.

Teichman, J. M. H., Long, R. D., and Hulbert, J. C. (1995). Long-term renal fate and prognosis after staghorn calculus management. *J Urol*, 153(5), 1403–7.

Trinchieri, A., Ostini, F., Nespoli, R., *et al.* (1999). A prospective study of recurrence rate and risk factors for recurrence after a first renal stone. *J Urol*, 162(1), 27–30.

Uribarri, J., Oh, M. S., and Carroll, H. J. (1989). The first kidney stone. *Ann Intern Med*, 111(12), 1006–9.

Vezzoli, G., Soldati, L., and Gambaro, G. (2008). Update on primary hypercalciuria from a genetic perspective. *J Urol*, 179(5), 1676–82.

Williams, J. C., Jr., Paterson, R. F., Kopecky, K. K., *et al.* (2002). High resolution detection of internal structure of renal calculi by helical computerized tomography. *J Urol*, 167(1), 322–6.

Worcester, E. M. and Coe, F. L. (2010). Calcium kidney stones. *N Engl J Med*, 363(10), 954–63.

Zarse, C. A., McAteer, J. A., Sommer, A. J., *et al.* (2004). Nondestructive analysis of urinary calculi using micro computed tomography. *BMC Urol*, 4(1), 15.

Zilberman, D. E., Ferrandino, M. N., Preminger, G. M., *et al.* (2010). In vivo determination of urinary stone composition using dual energy computerized tomography with advanced post-acquisition processing. *J Urol*, 184(6), 2354–9.

Zilberman, D. E., Tsivian, M., Lipkin, M. E., *et al.* (2011). Low dose computerized tomography for detection of urolithiasis—its effectiveness in the setting of the urology clinic. *J Urol*, 185(3), 910–14.

CHAPTER 201

Calcium stones

David A. Bushinsky and Orson Moe

Idiopathic hypercalciuria

Idiopathic hypercalciuria is defined as an excess of urine calcium excretion without a discernible metabolic cause. This excess urine calcium excretion is thought to occur when there is dysregulation of calcium transport at the major sites of calcium transport: the intestine, kidney, and bone (Frick and Bushinsky, 2003; Bushinsky et al., 2006, 2012; Bushinsky, 2008; Monk and Bushinsky, 2010, 2011). Idiopathic hypercalciuria is a heterogeneous disorder with varying and overlapping underlying pathogenic mechanisms.

An increase in intestinal calcium absorption may occur though either a direct increase in calcium absorption or through excess 1,25 dihydroxyvitamin D_3 ($1,25(OH)_2D_3$)-mediated calcium absorption. Decreased renal mineral reabsorption of calcium will lead to hypercalciuria. An increase in urinary phosphorus will lead to hypercalciuria through a hypophosphataemia-mediated increase in $1,25(OH)_2D_3$ leading to increased intestinal calcium absorption. Enhanced bone mineral resorption will increase serum calcium, suppress parathyroid hormone (PTH) and $1,25(OH)_2D_3$, and lead to hypercalciuria. Excess PTH and/or $1,25(OH)_2D_3$ will result in hypercalcaemia, an increased filtered load of calcium, and will result in hypercalciuria.

Although ion and hormone measurements may help to differentiate between these alternative mechanisms, small changes in these measurements may be beyond our ability to detect. We not only measure imprecisely but there are excellent homeostatic control mechanisms to restore ion concentration to the normal range. Often the system must be stressed, perhaps by withholding a particular ion such as calcium, to better understand mechanisms. Even then, with the polygenic nature of hypercalciuria, dysregulation at multiple sites may be involved to produce the phenotype (Frick and Bushinsky, 2003; Coe et al., 2005; Moe, 2006a). Indeed, as we argue below, idiopathic hypercalciuria may be a systemic dysregulation of calcium transport.

Effects of increased intestinal calcium absorption

With increased ingestion of dietary calcium, a primary increase in intestinal calcium absorption will result in an increase in serum calcium resulting in decreased serum PTH and $1,25(OH)_2D_3$. The filtered load of calcium will increase, which with decreased PTH, which increases tubular reabsorption of calcium, will result in hypercalciuria. With fasting or a low-calcium diet, serum and urine calcium will move into the normal range since there is little intestinal calcium for the excess absorption to be manifest.

With excess $1,25(OH)_2D_3$, intestinal calcium absorption will increase, as will serum calcium, and PTH will fall. However, after fasting or a low-calcium diet, urine calcium will remain elevated as increased $1,25(OH)_2D_3$ stimulates bone resorption. Serum calcium and phosphorus will remain normal or even elevated depending on the magnitude of the renal tubule calcium reabsorption compared to the bone mineral resorption.

Effects of decreased renal tubule mineral reabsorption

Decreased renal calcium reabsorption will lead directly to hypercalciuria resulting in a fall in serum calcium and increase in serum PTH and $1,25(OH)_2D_3$, the latter leading to increased intestinal calcium absorption. The hypercalciuria will persist after an overnight fast or while consuming a low-calcium diet. Decreased renal phosphorus reabsorption will decrease serum phosphorus and stimulate serum $1,25(OH)_2D_3$ leading to enhanced calcium absorption and an increase in the filtered load of calcium. The increased serum calcium will suppress PTH, resulting in hypercalciuria.

Worcester et al. has shown in patients with idiopathic hypercalciuria that the hypercalciuria is not due to alterations in PTH (Worcester et al., 2007). She has also shown that in patients with idiopathic hypercalciuria, there is reduced calcium absorption in the proximal tubule (Worcester et al., 2008).

Effects of enhanced bone demineralization

Enhanced bone demineralization will lead to an increase in serum calcium concentration, a fall in PTH, and an increase in the filtered load of calcium resulting in hypercalciuria.

This analysis of ion and hormone levels lead to testable hypotheses. For example, intestinal calcium absorption will be low only with enhanced bone resorption. Serum PTH should be elevated and fasting serum calcium suppressed only with a defect in renal calcium reabsorption and fasting serum phosphorus depressed only with a defect in renal phosphorus reabsorption. On a low-calcium diet, fasting urine calcium excretion will normalize only with a direct increase in intestinal calcium absorption.

Human data on pathophysiology of idiopathic hypercalciuria

Parathyroid hormone

Several studies have reported elevated levels of PTH in hypercalciuric stone formers, which suggest that hypercalciuria leads to a fall in blood ionized calcium leading to an increase in PTH (Coe et al., 1973; Bordier et al., 1977). However, in most reports fasting PTH levels are not elevated and are even low in some, suggesting that most patients do not have decreased tubule calcium reabsorption as the primary mechanism for the hypercalciuria (Bordier et al., 1977; Burckhardt and Jaeger, 1981; Bataille et al., 1991). Bataille et al. found only 1 of 42 patients with idiopathic hypercalciuria who had fasting hypercalciuria and an elevated level of PTH (Bataille et al., 1991), again suggesting that this mechanism for idiopathic

hypercalciuria is unusual. However, most of these studies were done before specific two-site PTH assays, which are more specific for the biologically intact hormone, became available.

Calcitriol

Serum levels of $1,25(OH)_2D_3$ in patients with idiopathic hypercalciuria have been reported in a number of studies (Gray et al., 1977; Shen et al., 1977; Coe et al., 1982; Broadus et al., 1984; Insogna et al., 1985; Bataille et al., 1991). Often, but not invariably, the levels are elevated suggesting either (a) a primary increase in calcitriol leading to hypercalciuria or (b) a secondary response to decreased tubule reabsorption of calcium or phosphorus. Elevated levels of calcitriol are inconsistent with increased bone resorption as a primary mechanism for the hypercalciuria. Elevated levels of calcitriol suggest that increased intestinal calcium absorption is responsible for hypercalciuria in the many patients with idiopathic hypercalciuria.

Increased urinary phosphorus

Nine of 59 members of a Bedouin tribe had hyperphosphaturia, hypophosphataemic rickets, elevated levels of calcitriol, and hypercalciuria (Tieder et al., 1987). Almost half of the remaining patients had hypercalciuria with a small reduction in serum phosphate levels and a mild increase in calcitriol levels, all indicating that a loss of urinary phosphate could be the cause of the hypercalciuria. In the study by Worcester et al., there is evidence of increased post-prandial phosphaturia in hypercalciuric stone formers (Worcester et al., 2008). One study suggested the mutation in the gene coding for the proximal tubule sodium phosphate cotransporter NaPi-2a leads to hyperphosphaturia and subsequent hypercalciuria (Prie et al., 2002) but the functional data that was critical to support this hypothesis does not appear to be reproducible (Virkki et al., 2003). A recent study of 98 pedigrees with multiple hypercalciuric stone formers found that although variants of this gene are not rare, these variants do not appear to be associated with clinically significant renal phosphate or calcium handling anomalies (Lapointe et al., 2006). In contrast to NaPi-2a, NaPi-2c mutations in humans are linked to phosphate wasting and secondary hypercalciuria.

Human evidence for a systemic dysregulation of calcium transport in idiopathic hypercalciuria

To help determine the mechanism of hypercalciuria in patients with idiopathic hypercalciuria Coe et al. (1982) studied 24 patients and 9 controls each fed a low-calcium diet (2 mg/kg per day) for over a week. Neither serum calcium nor calcitriol levels differed between the groups; however, the hypercalciurics had a mild decrease in PTH levels. On this low-calcium diet the controls excreted less calcium then they consumed while 16 of the 24 hypercalciuric patients excreted more calcium, indicating probable loss of bone mineral. There was a smooth transition between those who retained calcium, suggesting enhanced intestinal calcium absorption, and those who lost calcium, suggesting a failure of the kidney to adequately reabsorb calcium. This smooth transition in urine calcium excretion and in net calcium retention suggested that there were not specific, well-defined pathophysiologic aetiologies for the cause of the hypercalciuria. Thus, especially in clinical setting, patients cannot be accurately and consistently classified, the prescription of a diet low in calcium to those who are thought to absorb excessive amounts of dietary calcium can potentially lead to a dangerous reduction of bone mineral density (BMD) (Coe et al., 1982; Freundlich et al., 2002; Asplin et al., 2003). We also know that a normal calcium diet when given with sodium and protein restriction retards stone formation (Borghi et al., 2002).

Adams et al. (1982) showed that giving calcitriol to normal men on a normal- or high-calcium diet lead to increased intestinal calcium absorption and urine excretion, in the absence of hypercalcaemia, similar to observations in patients with idiopathic hypercalciuria. When normal men were fed a very low-calcium diet, administration of calcitriol also led to an increase in intestinal calcium absorption and to increased urine calcium excretion (Maierhofer et al., 1983). Calcium retention fell, which can only result from enhanced bone resorption (Maierhofer et al., 1983).

In men with idiopathic hypercalciuria, Favus et al. has found an increased number of vitamin D receptors in peripheral blood monocytes, in the absence of elevated levels of calcitriol, in comparison to age-matched controls (Favus et al., 2004).

These studies indicate that idiopathic hypercalciuria can be modelled by calcitriol administration, suggesting that an excess of this hormone, or its activity through an increased number of vitamin D receptors, may be responsible, at least in part, for this disorder.

Bone resorption in patients with idiopathic hypercalciuria

Patients with hypercalciuria are often in negative calcium balance, that is, they excrete more calcium than they absorb, reflecting a net loss of total body calcium (Coe and Bushinsky, 1984; Pak et al., 1992; Bushinsky, 1998, 2000, 2002; Monk and Bushinsky, 2010). The source of this additional urine calcium is almost certainly the skeleton which is the largest store of calcium in the body (Asplin et al., 2003). The bone lesion, if any, in idiopathic hypercalciuria is not well defined and there are conflicting reports in the literature. What is clear is that patients with nephrolithiasis have a bone fracture rate that is higher than those without nephrolithiasis (Melton et al., 1998).

The measurement of bone resorption, formation, and turnover in idiopathic hypercalciuria has produced mixed results. Some studies showed increase in markers indicating increased bone turnover (Sutton and Walker, 1986; Heilberg and Weisinger, 2006). Urinary hydroxyproline is increased in unselected patients with idiopathic hypercalciuria (Sutton and Walker, 1986) and serum osteocalcin levels are elevated in stone formers with abnormal renal tubule calcium reabsorption but not in those with increased intestinal calcium absorption (Urivetzky et al., 1988). Using ^{47}Ca bone turnover studies, increased formation and resorption have been demonstrated (Liberman et al., 1968). Cytokines which increase bone resorption are elevated in patients with idiopathic hypercalciuria (Pacifici et al., 1990; Weisinger et al., 1996; Ghazali et al., 1997; Misael da Silva et al., 2002). Interleukin (IL)-1 is elevated in the monocytes of patients with fasting hypercalciuria but not in those with increased intestinal calcium absorption (Pacifici et al., 1990). Not only is IL-1 elevated but there are also increases in IL-6 and tumour necrosis factor alpha (Weisinger et al., 1996; Ghazali et al., 1997; Misael da Silva et al., 2002). Bone biopsies generally reveal low bone formation and turnover but increased resorption has also been described (Malluche et al., 1980; Steiniche et al., 1989; Heilberg et al., 1998).

Reductions in BMD, though generally modest, have been reported in patients with nephrolithiasis compared to matched controls (Coe et al., 1982; Pietschmann et al., 1992; Jaeger et al., 1994; Giannini et al., 1998; Heilberg et al., 1998; Bushinsky, 2002; Misael

da Silva et al., 2002; Tasca et al., 2002; Heilberg and Weisinger, 2006). Reductions in spinal bone density have been observed in patients with fasting hypercalciuria (Pacifici et al., 1990; Pietschmann et al., 1992). There was lower spinal BMD in hypercalciuric, compared to normocalciuric, patients (Pietschmann et al., 1992). Stone formers were found to be slightly shorter and had a significantly lower BMD at the tibial diaphysis and the tibial epiphysis compared to controls (Jaeger et al., 1994). Forty nine recurrent stone formers with idiopathic hypercalciuria had a lower lumbar spine Z-score than controls (Giannini et al., 1998). Bone formation and resorption in 40 stone formers led to the classification of 10 being osteopenic (Misael da Silva et al., 2002). There was a more negative Z-score in L1–L2 in hypercalciuric patients than in controls (Tasca et al., 2002), after adjustment data from the third National Health and Nutrition Examination Survey (NHANES III) demonstrated that men with a history of kidney stones have a lower femoral neck BMD than those without a history of stones (Lauderdale et al., 2001). This analysis was supported by analysis of almost 6000 older man which demonstrated an association of kidney stones with decreased BMD (Cauley et al., 2005).

BMD has been found to be correlated inversely with urine calcium excretion (Giannini et al., 2003; Vezzoli et al., 2005) and in stone formers but not in non-stone formers (Asplin et al., 2003). Individuals who form kidney stones have an increased risk of fractures (Melton et al., 1998; Lauderdale et al., 2001). Using data from NHANES III there was found to be an increased risk of wrist and spine fractures in stone formers (Lauderdale et al., 2001). A retrospective analysis of stone formers reveals an increased incidence of vertebral fractures, but not fractures at other sites (Melton et al., 1998).

Animal data supporting a systemic dysregulation of calcium transport in idiopathic hypercalciuria

Genetic hypercalciuric stone-forming rats

To help understand the mechanism of idiopathic hypercalciuria in man, we developed an animal model of this disorder (Kim et al., 1993; Li et al., 1993; Bushinsky, 1996, 1999, 2000; Krieger et al., 1996; Asplin et al., 1997, 2009; Tsuruoka et al., 1997; Yao et al., 1998, 2005; Bushinsky et al., 1988, 1994, 1995, 1999a, 1999b, 2000, 2001, 2002, 2006; Scheinman et al., 2000; Hoopes et al., 2003, 2006; Evan et al., 2004; Bushinsky and Asplin, 2005; Grynpas et al., 2009). Through almost 100 generations of successive inbreeding of the most hypercalciuric progeny of the most hypercalciuric Sprague-Dawley rats found in a large screening, we achieved a strain of rats whose urinary calcium excretion is approximately eight to ten times that of controls. Regions of five chromosomes, 1, 4, 7, 10, and 14, were found to be associated with the hypercalciuria (Hoopes et al., 2003). The specific genes responsible for the hypercalciuria have not yet been identified. Normocalciuric Wistar-Kyoto rats were then bred with the genetic hypercalciuric stone-forming (GHS) rats to yield congenic rats with the chromosome 1 locus on the Wistar-Kyoto background (Hoopes et al., 2006). These congenic rats were also hypercalciuric; but to a lesser extent than the parental GHS rats supporting the importance of this locus and that the hypercalciuria in the GHS rats is due to a number of genes.

When compared to controls these genetic hypercalciuric rats absorb more dietary calcium at lower levels of calcitriol (Bushinsky and Favus, 1988; Bushinsky et al., 2006) similar to observations in humans with idiopathic hypercalciuria (Monk and Bushinsky, 2010). This increased intestinal calcium absorption is due to an increase in the mucosal to serosal (absorptive) calcium flux with no change in the serosal to mucosal (secretory) flux (Bushinsky and Favus, 1988). When these genetic hypercalciuric rats are fed a diet with very limited calcium, urinary calcium excretion remains significantly elevated compared with that of similarly treated controls, indicating that there is a defect in renal tubule calcium reabsorption and/or an increase in bone resorption (Kim et al., 1993), again similar to observations in humans with idiopathic hypercalciuria (Coe et al., 1982; Pak, 1997). When exposed to increasing amounts of calcitriol cultured neonatal mouse bone, the hypercalciuric rats released more calcium than the bone of control rats (Krieger et al., 1996). The BMD of the hypercalciuric rats is lower than that of controls (Grynpas et al., 2009). Administration of a bisphosphonate, an inhibitor of bone resorption, to hypercalciuric rats fed a low-calcium diet significantly reduces urinary calcium excretion (Bushinsky et al., 1999). Utilizing clearance studies, a primary defect in renal tubular calcium reabsorption has been reported (Tsuruoka et al., 1997). Thus, these hypercalciuric rats have a clear systemic abnormality in calcium homeostasis; they absorb more intestinal calcium, they resorb more bone, and they do not adequately reabsorb filtered calcium. As each of the hypercalciuric rats forms renal stones (Bushinsky et al., 1995, 2000, 2002; Evan et al., 2004), they have been termed *genetic hypercalciuric stone-forming* (GHS) rats (Bushinsky, 1996, 1999; Bushinsky et al., 2000, 2002, 2006). The bone, kidney, and intestine of the GHS rats have an increased number of vitamin D and calcium-sensing receptors (Li et al., 1993; Krieger et al., 1996; Karnauskas et al., 2005; Yao et al., 2005; Hoopes et al., 2006), suggesting potential underlying mechanism(s) for the hypercalciuria. The increased levels of the vitamin D receptor are due to an increase in half-life of this receptor (Yao et al., 2005). Neither the vitamin D receptor nor the calcium receptor is located on any of the five chromosomes which have been associated with the hypercalciuria.

Thus available evidence from humans and a genetic animal model of hypercalciuric stone formation both suggest that there is a systemic dysregulation of calcium transport, rather than a specific organ-centred defect, responsible for the hypercalciuria. A small excess of calcitriol in man replicates many aspects of this disorder suggesting that a slight excesses of this hormone, or its receptor as shown in the rat studies, may be central to the pathophysiology of idiopathic hypercalciuria.

Site of initial solid phase

The site of the initial solid phase in kidney stone forms have been studied utilizing direct observation of the renal papilla in conjunction with papillary biopsies (Bushinsky et al., 2012). Years ago, on post-mortem examinations, Randall noticed that kidney stones grew on plaque on renal papillas, now called Randall's plaque (Randall, 1937; Matlaga et al., 2006). The plaque has been shown to form in the basement membranes of the thin limbs of Henle's loop (Evan et al., 2003, 2005a). This crystal then migrates to the suburothelial space where can be observed as plaque. The plaque, composed of the calcium phosphate crystal and an organic matrix which includes osteopontin, enlarges and erodes through the urothelium (Bushinsky, 2003). If urine is supersaturated with respect to calcium oxalate this crystal will grow on the calcium phosphate surface resulting in a kidney stone. If this stone enlarges and breaks off from the plaque mooring it will pass into the urinary space and

cause clinically significant stone disease (Evan et al., 2005b). The number of observed plaques has been shown to be increased in patients who form kidney stones (Kuo et al., 2003). The amount of papillary surface covered by plaque has been seen to vary directly with urine calcium excretion and inversely with urinary pH and volume (Kuo et al., 2003).

The basement membrane of the thin limb initially does not appear to be a likely site for the initial crystallization in patients with idiopathic hypercalciuria. There is not vectorial transport of either calcium or phosphorus at this site (Rocha et al., 1977). Transtubular permeabilities of these ions are extremely low in this region (Rocha et al., 1977) so it is difficult to link supersaturation within the thin limbs (Asplin et al., 1996) to the surrounding interstitium. However, the thin limbs are in close proximity to the vasa recta and the collecting ducts and all are situated in a highly concentrated, hypertonic environment. One could hypothesize the following sequence of events, which might lead to increased supersaturation and subsequent crystal formation (Bushinsky, 2003). Following a meal and absorption of calcium, the renal filtered load of calcium increases, resulting in increased tubular calcium concentration (Bushinsky and Monk, 1998). The medullary countercurrent mechanism concentrates the calcium which was extracted from the thick ascending limb towards the papilla which are hypertonic. The vasa rectum, also with an increased calcium concentration, does not readily remove calcium from the interstitium. The increased serum calcium stimulates the calcium receptor and decreases water reabsorption in the collecting duct (Hebert et al., 1997), which further increases the tonicity of the interstitium. During acidification of the urine, vectorial proton transport into the collecting duct alkalinizes the interstitium. The pH of the vasa recta also increases following gastric proton secretion, the so-called alkaline tide, resulting in less bicarbonate removal from the medullary interstitium. The increased pH leads to a decrease in the solubility of calcium phosphate, which is the crystal phase of the first complexes. Local collagen fibrils might provide the initial nucleating sites allowing for heterogeneous nucleation, which occurs with a lower degree of supersaturation than homogeneous nucleation (Bushinsky, 2003).

Primary hyperparathyroidism

PTH is critical to the precise regulation of blood ionized calcium. An elevation of PTH will promptly and significantly increase blood calcium. Primary hyperparathyroidism is due to chronic, excess secretion of PTH (Bushinsky and Monk, 1998; Bringhurst et al., 2002) and along with malignancy are the most common causes of hypercalcaemia (Bushinsky and Monk, 1998). Primary hyperparathyroidism has an incidence of 1 in 500 to 1 in 1000 (Bilezikian and Silverberg, 2006) and may lead to nephrolithiasis (Bushinsky and Monk, 1998). A single benign adenoma is present in approximately 75% of patients, four-gland hyperplasia in approximately 20% of patients, and parathyroid carcinoma in < 1% of patients (Rosen et al., 1994; Weber et al., 1994).

Effect on urine calcium excretion

In primary hyperparathyroidism, the elevation in blood ionized calcium, and generally total serum calcium, appears due, in large part, to the PTH-induced increase in calcium reabsorption in the distal tubule, while increased bone resorption appears to play a minor role (Nordin and Peacock, 1969; Bushinsky et al., 2012). In spite of the PTH-induced increased renal tubular calcium reabsorption, primary hyperparathyroidism generally leads to an increase in urine calcium excretion (Transbol et al., 1970). However, in patients with primary hyperparathyroidism, urine calcium excretion appears to be lower, at a similar level to serum calcium, when compared to either normal individuals or those with other hypercalcaemic disorders such as bone metastasis, sarcoidosis, vitamin D intoxication, or myeloma (Nordin and Peacock, 1969; Transbol et al., 1970).

Urine calcium excretion may be substantially elevated even in those patients who have slight elevations in serum calcium (Parks et al., 1980). In 48 stone formers with documented hyperparathyroidism, 30 had mild hypercalcaemia with serum calcium in the range of 10.1–11.0 mg/dL and urine calcium excretion exceeded the upper limit of normal in most. However, there was no difference in levels of serum calcium, PTH, $1,25(OH)_2D_3$, or urinary calcium in those hyperparathyroid patients who formed stones compared to those that did not (Pak et al., 1981).

Hypercalciuria in patients with primary hyperparathyroidism appears secondary to the increased filtered load of calcium and to the hypercalcaemia activating the calcium-sensing receptor on the basolateral membrane of the thick ascending loop leading to a suppression of potassium secretion in the lumen, a decrease in the lumen positive charge, and decreased calcium reabsorption in this segment (Riccardi et al., 1996; Frick and Bushinsky, 2003).

Kidney stones in patients with primary hyperparathyroidism are composed of hydroxyapatite, calcium oxalate, or brushite (Hodgkinson and Marshall, 1975). Pak et al. has found that as the phosphate content of the stones increased from calcium oxalate to mixed calcium oxalate–apatite, and finally to apatite, the percentage of patients with primary hyperparathyroidism increased from 2% to 10% (Porits et al., 2004).

Diagnosis

With more routine measurements of blood chemistry most patients with primary hyperparathyroidism are diagnosed prior to overt signs and symptoms of hypercalcaemia (Wermers et al., 1997). In Olmstead County, Minnesota only 2% of patients had classic symptoms of hypercalcaemia at the time of diagnosis (Wermers et al., 1997). For unknown reasons women are affected approximately three times more commonly than men, the peak incidence is between 50 and 60 years of age, and the serum calcium elevation is most often < 1 mg/dL over the upper range of normal (Bilezikian et al., 2005; Bilezikian and Silverberg, 2006). Diagnosis is generally made by the finding of hypercalcaemia, which should be confirmed by the measurement of blood ionized calcium (Calvi and Bushinsky, 2008), the exclusion of other causes of hypercalcaemia (Bushinsky, 2005), in the presence of an elevated level of serum PTH. It is important to note that a PTH level within the normal range in the presence of hypercalcaemia signifies inappropriate suppression and hyperparathyroidism. Nephrolithiasis is present in 10–25% of patients with primary hyperparathyroidism and is the most common complication of this disorder (Bilezikian et al., 2005; Bilezikian and Silverberg, 2006). The incidence of nephrolithiasis does not appear to increase with time in untreated patients (Silverberg et al., 1990, 1999). When compared to the 1970s and 1980s, the incidence of nephrolithiasis in patients with primary hyperparathyroidism appears to be decreasing (Silverberg et al., 1990; Bilezikian et al., 2005; Bilezikian and Silverberg, 2006).

It is often difficult to separate patients with primary hyperparathyroidism from those with familial hypocalciuric hypercalcaemia (Mark et al., 1978). This autosomal dominant disorder results from a mutation of the calcium-sensing receptor gene (Brown et al., 1998; Scheinman et al., 1999) leading to a decrease in the sensitivity of the receptor for calcium (Khosla et al., 1993) and excessive secretion of PTH at a normal blood ionized calcium. The decrease in sensitivity of the calcium-sensing receptor to calcium in the thick ascending limb leads to excessive renal tubular calcium reabsorption and relative hypocalciuria. The distinction is important, as hypercalcaemia always recurs after parathyroidectomy in patients with familial hypocalciuric hypercalcaemia and thus surgery is contraindicated. Marx et al. found that the excretion rate for calcium as a function of creatinine clearance provided reasonable separation between the two groups of patients (Marx et al., 1981). Patients generally have a reduced calcium clearance such that even in the presence of overt hypercalcaemia urinary calcium excretion is generally < 200 mg/day (5 mmol/day) (Marx et al., 1978). The ratio of calcium clearance to creatinine clearance in patients with familial hypocalciuric hypercalcaemia is generally < 0.013 while it is greater in patients with routine primary hyperparathyroidism (Marx et al., 1978). Bilezikian et al. (2005) suggest that the distinction between primary hyperparathyroidism and familial hypocalciuric hypercalcaemia be made on the following four criteria: (1) family history, (2) onset of hypercalcaemia early in life, (3) exceedingly low urinary calcium excretion, and (4) a specific gene abnormality. If there is any question, the diagnosis of primary hyperparathyroidism can be supported by the finding of a single enlarged gland on imaging studies. If there is still a question of diagnosis, surgery should be delayed until the correct diagnosis is clear.

Whether to operate

In primary hyperparathyroidism, surgery to remove the adenoma or hyperplastic gland is curative. However, especially in older asymptomatic patients, surgery is not always indicated. Two National Institutes of Health consensus conferences (National Institutes of Health, 1991; Bilezikian et al., 2002) suggested that in patients with primary hyperparathyroidism surgery is recommended for all with nephrolithiasis, whether the patients are symptomatic or the stone is found on a radiograph (Bilezikian et al., 2002; Bringhurst et al., 2002). Successful surgery requires a skilled, experienced surgeon; experienced as this is a difficult procedure. There are a variety of surgical approaches from minimally invasive parathyroidectomy following localization of the parathyroid gland with technetium-99m-sestamibi and/or ultrasound to a full neck exploration under general anaesthesia.

In general, patients who do not require surgery do well with careful medical follow-up (Silverberg et al., 1999) and have constant biochemical measurements and BMD. However, over 10 years 25% of these previously asymptomatic patients will develop symptoms and patients < 50 years of age have a greater likelihood of progression to symptoms. Symptomatic patients, who initially should have had surgery but did not, often do not fare well.

Effect of surgery

Following successful surgery, serum calcium and urine calcium excretion normalize (Anderson et al., 1964; Pyrah et al., 1966) and the incidence of kidney stones decreases markedly (Britton et al., 1971; Pratley et al., 1973; Parks et al., 1980; Silverberg et al., 1999; Mollerup et al., 2002). Mollerup et al. followed 674 consecutive patients after surgical parathyroidectomy (Mollerup et al., 2002) and found that the relative risk of stone formation was 40 prior to surgery and 16 after surgery. Ten years after surgery the risk of stone formation was no different from controls and stone-free survival 20 years after surgery was 90.4%. Parks et al. found that in 48 patients following successful surgery a stone recurred in only a single patient who remained hypercalciuric (Parks et al., 1980). Silverberg et al. found that of 20 patients with primary hyperparathyroidism, the 12 who had surgery did not have recurrent stones while there was recurrence in six of the eight who did not have surgery (Silverberg et al., 1999). However, Pratley et al. found that after successful parathyroid surgery, eight of 54 patients had either recurrent stones, nephrocalcinosis or increasing blood urea nitrogen (Pratley et al., 1973). Britton et al. also found that after surgery 32 of 52 patients had evidence of recurrent stones (Britton et al., 1971).

Hypocitraturia

Citrate

Citrate inhibits the nucleation, growth, and aggregation of calcium oxalate crystals (Robertson and Scurr, 1986; Hallson and Kasidas, 1995; Bushinsky et al., 2012). Citrate inhibits crystallization not only by complexing with calcium and but also by directly inhibiting crystallization though this effect is smaller in magnitude (Nicar et al., 1987; Tiselius et al., 1993). In patients with idiopathic hypercalciuria, potassium citrate has been shown to effectively inhibit recurrent calcium nephrolithiasis (Pak et al., 1985; Preminger et al., 1985; Bushinsky et al., 1993; Hofbauer et al., 1994; Pak, 1994; Whalley et al., 1996; Ettinger et al., 1997). Potassium citrate also reduces stone recurrence in patients with distal renal tubular acidosis (RTA) (Preminger et al., 1985) and in normocitrauric stone formers (Ettinger et al., 1997). Citrate increases the upper limit of meta-stability by increasing both urine citrate and pH (Greischar and Coe, 2003). During metabolic acidosis, proximal citrate reabsorption increases, leading to a reduction of urinary citrate excretion (Hamm, 1990). A reduction of urinary citrate, due to the increased acid load generated from dietary protein ingestion, promotes formation of both calcium oxalate and uric acid stones (Bataille et al., 1991; Lemann et al., 2003).

Urinary citrate and renal citrate handling

The two most important functions of urinary citrate are as a chelator for urinary calcium and as a physiologic urinary base (Pak, 1994; Moe and Preisig et al., 2006). Citrate is freely filtered at the glomerulus, and 65–90% is reabsorbed in the proximal tubule (Hamm, 1990). In physiologic urine pH, bivalent ($citrate^{2-}$) and trivalent citrate ($citrate^{3-}$) exist in equilibrium (pK of the $citrate^{2-}$/$citrate^{3-}$ pair 5.4–5.7). Citrate is a component of the tricarboxylic acid cycle, and the majority of citrate reabsorbed by the kidney is oxidized to electroneutral end products indicating that H^+ must be consumed in the process rendering citrate a urinary base. After a very large alkali load, bicarbonate assumes the most important role of base excretion; however, generally when bicarbonaturia is minimal, citrate is the most important and highly regulated urinary base.

While calcium associates with all species of citrate in a one-to-one stoichiometry, the highest affinity is a monovalent anionic

$CaCitrate^-$ complex. In addition to being stable, CaCitrate is quite soluble in an aqueous environment. With adequate amounts of citrate in the urine, most calcium will not be free to bind with other less soluble complexes (Moe and Preisig, 2006). In addition to forming a soluble complex, citrate also inhibits the spontaneous nucleation of calcium oxalate and brushite (Nicar et al., 1987) and retards agglomeration of preformed calcium oxalate (Kok et al., 1986). Citrate appears to increase the inhibitory activity of urine macromolecules. Citrate increases the calcium oxalate aggregation inhibition by Tamm–Horsfall protein *in vitro* (Tiselius et al., 1995). Citrate appears to be the most important inhibitor of calcium crystallization in urine.

Urinary citrate excretion is determined solely by reabsorption in the proximal tubule and the important regulator of citrate reabsorption is proximal tubule cell pH. Acidosis increases proximal tubule citrate reabsorption by a number of mechanisms. Acutely, a low lumimal pH titrates $citrate^{3-}$ to $citrate^{2-}$ which is the preferred transported species (Brennan et al., 1988). The Na-citrate transporter is also gated by pH and a low pH stimulates its activity (Wright et al., 1982). Chronically, intracellular acidosis increases expression of the transporter and stimulates metabolism of citrate in the cytoplasm and mitochondria (Melnick et al., 1996; Melnick et al., 1998; Aruga et al., 2000). Thus the response of the proximal tubule to cellular acidification is hypocitraturia.

Clinical hypocitraturia

Hypocitraturia has been reported in 15–60% of stone formers (Nicar et al., 1983; Hosking et al., 1985; Pak et al., 1985; Minisola et al., 1989). This wide range in the prevalence almost certainly reflects differences in the populations studied, in dietary background, and in the definition of hypocitraturia. Isolated hypocitraturia is not common, more typically hypocitraturia is accompanied by other abnormalities such as hypercalciuria in stone formers. While some groups report differences in normals related to age and sex (Parks and Coe, 1986; Minisola et al., 1989); others have not found these differences (Hosking et al., 1985; Nikkila et al., 1989). While a rigid cut-off of this continuous variable into a range of 'hypocitraturia' is impossible and physiologically inappropriate (Pak, 1994), a clinically useful guideline can be set at 320 mg/day (1.68 mmoles/day) assuming about 1 L of urine.

Causes of hypocitraturia in the context of nephrolithiasis are those that acidify the proximal tubule cell. Metabolic acidosis (a reduction in pH caused by a low plasma $[HCO_3^-]$) is not always detectable in these settings. Some of these conditions, such as RTA, have clear systemic metabolic acidosis while most of the other conditions have normal plasma $[HCO_3^-]$. In K^+ depletion, plasma $[HCO_3^-]$ may be high. Occasionally, no underlying defect can be uncovered and the patient is thought to have idiopathic hypocitraturia.

Treatment

Therapy for hypocitraturia is directed at the correction of the disorders that reduce urine citrate, such as acidosis, an increased acid load, or hypokalaemia. Alkali supplementation will raise urine citrate levels. However, using sodium as the cation for the alkali will lead to an increased urine calcium excretion which will offset the benefits of increased urine citrate (Lemann et al., 1989, 1991). Either potassium bicarbonate or potassium citrate may be used. However, citrate requires less frequent dosing than bicarbonate as it generates a bicarbonate equivalent when metabolized. Citrate also raises urine pH much less than bicarbonate for the same molar equivalents of base administered. Potassium citrate is the most frequently used alkali for hypocitraturic patients and has been proven to be efficacious in hypocitraturia induced by distal RTA (Preminger et al., 1985), chronic diarrhoeal syndrome (Pak et al., 1985), thiazide-induced hypokalaemia (Pak et al., 1985), hyperuricosuric calcium nephrolithiasis (Pak and Peterson, 1986), and uric acid nephrolithiasis (Pak et al., 1986). While the maximal recommended dose is 60 mEq/day, milder cases can be started on 30–40 mEq/day.

There are three randomized trials of citrate therapy to prevent recurrent stone formation in calcium oxalate stone formers. A 3-year, double-blinded trial of potassium citrate in calcium oxalate stone formers with hypocitraturia demonstrated no change in the stone formation rate in the placebo group, while there was a decrease from 1.2 to 0.1 stones per patient-year in those treated with potassium citrate ($P < 0.001$) (Barcelo et al., 1993). Twenty per cent of the placebo group remained stone-free compared to 72% of the treated group. A 3-year, double-blind study of potassium magnesium citrate also demonstrated a reduction in calcium oxalate stone formation rate in the treated group (Ettinger et al., 1997). The patients in this second study had a variety of metabolic abnormalities with only 20% having hypocitraturia and the benefit of citrate therapy was not limited to those with hypocitraturia. A third 3-year randomized study using sodium-potassium citrate in calcium oxalate stone formers did not demonstrate a reduction in the rate of stone recurrence (Hofbauer et al., 1994). The majority of patients had hypocitraturia on entry into the study and treatment resulted in urinary citrate. The reason for the different outcomes in these studies is not clear; however it is possible that the mixed sodium-potassium salt blunted the antilithogenic response to citrate (Hofbauer et al., 1994). Thus there are two out of three positive controlled trials and a number of uncontrolled trials showing reduction in stone formation with citrate therapy.

A formulation of potassium magnesium citrate has been developed as an alternative to potassium citrate. The formulation of $4K^+$: $2Mg^{2+}$: 2 $citrate^{3-}$ will deliver 7, 3.5, and 10.5 mEq of K^+, Mg^{2+}, and $citrate^{3-}$ respectively per tablet. This formulation of potassium magnesium citrate has been shown to be effective in treating thiazide-induced electrolyte deficits (Ruml and Pak, 1999; Odvina et al., 2006), is more effective than potassium citrate in raising urinary pH (Pak et al., 1992), and is effective in preventing stone recurrence (Ettinger et al., 1997). However there have been no studies to compare the clinical effectiveness of potassium magnesium citrate to potassium citrate in preventing recurrent stone formation.

Hyperuricosuria

Hyperuricosuria refers to an excess of the sum of urinary urate and uric acid regardless of the relative partition between these two species (Bushinsky et al., 2012). Hyperuricosuria can not only be a cause of uric acid nephrolithiasis but it is also a well-documented risk factor for calcium oxalate stones. About 5% of stones analysed contain both calcium and uric acid (Herring, 1962). In patients with both gout and uric acid nephrolithiasis, 17% of stones contain calcium (Gutman and Yü, 1965). In > 800 calcium stone formers, 15% have hyperuricosuria as the sole metabolic abnormality and 14% meet the criteria for both hyperuricosuria and hypercalciuria

(Millman et al., 1982). However, the majority of pure uric acid stones are not due to hyperuricosuria but rather are due to an unduly acidic urine (Moe, 2006b).

When urine pH is not acidic, hyperuricosuria leads to excessive urinary urate rather than uric acid and urate is far more soluble than is uric acid. The most abundant cation in urine is usually sodium and sodium urate has a lower solubility than potassium urate. In the presence of hyperuricosuria and absence of excessively acidic urine, sodium urate crystallizes and forms a clinically significant kidney stone.

Pathogenesis of hyperuricosuria-associated calcium urolithiasis

While the clinical entity of hyperuricosuric calcium urolithiasis is well established, the mechanism by which this occurs has not been firmly established. The role of hyperuricosuria in calcium stone formation can be attributed to several of its effects on urinary supersaturation and crystallization (Sorensen and Chandhoke, 2002). One mechanism that has been proposed is heterogeneous nucleation or epitaxy. The crystal lattice dimensions for uric acid, sodium urate, calcium oxalate monohydrate, and calcium oxalate dehydrate are very similar so the presence of one in solid phase will promote the precipitation of the other (Lonsdale, 1968). Both Coe and co-workers and Pak and co-workers showed that *in vitro*, sodium urate, rather than uric acid, is responsible for epitaxic crystal growth (Coe et al., 1975; Pak et al., 1975). However, another *in vitro* study demonstrated that urate-induced calcium oxalate crystallization does not decrease urate concentration in solution so the authors questioned the epitaxy theory (Grover et al., 1990, 1992). A second model is termed the 'salting out' phenomenon. While epitaxy requires a solid phase to initiate the crystallization, salting out refers to the ability of urate to lower the formation product of calcium oxalate by as yet unknown mechanisms (Grover et al., 1990, 1992; Kallistratos et al., 1970). This theory was supported by studying clinical samples where it was found that the formation product of calcium oxalate increased with allopurinol therapy (Pak et al., 1977, 1978). A third model proposes that uric acid or sodium urate sequesters inhibitors of calcium crystallization. The addition of sodium urate to urine lowers the activity of inhibitors (Fellstrom et al., 1982; Zerwekh et al., 1983). Sodium urate has also been shown to bind to polyanionic macromolecules, many of which are functional crystallization inhibitors (Hesse et al., 1987). However this finding is not universally consistent, as Ryall and co-workers used undiluted urine to show that sodium urate crystals did not alter metastable limits, size, and growth rate of calcium oxalate crystals upon an oxalate challenge (Ryall et al., 1986).

Causes

As with the excretion of other components of kidney stones, urinary uric acid excretion is a continuous variable and there are not have rigid definitions of hyperuricosuria. Gutman and Yü suggest that the upper limits of excretion as 750 mg or 4.5 mmol/day for adult females and 800 mg or 4.8 mmol/day for adult males (Gutman and Yü, 1965). At the steady state, that is when plasma uric acid is constant, uric acid production must equal the sum of urinary uric acid excretion and intestinal uricolysis where uricase-producing bacteria degrade the luminal uric acid. An increase in uric acid production is generally accompanied by hyperuricaemia and is compensated for by increased renal excretion and intestinal uricolysis. An increase in renal excretion should be accompanied by hypouricaemia in the absence of changes in production and intestinal degradation. The single most prevalent cause of hyperuricosuria from the view of kidney stones is excessive dietary purine.

Treatment

Hyperuricosuria is often not caused by a single risk factor, thus it is important to address all causative factors of nephrolithiasis in any given individual. Approximately 70% of hyperuricosuric patients have high purine intake as the cause of hyperuricosuria since their uric acid excretion falls with dietary purine restriction (Coe, 1978; Coe and Parks, 1981). Dietary modification should be the first line of therapy though, as with any dietary modification, compliance is variable. The non-compliant patients, along with the 30% which represents the non-responders to dietary changes, require other approaches.

The cornerstone of xanthine oxidase inhibition continues to be allopurinol which was approved by the US Food and Drug Administration in 1966 for treatment of gout (Terkeltaub, 2003). Allopurinol is oxidized by xanthine oxidase to oxypurinol which inhibits xanthine oxidase. At low concentrations, allopurinol is both a substrate and a competitive inhibitor of the enzyme while at high concentrations, it functions as a non-competitive inhibitor. The side effects of allopurinol include rash, gastrointestinal upset, abnormal liver enzymes, and an increased half-life in chronic kidney disease.

More recently a number of other xanthine oxidase inhibitors have been marketed which have a more favourable toxicology profile, improved bioavailability, and more potent and persistent action than allopurinol (Pacher et al., 2003). Febuxostat is a thiazolecarboxylic acid derivative that is a potent xanthine oxidase inhibitor (Takano et al., 2005; Terkeltaub et al., 2006). While the efficacy of febuxostat to reduce hyperuricaemia and gouty episodes is established (Schumacher et al., 2009) its ability to decrease uric acid excretion and reduce stone formation is not yet clear. Although xanthine oxidase inhibitors reduce hyperuricosuria and stone recurrence (Coe, 1977), whether they reduce stone recurrence in patients with combined hyperuricosuria and hypercalciuria remains to be determined. Other measures targeted at reducing urinary calcium, such as thiazides, are effective in reducing calcium stone recurrence in patients with or without hyperuricosuria (Pak and Peterson, 1986; Meschi et al., 2004).

Calcium phosphate stones and renal tubular acidosis

Mechanisms of calcium phosphate stone formation

Some patients form calcium phosphate stones principally because they have a higher urinary pH leading to a higher calcium phosphate supersaturation compared to patients whose stones are mainly calcium oxalate (Parks et al., 2004; Bushinsky et al., 2012). Urine pH and calcium phosphate supersaturation increase progressively as the fraction of calcium phosphate salts in formed stones increases. Patients with predominantly calcium phosphate stones tend to be as hypercalciuric as other stone formers and may have higher urine volumes where there is no difference in urine phosphate and citrate excretion.

Renal pathology of calcium phosphate stones in renal tubular acidosis

Biopsy of renal papillae in patients with brushite (calcium monohydrogen phosphate) stones reveal that the papillae are grossly and variably deformed with dilated terminal ducts of Bellini out of which there are often projections of calcium phosphate plugs. Histology reveals that the ducts are tremendously dilated and contain apatite crystals. Epithelial cells are damaged and often obliterated. The surrounding interstitium is often fibrotic, with glomerular obsolescence in the cortex (Evan et al., 2005c). This damage to the inner medullary collecting duct is thought to decrease the ability to acidify the urine. However the sequence of events is not clear: was there a decrease in the ability to acidify leading to crystal deposition and cellular injury and, if so, what led to the initial decrease in acidification?

Renal tubular acidosis

Hereditary RTA is caused by mutations in critical membrane transporter units. Autosomal dominant or recessive RTA is caused by defects in *SLC4A1*; its protein product, AE1, is a chloride-bicarbonate exchanger on the basolateral surface of type A intercalated cells (Karet et al., 1998; Quilty et al., 2002; Devonald et al., 2003). These patients often have stones and/or nephrocalcinosis (Parks et al., 2004). Autosomal recessive RTA, with and without hearing loss, is caused by defects of the B1 and a4 subunits of vacuolar ATPase of the intercalated cells and also causes stones and nephrocalcinosis (Karet et al., 1999; Stehberger et al., 2003). In these rare diseases, the inability to acidify the urine leads to the formation of apatite within inner medullary collecting duct leading to calcium phosphate crystal formation and a cycle of cell injury, interstitial inflammation, and further calcium deposition.

While some cases of acquired distal RTA reflect direct cell injury from diseases such as Sjögren syndrome (Aasarod et al., 2000), hyper-gamma globulinaemic diseases (Spruce et al., 1984), sickle cell disease (Kurtzman and Kurtzman, 1983), lithium treatment (Kurtzman and Kurtzman, 1983), and obstructive uropathy (Sharma et al., 1997), the majority, which also lead to calcium phosphate stone formation, occur without any obvious inciting cause. Often, there is reduced urine citrate excretion and a urine pH > 6 with possibly variably amount of hypercalciuria, suggesting an abnormality of acid base homeostasis although there is no reduction of bicarbonate. These patients are often said to have an 'incomplete RTA'. When challenged with an acid load, patients with 'incomplete RTA' often cannot lower urine pH below 5.5 (Pongchaiyakul et al., 2004).

Carbonic anhydrase inhibition with acetazolamide or topiramate leads to a condition resembling RTA. Topiramate is used in the treatment of a number of neurological disorders such as seizures and migraine headaches. Topiramate leads to elevated urinary pH and hypocitraturia and increased stone risk though there is minimal or no systemic acidosis (Welch et al., 2006).

Clinical management

Most calcium phosphate stone formers with RTA are hypercalciuric, due, in part, to the acid loading which directly decreases tubular calcium reabsorption (Lemann et al., 2003). Alkali supplements will increase blood bicarbonate and decrease urine calcium excretion and calcium phosphate supersaturation will fall. Urine citrate, which is reduced by metabolic acidosis, will increase with bicarbonate therapy (Hamm and Alpern, 1996). The increase in citrate will directly increase the upper limit of meta-stability for calcium phosphate (Greischar and Coe, 2003). Often the urine calcium excretion will not fall, because the hypercalciuria is due to causes other than the acidosis. In this case thiazide treatment, as in idiopathic hypercalciuria, is often beneficial. While the alkali supplements may be beneficial in raising urine citrate, they may also raise urine pH, leading to a worsening calcium phosphate supersaturation. In this complex disorder, with the potential beneficial and deleterious effects of bicarbonate therapy it is essential to measure, and treat to lower, calcium phosphate supersaturation. Unfortunately there are no high-quality trials to establish the optimal treatment for calcium phosphate stones.

Hyperoxaluria

Hyperoxaluria is as important as hypercalciuria in conferring stone risk (Pak et al., 2004; Bushinsky et al., 2012). Although one primarily encounters renal pathology (nephrolithiasis, oxalosis, etc.) in oxalate-related diseases, the pathogenesis is often more from gastrointestinal than renal origin. The levels of oxalate in plasma in normal subjects range from 1.3 to 3.1 mM (mean 2.03) with females having slightly levels than males (Kasidas and Rose, 1986). Plasma oxalate is higher in patients with primary hyperoxaluria and in those with chronic renal failure (Constable et al., 1979).

Oxalate is the simplest dicarboxylic acid with pK_1 = 4.2 and pK_2 = 1.2 so it exists largely as a divalent anion at normal systemic pH. The only body fluid where there is significant undissociated oxalic acid is in the very acidic gastric lumen. Oxalate is a complex anion to consider in terms of external balance and determinants of urinary excretion as is undergoes synthesis and degradation as well as absorption and excretion. Our diets contain oxalate and it is absorbed, secreted, and broken down in the intestine. It is also synthesized by humans and is filtered, reabsorbed, and secreted by the kidneys (Jaeger and Robertson, 2004; Hatch and Freel, 2005). Oxalate is an end product of metabolism and cannot be further metabolized in the body; however, intestinal bacteria are capable of oxalate degradation.

A number of factors make it difficult to understand the complex physiology of this metabolite. Because of intestinal metabolism and endogenous production, it is very difficult to establish a steady state. The ratio of urinary to ingested oxalate in a given time period ranges from 0.05 to 0.5 (Holmes et al., 2001). In the epithelia, oxalate transport occurs through paracellular, transcellular carrier-mediated, and putative non-ionic diffusive pathways which make modelling of the epithelia extremely difficult. In the intestine and the renal epithelia there is bidirectional transport with a highly variable net flux. Finally, the lack of definitive identification of candidate transporters and specific reagents precludes unequivocal conclusions to be drawn. However it is important to attempt to understand oxalate transport due to its important role for increasing risk for kidney stone formation.

Intestinal transport and metabolism

Dietary oxalate comes from a wide variety of foods. The typical food chart lists rhubarb, spinach, beetroot, parsley, okra, soy products, sesame seeds, pepper, chocolate products, and tea. Precursors of oxalate, such as animal product containing collagen which is rich

in hydroxyproline, can also cause 'dietary hyperoxaluria'. There is no evidence for *de novo* intraluminal generation of oxalate in the intestine.

Oxalate is both absorbed and secreted in the gastrointestinal tract. Both paracellular and transcellular pathways are believed to contribute to oxalate movement. Paracellular permeability has been documented *in vitro* and quantitative estimates for the paracellular flux range from 70% to 400% of the total net flux (Hatch et al., 1993, 1994). However, paracellular oxalate transport cannot be confirmed *in vivo* as yet.

Transcellular transport of oxalate is present in the intestine. In the gastric lumen the pH is low and titrates part of dietary oxalate into small hydrophobic molecules and this undissociated oxalic acid can theoretically diffuse through the lipid bilayer (Chen et al., 2003; Hatch and Freel, 2005). There is circumstantial evidence for this 'gastric phase' of non-ionic oxalic acid absorption. It is not clear whether acid in the colon, which is generated by certain bacteria on carbohydrate substrate, can contribute significant non-ionic absorption of oxalic acid (Diamond et al., 1988).

The most important component of oxalate transport is mediated through transcellular movement of ionized oxalate. Definitive cell models of intestinal oxalate transport are not yet available. *In vitro* measurements utilizing inhibitors of oxalate transport in isolated bowel segments have shown dependence on ATP (metabolic inhibitors), anion exchange (stilbenes), and coupling with other cation exchangers (amiloride analogues) (Hatch et al., 1984, 1993, 1994; Knickelbein et al., 1986). Candidate anion exchangers are selected on the basis of two requisites: expression in the intestine, and ability to transport oxalate based on expression in *Xenopus* oocytes. Some members of the SLC26 family are potential contenders for oxalate transport in the gut and kidney.

Similar to transport of organic solutes in the proximal tubule, which can be reabsorptive or secretory, the intestine can also absorb or secrete oxalate. *In vitro* studies suggest that net secretion predominates in the proximal small intestine while transport shifts to net absorption in the colon (Freel et al., 1980; Hatch et al., 1993, 1994; Hatch and Freel, 2005). However, it is unclear if this is the case in the intact animal. That probiotics decrease serum oxalate in primary hyperoxaluria suggests that there is significant secretion in the colon as well (Hoppe et al., 2005; Hatch et al., 2006).

The most definitive evidence of intestinal oxalate secretion and of the role of a specific transporter comes from the *slc26a6* null mouse where hyperoxaluria and calcium oxalate cystoliths result from impaired intestinal oxalate secretion (Wang et al., 2005; Freel et al., 2006). In contrast, polymorphic variants of SLC26A6 in humans have not provided conclusive evidence for the role of this transporter in human hyperoxaluria (Monico et al., 2008). The few studies in humans suggest that in contrast to rodents, humans have little intestinal oxalate secretion (Hodgkinson, 1974).

Clinically a number of factors can affect oxalate absorption. Since complexes containing oxalate cannot be absorbed, increased luminal calcium, which binds oxalate, will decrease oxalate absorption. Clinicians have suggested this is why increased dietary calcium intake does not necessarily increase the risk for stone formation; however, there is no direct evidence supporting this proposed model (Barilla et al., 1978; Curhan et al., 1993, 1997; Hess et al., 1998; Liebman and Costa, 2000).

Sequestration of intestinal calcium by fatty acids may also contribute to enhanced oxalate absorption (Barilla et al., 1978; Liebman and Costa, 2000). Bile acids increase bidirectional flux of oxalate *in vitro* via paracellular pathways and increase transcellular secretion in the colon which has been postulated to increase paracellular oxalate absorption in malabsorptive states (Hatch et al., 1981; Kathpalia et al., 1984). A more complete discussion of intestinal oxalate transport can be found in reviews (Hatch and Freel, 2005, 2008).

Microbial metabolism of luminal oxalate

Oxalobacter formigenes is a Gram-negative obligate anaerobe that utilizes oxalate as a main source of energy for cellular biosynthesis (Jonsson et al., 2004). *O. formigenes* degrades oxalate from the bowel lumen to formate. While it was first isolated in ruminates (Dawson et al., 1980), it has now been isolated in many other animal species (Allison et al., 1995). *O. formigenes* is found in stool specimens of approximately two-thirds of the general adult population (Allison et al., 1995; Sidhu et al., 1997). The role of *O. formigenes* in intestinal oxalate absorption from dietary sources and oxalate secretion from hepatic production has received attention as a potential probiotic therapy or enzyme supplementation (Hatch et al., 2006).

The clinical significance of *O. formigenes* colonization has been studied in relatively small numbers of recurrent calcium oxalate nephrolithiasis patients (Sidhu et al., 1999; Kwak et al., 2003; Troxel et al., 2003), those with inflammatory bowel disease (Kumar et al., 2003), and those with cystic fibrosis (Sidhu et al., 1998). Some studies found a lower prevalence of *O. formigenes* colonization in kidney stone formers when compared to non-stone formers while others showed lower oxalate excretion in patients with nephrolithiasis who had *O. formigenes* in their stool than in those without (Kumar et al., 2003; Kwak et al., 2003; Troxel et al., 2003). Two major limitations of these studies are the small study population and potential for frequent use of antibiotics among the study subjects. A recent, larger-scale case-controlled study excluded antibiotic use. In this study, 17% of stone formers were positive for *O. formigenes* compared to 38% of normal subjects (Kaufman et al., 2008).

O. formigenes may degrade intestinal luminal oxalate thereby creating a favourable concentration gradient for intestinal oxalate secretion. Supporting this hypothesis is a study using Ussing chambers which demonstrated that colonization by *O. formigenes* increases oxalate secretion (Hatch et al., 2006). In a small number of patients with type 1 primary hyperoxaluria, there was a reduction of both urinary and plasma oxalate following oral administration of *O. formigenes* in some of the patients (Hoppe et al., 2006; Milliner, 2006). Because of these findings, *O. formigenes* has been proposed as a potential probiotic to lower urinary oxalate excretion in patients with enteric hyperoxaluria (Lieske et al., 2005). However, the associations in the above examples, even when present, are quite weak. Under controlled experimental conditions *O. formigenes* status correlates poorly with stool oxalate levels and does not correlate with urinary oxalate levels (Holmes et al., 2001; Prokopovich et al., 2007).

Renal transport

Renal transport of oxalate is not well understood. The ratio of oxalate clearance to creatinine clearance (or fractional excretion $FE_{oxalate}$) varies from 0.5 to 3 indicating net that the tubules may absorb or secrete oxalate (Constable et al., 1979; Boer et al.,

1985; Prenen et al., 1985; Chen et al., 1990; Kasidas et al., 1990). Reduction of glomerular filtration rate increases $FE_{oxalate}$ in some, but not all, studies while primary hyperoxaluria appears to increase $FE_{oxalate}$ (Boer et al., 1985; Prenen et al., 1985; Kasidas et al., 1990). Plasma oxalate may promote tubular secretion in primary hyperoxaluria; however, in chronic kidney disease the hyperoxalaemia may be the result of reduced oxalate excretion. Oxalate is 86% ultrafiltrable making regulation by altering filtration unlikely (Constable et al., 1979). The tubular handling of oxalate is not well defined. Tubular oxalate flux has demonstrated both luminal and basolateral uptake are possible and are mediated by anion exchangers, principally SLC26 on both sides of the membrane (Wareing and Green, 1994; Brandle et al., 1998).

Enteric hyperoxaluria

Hyperoxaluria is often present in patients with bowel disease such as patients with Crohn disease, coeliac sprue, pancreatic insufficiency, and bypass surgery for obesity (Dobbins, and Binder, 1977; Hylander et al., 1980; Canos et al., 1981). The risk of nephrolithiasis in patients with inflammatory bowel disease appears to be 10–100 times that of the normal population (Pardi et al., 1998). These patients often have multiple stones which are composed of calcium oxalate when the ileum is involved and uric acid when the patients have large amounts of diarrhoea (Rudman et al., 1980; Pardi et al., 1998).

The mechanism by which these disorders enhance urine oxalate excretion involves dietary fat malabsorption with steatorrhoea. Increased luminal free fatty acids are important and the amounts of faecal fat and hyperoxaluria are correlated (Dobbins and Binder, 1977; Modigliani et al., 1978; McLeod and Churchill, 1992). The presence of bile acids and long-chain fatty acids enhance the absorption of oxalate through increasing the permeability of the colon to oxalate (Marangella et al., 1982; Kathpalia et al., 1984). The free fatty acids also bind calcium, in preference to oxalate, allowing free oxalate to be absorbed. The bile acid-induced diarrhoea causes a loss of base resulting in metabolic acidosis and reduced citrate excretion. Following bariatric surgery, patients develop hyperoxaluria and nephrolithiasis (Asplin and Coe, 2007; Kleinman, 2007; Sinha et al., 2007; Duffey et al., 2008; Lieske et al., 2008) and in the most serious cases chronic kidney disease from extensive oxalosis (Hassan et al., 2001; Nasr et al., 2008; Nelson et al., 2009). The mechanism of hyperoxaluria is not known although fat malabsorption has been proposed but not proven.

Primary hyperoxaluria

Primary hyperoxaluria (PH) results from hepatic enzyme deficiencies and leads to massive endogenous oxalate production and nephrolithiasis (Petrarulo et al., 1998; Danpure, 2005; Milliner, 2005). PH patients have severe hyperoxaluria (80–300 mg/day (1–4 mmol/day)) and widespread deposition of oxalate in tissues including the heart, bone marrow, muscle, and renal parenchyma at a young age. Cardiomyopathy, bone marrow suppression, and renal failure may result. In type 1 PH, the deficient hepatic enzyme is alanine:glyoxylate aminotransferase (AGT), and deficiency is caused by one of several mutations found in the AGT gene *AGXT*. In type 2 PH, which is an even more uncommon disorder, patients lack D-glycerate reductase and glyoxylate reductase due to mutations in the *GRHPR* gene.

All patients with PH should be treated with the goal of reducing urinary calcium oxalate supersaturation. Patients should be instructed to drink ample fluids and the physician should consider administration of potassium citrate, magnesium, and orthophosphate. Orthophosphate effectively inhibitors calcium oxalate crystallization but should be avoided in patients with a glomerular filtration rate of < 50 mL/min. In some patients with type 1 PH pyridoxine (vitamin B_6) can increase enzyme activity, thereby reducing oxalate production. As discussed above, *Oxalobacter formigenes* relies on oxalate for its metabolism (Kwak et al., 2003; Taylor and Curhan, 2007). Administration of these bacteria to small numbers of patients with PH type 1 resulted in a very modest reduction in urinary oxalate excretion (Taylor and Curhan, 2007). Provision of these bacteria, if they ultimately win government approval, may provide additional therapy in the treatment of PH. Patients with renal failure might benefit from renal transplantation as dialysis is not as effective as a functioning kidney in removal of oxalate.

After transplantation, these measures should be continued to prevent rapid loss of the allograft caused by calcium oxalate deposition. For patients with type 1 PH, liver transplantation supplies the missing AGT and may be curative, especially if it is performed prior to the development of end-stage renal failure. Some patients require combined liver and kidney transplantation (Watts et al., 1991; Danpure, 2005; Milliner, 2005).

Site of the initial solid phase

Calcium oxalate urolithiasis secondary to intestinal hyperoxaluria stones does not form on Randall's plaque. Rather crystallization occurs through nucleation and growth in the urine. Inner medullary collecting ducts are filled with apatite plugs that resemble those found in calcium phosphate stone formers. Although the apatite plugging indicates that the inner modularly collecting duct lumen fluid must have been at a pH > 6, the average urine pH of these patients was < 5.5 (Evan et al., 2003), meaning that tubule fluid and urine pH were not the same. Although stones are always pure calcium oxalate in these patients, tubules do not contain calcium oxalate crystals (Parks et al., 2003).

Treatment

Therapy should be directed at the underlying disorder with the goal of reducing the amount of free fatty acids in the intestine. For example, the provision of a gluten-free diet should reduce hyperoxaluria in patients with sprue; however there are no clinical trials to support this approach (Hylander et al., 1980; McLeod and Churchill, 1992; Lemann et al., 1996; Parivar et al., 1996; Worcester, 2002; Parks et al., 2003). In addition to reducing intestinal free fatty acidosis dietary oxalate should be reduced. Increasing dietary calcium will bind intestinal oxalate and decrease its absorption. Cholestyramine 2–4 g with each meal is an effective oxalate binder but has an unpleasant taste and may induce vitamin K deficiency. Malabsorption causes bowel losses of bicarbonate, thus urine pH and citrate will be low and potassium citrate supplementation may be beneficial. Increasing urinary pH will decrease uric acid crystallization, and the higher urine citrate will bind urine calcium and reduce calcium oxalate supersatuation, and also will inhibit calcium oxalate crystallization directly. Increasing fluid intake is always beneficial in preventing stone formation.

References

Aasarod, K., Haga, H. J., and Berg, K. J. (2000). Renal involvement in primary Sjogren's syndrome. *QJM*, 93, 297–304.

Adams, N. D., Gray, R. W., and Lemann, J. J. (1982). Effects of calcitriol administration on calcium metabolism in healthy men. *Kidney Int*, 21, 90–7.

Allison, M. J., Daniel, S. L., and Cornick, N. A. (1995). Oxalate-degrading bacteria. In S. R. Khan (ed.) *Calcium Oxalate in Biological Systems*, pp. 131–68. Boca Raton, FL: CRC.

Anderson, J., Osborn, S. C., Tomlinson, R. W. S., *et al.* (1964). Calcium dynamics of the gastrointestinal tract and bone in primary hyperparathyroidism. *QJM*, 33, 42.

Aruga, S., Wehrli, S., Kaissling, B., *et al.* (2000). Chronic metabolic acidosis increases NaDC-1 mRNA and protein abundance in rat kidney. *Kidney Int*, 58, 206–15.

Asplin, J. R., Bauer, K. A., Kinder, J., *et al.* (2003). Bone mineral density and urine calcium excretion among subjects with and without nephrolithiasis. *Kidney Int*, 63, 662–9.

Asplin, J. R. and Coe, F. L. (2007). Hyperoxaluria in kidney stone formers treated with modern bariatric surgery. *J Urol*, 177, 565–9.

Asplin, J. R., Bushinsky, D. A., Singharetnam, W., *et al.* (1997). Relationship between supersaturation and crystal inhibition in hypercalciuric rats. *Kidney Int*, 51, 640–5.

Asplin, J. R., Donahue, S. E., Lindeman, C., *et al.* (2009). Thiosulfate reduces calcium phosphate nephrolithiasis. *J Am Soc Nephrol*, 20, 1246–53.

Asplin, J. R., Mandel, N. S., and Coe, F. L. (1996). Evidence for calcium phosphate supersaturation in the loop of Henle. *Am J Physiol*, 270, F604–13.

Barcelo, P., Wuhl, O., Servitge, E., *et al.* (1993). Randomized double-blind study of potassium citrate in idiopathic hypocitraturic calcium nephrolithiasis. *J Urol*, 150, 1761–4.

Barilla, D. E., Notz, C., Kennedy, D., *et al.* (1978). Renal oxalate excretion foloowing oral oxalate loads in patients with ileal disease and with renal and absorptive hypercalciurias. Effect of calcium and magnesium. *Am J Med*, 64(4), 579–85.

Bataille, P., Achard, J. M., Fournier, A., *et al.* (1991). Diet, vitamin D and vertebral mineral density in hypercalciuric calcium stone formers. *Kidney Int*, 39, 1193–205.

Bilezikian, J. P., Brandi, M. L., Rubin, J., *et al.* (2005). Primary hyperparathyroidism: new concepts in clinical, densitometric and biochemical features. *J Inter Med*, 257, 6–17.

Bilezikian, J. P., Potts J. T., Jr., El-Hajj Fuleihan, G., *et al.* (2002). Summary statement from a workshop on asymptomatic primary hyperparathyroidsim: a perspective for the 21st century. *J Bone Min Res*, 17, N2–11.

Bilezikian, J. P. and Silverberg, S. J. (2006). Primary hyperparathyroidism. In M Favus (ed.) *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*, pp. 181–5. Washington, DC: American Society of Bone and Mineral Research.

Boer, P., Prenen, J. A., Koomans, H. A., *et al.* (1985). Fractional oxalate clearance in subjects iwth normal and impaired renal function. *Nephron*, 41, 78–81.

Bordier, P., Ryckewart, A., Gueris, J., *et al.* (1977). On the pathogenesis of so-called idiopathic hypercalciuria. *Am J Med*, 63, 398.

Borghi, L., Schianchi, T., Meschi, T., *et al.* (2002). Comparison of two diets for the prevention of recurrent stones in idiopathic hypercalciuria. *N Eng J Med*, 346, 77–84.

Brandle, E., Bernt, U., and Hautmann, R. E. (1998). In situ characterization of oxalate transport across the basolateral membrane of the proximal tubule. *Pfleugers Arch*, 435, 840–9.

Brennan, S., Hering-Smith, K., and Hamm, L. L. (1988). Effect of pH on citrate reabsorption in the proximal convoluted tubule. *Am J Physiol*, 255 (2 Pt 2), F301–6.

Bringhurst, F. R., Demay, M. B., and Kronenberg, H. M. (2002). Hormones and disorders of mineral metabolism. In P. R. Larsen, H. M. Kronenberg, S. Melmed, *et al.* (eds.) *Williams Textbook of Endocrinology*, pp. 1303–71. Philadelphia, PA: Saunders.

Britton, D. C., Thompson, M. H., Johnston, I. D. A., *et al.* (1971). Renal function following parathyroid surgery in primary hyperparathyroidism. *Lancet*, 2, 74.

Broadus, A. E., Insogna, K. L., Lang, R., *et al.* (1984). Evidence for disordered control of 1, 25-dihydroxyvitamin D production in absorptive hypercalciuria. *N Engl J Med*, 311, 73–80.

Brown, E. M., Pollock, M., and Hebert, S. C. (1998). The extracellular calcium-sensing receptor: its role in health and disease. *Annu Rev Med*, 49, 15–29.

Burckhardt, P. and Jaeger, P. (1981). Secondary hyperparathyroidism in idiopathic renal hypercalciuria: fact or theory? *J Clin Endocrinol Metab*, 55, 550.

Bushinsky, D. A. (1996). Genetic hypercalciuric stone forming rats. *Semin Nephrol*, 16, 448–57.

Bushinsky, D. A. (1998). Nephrolithiasis. *J Am Soc Nephrol*, 9, 917–24.

Bushinsky, D. A. (1999). Genetic hypercalciuric stone-forming rats. *Curr Opin Nephrol Hypertens*, 8, 479–88.

Bushinsky, D. A. (2000). Bench to bedside: lessons from the genetic hypercalciuric stone forming rat. *Am J Kidney Dis*, 36, 61–4.

Bushinsky, D. A. (2000). Renal lithiasis. In H. D. Humes (ed.) *Kelly's Textbook of Medicine*, pp. 1243–8. New York: Lippincott Williams & Wilkins.

Bushinsky, D. A. (2002). Recurrent hypercalciuric nephrolithiasis—does diet help? *N Eng J Med*, 346, 124–5.

Bushinsky, D. A. (2003). Nephrolithiasis: site of the initial solid phase. *J Clin Invest*, 111, 602–5.

Bushinsky, D. A. (2005). Disorders of calcium and phosphorus homeostasis. In A. Greenberg (ed.) *Primer on Kidney Diseases*, pp. 120–30. San Diego, CA: Academic Press.

Bushinsky, D. A. (2008). Calcium nephrolithiasis. In C. J. Rosen (ed.) *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*, pp. 460–4. Washington, DC: American Society of Bone and Mineral Research.

Bushinsky, D. A. and Asplin, J. R. (2005). Thiazides reduce brushite, but not calcium oxalate, supersaturation and stone formation in genetic hypercalciuric stone-forming rats. *J Am Soc Nephrol*, 16, 417–24.

Bushinsky, D. A., Asplin, J. R., Grynpas, M. D., *et al.* (2002). Calcium oxalate stone formation in genetic hypercalciuric stone-forming rats. *Kidney Int*, 61, 975–87.

Bushinsky, D. A., Bashir, M. A., Riordon, D. R., *et al.* (1999a). Increased dietary oxalate does not increase urinary calcium oxalate saturation in hypercalciuric rats. *Kidney Int*, 55, 602–12.

Bushinsky, D. A., Coe, F. L., and Moe, O. W. (2012). Nephrolithiasis. In B. M. Brenner (ed.) *The Kidney*, pp. 1455–507. Philadelphia, PA: W. B. Saunders.

Bushinsky, D. A. and Favus, M. J. (1988). Mechanism of hypercalciuria in genetic hypercalciuric rats: inherited defect in intestinal calcium transport. *J Clin Invest*, 82, 1585–91.

Bushinsky, D. A., Frick, K. K., and Nehrke, K. (2006). Genetic hypercalciuric stone-forming rats. *Curr Opinion Nephrol Hypertens*, 15, 403–18.

Bushinsky, D. A., Grynpas, M. D., and Asplin, J. R. (2001). Effect of acidosis on urine supersaturation and stone formation in genetic hypercalciuric stone forming rats. *Kidney Int*, 59, 1415–23.

Bushinsky, D. A., Grynpas, M. D., Nilsson, E. L., *et al.* (1995). Stone formation in genetic hypercalciuric rats. *Kidney Int*, 48, 1705–13.

Bushinsky, D. A., Kim, M., Sessler, N. E., *et al.* (1994). Increased urinary saturation and kidney calcium content in genetic hypercalciuric rats. *Kidney Int*, 45, 58–65.

Bushinsky, D. A. and Monk, R. D. (1998). Calcium. *Lancet*, 352, 306–11.

Bushinsky, D. A., Neumann, K. J., Asplin, J., *et al.* (1999b). Alendronate decreases urine calcium and supersaturation in genetic hypercalciuric rats. *Kidney Int*, 55, 234–43.

Bushinsky, D. A., Parker, W. R., and Asplin, J. R. (2000). Calcium phosphate supersaturation regulates stone formation in genetic hypercalciuric stone-forming rats. *Kidney Int*, 57, 550–60.

Calvi, L. M. and Bushinsky, D. A. (2008). When is it appropriate to order an ionized calcium? *J Am Soc Nephrol*, 19, 1257–60.

Canos, H. J., Hogg, G. A., and Jeffery, J. R. (1981). Oxalate nephropathy due to gastrointestinal disorders. *Can Med Assoc J*, 124, 729–33.

Cauley, J. A., Fullman, R. L., Stone, K. L., *et al.* (2005). Factors associated with the lumbar spine and proximal femur bone mineral density in older men. *Osteoporos Int*, 16, 1525–37.

Chen, S. M., Chen, T. W., Lee, Y. H., *et al.* (1990). Renal excretion of oxalate in patients with chronic renal failure or nephrolithiasis. *J Formos Med Assoc*, 89, 651–6.

Chen, Z., Ye, Z., Zeng, L., *et al.* (2003). Clinical investigation on gastric oxalate absorption. *Chin Med J*, 116, 1749.

Coe, F. L. (1977). Treated and untreated recurrent calcium nephrolithiasis in patients with idiopathic hypercalciuria, hyperuricosuria, or no metabolic disorder. *Ann Int Med*, 87, 404–10.

Coe, F. L. (1978). Hyperuricosuric calcium oxalate nephrolithiasis. *Kidney Int*, 13, 418–26.

Coe, F. L. and Bushinsky, D. A. (1984). Pathophysiology of hypercalciuria. *Am J Physiol*, 247, F1–3.

Coe, F. L., Canterbury, J. M., Firpo, J. J., *et al.* (1973). Evidence for secondary hyperparathyroidism in idiopathic hypercalciuria. *J Clin Invest*, 52, 134–42.

Coe, F. L., and Parks, J. H. (1981). Hyperuricosuria and calcium nephrolithiasis. *Urol Clin North Am*, 8, 227–44.

Coe, F. L., Evan, A., and Worcester, E. (2005). Kidney stone disease. *J Clin Invest*, 115, 2598–608.

Coe, F. L., Favus, M. J., Crockett, T., *et al.* (1982). Effects of low-calcium diet on urine calcium excretion, parathyroid function and serum 1, $25(OH)_2D_3$ levels in patients with idiopathic hypercalciuria and in normal subjects. *Am J Med*, 72, 25–32.

Coe, F. L., Lawton, R. L., Goldstein, R. B., *et al.* (1975). Sodium urate accelerates precipitation of calcium oxalate in vitro. *Proc Soc Exp Biol Med*, 149, 926–9.

Constable, A. R., Joekes, A. M., Kasidas, G. P., *et al.* (1979). Plasma level and renal clearance of oxalate in normal subjects and in patients with primary hyperoxaluria or chronic renal failure or both. *Clin Sci (Lond)*, 56, 299–304.

Curhan, G. C., Willett, W. C., Rimm, E. B., *et al.* (1993). A prospective study of dietary calcium and other nutrients and the risk of symptomatic kidney stones. *N Engl J Med*, 328, 833–8.

Curhan, G. C., Willett, W. C., Speizer, F. E., *et al.* (1997). Comparison of dietary calcium with supplemental calcium and other nutrients as factors affecting the risk for kidney stones in women. *Ann Intern Med*, 126, 497–504.

Danpure, C. J. (2005). Molecular etiology of primary hyperoxaluria type 1: new direction for treatment. *Am J Nephrol*, 25, 303–10.

Dawson, K. A., Allison, M. J., and Hartman, P. A. (1980). Isolation and some characteristics of anaerobic oxalate-degrading bacteria from the rumen. *Appl Environ Microbiol*, 40, 833–9.

Devonald, M. A., Smith, A. N., and Poon, J. P. (2003). Non-polarized targeting of AE1 causes autosomal dominant distal renal tubular acidosis. *Nat Genet*, 33, 125–7.

Diamond, K. L., Fox, C. C., and Barch, D. H. (1988). Role of cecal pH in intestinal oxalate absorption in the rat. *J Lab Clin Med*, 112, 352.

Dobbins, J. W. and Binder, H. J. (1977). Importance of colon in enteric hyperoxaluria. *N Eng J Med*, 296(6), 298–301.

Duffey, B. G., Pedro, R. N., Makhlouf, A., *et al.* (2008). Roux-en-Y gastric bypass is associated with early increased risk factors for development of calcium oxalate nephrolithiasis. *J Am Coll Surg*, 206, 1145–53.

Ettinger, B., Pak, C. Y., Citron, J. T., *et al.* (1997). Potassium-magnesium citrate is an effective prophylaxis against recurrent calcium oxalate nephrolithiasis. *J Urol*, 158, 2069–73.

Evan, A. P., Bledsoe, S. B., Smith, S. B., *et al.* (2004). Calcium oxalate crystal localization and osteopontin immunostaining in genetic hypercalciuric stone-forming rats. *Kidney Int*, 65, 154–61.

Evan, A. P., Coe, F. L., and Rittling, S. R. (2005a). Apatite plaque particles in inner medulla of kidneys of calcium oxalate stone formers: osteopontin localization. *Kidney Int*, 68, 145–54.

Evan, A. P., Coe, F. L., Lingeman, J., *et al.* (2005b). Insights on the pathology of kidney stone formation. *Urol Res*, 33, 383–9.

Evan, A. P., Lingeman, J. E., and Coe, F. L. (2005c). Crystal-associated nephropathy in patients with brushite nephrolithiasis. *Kidney Int*, 67, 576–91.

Evan, A. P., Lingeman, J. E., Coe, F. L., *et al.* (2003). Randall plaque of patients with nephrolithiasis begins in basement membranes of thin loops of Henle. *J Clin Invest*, 111, 607–16.

Favus, M. J., Karnauskas, A. J., Parks, J. H., *et al.* (2004). Peripheral blood monocyte vitamin D receptor levels are elevated in patients with idiopathic hypercalciuria. *J Clin Endocrinol Metab*, 89, 4937–43.

Fellstrom, B., Backman, U., Danielson, B. G., *et al.* (1982). Inhibitory activity of human urine on calcium oxalat crystal growth: effects of sodium urate and uric acid. *Clin Sci (Lond)*, 62, 509–14.

Freel, R. W., Hatch, M., Earnest, D. L., *et al.* (1980). Oxalate transport across the isolated rat colon. *Biochem Biophys Acta*, 600, 800.

Freel, R. W., Hatch, M., Green, M., *et al.* (2006). Ileal oxalate absorption and urinary oxalate excretion are enhanced in Slc26a6 null mice. *Am J Physiol*, 290, G719–28.

Freundlich, M., Alonzo, E., Bellorin-Font, E., *et al.* (2002). Reduced bone mass in children with idiopathic hypercalciuria and in their asymptomatic mothers. *Nephrol Dial Transplant*, 17, 1396–401.

Frick, K. K. and Bushinsky, D. A. (2003). Molecular mechanisms of primary hypercalciuria. *J Am Soc Nephrol*, 14, 1082–95.

Ghazali, A., Fuentes, V., Desaint, C., *et al.* (1997). Low bone mineral density and peripheral blood monocyte activation profile in calcium stone formers with idiopathic hypercalciuria. *J Clin Endocrinol Metab*, 82, 32–8.

Giannini, S., Nobile, M., Dalle Carbonare, L., *et al.* (2003). Hypercalciuria is a common and important finding inpostmenopausal women with osteoporosis. *Eur J Endocrinol*, 149, 209–13.

Giannini, S., Nobile, M., Sartori, L., *et al.* (1998). Bone density and skeletal metabolism are altered in idiopathic hypercalciuria. *Clin Nephrol*, 50, 94–100.

Gray, R. W., Wilz, D. R., Caldas, A. E., *et al.* (1977). The importance of phosphate in regulating plasma 1, $25(OH)_2$ vitamin D levels in humans: studies in healthy subjects, in calcium stone formers and in patients with primary hyperparathyroidism. *J Clin Endocrinol Metab*, 45, 299–306.

Greischar, A. N. Y. and Coe, F. L. (2003). Influence of urine pH and citrate concentration on the upper limit of metastability for calcium phosphate. *J Urol*, 169, 867–70.

Grover, P. K., Ryall, R. L., and Marshall, V. R. (1990). Effect of urate on calcium oxalate crystallization in human urine: evidence for a promotory role of hyperuricosuria in urolithiasis. *Clin Sci (Lond)*, 79, 9–15.

Grover, P. K., Ryall, R. L., and Marshall, V. R. (1992). Calcium oxalate crystallization in urine: role of urate and glycosaminoglycans. *Kidney Int*, 41, 149–54.

Grynpas, M., Waldman, S., Holmyard, D., *et al.* (2009). Genetic hypercalciuric stone-forming rats have a primary decrease in bone mineral density and strength. *J Bone Min Res*, 24, 1420–6.

Gutman, A. and Yü, T. F. (1965). Urine NH4+ excretion in primary gout. *J Clin Invest*, 92, 1474–81.

Hallson, P. C. and Kasidas, G. P. (1995). The inhibitory activity of some citrate analogues upon calcium crystalluria: Observations using an improved evaporation technique. *Urol Int*, 57, 43–7.

Hamm, L. L. (1990). Renal handling of citrate. *Kidney Int*, 38, 728–35.

Hamm, L. L. and Alpern, R. J. (1996). Regulation of acid-base balance, citrate, and urine pH. In F. L. Coe, M. Favus, and C. Y. C. Pak (eds.) *Kidney Stones: Medical and Surgical Management*, pp. 289–302. Philadelphia, PA: Lippincott-Raven.

Hassan, I. M., Juncos, L. A. M., Milliner, D. S. M., *et al.* (2001). Chronic renal failure secondary to oxalate nephropathy: a preventable complication after jejunoileal bypass. *Mayo Clinic Proc*, 76, 758–60.

Hatch, M., Cornelius, M., Allison, H., *et al.* (2006). Oxalobacter sp. reduces urinary oxalate excretion by promoting enteric oxalate secretion. *Kidney Int*, 69, 691–8.

Hatch, M. and Freel, R. W. (2005). Intestinal transport of an obdurate anion: Oxalate. *Urol Res*, 33, 1–16.

Hatch, M. and Freel, R. W. (2008). The roles and mechanisms of intestinal oxalate transport in oxalate homeostasis. *Sem Nephrol*, 28, 143–51.

Hatch, M., Freel, R. W., Goldner, A. M., *et al*. (1981). Effect of bile salt on active oxalste transport in the colon. In H. Kasper and H. Goebell (eds.) *Colon and Nutrition*, pp. 299–305. Lancaster: MTP.

Hatch, M., Freel, R. W., Goldner, A. M., *et al*. (1984). Oxalate and chloride absorption by the rabbit colon: sensitivity to metabolic and anion transport inhibitors. *Gut*, 25, 232.

Hatch, M., Freel, R. W., and Vaziri, N. D. (1993). Characteristics of the transport of oxalate and other ions across rabbit proximal colon. *Pfleugers Arch*, 423, 206.

Hatch, M., Freel, R. W., and Vaziri, N. D. (1994). Mechanisms of oxalate absorption and secretion across the rabbit distal colon. *Pfleugers Arch*, 426, 101.

Hebert, S. C., Brown, E. M., and Harris, H. W. (1997). Role of the Ca^{2+}-sensing receptor in divalent mineral ion homeostasis. *J Exp Biol*, 200, 295–302.

Heilberg, I. P., Martini, L. A., Teixeira, S. H., *et al*. (1998). Effect of etidronate treatment on bone mass of male nephrolithiasis patients with idiopathic hypercalciuria and osteopenia. *Nephron*, 79, 430–7.

Heilberg, I. P. and Weisinger, J. R. (2006). Bone disease in idiopathic hypercalciuria. *Curr Opin Nephrol Hypertens*, 15, 394–402.

Herring, L. C. (1962). Observations on the analysis of ten thousand urinary calculi. *J Urol*, 88, 545–62.

Hess, B., Jost, C., Zipperle, L., *et al*. (1998). High calcium intake abolishes hyperoxaaluria and reduces urinary crystallization during a 20-foldnormal oxalate load in humans. *Nephrol Dial Transplant*, 13, 2241.

Hesse, A., Wurzel, H., Krampitz, G., *et al*. (1987). Experimental determination of the kinetics of calcium-binding with chondroitin sulphate and the effects of uric acid on this process. *Urol Res*, 15, 93–7.

Hodgkinson, A. (1974). Relations between oxalic acid, calcium, magnesium and creatinine excretion in normal men and male patients with calcium oxalate kidney stones. *Clin Sci Mol Med*, 46, 357–67.

Hodgkinson, A. and Marshall, R. W. (1975). Changes in the composition of urinary tract stones. *Invest Urol*, 13, 13F951–6.

Hofbauer, J., Hobarth, K., Szabo, N., *et al*. (1994). Alkali citrate prophylaxis in idiopathic recurrent calcium oxalate urolithiasis—a prospective randomized study. *Br J Urol*, 73, 362–5.

Holmes, R. P., Goodman, H. O., and Assimos, D. G. (2001). Contribution of dietary oxalate to urinary oxalate excretion. *Kidney Int*, 59, 270–6.

Hoopes, R. R., Jr., Middleton, F. A., Sen, S., *et al*. (2006). Isolation and confirmation of a calcium excretion quantitative trait locus on chromosome 1 in genetic hypercalciuric stone-forming congenic rats. *J Am Soc Nephrol*, 17, 1292–304.

Hoopes, R. R., Reid, R., Sen, S., *et al*. (2003). Quantitative trait loci for hypercalciuria in a rat model of kidney stone disease. *J Am Soc Nephrol*, 14, 1844–50.

Hoppe, B., Beck, B., Gatter, N., *et al*. (2006). Oxalobacter formigenes: a potential tool for the treatment of primary hyperoxaluria type 1. *Kidney Int*, 70, 1305–11.

Hoppe, B., vonUnruh, G., Laube, N., *et al*. (2005). Oxalate degrading bacteria: new treatment option for patients with primary and secondary hyperoxaluria. *Urol Res*, 33, 372–5.

Hosking, D. H., Wilson, J. W., and Liedtke, R. R. (1985). Urinary citrate excretion in normal persons and patients with idiopathic calcium urolithiasis. *J Lab Clin Med*, 106, 682–9.

Hylander, E., Jarnum, S., and Nielsen, K. (1980). Calcium treatment of enteric hyperoxaluria after jejunoileal bypass for morbid obesity. *Scand J Gastroent*, 15, 349–52.

Insogna, K. L., Broadus, A. E., Dryer, B. E., *et al*. (1985). Elevated production rate of 1,25-dihydroxyvitamin D in patients with absorptive hypercalciuria. *J Clin Endocrinol Metab*, 61, 490–5.

Jaeger, P., Lippuner, K., Casez, J. P., *et al*. (1994). Low bone mass in idiopathic renal stone formers: magnitude and significance. *J Bone Min Res*, 9, 1525–32.

Jaeger, P. and Robertson, W. G. (2004). Role of dietary intake and intestinal absorption of oxalate in calcium stone formation. *Nephron Phys*, 98, 64–71.

Jonsson, S., Ricagno, S., Lindqvist, Y., *et al*. (2004). Kinetic and mechanistic characterization of the formyl-coa transferase from Oxalobacter formigenes. *J BiolChem*, 279, 36003–12.

Kallistratos, G., Timmerman, A., and Fenner, O. (1970). Influence of the salting-out effect on the formation of calcium oxalate crystals in human urine. *Natturwissenschaften*, 57, 198.

Karet, F. E., Finberg, K. E., and Nelson, R. D. (1999). Mutations in the gene encoding B1 subunit of H+- ATPhase cause renal tubular acidosis with sensorineural deafness. *Nat Genet*, 21, 84–90.

Karet, F. E., Gainza, F. J., and Gyory, A. Z. (1998). Mutations in the chloride-bicarbonate exchanger gene AE1 caouse autosomal dominant but not autosomal recessive distal renal tubular acidosis. *Proc Natl Acad Sci U S A*, 95, 6337–42.

Karnauskas, A. J., van Leeuwen J. P., van den Bemd, G. J., *et al*. (2005). Mechanism and function of high vitamin D receptor levels in genetic hypercalciuric stone-forming rats. *J Bone Min Res*, 20, 447–54.

Kasidas, G. P., Nmat, S., and Rose, G. A. (1990). Plasma oxalate and creatinine and oxalate/creatinine clearance ratios in normal subjects and in primary hyperoxaluria. Evidence for renal hyperoxaluria. *Clin Chim Acta*, 191, 67–77.

Kasidas, G. P. and Rose, G. A. (1986). Measurement of plasma oxalate in healthy subjects and in patients with chronic renal failure using immobilized oxalate oxidase. *Clin Chim Acta*, 154, 49–58.

Kathpalia, P., Favus, M. J., and Coe, F. L. (1984). Evidence for size and charge permselectivity of rat ascending colon: effects of ricinoleate and bile salts on oxalic acid and neutral sugar transport. *J Clin Invest*, 74, 805–11.

Kaufman, D. W., Kelly, J. P., Curhan, G. C., *et al*. (2008). Oxalobacter formigenes may reduce the risk of calcium oxalate kidney stones. *J Am Soc Nephrol*, 19, 1197–203.

Khosla, S., Ebeling, P. R., Firek, A. F., *et al*. (1993). Calcium infusion suggests a 'set-point' abnormality of parathyroid gland function in familial benign hypercalcemia and more complex disturbances in familial benign hypercalcemia and more complex distrubances in primary hyperparathyroidism. *J Clin Endocrinol Metab*, 76, 715–20.

Kim, M., Sessler, N. E., Tembe, V., *et al*. (1993). Response of genetic hypercalciuric rats to a low calcium diet. *Kidney Int*, 43, 189–96.

Kleinman, J. G. (2007). Bariatric surgery, hyperoxaluria, and nephrolithiasis: a plea for close postoperative management of risk factors. *Kidney Int*, 72, 8–10.

Knickelbein, R. G., Aronson, P. S., and Dobbins, J. W. (1986). Oxalate transport by anion exchange across rabbit ileal brush border. *J Clin Invest*, 77, 170.

Kok, D. J., Papapoulos, S. E., and Bijvoet, O. L. (1986). Excessive crystal agglomeration with low citrate excretion in recurrent stone-formers. *Lancet*, 10, 1056–8.

Krieger, N. S., Stathopoulos, V. M., and Bushinsky, D. A. (1996). Increased sensitivity to 1, $25(OH)_2D_3$ in bone from genetic hypercalciuric rats. *Am J Physiol (Cell Physiol)*, 271, C130–5.

Kumar, R., Ghoshal, U. C., Singh, G., *et al*. (2003). Infrequency of colonization with Oxalobacter formigenes in inflammatory bowel disease: possible role in renal stone formation. *J Gastroenterol Hepatol*, 19, 14032–9.

Kuo, R. L., Lingeman, J. E., Evan, A. P., *et al*. (2003). Urine calcium and volume predict coverage of renal papilla by Randall's plaque. *Kidney Int*, 64, 2150–4.

Kurtzman, N. A. and Kurtzman, N. (1983). Acquired distal renal tubular acidosis. *Kidney Int*, 24, 807–19.

Kwak, C., Kim, H. K., Kim, E. C., *et al*. (2003). Urinary oxalate levels and the enteric bacterium Oxalobacter formigenes in patients with calcium oxalate urolithiasis. *Eur Urol*, 44, 475–81.

Lapointe, J. Y., Tessier, J., Paquette, Y., *et al*. (2006). NPT2a gene variation in calcium nephrolithiasis with renal phosphate leak. *Kidney Int*, 69, 2261–7.

Lauderdale, D. S., Thisted, R. A., Wen, M., *et al*. (2001). Bone mineral density and fracture among prevalent kidney stone cases in the Third National Health and Nutrition Examination Survey. *J Bone Miner*, 16, 1893–8.

Lemann, J. Jr., Bushinsky, D. A., and Hamm, L. L. (2003). Bone buffering of acid and base in humans. *Am J Physiol Renal Physiol*, 285, F811–32.
Lemann, J., Jr., Gray, R. W., and Pleuss, J. A. (1989). Potassium bicarbonate, but not sodium bicarbonate, reduces urinary calcium excretion and improves calcium balance in healthy men. *Kidney Int*, 35, 688–95.
Lemann, J., Jr., Pleuss, J. A., Gray, R. W., *et al.* (1991). Potassium administration decreases and potassium deprivation increases urinary calcium excretion in healthy adults. *Kidney Int*, 39, 973–83.
Lemann, J., Jr., Pleuss, J. A., Worcester, E. A., *et al.* (1996). Urinary oxalate excretion increases with body size and decreases with increasing dietary calcium intake among healthy adults. [Erratum: *Kidney Int*, 1996; 50, 341] *Kidney Int*, 49, 200–18.
Li, X. -Q., Tembe, V., Horwitz, G. M., *et al.* (1993). Increased intestinal vitamin D receptor in genetic hypercalciuric rats: a cause of intestinal calcium hyperabsorption. *J Clin Invest*, 91, 661–7.
Liberman, U. A., Sperling, O., Atsmon, A., *et al.* (1968). Metabolic and calcium kinetic studies in idiopathic hypercalciuria. *J Clin Invest*, 47, 2580–90.
Liebman, M. and Costa, G. (2000). Effects of calcium and magnesium on urinary oxalate excretion after oxalate loads. *J Urol*, 163, 1565.
Lieske, J. C., Goldfarb, D., DeSimone, C., *et al.* (2005). Use of a probioitic to decrease enteric hyperoxaluria. *Kidney Int*, 68, 1244–9.
Lieske, J. C., Kumar, R., and Collazo-Clavell, M. L. (2008). Nephrolithiasis after bariatric surgery for obesity. *Kidney Int*, 28, 163–73.
Lonsdale, K. (1968). Epitaxy as a growth factor in urinary calculi and gallstones. *Nature*, 217, 56–8.
Maierhofer, W. J., Gray, R. W., Cheung, H. S., *et al.* (1983). Bone resorption stimulated by elevated serum 1, 25-$(OH)_2$-vitamin D_3 concentrations in healthy men. *Kidney Int*, 24, 555–60.
Malluche, H., Tschoepe, W., Ritz, E., *et al.* (1980). Abnormal bone histology in idiopathic hypercalciuria. *J Clin Endocrinol Metab*, 50, 654–8.
Marangella, M., Frutero, B., Bruno, M., *et al.* (1982). Hyperoxaluria in idiopathic calcium stone disease: further evidence of intestinal hyperabsorption of oxalate. *Clin Sci*, 63, 381–5.
Mark, S. J., Spiegel, A. M., and Brown, E. M. (1978). Divalent cation metabolism: Familial hypocalciuric hypercalcemia vs. typical primary hyperparathyroidism. *Am J Med*, 65, 235–42.
Marx, S. J., Attie, M. F., Levine, M., *et al.* (1981). The hypocalciuric or benign variant of familial hypercalcemia: clinical and biochemical features in fifteen kindreds. *Medicine*, 60, 397–412.
Marx, S. J., Spiegel, A. M., Brown, E. M., *et al.* (1978). Divalent cation metabolism: famalial hypocalciuric hypercalcemia veersus typical primary hyperparathyroidism. *Am J Med*, 65, 235–42.
Matlaga, B. R., Williams, J. C., Jr., and Kim, S. C. (2006). Endoscopic evidence of calculus attachment to Randall's plaque. *J Urol*, 175, 1720–4.
McLeod, R. S. and Churchill, D. N. (1992). Urolithiasis complicating inflammatory bowel disease. *J Urol*, 148, 974–8.
Melnick, J. Z., Preisig, P. A., Moe, O. W., *et al.* (1998). Renal cortical mitochondrial aconitase is regulated in hypo- and hypercitraturia. *Kidney Int*, 54, 160–5.
Melnick, J. Z., Srere, P., Elshourbagy, N. A., *et al.* (1996). Adenosine triphosphate citrate lyase mediates hypocitraturia in rats. *J Clin Invest*, 98, 2387.
Melton, L. J. I., Crowson, C. S., Khosla, S., *et al.* (1998). Fracture risk among patients with urolithiasis: a population based cohort study. *Kidney Int*, 53, 459–64.
Meschi, T., Maggiore, U., Fiaccadori, E., *et al.* (2004). The effect of fruits and vegetables on urinary stone risk factors. *Kidney Int*, 66, 2402–10.
Milliner, D. (2006). Treatment of the primary hyperoxalurias: a new chapter. *Kidney Int*, 70, 1198–200.
Milliner, D. S. (2005). The primary hyperoxalurias: an algorithm for diagnosis. *Am J Nephrol*, 25, 154–60.
Millman, S., Strauss, A. L., Parks, J. H., and Coe, F. L. (1982). Pathogenesis and clinical course of mixed calcium oxalate and uric acid nephrolithiasis. *Kidney Int*, 22(4), 366–70.
Minisola, S., Rossi, W., Pacitti, M. T., *et al.* (1989). Studies on citrate metabolism in normal subjects and kidney stone patients. *Miner Electrolyte Metab*, 15, 303–8.
Misael da Silva, A. M., dos Reis, L. M., Pereira, R. C., *et al.* (2002). Bone involvement in idiopathic hypercalciuria. *Clin Nephrol*, 57, 183–91.
Modigliani, R., Labayle, D., Aymes, C., *et al.* (1978). Evidence for excessive absorption of oxalate by the colon in enteric hyperoxaluria. *Scand J Gastroent*, 13, 187–92.
Moe, O. W. (2006a). Kidney stones: pathophysiology and medical management. *Lancet*, 367, 333–44.
Moe, O. W. (2006b). Uric acid nephrolithiasis: protonation of an essential molecule. *Curr Opin Nephrol Hypertens*, 15, 366–73.
Moe, O. W. and Preisig, P. A. (2006). Duel role of citrate in urine. *Curr Opin Nephrol Hypertens*, 15, 419–24.
Mollerup, C. L., Vestergaard, P., Frokjaer, V. G., *et al.* (2002). Risk of renal stone events in primary hyperparathyroidism before and after parathyroid surgery: controlled retrospective follow up study. *Br Med J*, 325, 785–6.
Monico, C. G., Weinstein, A., Jiang, Z., *et al.* (2008). Phenotypic and functional analysis of human SLC26A6 variants in patients with familial hyperoxaluria and calcium oxalate nephrolithiasis. *Am J Kidney Dis*, 52, 1096–103.
Monk, R. D. and Bushinsky, D. A. (2010). Nephrolithiasis and nephrocalcinosis. In J. Frehally, J. Floege, and R. J. Johnson (eds.) *Comprehensive Clinical Nephrology*, pp. 687–701. St. Louis, MO: Elsevier.
Monk, R. D. and Bushinsky, D. A. (2011). Kidney stones. In H. M. Kronenberg, S. Melmed, K. S. Polonsky, *et al.* (eds.) *Williams Textbook of Endocrinology*, pp. 1350–67. Philadelphia, PA: W. B. Saunders.
Nasr, S. H., D'Agati, V. D., Said, S. M., *et al.* (2008). Oxalate nephropathy complicating roux-en-Y gastric bypass: an underrecognized cause of irreversible renal failure. *Clin J Am Soc Nephrol*, 3, 1676–83.
National Institutes of Health (1991). Consensus development conference statement on primary hyperparathyroidism. *J Bone Min Res*, 6, s9–13.
Nelson, W. K., Houghton, S. G., Milliner, D. S., *et al.* (2009). Enteric hyperoxaluria, nephrolithiasis, and oxalate nephropathy: potentially serious and unappreciated complications of Roux-en-Y gastric bypass. *Surg Obes Relat Dis*, 1, 481–5.
Nicar, M. J., Hill, K., and Pak, C. Y. C. (1987). Inhibition by citrate of spontaneous precipitation of calcium oxalate *in vitro*. *J Bone Min Res*, 2, 215–20.
Nicar, M. J., Skurla, C., Sakhaee, K., *et al.* (1983). Low urinary citrate excretion in nephrolithiasis. *Urology*, 21, 8–14.
Nikkila, M., Koivula, T., and Jokela, H. (1989). Urinary citrate excretion in patients with urolithiasis and normal subjects. *Eur Urol*, 16, 382–5.
Nordin, B. E. C. and Peacock, M. (1969). Role of kidney in regulation of plasma-calcium. *Lancet*, 2, 1280–3.
Odvina, C. V., Mason, R. P., and Pak, C. Y. (2006). Prevention of thiazide-induced hypokalemia without magnesium depletion by potassium-magnesium-citrate. *Am J Ther*, 13, 101–8.
Pacher, P., Nivorozhkin, A., and Szabo, C. (2003). Therapeutic effects of xanthine oxidase inhibitors: renaissance half a century after the discovery of allopurinol. *Pharmacol Rev*, 58, 87–114.
Pacifici, R., Rothstein, M., Rifas, L., *et al.* (1990). Increased monocyte interleukin-1 activity and decreased vertebral bone density in patients with fasting idiopathic and hypercalciuria. *J Clin Endocrinol Metab*, 71, 138–45.
Pak, C. Y. C. (1992). Pathophysiology of calcium nephrolithiasis. In D. W. Seldin and G. Giebisch (eds.) *The Kidney: Physiology and Pathophysiology*, pp. 2461–80. New York: Raven Press, Ltd.
Pak, C. Y. (1994). Citrate and renal calculi: an update. *Min Electrolyte Metab*, 20, 371–7.
Pak, C. Y. (1997). Nephrolithiasis. *Current Ther Endo Metab*, 6, 572–6.
Pak, C. Y. C., Adams-Huet, B., Poindexter, J., *et al.* (2004). Relative effect of urinary calcium and oxalate on saturation of calcium oxalate. *Kidney Int*, 66, 2032–37.
Pak, C. Y. and Arnold, L. H. (1975). Heterogeneous nucleation of calcium oxalate by seeds of monosodium urate. *Proc Soc Exp Biol Med*, 149, 930–2.
Pak, C. Y., Barilla, D. E., Holt, K., *et al.* (1978). Effect of oral purine load and allopurinol on the crystallization of calcium salts in urine of patients with hyperuricosuric calcium urolithiasis. *Am J Med*, 65, 593–9.

Pak, C. Y. C., Fuller, C., Sakhaee, K., *et al.* (1985). Long-term treatment of calcium oxalate nephrolithiasis with potassium citrate. *J Urol*, 134, 11–19.
Pak, C. Y., Koenig, K., Khan, R., *et al.* (1992). Physicochemical action of potassium-magnesium citrate in nephrolithiasis. *J Bone Min Res*, 7, 281–5.
Pak, C. Y. C., Nicar, M. J., Peterson, R., *et al.* (1981). A lack of unique pathophysiologic background for nephrolithiasis of primary hyperparathyroidism. *J Clin Endocrinol Metab*, 53, 536–42.
Pak, C. Y. and Peterson, R. (1986). Successful treatment of hyperuricosuric calcium oxalate nephrolithiasis with potassium citrate. *Arch Intern Med*, 146, 863–7.
Pak, C., Peterson, R., Sakhaee, K., *et al.* (1985). Correction of hypocitraturia and prevention of stone formation by combined thiazide and potassium citrate therapy in thiazide-unresponsive hypercalciuric nephrolithiasis. *Am J Med*, 79, 284–8.
Pak, C. Y. C., Sakhaee, K., and Fuller, C. (1986). Successful management of uric aid nephrolithiasis with potassium citrate. *Kidney Int*, 30, 422–8.
Pak, C. Y., Waters, C. O., Arnold, L., *et al.* (1977). Mechanism for calcium urolithiasis among patients with hyperuricosuria. *J Clin Invest*, 59, 426–31.
Pardi, D. S., Tremaine, W. J., Sandborn, W. J., *et al.* (1998). Renal and urological complications of inflammatory bowel disease. *Am J Gastroenterol*, 93, 500–14.
Parivar, F., Low, R. K., and Stoller, M. L. (1996). The influence of diet on urinary stone disease. *J Urol*, 155, 432–40.
Parks, J. H. and Coe, F. L. (1986). A urinary calcium-citrate index for the evaluation of nephrolithiasis. *Kidney Int*, 30, 85–90.
Parks, J. H., Coe, F. L., and Favus, M. J. (1980). Hyperparathyroidism in nephrolithiasis. *Arch Intern Med*, 140, 1479.
Parks, J. H., Worcester, E. M., and Coe, F. L. (2004). Clinical implications of abundant calcium phosphate in routinely analyzed kidney stones. *Kidney Int*, 64, 2150–4.
Parks, J. H., Worcester, E. M., O'Connor, R. C., *et al.* (2003). Urine stone risk factors in nephrolithiasis patients with and without bowel disease. *Kidney Int*, 63, 255–65.
Petrarulo, M., Vitale, C., Facchini, P., *et al.* (1998). Biochemical approach to diagnosis and differentiation of primary hyperoxalurias: an update. *J Nephrol*, 11, 23–8.
Pietschmann, F., Breslau, N. A., and Pak, C. Y. C. (1992). Reduced vertebral bone density in hypercalciuric nephrolithiasis. *J Bone Miner Res*, 7, 1383–8.
Pongchaiyakul, C., Domrongkitchaiporn, S., and Stichantrakul, W. (2004). Incomplete renal tubular acidosis and bone mineral density: a population survey in an area of endemic renal tubular acidosis. *Nephrol Dial Transplant*, 19, 3029–33.
Porits, A. J., Hermans, K., Culhane-Pera, K. A., *et al.* (2004). Stone disease in the Hmong of Minnesota: initial description of a high-risk population. *J Endourol*, 18, 853–7.
Pratley, S. K., Posen, S., and Reeve, T. S. 1973. Primary hyperparathyroidism: Experience with 60 patients. *Med J Aust*, 1, 42.
Preminger, G. M., Sakhaee, K., Skurla, C., *et al.* (1985). Prevention of recurrent calcium stone formation with potassium citrate therapy in patients with distal renal tubular acidosis. *J Urol*, 134, 20–3.
Prenen, J. A., Dorhout Mees, E. J., and Boer, P. (1985). Plasma oxalate concentration and oxalate distribution volume in patients with normal and decreased renal function. *Eur J Clin Invest*, 15, 45–9.
Prie, D., Huart, V., Bakouh, N., *et al.* (2002). Nephrolithiasis and osteoporosis associated with hypophosphatemia caused by mutations in the type 2a sodium-phosphate cotransporter. *N Eng J Med*, 347, 983–91.
Prokopovich, S., Knight, J., Assimos, D. G., *et al.* (2007). Variability of oxalobacter formigenes and oxalate in stool samples. *J Urol*, 178, 2186–90.
Pyrah, L. N., Hodgkinson, A., and Anderson, C. K. (1966). Primary hyperparathyroidism. *Br J Surg*, 53, 245–316.
Quilty, J. A., Cordat, E., and Reithmeier, R. A. (2002). Impaired trafficking of human kidney anion exchanger (kAE1) caused by hetero-oligomer formation with a truncated mutant assoiciated with distal renal tubular acidosis. *Biochem J*, 368, 895–903.
Randall, A. (1937). The origin and growth of renal calculi. *Ann Surg*, 105, 1009–27.
Riccardi, D., Lee, W. S., Lee, K., *et al.* (1996). Localization of the extracellular Ca^{2+}-sensing receptor and PTH/PTHrP receptor in rat kidney. *Am J Physiol*, 271, F951–6.
Robertson, W. G. and Scurr, D. S. (1986). Modifiers of calcium oxalate crystallization found in urine. *J Urol*, 135, 1322–6.
Rocha, A. S., Magaldi, J. B., and Kokko, J. P. (1977). Calcium and phosphate transport in isolated segments of rabbit Henle's loop. *J Clin Invest*, 59, 975–83.
Rosen, I. B., Yong, J. E. M., Archibald, S. D., *et al.* (1994). Parathyroid cancer: clinical variations and relationships to autotransplantation. *Can J Cancer*, 37, 465–9.
Rudman, D., Dedonis, J. L., Fountain, M. T., *et al.* (1980). Hypocituria in patients with gastrointestinal malabsorption. *N Engl J Med*, 303, 657–61.
Ruml, L. A. and Pak, C. Y. (1999). Effect of potassium magnesium citrate on thiazide-induced hypokalemia and magnesium loss. *Am J Kid Dis*, 34, 107–13.
Ryall, R. L., Hibberd, C. M., and Marshall, V. R. (1986). The effect of crystalline monosodium urate on the crystallization of calcium oxalate in whole human urine. *Urol Res*, 14, 63–5.
Scheinman, S. J., Cox, J. P. D., Lloyd, S. E., *et al.* (2000). Isolated hypercalciuria with mutation in CLCN5: relevance to idiopathic hypercalciuria. *Kidney Int*, 57, 232–9.
Scheinman, S. J., Guay-Woodford, L. M., Thakker, R. V., *et al.* (1999). Genetic disorders of renal electrolyte transport. *N Eng J Med*, 340, 1177–87.
Schumacher, H. R., Jr., Becker, M. A., Lloyd, E., *et al.* (2009). Febuxostat in the treatment of gout: 5-yr findings of the FOCUS efficacy and safety study. *Rheumatology*, 48, 188–94.
Sharma, R. K., Jha, R., and Bhatia, V. L. (1997). Secondary distal renal tubular acidosis in association with urological abnormalities. *Nephrol Dial Transplant*, 12, 233.
Shen, F. H., Baylink, D. J., Nielsen, R. L., *et al.* (1977). Increased serum 1, 25-dihydroxyvitamin D in idiopathic hypercalciuria. *J Lab Clin Med*, 90, 955–62.
Sidhu, H., Enatska, L., Ogden, S., *et al.* (1997). Evaluating children in the Ukraine for colonization with the intestinal bacterium *Oxalobacter formigenes*, using a polymerase chain reaction-based detection system. *Moll Diagn* 2, 89–97.
Sidhu, H., Hoppe, B., Hesse, A., *et al.* (1998). Absence of Oxalobacter formigenes in cystic fibrosis patients: a risk factor for hyperoxaluria. *Lancet*, 352, 1026–9.
Sidhu, H., Schmidt, M. E., Cornelius, J. G., *et al.* (1999). Direct correlation between hyperoxaluria/oxalate stone disease and the absence of the gastrointestinal tract-dwelling bacterium Oxalobacter formigenes: possible prevention by gut recolonization or enzyme replacement therapy. *J Am Soc Nephrol*, 10, S334–40.
Silverberg, S. J., Shane, E., and Jacobs, T. P. (1999). Primary hyperparathyroidism: 10-year course with or without parathyroid surgery. *N Eng J Med*, 341, 1249–55.
Silverberg, S. J., Shane, E., Jacobs, T. P., *et al.* (1990). Nephrolithiasis and bone involvement in primary hyperparathyroidism, 1985–1990. *Am J Med*, 89, 334.
Sinha, M. K., Collazo-Clavell, M. L., Rule, A., *et al.* (2007). Hyperoxaluric nephrolithiasis is a complication of Roux-en-Y gastric bypass surgery. *Kidney Int*, 72, 100–7.
Sorensen, C. M. and Chandhoke, P. S. (2002). Hyperuricosuric calcium nephrolithiasis. *Endocrinol Metlab Clin North Am*, 31, 915–25.
Spruce, B. A., Baylis, P. H., Kerr, D. N., *et al.* (1984). Idiopathic hypergammaglobulinaemia associated with nephrogenic diabetes insipidus and distal renal tubular acidosis. *Postgrad Med J*, 60, 493–4.
Stehberger, P. A., Schulz, N., and Finberg, K. E. (2003). Localization and regulation of the ATP6V0A4(a4) vacuolar H+-ATPase subunit defective

in an inherited form of distal renal tubular acidosis. *J Am Soc Neph*, 14, 3027–38.
Steiniche, T., Mosekilde, L., Christensen, M. S., *et al.* (1989). Histomorphometric analysis of bone in idiopathic hypercalciuria before and after treatment with thiazide. *APMIS*, 97, 302–8.
Sutton, R. A. L. and Walker, V. R. (1986). Bone resorption and hypercalciuria in calcium stone formers. *Metabolism* 35, 485–8.
Takano, Y., Hase-Aoki, K., Horiuchi, H., *et al.* (2005). Selectivity of febuxostat, a novel non-purine inhibitor of xanthine oxidase/xanthine dehydrogenase. *Life Sci*, 76, 1835–47.
Tasca, A., Cacciola, A., Ferrarese, P., *et al.* (2002). Bone alterations in patients with idiopathic hypercalciuria and calcium nephrolithiasis. *Urology*, 59, 865–9.
Taylor, E. N. and Curhan, G. C. (2007). Fructose consumption and the risk of kidney stones. *Kidney Int*, 73, 207–12.
Terkeltaub, R. A. (2003). Clinical practice. Gout. *N Eng J Med*, 349, 1647–55.
Terkeltaub, R., Bushinsky, D., and Becker, M. (2006). Recent developments in our understanding of the renal basis of hyperuricemia and the development of novel antihyperuricemic therapeutics. *Arthritis Res Ther*, 8, S4.
Tieder, M., Modai, D., Shaked, U., *et al.* (1987). 'Idiopathic' hypercalciuria and hereditary hypophosphatemic rickets: two phenotypical expressions of a common genetic defect. *N Engl J Med*, 316, 125–9.
Tiselius, H. G., Bek-Jensen, H., Fornander, A. M., *et al.* (1995). Crystallization properties in urine from calcium oxalate stone formers. *J Urol*, 154, 940–6.
Tiselius, H. G., Fornander, A., and Nilsson, M. A. (1993). The effects of citrate and urine on calcium oxalate crystal aggregation. *Urol Res*, 21, 363–6.
Transbol, I., Hornum, I., Hahnemann, S., *et al.* (1970). Tubular reabsorption of calcium in the differential diagnosis of hypercalcemia. *Acta Med Scand*, 188, 505.
Troxel, S. A., Sidhu, H., Kaul, P., *et al.* (2003). Intestinal Oxalobacter formigenes colonization in calcium oxalate stone formers and its relation to urinary oxalate. *J Endourol*, 17, 173.
Tsuruoka, S., Bushinsky, D. A., and Schwartz, G. J. (1997). Defective renal calcium reabsorption in genetic hypercalciuric rats. *Kidney Int*, 51, 1540–7.
Urivetzky, M., Anna, P. S., and Smith, A. D. (1988). Plasma osteocalcin levels in stone disease: a potential aid in the differential diagnosis of calcium nephrolithiasis. *J Urol*, 139, 12–4.
Vezzoli, G., Soldati, L., Ardila, M., *et al.* (2005). Urinary calcium is a determinant of bone mineral density in elderly men participating in the InCHIANTI study. *Kidney Int*, 67, 2006–14.
Virkki, L. V., Forster, I. C., Hernando, N., *et al.* (2003). Functional characterization of two naturally occurring mutations in the human sodium-phosphate contransporter type IIa. *J Bone Min Res*, 18, 2135–41.
Wang, Z., Wang, T., Petrovic, S., *et al.* (2005). Renal and intestinal transport defects in slc26a6-null mice. *Am J Physiol Cell Physiol*, 288, C957–65.
Wareing, M. and Green, R. (1994). Effect of formate and oxalate on fluid reabsorption from the proximal convoluted tubule of the anaesthetized rat. *J Physiol*, 477, 347–54.
Watts, R. W. E., Danpure, C. J., de Pauw, L., *et al.* (1991). Combined liver-kidney and isolated liver transplantations for primary hyperoxaluria type 1. The European experience. *Nephrolol Dial Transplant*, 6, 502–11.
Weber, C. J., Sewell, C. W., and McGarity, W. C. (1994). Persistent and recurrent sporadic primary hyperparathyroidism: histopathology, complications, and results of reoperation. *Surgery*, 116, 991–8.
Weisinger, J. R., Alonzo, E., Bellorin-Font, E., *et al.* (1996). Possible role of cytokines on the bone mineral loss in idiopathic hypercalciuria. *Kidney Int*, 49, 244–50.
Welch, B. J., Graybeal, D., Moe, O. W., *et al.* (2006). Biochemical and stone risk profiles with topiramate treatment. *Am J Kid Dis*, 48(4), 555–63.
Wermers, R. A., Khosla, S., Atkinson, E. J., *et al.* (1997). The rise and fall of primary hyperparathyroidism: a population-based study in Rochester, Minnesota, 1965–1992. *Ann Intern Med*, 126, 433–40.
Whalley, N. A., Meyers, A. M., Martins, M., *et al.* (1996). Long term effects of potassium citrate therapy on the formation of new stones in groups of recurrent stone formers with hyprocitraturia. *Br J Urol*, 78, 10–14.
Worcester, E. M. (2002). Stones from bowel disease. *Endocrinol Metab Clin North Am*, 31, 979–99.
Worcester, E. M., Coe, F. L., Evan, A. P., *et al.* (2008). Evidence for increased postprandial distal nephron calcium delivery in hypercalciuric stone-forming patients. *AJP Renal Physiol*, 295, F1286–94.
Worcester, E. M., Gillen, D. L., Evan, A. P., *et al.* (2007). Evidence that postprandial reduction of renal calcium reabsorption mediates hypercalciuria of patients with calcium nephrolithiasis. *AJP Renal Physiol*, 292, F66–75.
Wright, S. H., Kippen, I., and Wright, E. M. (1982). Effect of pH on the transport of Krebs cycle intermediates in renal brush border membranes. *Biochem Biophys Acta*, 684, 287–90.
Yao, J., Karnauskas, A. J., Bushinsky, D. A., *et al.* (2005). Regulation of renal calcium-sensing receptor gene expression in response to 1, 25$(OH)_2D_3$ in genetic hypercalciuric stone forming rats. *J Am Soc Nephrol*, 16, 1300–8.
Yao, J., Kathpalia, P., Bushinsky, D. A., *et al.* (1998). Hyperresponsiveness of vitamin D receptor gene expression to 1, 25-dihydroxyvitamin D_3: a new characteristic of genetic hypercalciuric stone-forming rats. *J Clin Invest*, 101, 2223–32.
Zerwekh, J. E., Holt, K., and Pak, C. Y. (1983). Natural urinary macromolecular inhibitors: attenuation of inhibitory activity by urate salts. *Kidney Int*, 23, 838–41.

CHAPTER 202

Uric acid stones

Michel Daudon and Paul Jungers

Introduction

Uric acid nephrolithiasis (UANL) was the commonest form of nephrolithiasis in adults in past centuries, affecting especially kings, nobles, and the wealthy, and more generally overeating, affluent people, who were often also afflicted with gout. Gouty attacks either preceded onset of renal colics, as was the case for Michelangelo, or followed gravel, as was the case for Montaigne who gave unsurpassable descriptions of the pain associated with stone passage. In the twentieth century, calcium oxalate nephrolithiasis became the predominant form of stone disease especially since the end of World War II, in parallel with lifestyle modification and generalized nutritional affluence in industrialized countries. However, UANL subsisted, and now affects all classes of society because it is favoured by the same factors that have resulted in the epidemic development of obesity in recent decades (Zilberman et al., 2010).

UA stones now globally account for about 10% of analysed kidney stones, with a prevalence of 20% or more in countries such as Germany (Hesse et al., 2003) or in Asian populations, which suggests the cumulative role of genetic and dietary factors.

As recently highlighted, the relative proportion of UA stones is higher in obese people and type 2 diabetics than in lean stone-formers of similar age and gender (Ekeruo et al., 2004; Daudon et al., 2006b). Among 1931 non-diabetic stone formers, calculi were made of UA in 28.7% of men with a body mass index (BMI) ≥ 30 kg/m^2 and only 7.1% in those with a BMI < 25, the corresponding figures being 17.1% and 6.1% in women (Daudon et al., 2006a). UA was the main component of calculi in 29% of type 2 diabetics as compared with 6% in non-diabetics in a US population (Lieske et al., 2006) and in 35.7% of type 2 diabetics as compared with 11.3% in non-diabetics in our experience (Daudon et al., 2006b). Reciprocally, type 2 diabetes is disproportionately frequent among UA stone formers: 25.6–33% had overt type 2 diabetes (Sakhaee et al., 2002; Pak et al., 2003). In our series, 27.8% of UA stone formers had type 2 diabetes compared with 6.9% of calcium stone formers (Daudon et al., 2006b). BMI and type 2 diabetes were independent, significant risk factors associated with UANL, the specific effect of diabetes being more marked in women than in men and the more apparent at younger age in either gender (Daudon et al., 2006b).

Factors of lithogenesis in uric acid nephrolithiasis

Factors that induce the formation of UA stones are a low urine volume (as in every type of nephrolithiasis), a high uricosuria (fractional UA excretion > 10%), and a low urine pH (< 5.5) which appears as the major factor (Pak et al., 2001; Sakhaee et al., 2002; Sakhaee, 2009). Indeed, under its pKa (5.35 at 37°C), UA is in undissociated form, whose solubility is at best 100 mg/L whereas UA excretion commonly is > 600 mg/day. Of note, UA solubility markedly increases with increasing pH > 6, this pH dependence offering a therapeutic opportunity.

UANL results in a minority of cases from innate or acquired causes leading to UA overproduction or urine acidity, but in the great majority of cases presents as idiopathic or primary. The main causes and mechanisms involved in UANL are summarized in Table 202.1.

Secondary forms of uric acid nephrolithiasis

Hyperuricosuria from UA overproduction

Inborn errors of purine metabolism

Two hereditary enzymatic disorders cause endogenous UA overproduction leading to hyperuricaemia (with gout and infiltration of the renal parenchyma with UA crystals) and massive hyperuricosuria with UA stone formation. Deficiency in hypoxanthine guanine phosphoribosyltransferase (HGPRT) in its complete form (Lesch–Nyhan syndrome) associates with early gout, UA stones, and characteristic, severe neurological symptoms which are absent in partial deficiency (Jinnah and Friedman, 2001). Hyperactivity of phosphoribosyl pyrophosphate synthase (PRPPS) results in gout and UA stone formation in adolescents (Nyhan, 2005). Both diseases are X-linked and affect primarily males. Treatment relies on hyperdiuresis, alkalinization, and allopurinol with prudent dosing to avoid the formation of stones made of xanthine (owing to metabolic deviation) and oxypurinol (the main metabolite of allopurinol).

Myelo- and lymphoproliferative diseases

In Vaquez polycythemia and acute or chronic leukaemias, accelerated nucleoprotein turnover results in UA overexcretion in untreated patients, whereas the massive nucleoprotein destruction induced by chemotherapy (tumour lysis syndrome) provokes acute precipitation of UA in kidney tubules (Davidson et al., 2004). Prevention relies on alkalinization, hyperhydration, and allopurinol, or, in acute massive hyperuricaemia, uricase (Howard et al., 2011).

Hyperuricosuria from defective renal urate reabsorption

Inactivating mutations of genes encoding the tubular urate transporters URAT1 (*SLC22A12*) or GLUT9 (*SLC2A9*) (Wright et al.,

Table 202.1 Pathophysiological mechanisms of uric acid nephrolithiasis

Hyperuricosuria from UA overproduction	Inborn errors of metabolism: ◆ Deficiency of HGPRT (Lesch–Nyhan syndrome) ◆ Overactivity of PRPPS ◆ Deficiency of glucose-6-phosphatase (type I glycogenosis) Nucleolysis in myelo- or lymphoproliferative disorders Purine gluttony High fructose intake Gouty diathesis
Hyperuricosuria from decreased tubular UA reabsorption	Proximal tubular defects (Fanconi syndrome) Inactivating mutations of urate tubular transporters (renal hypouricaemia) Uricosuric drugs
Unduly low urine pH	Chronic diarrhoea (enteropathies, ileostomy) High protein intake, acidifying beverages Obesity, metabolic syndrome, type 2 diabetes, ageing (insulin resistance with defective ammoniagenesis)

2010) result in decreased proximal tubular reabsorption of urates leading to hyperuricosuria (excretion fraction often > 50%) and hypouricaemia, an unusual finding highly suggestive of the diagnosis of familial renal hypouricaemia (Enomoto et al., 2002; Iwai et al., 2004; Dinour et al., 2010). Familial renal hypouricaemia is an essentially benign condition, although it may be associated with reversible, exercise-induced acute kidney injury episodes (Ohta et al., 2004). Membrane transporters of urate in kidney are summarized in Fig. 202.1.

Fanconi syndrome, either hereditary or acquired, may result in UA stones formation. Uricosuric drugs, most of which are URAT1 inhibitors, by destination augment UA excretion and may induce UA stone formation in patients with heavy hyperuricaemia and therefore were withdrawn from clinical use.

Intestinal base loss

Chronic diarrhoea in patients suffering from inflammatory intestinal diseases or with ileostomy results in oliguria and very low urine pH (often < 5) due to water and base losses, which may be prevented by adequate fluid and alkali supply (Parks et al., 2003). Chronic occult diarrhoea associated with laxative abuse favours ammonium urate calculi in anorectic patients with protein deficiency and reduced phosphaturia (Dick et al., 1990).

Altogether, all of these causes account for at best 1–2% of UANL, which in the vast majority of cases present without a specific cause.

Primary, idiopathic uric acid nephrolithiasis

Idiopathic UANL is by far the commonest form of UANL encountered in clinical practice

Epidemiology

Currently, UA is the main component of about 10% of urinary calculi in men and 5% in women in Western countries (Daudon et al., 2004) with an even higher prevalence in some middle-East populations such as in Lebanon or southern Israel and in Asian populations, suggesting an influence of genetic factors.

In most countries, UA stones are twice as frequent in men than in women at any age, and their prevalence markedly increases with age (Gentle et al., 1997; Daudon et al., 2004). In our series, nearly 35% of incident stones in men and 20% in women aged ≥ 70 years were UA stones. UANL is often observed in association with gout (Gutman and Yü, 1968). Helical tomodensitometry recently showed 27–39% of gouty patients having UA stones (Alvarez-Nemegyei et al., 2005; Shimizu and Hori, 2009). However, in an increasing number of cases, UANL now presents without a personal or familial history of gout, but rather is associated with obesity, type 2 diabetes, or features of the metabolic syndrome, in parallel with the

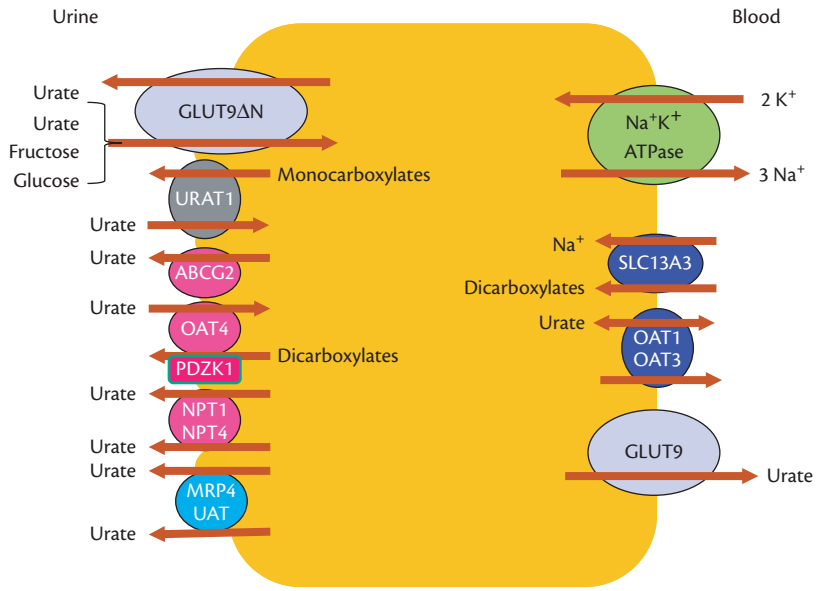


Fig. 202.1 Kidney transport of urate.

relentless increasing prevalence of obesity, metabolic syndrome, and type 2 diabetes in Western countries.

Pathophysiology of idiopathic UANL

Overly acidic urine, with urine pH < 5.5 permanently over night and day is the determinant factor of idiopathic UANL (Pak et al., 2001; Cameron et al., 2007), consistently found in all UA stone formers, although subjects with similarly low urine pH may remain without UA stone formation. Abundant consumption of animal proteins with their acid load contributes to lowering urine pH, and overindulgence with purine-rich foods increases uricosuria. However, both of these factors are dispensable, because the acidifying effect of animal protein load is weak and urine always contains a sufficient amount of UA to crystallize in acidic urine (Sakhaee et al., 2002). Thus, abnormally low urine pH remains as the major factor of UA formation, as known for a long time, although its mechanism has only recently been identified.

Mechanism of urine acidity

Recent studies have evidenced a relationship between urine pH, obesity, and type 2 diabetes. Urinary pH decreases with higher body weight (Maalouf et al., 2004; Taylor and Curhan, 2006), as well as with the number of features of the metabolic syndrome (Maalouf et al., 2007) and with the presence of type 2 diabetes (Cameron et al., 2006; Daudon et al., 2006b), all conditions characterized by insulin resistance (Eckel et al., 2005).

Defective ammoniagenesis was evidenced 50 years ago as the mechanism of low urine pH in patients with UANL (Henneman et al., 1962) and is also present in older subjects (Agarwal and Cabebe, 1980). Reduced ammoniagenesis was demonstrated in patients with type 2 diabetes (Sakhaee et al., 2002; Cameron et al., 2006) or the metabolic syndrome (Abate et al., 2004), thus suggesting a pathogenic role of insulin resistance. Indeed, physiologically insulin stimulates renal ammoniagenesis in proximal tubule cells (Chobanian and Hammerman, 1987) whereas insulin resistance at the kidney level abrogates this effect (Xu et al., 2003). Moreover, obesity results in the ectopic accumulation of saturated fatty acids in proximal tubule cells, inducing a cellular lipotoxicity which abolishes the stimulation of the Na-H+ exchanger NHE3, thus enhancing sodium and UA reabsorption (Bobulescu et al., 2008), contributing to hypertension and hyperuricaemia, both features of the metabolic syndrome (Reaven, 2002). In addition, high fructose consumption, a factor of obesity, induces hepatic UA production and insulin resistance (Johnson et al., 2010). In the elderly, age-related mitochondrial dysfunction induces insulin resistance (Petersen et al., 2003), a fact in keeping with the increased prevalence of UANL in older subjects irrespective of the body weight.

Mechanisms involved in the formation of UA stones are summarized in Fig. 202.2.

Clinical manifestations and diagnosis

Patients with UANL typically produce red-orange stones, often in the form of coloured sands or gravels. UANL may also manifest as isolated microscopic haematuria with presence of characteristic UA crystals at sediment examination. UA stones, sometimes bilateral, may silently develop to form large, obstructive calculi, even staghorn stones, which may be revealed by sudden anuria.

Pure UA calculi are totally radiolucent but detectable by echography and easily identified as UA by non-contrast, helical CT, which also differentiates UA stones from pyelic urothelial tumours (Bellin et al., 2004). The association of radiolucent stones and acidic urine is suggestive of UANL. However, diagnosis should be confirmed by means of infrared spectroscopy (or X-ray diffraction) in order to exclude other radiolucent stones, especially 2,8-dihydroxyadenine stones which, at variance with UA stones, are insoluble whatever the pH and require allopurinol therapy to prevent progressive alteration of renal function (Bollée et al., 2010).

Management of the patient with uric acid nephrolithiasis

UA solubility markedly increases at urine pH > 6, with about 1 g/L (6 mmol/L) AU solved at pH 7. Thus alkalinization is the

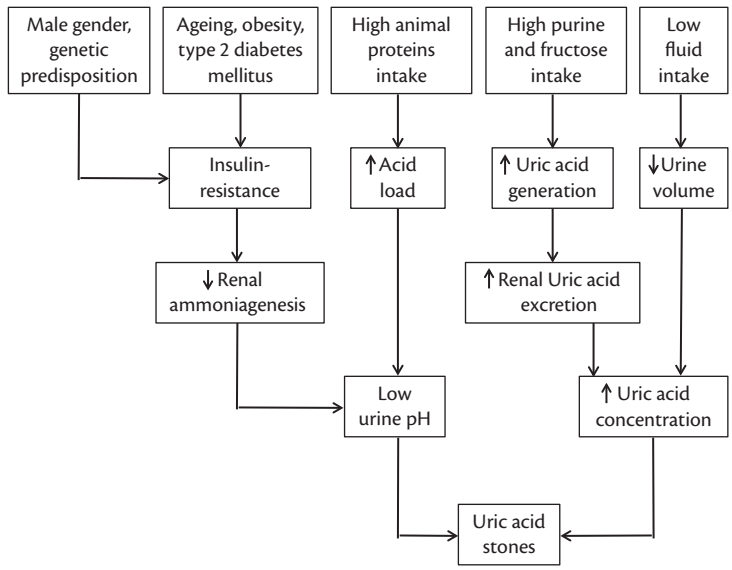


Fig. 202.2 Mechanisms involved in uric acid stone formation.

cornerstone of medical therapy, allowing dissolution of existing stones and prevention of stone recurrence (Preminger, 1987).

Treatment of existing stones

Medical dissolution of UA stones may be obtained in the majority of patients with non-obstructing calculi by increasing urine pH up to 6.5–7. Active alkalinization is achieved by oral administration of potassium citrate (60–80 mEq/day in divided doses) or more rapidly by means of an intravenous infusion of sodium bicarbonate (Preminger, 1987). Urological intervention is infrequently needed and reserved for patients with complete obstruction, especially if bilateral calculi, anuria, or insufficient response to alkalinization. Urine diversion will use pyelostomy or ureteric catheterization. UA stones are easily fragmented by extracorporeal shock wave lithotripsy or removed by ureteroscopy.

Preventive medical therapy

Prevention of UA stone recurrence aims at rising urine dilution, decreasing UA excretion, and increasing UA solubility through urine alkalinization which is the most important component of the treatment. For the purpose of chronic prevention, moderate alkalinization maintaining urinary pH at about 6.5 is usually sufficient. Potassium citrate (40–60 mEq/day in divided doses) is the preferred agent because potassium urate is twice as soluble as sodium urate (Pak et al., 1986). In patients who poorly tolerate potassium citrate or have contraindications to potassium salts, sodium bicarbonate is an acceptable alternative.

High fluid intake should increase urine volume at 2–2.5 L/day. Mineral waters rich in bicarbonate, as well as orange juice (Odvina, 2006) increase both urine volume and pH. Consumption of purine-rich foods, including beer, animal proteins, and alcoholic beverages should be reduced (Siener and Hesse, 2003; Choi et al., 2004a, 2004b). Beverages ('soft drinks') and foods rich in fructose should be avoided (Johnson et al., 2007). Allopurinol or febuxostat should be given only in cases of persistent hyperuricosuria or hyperuricaemia (Coe et al., 2005).

Nowadays, management of the patient with UANL should not be limited to the treatment of UA stones but also take into account the recently highlighted relationship between UA stone disease and insulin resistance. Accordingly, patients diagnosed with UANL should be evaluated for components of the metabolic syndrome (Maalouf, 2011) and treated appropriately in order to prevent the development of type 2 diabetes and atherosclerosis, whose consequences are much more deleterious than those of UANL itself.

References

Abate, N., Chandalia, M., Cabo-Chan, A.V., Jr., *et al.* (2004). The metabolic syndrome and uric acid nephrolithiasis: novel features of renal manifestation of insulin resistance. *Kidney Int*, 65, 386–92.

Agarwal, B. N. and Cabebe, F. G. (1980). Renal acidification in elderly subjects. *Nephron*, 26, 291–5.

Alvarez-Nemegyei, J., Medina-Escobedo, M., Villanueva-Jorge, S., *et al.* (2005). Prevalence and risk factors for urolithiasis in primary gout: is a reappraisal needed? *J Rheumatol*, 32, 2189–91.

Bellin, M. F., Renard-Penna, R., Conort, P., *et al.* (2004). Helical CT evaluation of the chemical composition of urinary tract calculi with a discriminant analysis of CT-attenuation values and density. *Eur Radiol*, 14, 2134–40.

Bobulescu, I. A., Dubree, M., Zhang, J., *et al.* 2008). Effect of renal lipid accumulation on proximal tubule Na+/H+ exchange and ammonium secretion. *Am J Physiol Renal Physiol*, 294, F1315–22.

Bollée, G., Dollinger, C., Boutaud, L., *et al.* (2010). Phenotype and genotype characterization of adenine phosphoribosyltransferase deficiency. *J Am Soc Nephrol*, 21, 679–88.

Cameron, M. A., Baker, L. A., Maalouf, N. M., *et al.* (2007). Circadian variation in urine pH and uric acid nephrolithiasis risk. *Nephrol Dial Transplant*, 22, 2375–8.

Cameron, M. A., Maalouf, N. M., Adams-Huet, B., *et al.* (2006). Urine composition in type 2 diabetes: predisposition to uric acid nephrolithiasis. *J Am Soc Nephrol*, 17, 1422–8.

Chobanian, M. C. and Hammerman, M. R. (1987). Insulin stimulates ammoniagenesis in canine renal proximal tubular segments. *Am J Physiol*, 253, F1171–7.

Choi, H. K., Atkinson, K., Karlson, E. W., *et al.* (2004a). Purine-rich foods, dairy and protein intake, and the risk of gout in men. *N Engl J Med*, 350, 1093–103.

Choi, H. K., Atkinson, K., Karlson, E. W., *et al.* (2004b). Alcohol intake and risk of incident gout in men. *Lancet*, 363, 1277–81.

Coe, F. L., Evan, A., and Worcester, E. (2005). Kidney stone disease. *J Clin Invest*, 115, 2598–608.

Daudon, M., Dore, J. C., Jungers, P., *et al.* (2004). Changes in stone composition according to age and gender of patients: a multivariate epidemiological approach. *Urol Res*, 32, 241–7.

Daudon, M., Lacour, B., and Jungers, P. (2006a). Influence of body size on urinary stone composition in men and women. *Urol Res*, 34, 193–9.

Daudon, M., Traxer, O., Conort, P., *et al.* (2006b). Type 2 diabetes increases the risk for uric acid stones. *J Am Soc Nephrol*, 17, 2026–33.

Davidson, M. B., Thakkar, S., Hix, J. K., *et al.* (2004). Pathophysiology, clinical consequences and treatment of tumor lysis syndrome. *Am J Med*, 116, 546–54.

Dick, W. H., Lingeman, J. E., Preminger, G. M., *et al.* (1990). Laxative abuse as a cause for ammonium urate renal calculi. *J Urol*, 143, 244–7.

Dinour, D., Gray, N. K., Campbell, S., *et al.* (2010). Homozygous SLC2A9 mutations cause severe renal hypouricemia. *J Am Soc Nephrol*, 21, 64–72.

Eckel, R. H., Grundy, S. M., and Zimmet, P. Z. (2005). The metabolic syndrome. *Lancet*, 365, 1415–28.

Ekeruo, W. O., Tan, Y. H., Young, M. D., *et al.* (2004). Metabolic risk factors and the impact of medical therapy on the management of nephrolithiasis in obese patients. *J Urol*, 172, 159–63.

Enomoto, A., Kimura, H., Chairoungdua, A., *et al.* (2002). Molecular identification of a renal urate anion exchanger that regulates blood urate levels. *Nature*, 417, 447–52.

Gentle, D. L., Stoller, M. L., Bruce, J. E., *et al.* (1997). Geriatric urolithiasis. *J Urol*, 158, 2221–4.

Gutman, A. B. and Yü, T. F. (1968). Uric acid nephrolithiasis. *Am J Med*, 45, 756–79.

Henneman, P. H., Wallach, S., and Dempsey, E. F. (1962). The metabolism defect responsible for uric acid stone formation. *J Clin Invest*, 41, 537–42.

Hesse, A., Brändle, E., Wilbert, D., *et al.* (2003). Study on the prevalence and incidence of urolithiasis in Germany comparing the years 1979 vs. 2000. *Eur Urol*, 44, 709–13.

Howard, S. C., Jones, D. P. and Pui, C. H. (2011). The tumor lysis syndrome. *N Engl J Med*, 364, 1844–54.

Iwai, N., Mino, Y., Hosoyamada, M., Tago, N., *et al.* (2004). A high prevalence of renal hypouricemia caused by inactive SLC22A12 in Japanese. *Kidney Int*, 66, 935–44.

Jinnah, H. A. and Friedman, T. (2001). Lesch-Nyhan disease and its variants. In C. R. Scriver, A. L. Beaudet, W. S. Sly, *et al.* (eds.) *The Metabolic and Molecular Bases of Inherited Disease* (8th ed.), pp. 2537–70. New York: McGraw-Hill.

Johnson, R. J., Sanchez-Lozada, L. G., and Nakagawa, T. (2010). The effect of fructose on renal biology and disease. *J Am Soc Nephrol*, 21, 2036–9.

Johnson, R. J., Segal, M. S., Sautin, Y., *et al.* (2007). Potential role of sugar (fructose) in the epidemic of hypertension, obesity and the metabolic syndrome, diabetes, kidney disease, and cardiovascular disease. *Am J Clin Nutr*, 86, 899–906.

Lieske, J. C., de la Vega, L. S., Gettman, M. T., *et al.* (2006). Diabetes mellitus and the risk of urinary tract stones: a population-based case-control study. *Am J Kidney Dis*, 48, 897–904.
Maalouf, N. M. (2011). Metabolic syndrome and the genesis of uric acid stones. *J Ren Nutr*, 21, 128–31.
Maalouf, N. M., Cameron, M. A., Moe, O. W., *et al.* (2007). Low urine pH: a novel feature of the metabolic syndrome. *Clin J Am Soc Nephrol*, 2, 883–8.
Maalouf, N. M., Sakhaee, K., Parks, J. H., *et al.* (2004). Association of urinary pH with body weight in nephrolithiasis. *Kidney Int*, 65, 1422–5.
Nyhan, W. L. (2005). Disorders of purine and pyrimidine metabolism. *Mol Genet Metab*, 86, 25–33.
Odvina, C. V. (2006). Comparative value of orange juice versus lemonade in reducing stone-forming risk. *Clin J Am Soc Nephrol*, 1, 1269–74
Ohta, T., Sakano, T., Igarashi, T., *et al.* (2004). Exercise-induced acute renal failure associated with renal hypouricaemia: results of a questionnaire-based survey in Japan. *Nephrol Dial Transplant*, 19, 1447–53.
Pak, C. Y., Sakhaee, K., and Fuller, C. (1986). Successful management of uric acid nephrolithiasis with potassium citrate. *Kidney Int*, 30, 422–8.
Pak, C. Y., Sakhaee, K., Moe, O., *et al.* (2003). Biochemical profile of stone-forming patients with diabetes mellitus. *Urology*, 61, 523–7.
Pak, C. Y., Sakhaee, K., Peterson, R. D., *et al.* (2001). Biochemical profile of idiopathic uric acid nephrolithiasis. *Kidney Int*, 60, 757–61.
Parks, J. H., Worcester E. M., O'Connor, R. C., *et al.* (2003). Urine stone risk factors in nephrolithiasis patients with and without bowel disease. *Kidney Int*, 63, 255–65.
Petersen, K. F., Befroy, D., Dufour, S., *et al.* (2003). Mitochondrial dysfunction in the elderly: possible role in insulin resistance. *Science*, 300, 1140–2.
Preminger, G. M. (1987). Pharmacologic treatment of uric acid calculi. *Urol Clin North Am*, 14, 335–8.
Reaven, G. (2002). Metabolic syndrome: pathophysiology and implications for management of cardiovascular disease. *Circulation*, 106, 286–8.
Sakhaee, K. (2009). Recent advances in the pathophysiology of nephrolithiasis. *Kidney Int*, 75, 585–95.
Sakhaee, K., Adams-Huet, B., Moe, O. W., *et al.* (2002). Pathophysiologic basis for normouricosuric uric acid nephrolithiasis. *Kidney Int*, 62, 971–9.
Shimizu, T. and Hori, H. (2009). The prevalence of nephrolithiasis in patients with primary gout: a cross-sectional study using helical computed tomography. *J Rheumatol*, 36, 1958–62.
Siener, R. and Hesse, A. (2003). The effect of a vegetarian and different omnivorous diets on urinary risk factors for uric acid stone formation. *Eur J Nutr*, 42, 332–7.
Taylor, E. N. and Curhan, G. C. (2006). Body size and 24-hour urine composition. *Am J Kidney Dis*, 48, 905–15.
Wright, A. F., Rudan, I., Hastie, N. D., *et al.* (2010). A 'complexity' of urate transporters. *Kidney Int*, 78, 446–52.
Xu, H., Barnes, G. T., Yang, Q., *et al.* (2003). Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest*, 112, 1821–30.
Zilberman, D. E., Yong, D., and Albala, D. M. (2010). The impact of societal changes on patterns of urolithiasis. *Curr Opin Urol*, 20, 148–53.

CHAPTER 203

Cystine stones

Michel Daudon and Paul Jungers

Introduction

Historically, cystinuria was among the first inborn errors of metabolism described by Archibald Garrod a century ago. In fact, cystinuria is not an enzymatic disorder, but is caused by a genetic, autosomal recessive defect in the reabsorption of cystine and dibasic amino acids in the proximal tubule, resulting in increased urinary excretion of these amino acids and crystallization of the poorly soluble cystine, thus inducing stone formation. Indeed, cystine solubility is at best 250 mg/L (~1 mmol/L) up to pH 7, whereas its excretion reaches 600–1400 mg/day in homozygotes (Palacin et al., 2001).

Incidence of homozygotes is estimated at 1 in 7000 births worldwide, less in European countries and much higher (up to 1/2500 births) in countries with a high rate of consanguinity. Cystine stones represent 1–2% of all stones in adults and 5–8% in paediatric patients, with an equal distribution between males and females (Milliner and Murphy, 1993).

Classification of cystinuria

Biochemical classification

Based on urine excretion of cystine and dibasic amino acids in parents (obligatory heterozygotes) of affected homozygous patients and response to an oral cystine load, three phenotypes were initially described (Rosenberg et al., 1966). In all three types, homozygotes excrete ≥ 600 mg cystine per day and most become lithiasic. Heterozygotes in type I have normal aminoaciduria and do not form stones, whereas those in types II and III have variably elevated cystine excretion and some become lithiasic.

This biochemical classification was poorly correlated with the clinical phenotype, as heterozygotes bearing the same mutation had variable aminoaciduria. Therefore, a classification into types I and non-I (the latter including former types II and III) was subsequently introduced (Feliubadalo et al., 1999).

Genetic classification

However, because a coherent correlation between phenotype and genotype was still lacking, a new classification based on identification of mutations of the genes coding the proteins involved in the tubular transport of cystine and dibasic amino acids was proposed and is now universally accepted (Dello Strologo et al., 2002, Font-Llitjos et al., 2005; Chillaron et al., 2010):

In type A, homozygotes bear mutations of the two alleles of *SLC3A1*; heterozygotes have normal cystine excretion;

In type B, homozygotes bear mutations of the two alleles of *SLC7A9*; heterozygotes have normal or augmented cystinuria.

In the rare type AB, patients bear mutations in one allele of each gene; cystine excretion is 30% lower than in homozygotes of type A or B or mixed type (AAB or BBA).

Murine models of cystinuria reproduce features of human cystinuria A and B phenotypes (Chillaron et al., 2010).

Pathophysiology of cystine nephrolithiasis

Molecular mechanism of cystinuria

Reabsorption of cystine and dibasic amino acids in epithelial cells of the proximal tubule involves a complex heterodimeric transport system ($b^{0,+}$) composed of the heavy subunit rBAT, coded by the *SLC3A1* gene (located at 2p16.3), and the light catalytic subunit $b^{0,+}$AT coded by the *SLC7A9* gene (located at 19q13.1). Up to now, > 130 mutations in *SLC3A1* and nearly 100 in *SLC7A9* genes have been identified in cystinuric patients, some of these mutations being characteristic for given populations (Chillaron et al., 2010; Livrozet et al., 2014).

Normally, cystine and dibasic amino acids are taken from the tubular lumen by the apical transport system $b^{0,+}$ in exchange for neutral amino acids entering the tubular cells by a basolateral sodium-dependent transporter. Cystine is then intracellularly split into two cysteine moieties that are transferred into peritubular capillaries (Bouzidi and Daudon, 2007) (Fig. 203.1).

Patients harbouring both recessive deletions of *SLC3A1* and contiguous genes such as *PREPL* display the hypotonia-cystinuria syndrome or related syndromes, characterized by neonatal hypotonia, growth and mental retardation, and facial dysmorphy in addition to cystine nephrolithiasis (Martens et al., 2008).

Inactivating mutations of *SLC3A1* and *SLC7A9* result in defective tubular reabsorption of cystine. Although type A and B cystinuric patients differ with respect to their genetic background, their clinical phenotype is similar (Dello Strologo et al., 2002). Therefore, identification of the causal mutation, needed in genetic counselling and epidemiological studies, has no impact on therapeutic decision, which chiefly depends on the individual amount of daily cystine excretion.

Of note, the concomitant defect in intestinal absorption of cystine and dibasic amino acids has no nutritional consequence, because they can be absorbed as dipeptides through the apical transporter PEPT1 (Daniel, 2004).

Mechanism of cystine stone formation

Stone formation in cystinuric patients is the consequence of the excessive concentration of cystine in urine, which results in supersaturation leading to precipitation of cystine crystals which aggregate to form calculi. In addition, cystine crystals in collecting ducts

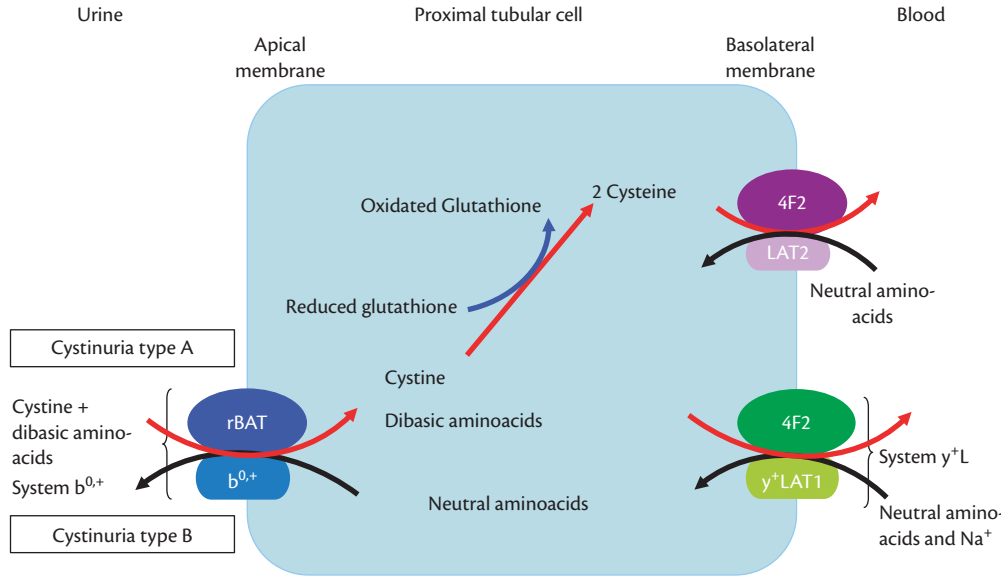


Fig. 203.1 Proximal tubular cellular handling of cystine and cysteine.

induce tubular atrophy and interstitial inflammatory fibrosis (Evan et al., 2006).

Clinical expression and evolution of cystine nephrolithiasis

Cystine stones may reveal in the first years of life, but do so predominantly in the second decade, and sometimes much later in life (Knoll et al., 2005; Haymann, 2015). In a recent nationwide inquiry including 400 French cystine stone formers, the first stone episode took place at a median age of nearly 20 years in both genders, with a peak of incidence between 15 and 25 years (Daudon and Conort, 2002).

Cystine calculi are most often bilateral and multiple, and may reach a staghorn-like development. They are highly recurrent with a mean activity index several times higher than in idiopathic calcium nephrolithiasis. As a consequence, cystine nephrolithiasis requires more urological interventions than in other forms of nephrolithiasis (Worcester et al., 2006). Multiple obstructive episodes and repeated urological procedures (especially open surgery) may lead to kidney atrophy, nephrectomy, and progressive reduction of renal function (Assimos et al., 2002; Prot-Bertoye et al., 2015) eventually progressing to end-stage renal failure (Jungers et al., 2004). In the French series of 400 cystinuric stone formers, 25% of patients had a non-functioning kidney (atrophy or previous nephrectomy) at presentation and five reached end-stage kidney disease (Daudon and Conort, 2002). Of note, because the genetic defect is limited to the kidneys, no recurrence of the disease occurs after kidney transplantation.

Diagnosis

Imaging of cystine stones

Cystine calculi, which contain sulphur, are weakly radio-opaque on X-ray and appear as faint, round opacities with a smooth contour, but small calculi often escape detection. Echography allows better detection and is widely used in clinical practice for repeat imaging as a non-irradiating method (Rogers et al., 2007). However, small calculi (< 3–5 mm) often escape detection while falsely positive images are not infrequent. Therefore, non-contrast, helical computed tomography is the reference method for identification of cystine stones of any size and in addition provides information on their structure and predicted response to shock waves (Kim et al., 2007).

Laboratory diagnosis

Stone analysis using infrared spectroscopy (or X-ray diffraction), by identifying cystine as the main component, constitutes the fastest and easiest way for accurate diagnosis. Following generalized morphoconstitutional analysis of urinary calculi (Daudon et al., 1993), the delay in diagnosis from first stone episode was shortened from 15 years before 1960 to < 3 months in the recent decade in our series. If no stone or fragment is available for analysis, microscopic examination of urine is contributive to diagnosis as in untreated patients urine often contains cystine crystals with their characteristic hexagonal, large size (20–70 µm), lamellar morphology (Bouzidi and Daudon, 2007).

The cyanide-nitroprusside test, formerly used as a screening test, lacks both sensitivity and specificity, and therefore is of limited interest.

Chromatography of urinary amino acids provides the formal diagnosis of cystinuria in showing the specific increase of cystine, ornithine, lysine, and arginine, and permits quantification of cystine excretion.

A search for genetic mutations is important for genetic counselling and detection of heterozygotes at risk of transmitting the disease and to develop stones. However, because homozygotes in types A and B cystinuria excrete the same amount of cystine and because cystine excretion is variable between patients harbouring the same mutation, genetic testing has no direct relevance for the therapeutic decision which mainly relies on the rate of cystine excretion.

Chromatography of urinary amino acids in children from an affected homozygous parent allows identification of infants with

increased cystine excretion who need prophylactic measures. Of note, diagnosis of cystinuria in infants may be falsely positive, due to transient neonatal cystinuria reflecting immaturity of the transporter SLC3A1 in the first months of life (Boutros et al., 2005). Therefore, when an abnormally high cystine excretion has been found at early determination, measurement should be repeated at age 18–24 months.

Urological treatment of cystine calculi

Because cystine nephrolithiasis is highly recurrent, urological intervention is often required. Fortunately, urological management of cystine stones was considerably improved with the development of extracorporeal shock wave lithotripsy (ESWL) and percutaneous nephrolithotomy (PCNL), and, more recently, flexible ureteroscopy, much less invasive techniques than the formerly used open surgery (Traxer et al., 2008; Tiselius, 2010). Although cystine stones are often resistant to shock waves, ESWL still is used as first-line treatment for renal calculi < 15 mm in diameter or for ureteral calculi. ESWL is especially efficient in children and infants whose high ureteral compliance allows expulsion of large fragments (Joly et al., 1999; Knoll et al., 2005). 'Rough' cystine calculi, with a heterogeneous internal structure, are more easily fragmented than 'smooth' ones (Bhatta et al., 1989; Kim et al., 2007). ESWL is successful in about 30% of renal calculi, and in > 80% of ureteral calculi which can receive more shock waves because they are not surrounded by renal parenchyma (Chow and Streem, 1998).

When no effective fragmentation is obtained at the first ESWL session, most urologists now recommend abandoning ESWL and turning to PCNL (for large renal stones), semi-rigid ureteroscopy (for lower or mid ureter calculi), or flexible ureterorenoscopy with Holmium laser (for upper ureteral, pyelic, or calyceal stones < 15–20 mm) (Rudnick et al., 1999; Traxer et al., 2008, Tiselius, 2010), this technique being usable in infants and young children (Schuster et al., 2002; Kim et al., 2008).

However, although urological treatment of cystine calculi is now much easier and with minimally invasive modern techniques (Trinchieri et al., 2007), prevention of recurrent stone formation by medical therapy should never be neglected in view of the high tendency of cystine nephrolithiasis to recur, inasmuch as stone recurrence rate and need for urological intervention is considerably reduced in patients compliant with medical treatment (Barbey et al., 2000; Pareek et al., 2005; Dello Strologo et al., 2007).

Medical preventive treatment

There is currently no aetiologic treatment able to correct the defect in renal cystine transport. Prevention of cystine formation and growth is based on measures aimed at reducing excretion, lowering urinary concentration, and increasing the solubility of cystine in urine.

Principles of medical treatment

Medical treatment uses a combination of several measures, both dietary and pharmacological (Table 203.1).

Limited intake of methionine and sodium

Restricting the dietary intake of animal proteins, most of which contain 500–600 mg of methionine per 100 g reduces the production of cystine by 0.5–1 mmol/day (Rodman et al., 1984). This may be achieved in adult patients by limiting intake of meat, fish, or poultry to about 150 g/day and to avoid foods very rich in methionine such as horse meat, eggs, and parmesan or gruyere cheese. No restriction of protein intake should be proposed for children (Knoll et al., 2005). However, methionine-rich foods should be avoided.

Table 203.1 Practical measures for medical treatment of cystine nephrolithiasis

Reducing cystine formation and excretion	Limit dietary methionine by restricting intake of meat, fish or poultry to about 150 g/day; avoid horse meat, eggs, parmesan and gruyere cheese, stock fish, and sardines in oil Reduce dietary sodium: sodium intake < 150 mmol/day
Increasing cystine solubility	Maximally dilute urine: increase oral fluid intake in order to achieve urine volume > 3 L/24 hours, well distributed over day and night, with large fluid intake at bedtime Alkalinize urine: potassium citrate (40–80 mEq/day) or sodium bicarbonate (8–12 g/day) diluted in 3 L water, with reinforced alkali intake at bedtime
Converting cystine into more soluble cysteine	Oral administration of D-penicillamine (600–1200 mg/day) or tiopronin (500–1000 mg/day) in divided doses, with half of daily dose taken at bedtime

Reducing sodium intake also contributes to lower cystine excretion (Jaeger et al., 1986). Strict sodium restriction markedly reduced cystinuria in some children (Rodriguez et al., 1995) but may be poorly tolerated.

Urine dilution

Reducing the concentration of cystine in urine is of primary importance. To achieve efficient dilution, daily volume should be increased above 3 L/day (Barbey et al., 2000) by increasing oral fluid intake accordingly at ≥ 3 L/24 hours (≥ 2 L/1.73 m^2 in children). Fluids should be well distributed throughout the day, with abundant fluid intake at bedtime and upon awakening in the night to avoid formation of crystals during the period of higher urine concentration (Chow and Streem, 1996; Joly et al., 1999; Monnens et al., 2000; Fjellstedt et al., 2001a).

Urine alkalinization

Because cystine solubility is only 200–250 mg/L at urine pH up to 7, adequate dilution in a patient with a daily cystine excretion of 1200 mg/day should be at least 5 L of water per day, which is not practicable. Therefore urine alkalinization should be associated, because cystine solubility rises to ≥ 500 mg/L (~ 2mmol/L) at urine pH ≥ 7.5 (Dent et al., 1965). Based on this finding, oral administration of alkali, in the form of potassium citrate or sodium bicarbonate, should increase urine pH to between 7.5–8, whilst avoiding exceeding pH 8 in order to prevent precipitation of calcium phosphate at the stone surface.

Potassium citrate (40–80 mEq/day in divided doses) is the preferred agent because, at variance with sodium bicarbonate, it does not increase natriuresis (Fjellstedt et al., 2001b). However, potassium salts may be contraindicated in patients with reduced renal function and potassium citrate often has poor gastric tolerance. In such cases, sodium bicarbonate (8–12 g/day, diluted in 3 L of water) is an acceptable alternative, because the beneficial effects of alkalinization outweigh the effects of sodium load on cystine excretion (Joly et al., 1999).

Acetazolamide taken at bedtime has been proposed as an adjunct to further increase urine pH during the night (Sterrett et al., 2008) but its prolonged use would induce chronic metabolic acidosis, so potassium citrate, with a reinforced dose at bedtime, appears preferable.

Thiol-containing agents

Thiol-containing agents (or sulphydryls) reduce the disulphide bonds that bridge the two cysteine moieties of the cystine molecule and form cysteine compounds 50 times more soluble than cystine itself (Lotz and Bartter, 1965). The most widely used are D-penicillamine and alpha-mercaptopropionyl glycine (or tiopronin). Because two molecules of sulfhydryl are needed to complex one molecule of cystine, with an efficacy < 100%, 500 mg of each can at best solubilize 200 mg cystine. Captopril, whose maximal authorized dose is 150 mg/day, is virtually ineffective (Dahlberg and Jones, 1989).

Both drugs induce adverse effects, especially when large doses are used, such as dysgeusia, mucocutaneous lesions, proteinuria, membranous nephropathy with nephritic syndrome, neutropenia and thrombocytopenia, or other immunoallergic manifestations requiring drug withdrawal. Incidence of adverse effects is comparable for both agents, but is perhaps slightly lower with tiopronin (Pak et al., 1986). Gradual titration and avoidance of large doses usually prevents such undesirable side effects (Joly et al., 1999).

New therapeutic perspectives

Pharmacologic chaperones, already used in diseases involving misfolded or mistargeted proteins (Amaral, 2006) are a promising approach but none is currently available for cystinuria. Structural analogues of cystine were shown *in vitro* to reduce growth and aggregation of cystine crystals by binding at their surface (Rimer et al., 2010). Extracts of plants used in traditional medicine were revealed to be able to solubilize cystine stones *in vitro* (Meiouet et al., 2011) but evidence of efficacy in humans is still to be demonstrated.

Strategy of medical therapy

Prevention of cystine stone formation is a life-long treatment, and as such has to be acceptable in the long term and devoid of deleterious adverse effects. Basic treatment relies on diet, hyperdiuresis, and urine alkalinization, and is sufficient in most patients with moderately severe cystinuria. Thiol derivatives should not be used as first-line treatment but added to basic measures in patients with more severe cystinuria when basic measures fail to prevent stone recurrence (Joly et al., 1999; Moe et al., 2011).

Clinical and laboratory monitoring

Clinical surveillance involves frequent measures of urinary specific gravity and pH, especially on morning urine. In patients on thiol derivatives, liver enzymes, proteinuria, and blood count should be regularly monitored, and vitamin B_6 supplementation (50 mg/day) should be given to patients treated with D-penicillamine.

Monitoring of cystine excretion has been proposed to adjust the dose of thiol derivatives. However, cystine concentration does not closely reflect the lithogenic power of urine and patients on thiol derivatives require free cystine measurements, which use complex, expensive, and not widely available methods (Goldfarb et al., 2006; Dello Strologo et al., 2007). We propose monitoring of crystalluria with determination of the crystal volume as an easier, cheaper, and more accurate method to directly assess the effect of therapy (Bouzidi and Daudon, 2007). Disappearance of crystals is unequivocal evidence of efficacy, whereas a crystal volume value > 3000 μ^3/mm^3 is indicative of the risk of recurrence (Daudon et al., 2003).

Importance of sustained compliance

The key to successful prevention of stone recurrence in cystinuric patients is regular compliance with the therapeutic programme (Tiselius, 2010) which has been proven effective in the long term (Barbey et al., 2000). Life-long compliance of the patient with cumbersome, complex measures is difficult to sustain (Pietrow et al., 2003) and may be obtained only in motivated patients adhering to the treatment. Proactive, individualized management with frequent visits and laboratory controls, up to two to four times per year in severe forms, is necessary for successful prevention of stone recurrence and protection of renal function.

References

Amaral, M. D. (2006). Therapy through chaperones: sense or antisense? Cystic fibrosis as a model disease. *J Inherit Metab Dis*, 29, 477–87.

Assimos, D. G., Leslie, S. W., Ng, C., *et al.* (2002). The impact of cystinuria on renal function. *J Urol*, 168, 27–30.

Barbey, F., Joly, D., Rieu, P., *et al.* (2000). Medical treatment of cystinuria: critical reappraisal of long-term results. *J Urol*, 163, 1419–23.

Bhatta, K. M., Prien, E. L., Jr., and Dretler, S. P. (1989). Cystine calculi—rough and smooth: a new clinical distinction. *J Urol*, 142, 937–40.

Boutros, M., Vicanek, C., Rozen, R., *et al.* (2005). Transient neonatal cystinuria. *Kidney Int*, 67, 443–8.

Bouzidi, H. and Daudon, M. (2007). Cystinuria: from diagnosis to follow-up. *Ann Biol Clin (Paris)*, 65, 473–81.

Chillaron, J., Font-Llitjos, M., Fort, J., *et al.* (2010). Pathophysiology and treatment of cystinuria. *Nat Rev Nephrol*, 6, 424–34.

Chow, G. K. and Streem, S. B. (1996). Medical treatment of cystinuria: results of contemporary clinical practice. *J Urol*, 156, 1576–8.

Chow, G. K. and Streem, S. B. (1998). Contemporary urological intervention for cystinuric patients: immediate and long-term impact and implications. *J Urol*, 160, 341–5.

Dahlberg, P. J. and Jones, J. D. (1989). Cystinuria: failure of captopril to reduce cystine excretion. *Arch Intern Med*, 149, 713–17.

Daniel, H. (2004). Molecular and integrative physiology of intestinal peptide transport. *Annu Rev Physiol*, 66, 361–84.

Daudon, M., Bader, C. A. and Jungers, P. (1993). Urinary calculi: review of classification methods and correlations with etiology. *Scanning Microsc*, 7, 1081–106.

Daudon, M., Cohen-Solal, F., Barbey, F., *et al.* (2003). Cystine crystal volume determination: a useful tool in the management of cystinuric patients. *Urol Res*, 31, 207–11.

Daudon, M. and Conort, P. (2002). Cystine urolithiasis: results of the national inquiry of the CLAFU on cystinuria. *Semin Urol Nephrol*, 28, 184–9.

Dello Strologo, L., Laurenzi, C., Legato, A., *et al.* (2007). Cystinuria in children and young adults: success of monitoring free-cystine urine levels. *Pediatr Nephrol*, 18, 69–73.

Dello Strologo, L., Pras, E., Pontesilli, C., *et al.* (2002). Comparison between SLC3A1 and SLC7A9 cystinuria patients and carriers: a need for a new classification. *J Am Soc Nephrol*, 13, 2547–53.

Dent, C. E., Friedman, M., Green, H., *et al.* (1965). Treatment of cystinuria. *Br Med J*, 1, 403–8.

Evan, A. P., Coe, F. L., Lingeman, J. E., *et al.* (2006). Renal crystal deposits and histopathology in patients with cystine stones. *Kidney Int*, 69, 2227–35.

Feliubadalo, L., Font, M., Purroy, J., *et al.* (1999). Non-type I cystinuria caused by mutations in SLC7A9, encoding a subunit ($b^{o,+}$AT) of rBAT. International Cystinuria Consortium. *Nat Genet*, 23, 52–7.

Fjellstedt, E., Denneberg, T., Jeppsson, J. O., *et al.* (2001a). Cystine analyses of separate day and night urine as a basis for the management of patients with homozygous cystinuria. *Urol Res*, 29, 303–10.

Fjellstedt, E., Denneberg, T, Jeppsson, J. O., *et al.* (2001b). A comparison of the effects of potassium citrate and sodium bicarbonate in the alkalinization of urine in homozygous cystinuria. *Urol Res*, 29, 295–302.

Font-Llitjos, M., Jimenez-Vidal, M., Bisceglia, L., *et al.* (2005). New insights into cystinuria: 40 new mutations, genotype-phenotype correlation, and digenic inheritance causing partial phenotype. *J Med Genet*, 42, 58–68.

Goldfarb, D. S., Coe, F. L., and Asplin, J. R. (2006). Urinary cystine excretion and capacity in patients with cystinuria. *Kidney Int*, 69, 1041–7.

Haymann, J.P. (2005). Metabolic disorders: stones as first manifestation of significant diseases. *World J Urol*, 33, 187–92.

Jaeger, P., Portmann, L., Saunders, A., *et al.* (1986). Anticystinuric effects of glutamine and of dietary sodium restriction. *N Engl J Med*, 315, 1120–3.

Joly, D., Rieu, P., Mejean, A., *et al.* (1999). Treatment of cystinuria. *Pediatr Nephrol*, 13, 945–50.

Jungers, P., Joly, D., Barbey, F., *et al.* (2004). ESRD caused by nephrolithiasis: prevalence, mechanisms, and prevention. *Am J Kidney Dis*, 44, 799–805.

Kim, S. C., Burns, E. K., Lingeman, J. E., *et al.* (2007). Cystine calculi: correlation of CT-visible structure, CT number, and stone morphology with fragmentation by shock wave lithotripsy. *Urol Res*, 35, 319–24.

Kim, S. S., Kolon, T. F., Canter, D., *et al.* (2008). Pediatric flexible ureteroscopic lithotripsy: the children's hospital of Philadelphia experience. *J Urol*, 180, 2616–19.

Knoll, T., Zollner, A., Wendt-Nordahl, G., *et al.* (2005). Cystinuria in childhood and adolescence: recommendations for diagnosis, treatment, and follow-up. *Pediatr Nephrol*, 20, 19–24.

Livrozet, M., Vandermeersch, S., Mesnard. L., *et al.* (2014). An animal model of type A cystinuria due to spontaneous mutation in 129S2/SvPasCrI mice. *Plos One*, 9 (7), e102700.

Lotz, M. and Bartter, F. C. (1965). Stone dissolution with D-penicillamine in cystinuria. *Br Med J*, 2, 1408–9.

Martens, K., Jaeken, J., Matthijs, G., *et al.* (2008). Multi-system disorder syndromes associated with cystinuria type I. *Curr Mol Med*, 8, 544–50.

Meiouet, F., El Kabbaj, S., and Daudon, M. (2011). In vitro study of litholytic activity of four medicinal plants against cystine stones. *Prog Urol*, 21, 40–7.

Milliner, D. S. and Murphy, M. E. (1993). Urolithiasis in pediatric patients. *Mayo Clinic Proc*, 68, 241–8.

Moe, O. W., Pearle, M. S., and Sakhaee, K. (2011). Pharmacotherapy of urolithiasis: evidence from clinical trials. *Kidney Int*, 79, 385–92.

Monnens, L. A., Noordam, K., and Trijbels, F. (2000). Necessary practical treatment of cystinuria at night. *Pediatr Nephrol*, 14, 1148–9.

Pak, C. Y., Fuller, C., Sakhaee, K., *et al.* (1986). Management of cystine nephrolithiasis with alpha- mercaptopropionylglycine. *J Urol*, 136, 1003–8.

Palacin, M., Goodyer, P., Nunes, V., *et al.* (2001). Cystinuria. In C. R. Scriver, A. Beaudet, W. S. Sly, *et al.* (ed). *The Metabolic and Molecular Bases of Inherited Disease* (8th ed.), pp. 4909–32. New York: McGraw-Hill.

Pareek, G., Steele, T. H. and Nakada, S. Y. (2005). Urological intervention in patients with cystinuria is decreased with medical compliance. *J Urol*, 174, 2250–2.

Pietrow, P. K., Auge, B. K., Weizer, A. Z., *et al.* (2003). Durability of the medical management of cystinuria. *J Urol*, 169, 68–70.

Prot-Bertoye, C., Lebbah, S., Daudon, M., *et al.* (2015). Chronic kidney disease and its determinants among cystinuric patients. *Clin J Am Soc Nephrol,* 10, 842–51.

Rimer, J. D., An, Z., Zhu, Z., *et al.* (2010). Crystal growth inhibitors for the prevention of L-cystine kidney stones through molecular design. *Science*, 330, 337–41.

Rodman, J. S., Blackburn, P., Williams, J. J., *et al.* (1984). The effect of dietary protein on cystine excretion in patients with cystinuria. *Clin Nephrol*, 22, 273–8.

Rodriguez, L. M., Santos, F., Malaga, S., *et al.* (1995). Effect of a low sodium diet on urinary elimination of cystine in cystinuric children. *Nephron*, 71, 416–18.

Rogers, A., Kalakish, S., Desai, R. A., *et al.* (2007). Management of cystinuria. *Urol Clin North Am*, 34, 347–62.

Rosenberg, L. E., Downing, S., Durant, J. L., *et al.* (1966). Cystinuria: biochemical evidence for three genetically distinct diseases. *J Clin Invest*, 45, 365–71.

Rudnick, D. M., Bennett, P. M., and Dretler, S. P. (1999). Retrograde renoscopic fragmentation of moderate-size (1.5–3.0-cm) renal cystine stones. *J Endourol*, 13, 483–5.

Schuster, T. G., Russell, K. Y., Bloom, D. A., *et al.* (2002). Ureteroscopy for the treatment of urolithiasis in children. *J Urol*, 167, 1813–16.

Sterrett, S. P., Penniston, K. L., Wolf, J. S., Jr., *et al.* (2008). Acetazolamide is an effective adjunct for urinary alkalinization in patients with uric acid and cystine stone formation recalcitrant to potassium citrate. *Urology*, 72, 278–82.

Tiselius, H. G. (2010). New horizons in the management of patients with cystinuria. *Curr Opin Urol*, 20, 169–73.

Traxer, O., Lechevallier, E. and Saussine, C. (2008). Cystinuria: diagnosis and management. *Prog Urol*, 18, 832–6.

Trinchieri, A., Montanari, E., Zanetti, G., *et al.* (2007). The impact of new technology in the treatment of cystine stones. *Urol Res*, 35, 129–32.

Worcester, E. M., Coe, F. L., Evan, A. P., *et al.* (2006). Reduced renal function and benefits of treatment in cystinuria vs other forms of nephrolithiasis. *Br J Urol Int*, 97, 1285–90.

CHAPTER 204

Cell biology of nephrocalcinosis/ nephrolithiasis

Benjamin A. Vervaet and Marc E. De Broe

Definitions

According to standard non-invasive visualization techniques, commonly based on X-ray radiology (i.e. plain radiographs and computed tomography (CT)) or ultrasonography, calcium nephrolithiasis is properly defined as 'the presentation of a macroscopic concrement of inorganic and organic material in the renal calyces and/or pelvis, either or not adhered to the papillae or pelvic urothelium'. On the same level of resolution, nephrocalcinosis refers to 'small, more electron dense or hyperechogenic lesions in the kidney', leading to definitions such as 'diffuse, fine, renal parenchymal calcification' or 'diffusely scattered foci of calcification in the kidneys' (Fig. 204.1). From a cell biological mechanistic point of view, however, these latter definitions lack sufficient detail as they fail to describe exactly where in the structural organization of the kidney the calcifications occur and what their chemical composition is.

Invasive microscopic studies in animals and humans, ranging in resolution from the visible light (0.2 μm) to the electron beam (0.2 nm) level, showed that the parenchymal mineral calcium deposits are particularly found/retained within the tubular lumen and/or residing in the interstitium (Khan and Hackett, 1980; Lieske et al., 1992; Markowitz et al., 2004; Verhulst et al., 2005; Vervaet et al., 2009) (Fig. 204.1). In addition, physicochemical analysis by infrared spectroscopy and μX-ray diffraction identified both calcium oxalate as well as calcium phosphate deposits at these sites, usually not co-localized (Markowitz et al., 2004; Evan et al., 2008b; Khan and Glenton, 2008; Vervaet et al., 2009). Importantly, in the last decade, both intratubular and interstitial calcifications have been identified as putative initial stages of calcium nephrolithiasis in an increasing number of calcium stone-forming conditions (Evan et al., 2003, 2005, 2007b, 2008a, 2008b, 2009). Although recent observations strongly suggest that *nephrocalcinosis* and *calcium nephrolithiasis* are to be considered two independent pathologies and that *nephrocalcinosis* may cause *calcium nephrolithiasis* only in particular conditions (Evan et al., 2003, 2005, 2007b, 2008b), for the sake of clarity, in this chapter all calcifications within the renal parenchyma (regardless of whether or not they may progress to nephrolithiasis) will be termed nephrocalcinosis. Distinction will be made between intratubular and interstitial nephrocalcinosis:

- *Intratubular nephrocalcinosis*: the histological observation of calcium oxalate and/or calcium phosphate deposits in the tubular lumen, lying adjacent to the tubular epithelial lining.
- *Interstitial nephrocalcinosis*: the histological observation of calcium oxalate and/or calcium phosphate deposits in the renal interstitium.

Pathologies presenting with nephrocalcinosis

The routine use of ultrasonography, X-ray, and CT scan revealed that nephrocalcinosis is associated with an amalgam of different disorders and conditions as illustrated in Table 204.1 (Alon, 1997; Sayer et al., 2004). Therefore, nephrocalcinosis, rather than presenting as an independent pathology, is to be considered a manifestation of a variety of underlying disorders. Determining which pathologies either present intratubular or interstitial nephrocalcinosis, or both, is not an easy task for several reasons. Early stages and mild forms of nephrocalcinosis tend to be asymptomatic and therefore are frequently left unnoticed by the patient as well as by the clinician. Furthermore, although renal biopsies would allow identification of the site of crystal deposition, they are not routinely obtained and, in contrast to necropsy tissue, represent only a small 'sample' of the kidney, suggesting that resident crystals can easily be missed with this procedure. On the other hand, when nephrocalcinosis can be observed histopathologically, precisely locating the crystal deposits may often be impossible due to tubular and interstitial deterioration, inflammation, and fibrosis. Also, from a technical point of view, Kummeling et al. demonstrated that standard preparation of biopsy material for histopathological evaluation, involving fixation, paraffin embedding, cutting, and staining of the tissue, can result in considerable loss of crystals from the renal samples (Kummeling et al., 2007). Despite these difficulties, the first section of Table 204.1 lists the available information concerning crystal localization and type related to particular pathologies.

Intratubular nephrocalcinosis: mechanism

Two key processes determine the development of intratubular nephrocalcinosis, *crystal formation* and *crystal retention*.

Crystal formation

Although crystals are regularly found in urine, it is important to note that the composition of the final urine might not always be representative of the original site of crystallization (Kok, 1996). Within normal physiological limits, *in vitro* crystallization experiments as

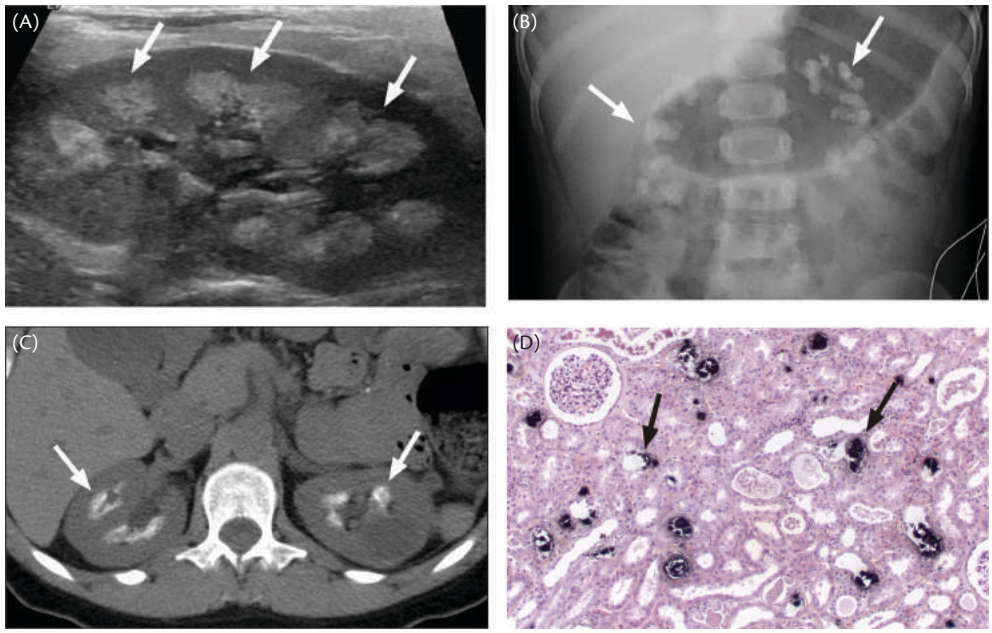


Fig. 204.1 Macroscopic visualization of medullary nephrocalcinosis by: (A) Ultrasound. Reproduced with permission from Fumagalli *et al. Arch Dis Child Fetal Neonatal Ed*, 2011 Sep; 96(5):F377. (B) Plain X-ray. Contributed to radiopaedia.org by Dr Hani Alsalam, King Abdulaziz Medical City—National Guard Hospital, Riyadh, Saudi Arabia (<http://radiopaedia.org/cases/renal-medullary-nephrocalcinosis>). (C) CT scan. Contributed to Imaging Science Today by Shweta Bhatt, University of Rochester Medical Center, Rochester, NY, USA (<http://www.dograrad.com/teaching-case/gu-radiology/20090703/medullary-nephrocalcinosis-273.html>). (D) Von Kossa stained renal section of a patient with primary hyperoxaluria. Arrows indicate renal crystal deposition. Tissue provided by C. Van Woerden.

well as mathematical models based on data from micropuncture and functional studies on isolated nephron segments, undoubtedly indicate that, in addition to final urine, the physicochemical properties of the tubular fluid at the nephron level can favour calcium salt crystal formation (Finlayson and Reid, 1978; Kok and Khan, 1994; Kok, 1996; Robertson, 2004). Driven by the requirement of body fluid, electrolytes, and acid–base homeostasis, the concerted actions of the different tubular epithelial cell types along the functional segments of the nephron drastically modulate ion composition, pH, osmolarity, and volume of the intratubular fluid before it is finally excreted (Fig. 204.2). These changing conditions may frequently challenge the solubility of the urinary calcium salts, calcium oxalate and calcium phosphate, and crystals may form in the tubular fluid as a result of supersaturation (Finlayson and Reid, 1978; Kok and Khan, 1994; Kok, 1996; Asplin et al., 1997; Robertson, 2004). In particular, the risk of calcium salt crystallization along the nephron is highest, where ion and water transport are uncoupled, that is, the loop of Henle and the collecting duct (Kok, 1996, 1997; Asplin et al., 1997; Tiselius, 1997) (Fig. 204.2). Critical determinants of the probability, extent, location, and type of crystals formed are the active concentrations of the respective ions and the pH of the tubular fluid (Asplin et al., 1997; Tiselius and Hojgaard, 1999; Coe et al., 2007; Parks et al., 2009; Renkema et al., 2009; Evan, 2010). Generally, calcium oxalate crystallization occurs under conditions of low pH, whereas calcium phosphate typically crystallizes under higher pH conditions. Importantly, the risk profile can extend upstream (in exceptional cases even up to the proximal tubule), and the level of crystallization can increase due to either insufficient water intake (Borghi et al., 1996) or increased filtered loads of crystal ions which are known to depend on diurnal variation (Carruthers et al., 1964; Ogawa, 1993a; Robert et al., 1994; Tiselius, 1997; Murayama et al., 2001), intake of diets rich in crystal constituents (Trohler et al., 1976; Massey et al., 1993), or the postprandial state (Hodgkinson and Heaton, 1965; Erickson et al., 1984). In addition, pathological conditions or drug (ab)use affecting proper tubular calcium, phosphate, oxalate, and acid–base handling at the molecular level may also greatly influence both the location and the probability of crystal formation along the nephron (Sayer et al., 2004; Unuma et al., 2009).

Crystal retention

Crystal retention refers to the process in which the kidney is unable to efficiently excrete urinary crystals, thereby leaving crystals behind in the tubular lumen and resulting in the histologic presentation of intratubular nephrocalcinosis. As illustrated in Table 204.1, crystals are deposited particularly in the more distal segments of the nephron, which is consistent with the risk of crystal formation along the nephron (Fig. 204.2). As crystal formation and excretion (crystalluria) are common, but harmless, in the healthy population (Robertson et al., 1969, 1977; Robertson and Peacock, 1972; Elliot et al., 1976; Elliot and Rabinowitz, 1980; Kok, 1996; Asplin et al., 1997; Ryall et al., 2000; Ryall, 2004), the question on how these crystals eventually are retained in the kidney has intrigued researchers for decades.

The 'free' and 'fixed' particle theory

Morphologically, retained intratubular crystals can either be found occluding the tubular lumen or, when their diameters are smaller than that of the tubular lumen, adhering to the tubular epithelium (Fig. 204.3). Although this difference was observed in the late nineteenth and early twentieth century (Henle, 1863; Lichtenstern, 1926; Huggins, 1933; Morgenroth et al., 1968; Oliver, 1968), mechanistic

Table 204.1 Several pathologies and conditions associated with nephrocalcinosis.

Pathology	Intratubular	Interstitial	Crystal type	Urinary supersaturation	Nephron segment	References
Transplant patients	Yes	(Yes)	CaP	CaOx/CaP	(Likely distal nephron)	Gwinner et al., 2005; Verhulst et al., 2005; Evenepoel et al., 2009; Vervaet et al., 2009;
Preterm infants	Yes	(Yes)	CaOx/CaP	CaOx/CaP	(Likely distal nephron)	Vervaet et al., 2009; Vervaet et al., 2009; 222
Primary hyperparathyroidism stone formers*	Yes	Yes	No CaOx/ CaP	CaOx/CaP	BD, IMCD, OMCD, CCD	Evan et al., 2008b; Coe et al., 2010a
Jejuno-ileal resection*	Yes	Yes	CaP	No CaP	BD, IMCD	Coe et al., 2010a
Jejuno-ileal bypass stone formers*	Yes	Yes	CaOx/CaP	CaOx/no CaP	BD, IMCD	Evan et al., 2008a; Coe et al., 2010a
Idiopathic calcium oxalate stone formers	No	Yes	No CaOx/ CaP	CaOx	BD, TLH	Evan et al., 2003
Brushite stone formers*	Yes	Yes	CaOx/CaP	CaOx/CaP	BD, IMCD, OMCD	Evan et al., 2005; Coe et al., 2010a
Acute phosphate nephropathy*	Yes	(Yes)	CaP	CaP	DCT, CD	Markowitz et al., 2004; Vervaet et al., 2009
Primary hyperoxaluria type 1 and 2*	Yes	(Yes)	CaOx	CaOx	Entire nephron	Lieske et al., 1992; Vervaet et al., 2009; Coe et al., 2010a
Ileostomy stone formers*	Yes	Yes	No CaOx/ CaP	CaOx/no CaP	BD, IMCD, TLH	Evan et al., 2009; Coe et al., 2010a
(Distal) Renal tubular acidosis*	Yes	No	CaOx/CaP	CaOx/CaP	BD, IMCD	Evan et al., 2007b; Coe et al., 2010a
Cystine stone formers*	Yes	Yes	Cystine/ CaP	Cystine/CaP	BD, IMCD, DCT, TLH	Evan et al., 2006; Coe et al., 2010a
Pseudo-Bartter syndrome	Yes	(Yes)			DTL	Unuma et al., 2009; Tojo et al., 2010
Normal population	No	Yes	CaP	Low CaOx/ no CaP	BD, LH	Low and Stoller , 1997

Other pathologies with limited specific information	
11β-hydroxysteroid dehydrogenase deficiency	Idiopathic hypercalciuria
Alkaptonuria	Liddle syndrome
Amelogenesis imperfect (MacGibbon–Lubinsky)	Lowe syndrome
Autosomal-dominant hypocalcaemia	Medullary sponge kidney
Bartter syndrome	Milk alkali syndrome
Bartter syndrome and sensorineural deafness	Multiple endocrine neoplasia type 1 and 2
Cystic fibrosis	Oxalosis
Dent disease	pseudohypoaldosteronism
Familial hypercholesterolaemia	Rapidly progressive osteoporosis
Glucocorticoid-suppressible aldosteronism	Sarcoidosis
Hypophosphatasia	Williams syndrome
Hypophosphataemia with nephrolithiasis	Wilson disease
Hypomagnesaemic hypercalciuric nephrocalcinosis	X-linked hypophosphataemia

Pathologies marked with an asterisk* can present plugging/occlusion of the IMCD and/or BD with crystal deposits.

(Yes) between brackets: interstitial nephrocalcinosis can be due to 'transepithelial uptake' (see 'Post-crystal-adhesion defence mechanisms').

The column 'Nephron segment' refers to the location where intratubular crystals can be observed.

BD, duct of Bellini; CaOx, calcium oxalate; CaP, calcium phosphate; CCD, cortical collecting duct; DTL, descending thin loop of Henle; IMCD, inner medullary collecting duct; LH, loop of Henle; OMCD, outer medullary collecting duct; TLH, thin loop of Henle.

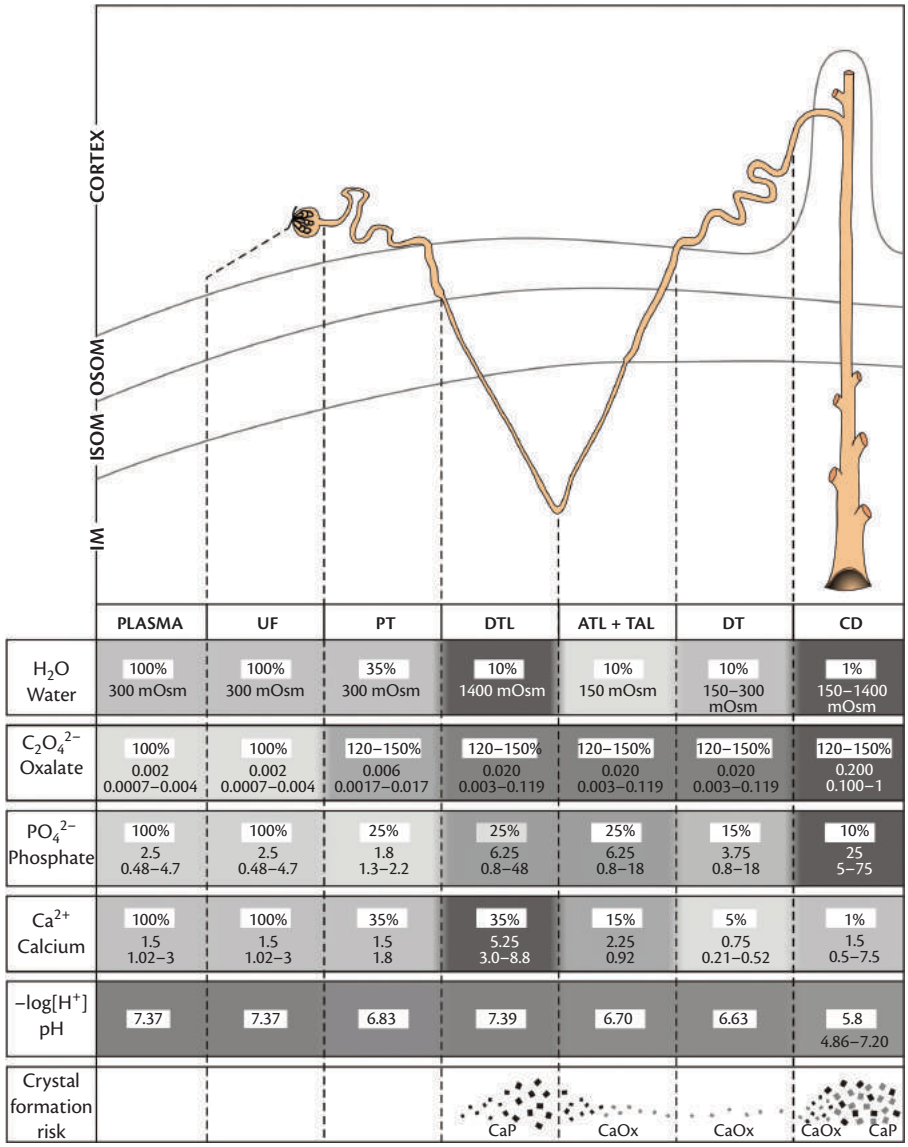


Fig. 204.2 Presentation of the major parameters determining the crystallization risk profiles of calcium oxalate and calcium phosphate along the different compartments of the nephron. Concentrations and values are obtained from four elaborate mathematical models, which combine data on fluid composition from micropuncture studies and functional studies on isolated nephron segments on the one hand, with morphological data on diameters and lengths of the different segments of the nephron on the other hand (Finlayson and Reid, 1978; Kok and Khan, 1994; Asplin et al., 1996; Tiselius, 1997; Robertson, 2004). (A) Percent substance of the filtered load remaining in the tubular fluid. (B) Theoretic concentration (mM) based on (A). (C) Observed concentration or calculated ranges of values (mM) based on reports of Finlayson and Reid (1978), Kok and Khan (1994), Robertson (2004), Asplin et al. (1996), and Tiselius (1997). For any parameter, the darker a segment along the nephron is coloured, the higher its contribution in the risk of crystallization at that nephron site. The bottom row visualizes this risk and summarizes where calcium oxalate and calcium phosphate crystal formation are most likely to initiate. It should be noted that extrapolations of animal data to the human situation should be interpreted with caution. However, the presented range of 'extreme low' to 'high values' of the mathematical models (C) will most likely cover the human situation. UF: ultrafiltrate, PT: proximal tubule, DTL: descending thin limb of loop of Henle, ATL: ascending thin limb of loop of Henle, TAL: thick ascending limb of loop of Henle, DT: distal tubule, CD: collecting duct, CaP: calcium phosphate, CaOx: calcium oxalate. IM: inner medulla, ISOM: inner stripe of medulla, OSOM: outer stripe of medulla.

concepts underlying these observations were not developed until the second half of the twentieth century. Two possible mechanisms were postulated by Vermeulen and Lyon and by Finlayson and Reid: (1) a 'free particle' mechanism, where crystals are retained by their size; and (2) a 'fixed particle' mechanism, where smaller crystals are retained by binding to the tubular epithelium (Vermeulen et al., 1959; Vermeulen and Lyon, 1968; Finlayson and Reid, 1978) (Fig. 204.3 and 204.7B, G). For reason of completeness, it should be noted that besides 'free' and 'fixed' crystal retention, direct nucleation of crystals onto tubular epithelial cells has been observed as well, albeit *in vitro*, suggestive for an alternative mechanism underlying intratubular nephrocalcinosis (Lieske et al., 2001). In this process, crystallization starts at particular sites on the epithelial surface instead of starting freely in the tubular fluid. However, as, up to now, few or no studies have elaborated on this putative mechanism, it will not be further discussed here.

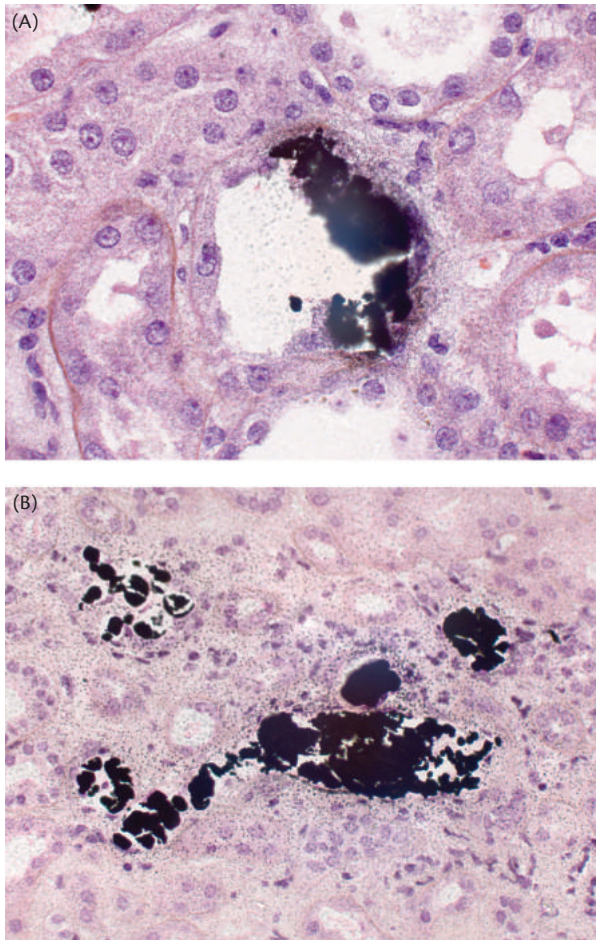


Fig. 204.3 (A) Von Kossa/HE stained renal section showing crystal adhesion in a patient with primary hyperoxaluria. Tissue provided by C. Van Woerden. (B) Von Kossa/HE stained renal section showing tubular obstruction in a patient with acute phosphate nephropathy.
Tissue provided by G. Markowitz.

Mechanism of 'free particle' retention

In the late 1970s, Finlayson and Reid theoretically inferred that intratubular crystals cannot grow large enough during their renal transit time to be retained in the tubules by their size (Finlayson and Reid, 1978). They therefore concluded that the development of intratubular nephrocalcinosis must depend on adhesion of urinary crystals to the tubular epithelium, thus via a 'fixed particle' mechanism. However, more detailed mathematical models with updated data sets for tubular dimensions, ion concentrations, and hydrodynamic factors (Kok and Khan, 1994; Kok, 1997; Robertson, 2004) could not exclude the possibility of crystal retention by size, in particular when aggregation of crystals is taken into account (Kok and Khan, 1994). Under pathological conditions this is likely to occur more often and one may reasonably assume that 'free particle' retention is determined by physical parameters such as the diameter of the tubular system, the rate of fluid flow and the extent of crystal formation, growth, and aggregation.

Histopathologically, 'free particle' retention typically presents itself as occlusion or plugging of the inner medullary collecting ducts and ducts of Bellini and has been observed in a wide variety of patients with stones of different aetiology (see Table 204.1, pathologies marked with asterisk). Whereas for most of these patients the type of intratubular crystal deposits is concordant with the crystal type expected to be found based on the urinary biochemistry, there are several discrepancies to be noted (Table 204.1). For patients with stones due to ileostomy, jejuno-ileal resection, or bypass, urine is acidic and not supersaturated with respect to calcium phosphate. Hence, based on these urinary characteristics, formation and retention of calcium phosphate crystals is highly unlikely, if not impossible. Yet, calcium phosphate deposits (i.e. apatite) are abundantly found in the lumina of the medullary collecting ducts in these patients. Also, patients with stones due to either ileostomy or hyperparathyroidism present significant urinary calcium oxalate supersaturation, indicating that calcium oxalate crystal deposition is to be expected. Yet, not calcium oxalate, but calcium phosphate is deposited in the collecting duct lumina. These and other discordances (described by Coe et al., 2010a) led to the hypothesis that defects in ion, water, but particularly acid/base handling by individual nephrons or nephron segments, which are unlikely to be noticed in the final urine, might be responsible for the observed phenotypes due to local increases in crystal formation.

Mechanism of 'fixed particle' retention

'Fixed particle' retention or crystal adhesion is particularly encountered in conditions presenting only moderate to even normal physiological levels of crystal formation as, for example, are suspected to occur in transplant patients and preterm infants (Khan et al., 1992; Schell-Feith et al., 2000; Nankivell et al., 2003; Gwinner et al., 2005; Schwarz et al., 2005; Verhulst et al., 2005; Vervaet et al., 2009). Nonetheless, putative crystal adhesion has been observed in excessive forms of nephrocalcinosis as well (Morgenroth et al., 1968; Oliver, 1968).

Ever since the 'fixed particle' concept was postulated, numerous studies have focused on identifying the mechanisms of crystal–cell interaction and crystal adhesion to the tubular epithelium. These studies indicated that the molecular phenotype of the tubular epithelium is a critical determinant. The main questions posed in this area of research are: (1) 'What is the (molecular) phenotype of crystal-binding epithelia?' and (2) 'If this epithelial phenotype is aberrant from the normal phenotype, is its presence cause or consequence of crystal adhesion?'

The molecular phenotype of crystal-binding epithelia

Several early histopathological observations in patients with nephrocalcinosis or nephrolithiasis of different aetiology reported the association between intratubular crystal deposits and renal morphological alterations related to renal injury (Huggins, 1933; De Albuquerque and De Paola, 1959; Oliver et al., 1966; King, 1967; Morgenroth et al., 1968; Haggitt and Pitcock, 1971). These histopathological reports actually were the first indication of a link between an altered tubular epithelial phenotype and the development of intratubular calcifications. Later investigations in animal models of 'nephrolithiasis', which emphasized the identification of the tubulointerstitial changes in the vicinity of intratubular crystals, further illustrated the association between intratubular crystal deposition and epithelial injury. These studies demonstrated that crystals can be found in contact with apoptotic and/or necrotic cells, denuded basement membranes, and the surface of injured epithelial cells (Dykstra and Hackett, 1979; Gill et al., 1979; Khan et al., 1979, 1981, 1982, 1984; Khan and Hackett, 1980; De Bruijn

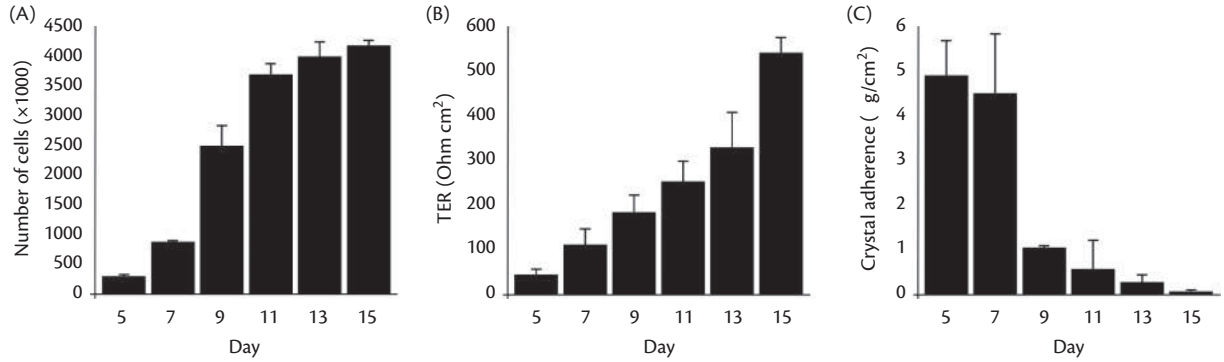


Fig. 204.4 Crystal binding to primary human tubular kidney cells in function of cell culture confluence. (A) Cell density and (B) transepithelial electrical resistances (i.e. two measures of cell culture confluence) were measured until 15 days after seeding human tubular cells on Transwell filters. Subconfluent cell cultures present proliferating, migrating cells, hence cells with a dedifferentiated phenotype, whereas confluent cultures show differentiated epithelial cells. (C) Crystal binding to human tubular cell cultures was quantified using [14C]-labelled calcium oxalate monohydrate (COM) crystals. Crystal binding is high on subconfluent cultures and gradually decreases in time to very low levels in confluent cultures.

Adapted from Verhulst *et al. J Am Soc Nephrol*, 2003 Jan; 14(1):107–15. Crystal retention capacity of cells in the human nephron: involvement of CD44 and its ligands hyaluronic acid and osteopontin in the transition of a crystal binding- into a nonadherent epithelium.

et al., 1993, 1996; Khan, 1995; Asselman et al., 2003; Sarica et al., 2004; Wiessner et al., 2009).

In vitro, mechanistic studies showed that crystals preferentially adhere to either injured, depolarized, immature, migrating, or proliferating tubular epithelial cells and not to fully differentiated tubular epithelia (Wiessner et al., 1987, 2001; Riese et al., 1988, 1992; Mandel et al., 1994; Verkoelen et al., 1995, 1998, 1999, 2003; Bigelow et al., 1998) (Fig. 204.4). Verkoelen et al. (1999, 2005), Schepers et al. (2003, 2005a), and Verhulst et al. (2003) showed that this is particularly true for epithelial cells of distal origin. Their experiments demonstrated that proximal tubular cells are prone to adhere to crystals *in vitro* independent of the differentiation status of these cells, whereas epithelial cells of distal origin acquire a non-crystal-adherent phenotype upon differentiation. As crystal formation in the tubular fluid particularly occurs in the distal parts of the nephron (see 'Crystal formation'), this latter feature is not required for proximal tubular cells as they only very rarely may encounter intratubular crystals. For reasons of completeness, it should be noted that even in the model describing nucleation of crystals directly onto the a renal epithelial cell layer, the composition of the cell surface appears to be a critical determinant as well (Nancollas et al., 1996; Verkoelen et al., 2000a; Lieske et al., 2001).

Crystal-binding molecules

As mild and early forms of intratubular nephrocalcinosis generally do not display overt renal deterioration resulting in loss of tubular epithelial cells, identifying the characteristics and molecular composition of the luminal membrane of crystal-binding epithelia gained particular interest. In this context, the concept of crystal-binding molecules was developed (Gill et al., 1979; Khan et al., 1984). Crystal-binding molecules are cell-surface molecules with affinity for crystals that are expressed or produced by renal tubular epithelial cells, particularly under pathological conditions, and allegedly are capable to anchor crystals to the luminal membrane of those cells. Importantly, these molecules are not or only scarcely present at the luminal membrane of fully differentiated epithelia, however, are upregulated or redistributed to the apical membrane under conditions of cellular dedifferentiation, that is, injury and repair (Bigelow et al., 1997; Verkoelen et al., 2000b, Verhulst et al., 2003; Sorokina et al., 2004; Asselman et al., 2005; Kleinman et al., 2010) (Fig. 204.5). Currently, four categories of crystal-binding molecules have been identified *in vitro* (Table 204.2): (1) terminal sialic acid residues, (2) phospholipids, (3) membrane bound proteins, and (4) glycosaminoglycans, of which hyaluronan (HA) appears to be the most potent crystal-binding polysaccharide. The variety in the nature of these molecules and the fact that no unique crystal-binding molecule has been identified so far indicates that the molecular composition of the crystal-binding membrane may depend on variations in pathophysiological and/or experimental conditions. It is intriguing, however, that all currently known crystal-binding molecules contribute to a negative cell-surface charge, that is, a feature which has been shown to be important in crystal adhesion to renal epithelial cells (Lieske et al., 1995, 1996, 1997). Therefore, it can be suggested that a spectrum of aberrant phenotypes may bind crystals as long as a sufficient amount and/or properly orientated negative charges are present on the luminal membrane.

Crystal adhesion as cause or consequence of epithelial phenotypical changes—in vitro

The question frequently arises whether crystal adhesion either is the consequence or the cause of the phenotypical alterations associated with epithelial injury/regeneration. The main difficulty in answering this question is the fact that there are two apparently conflicting observations on the interaction between crystals and epithelial cells. As described earlier (see 'The molecular phenotype of crystal-binding epithelia'), adhesion of crystals particularly occurs at injured/regenerating epithelia and not at normal cells, suggesting that a dedifferentiated phenotype causes crystal adhesion. On the other hand, crystals have been found to induce injury, proliferation, production of inflammatory mediators, and oxidative stress upon contact with epithelial cells *in vitro*, thereby suggesting that epithelial injury/dedifferentiation is a consequence of crystal adhesion (Lieske et al., 1992; Thamilselvan and Khan, 1998; Thamilselvan et al., 2003; Umekawa et al., 2002, 2003; Aihara et al., 2003; Khan, 2004b; Liang et al., 2006; Escobar et al., 2008). Thus,

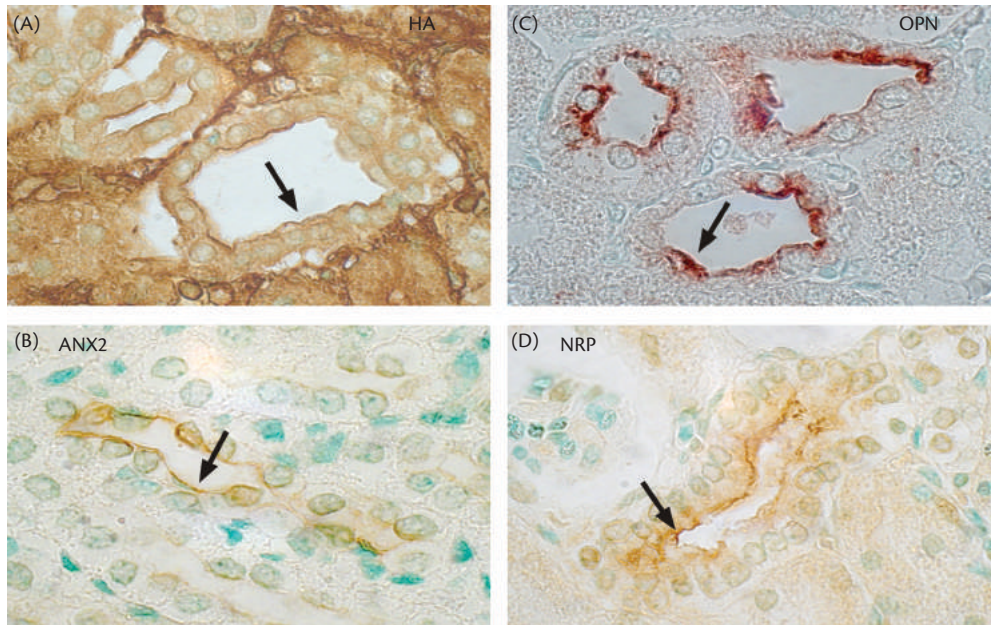


Fig. 204.5 Luminal expression of the crystal-binding molecules HA hyaluronan (A), annexin 2 (B), osteopontin (C), and nucleolin-related protein (D) in renal necropsy tissue of preterm infants, a condition with a high incidence of iatrogenic postnatal intratubular nephrocalcinosis.
Reproduced with permission from Vervaet *et al. Nephrol Dial Transplant*. 2009 Dec; 24(12):3659–68. Crystalluric and tubular epithelial parameters during the onset of intratubular nephrocalcinosis: illustration of the 'fixed particle' theory *in vivo*.

attachment of crystals to the tubular epithelium seems to be both a potential consequence as well as a potential cause of epithelial phenotypical changes. Two key aspects, however, suggest that these confusing observations do not necessarily contradict each other.

First, crystal adhesion is not to be considered the only type of crystal–cell interaction. In fact, three types of crystal–cell interactions can be envisioned (Fig. 204.6): (1) adhesion to cell-surface crystal-binding molecules which are expressed by injured/regenerating cells; (2) weak, transient interactions as expected during crystalluria when crystals passing with the fluid shortly contact the tubular wall; and (3) a forced crystal–cell contact as expected during obstruction of the tubular lumen. As crystals do not adhere to normal epithelial cells (see 'The molecular phenotype of crystal-binding epithelia'), it is highly unlikely that crystal adhesion is the primary cause of cellular injury and phenotypical alteration. Therefore, forced contacts and transient interactions, which can occur to epithelia with a normal phenotype, are to be considered the possible initial crystal–cell interactions with injury inducing potential. It can be suggested that obstructive crystals may harm the structural integrity of the plasma membrane primarily

Table 204.2 Crystal-binding molecules

Category	Crystal-binding molecule	References
Terminal sialic acid residues of the glycocalyx		Sachtleben and Ruhenstroth-Bauer, 1961; Eylar et al., 1962; Glaeser and Mel,1964, 1966; Haydon and Seaman, 1967
Plasma membrane phospholipids	Phosphatidylserine	Bigelow et al., 1997; Cao et al., 2001; Wiessner et al., 2001
Membrane bound proteins	Collagen IV	Kohri et al., 1991;
	Osteopontin (OPN)	Yamate et al., 1996, 1998, 1999; Konya et al., 2003
	Annexin 2 (ANX2)	Kumar et al., 2003; Carr et al., 2006;
	Nucleolin-related protein (NRP)	Sorokina and Kleinman, 1999; Sorokina et al., 2004
Glycosaminoglycans (GAGs)	Chondroitin sulphate	Verkoelen et al., 1995, 1998, 2000b
	Dermatan sulphate	
	Keratan sulphate	
	Heparin	
	Heparan sulphate	
	Hyaluronan (HA)	

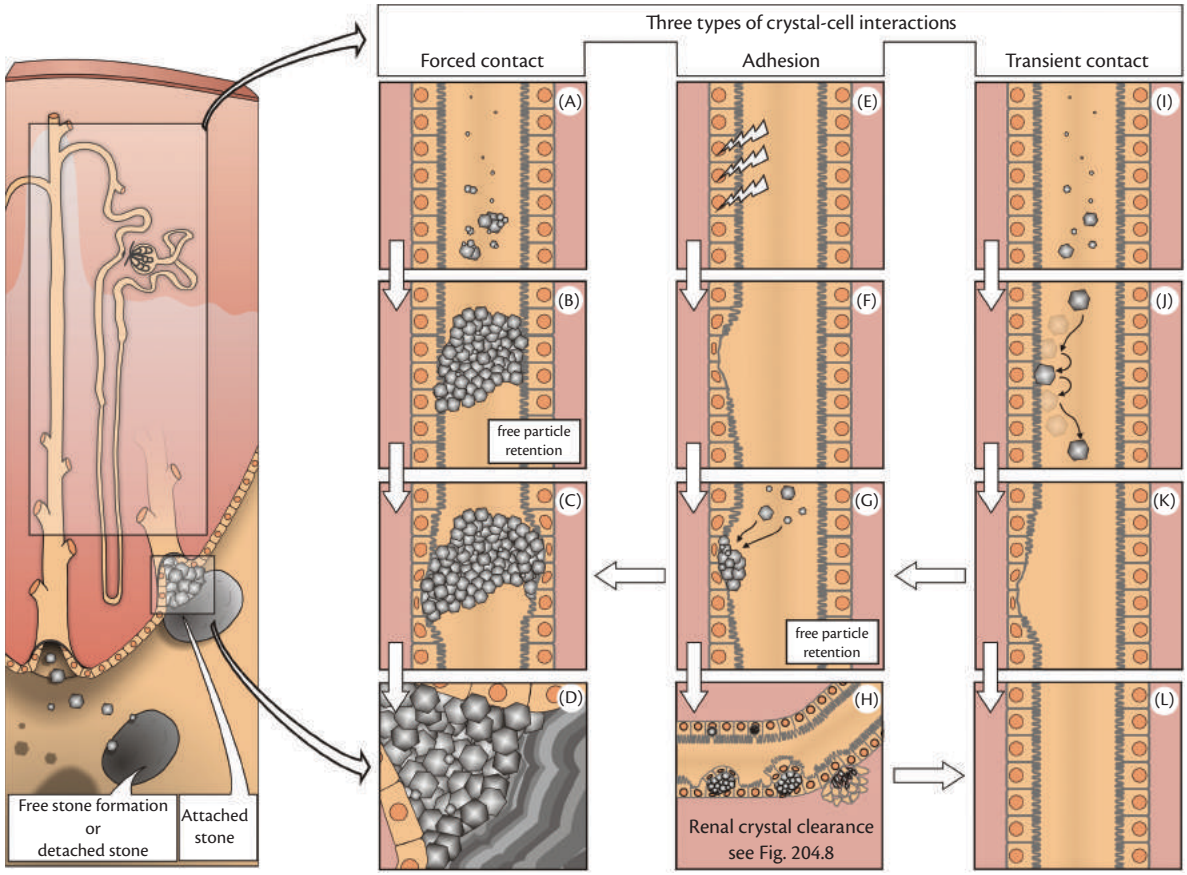


Fig. 204.6 Histopathological course of intratubular crystal–cell interactions and nephrocalcinosis. The scheme provides an overview of the different types of crystal–cell interactions and possible scenarios which are currently thought to be relevant in the development (and clearance) of intratubular nephrocalcinosis and/or nephrolithiasis. Although this figure has limitations it is an attempt to visually integrate our current understanding of intratubular crystal deposition processes. (A) Excessive crystal formation and/or aggregation in the tubular fluid. (B) Tubular obstruction exerting mechanical pressure on the tubular epithelium. (C) The tubular epithelium gets injured by this mechanical pressure. (D) Possible outcomes: loss of tubular function, inflammation, fibrosis, stone growth on crystal plug, etc. (E) Undefined insult to the tubular epithelium (e.g. ischaemia/reperfusion, prematurity, toxins, etc.). (F) This insult may direct the tubular epithelium to a dedifferentiated phenotype. (G) Upon crystal formation in the tubular fluid, crystals may adhere to an already dedifferentiated epithelium. Further crystal deposition on dedifferentiated cells and aggregation with adhered crystals may result in obstruction as shown in (C). (H) Adhered crystals can be cleared from the tubular lumen and kidney by endocytosis or transepithelial uptake, with restoration of epithelial integrity as shown in (L). See Fig. 204.8 for details. (I) Physiological crystal formation in the tubular fluid. (J) Transient non-adhesive contact between crystals and cells. (K) These contacts may temporarily direct the tubular epithelium to a dedifferentiated phenotype with crystal-binding properties. Passing crystals may adhere to this epithelium as shown in (G). (L) Recovery of the tubular epithelium.

Adapted with permission from Vervaet *et al. Nephrol Dial Transplant*, 2009 Jul; 24(7):2030–5. Nephrocalcinosis: new insights into mechanisms and consequences.; Vervaet *et al. Urol Res*, 2010 Aug; 38(4):249–56. The tubular epithelium in the initiation and course of intratubular nephrocalcinosis.

by denting the epithelial cells, whereas transient crystal–cell interactions might be injurious by scarring the tubular epithelium or even rip off certain membrane-bound constituents. Furthermore, prolonged non-adhesive contact between crystals and normal cells, as may occur when crystals are trapped in 'dead spots' at bends in the nephron where there is little or no fluid flow, may cause injury as well. Interestingly, this situation is actually mimicked in the vast majority of *in vitro* studies on the effect of crystals on cells where crystal suspensions are left on epithelial monolayers for several minutes to hours. Even so, it should be noted that in some histological studies in rat and man intratubular crystals were observed adjacent to epithelial cells which appear morphologically normal (Oliver et al., 1966; Evan et al., 2003; Vervaet et al., 2009). These observations may either suggest crystal adhesion to fully differentiated cells or alternatively may represent a 'snap-shot' of transient (non-adhesive) crystal–cell interactions. Although it is not known whether these epithelia actually present a normal apical membrane in terms of protein and phospholipid composition, this latter possibility cannot be excluded. Overall, although the exact mechanical and/or chemotoxical nature of crystal induced epithelial injury remains to be determined, non-adhesive interactions may direct the normal epithelium towards a crystal-binding phenotype in such a way that a crystal may induce its own adhesion or that of a subsequently passing crystal (Lieske et al., 1997). Once adhered, crystals may then further exert their putative toxic effects, aggravating epithelial injury.

A second aspect of this 'chicken or the egg' causality dilemma is based on the fact that crystal formation in solutions, such as original and artificial tubular fluid/urine, requires supersaturation of that solution with ions relative to the type of crystal. These ions

are most commonly oxalate, phosphate, and calcium. It has been hypothesized that not the crystals themselves, but rather the concomitant high concentrations of these ions might be injurious to the tubular epithelium. In particular, ionized oxalate has been considered to be the cause of oxidative stress and production of free radicals (Aihara et al., 2003; Thamilselvan et al., 2003; Khan, 2005). However, cell culture studies with emphasis on both oxalate and calcium concentration questioned the alleged toxicity of oxalate (Belliveau and Griffin et al., 2001; Umekawa et al., 2003; Schepers et al., 2005b; Khaskhali et al., 2009). It was found that free oxalate concentrations, required to exert toxic effects, cannot be achieved in the presence of physiologic amounts of calcium (Belliveau and Griffin et al., 2001; Schepers et al., 2005b). Furthermore, oxalate seems to be relatively harmless in the absence of calcium (Schepers et al., 2005b) and is only toxic at supraphysiological concentration levels (Schepers et al., 2005b; Verkoelen et al., 2005). In addition, accumulating evidence suggests that the assumed oxalate-induced cell injury often might be caused by crystals (Hammes et al., 1995; Thamilselvan and Khan, 1998; Umekawa et al., 2002; Khan, 2004a; Guo and McMartin, 2005; McMartin and Wallace, 2005; Guo et al., 2007; Tsujihata, 2008). Currently, as this issue has not been fully resolved, both passage and prolonged contact of crystals and high concentrations of oxalate, phosphate, and calcium with the tubular epithelium have still to be considered potential insults among the myriads of causes leading to epithelial injury and consecutive phenotypical alterations.

Crystal adhesion as cause or consequence of epithelial phenotypical changes—in vivo

Whereas *in vitro* crystal adhesion is to be considered a consequence and not an initial cause of epithelial injury, progress in our understanding of the pathophysiological mechanisms underlying intratubular nephrocalcinosis necessitates verification of these results in animals and humans. The following paragraphs present several observations in animal experiments from which the necessity of renal injury and its associated/subsequent epithelial changes in the development of crystal adhesion can be inferred.

A first indication is provided by experiments investigating the effect of deliberately induced epithelial injury on crystal retention. Gill et al. and Khan et al. investigated calcium oxalate crystal attachment to the normal and HCl- or triton-X-100-pretreated urothelium of the rat bladder and observed preferential adhesion of these crystals to the chemically injured bladder urothelium, whereas control bladders without urothelial injury remained free of adherent crystals (Gill et al., 1979; Khan et al., 1984). Although these studies did not involve the tubular epithelium, they provided a proof of principle that epithelial alteration can cause crystal adhesion. With respect to the renal epithelium, Kumar et al. observed that hyperoxaluric rats developed markedly more crystal deposition upon induction of renal injury by administration of gentamycin (Kumar et al., 1991). Finally, in a more relevant clinical setup, Xue et al. observed that shock wave-induced renal injury resulted in more crystal retention in the injured kidney than in the contralateral unshocked kidney upon administration of ethylene glycol (EG) (Xue et al., 2009).

A second line of indirect support comes from studies aiming at diminishing or abolishing crystal deposition by treating the animals with compounds that tend to prevent renal injury. As production of reactive oxygen species has been identified as an important aspect of renal injury (Baud and Ardaillou, 1986, 1993; Andreoli, 1991; Abid et al., 2005; Khan, 2005), prevention particularly focused on restoring the normal oxidation status of the renal tissue. For example, treatment with vitamin E, a fat-soluble antioxidant, prevents EG-induced calcium oxalate crystal deposition in the kidney by attenuation of tubular injury (Thamilselvan and Menon, 2005; Huang et al., 2006). Also, the cholesterol-lowering drug atorvastatin, which has anti-inflammatory and antioxidant activities, reduces both tubular injury and crystal deposition in the EG model (Wassmann et al., 2002). Likewise, Itoh et al. reported that the antioxidative effect of green tea decreased EG-induced renal calcium oxalate crystal retention in rat kidneys (Itoh et al., 2005). Furthermore, reduction of angiotensin II production via inhibition of angiotensin-converting enzyme (ACE) or blocking of angiotensin receptors (ARBs) has been shown to significantly reduce renal calcium oxalate crystal deposition as well as the development of interstitial inflammation and the level of oxidative stress in EG-treated rats (Antus et al., 2001; Toblli et al., 2001, 2002). Lastly, treatment with taurine, a naturally occurring sulfonic acid with renoprotective and antioxidant capacities, has been found to diminish crystal deposition in kidneys of the rat (Erdem et al., 2000; Sener et al., 2005; Li et al., 2009; Manna et al., 2009).

Finally, a third approach is to verify whether the crystal-binding molecules identified *in vitro* are also expressed in animal and human renal tissue. In this context, Asselman et al., Verhulst et al., and Vervaet et al. investigated the tubular epithelial phenotypical changes during the development of intratubular nephrocalcinosis both in rats (Asselman et al., 2003; Vervaet et al., 2009) and in humans (Verhulst et al., 2005; Vervaet et al., 2009). These studies not only demonstrated luminal expression of HA, OPN, ANX2, and NRP (Table 204.2) in kidneys with nephrocalcinosis, but also illustrated that alteration of the tubular epithelial phenotype can precede the onset of nephrocalcinosis. *First*, in EG-treated rats, calcium oxalate crystals are readily formed and excreted within 24 hours of EG administration. Interestingly, both intratubular crystal retention and epithelial phenotypical changes were negligible at that time (Asselman et al., 2003; Vervaet et al., 2009), thereby corroborating the *in vitro* well-documented finding that a normal differentiated monolayer of renal epithelial cells does not bind crystals (Bigelow et al., 1998; Verkoelen et al., 1998). *Second*, despite continuous crystal formation and excretion during a 4-day EG-administration period, crystal retention only gradually increased, in parallel with the extent of epithelial changes (Vervaet et al., 2009). This suggests that crystal retention progresses at the rate at which the tubular epithelium is altered, rather than being solely dependent on the presence of crystals in the tubular fluid. *Third*, at 2 days after arrest of a 4-day EG-administration, crystal retention markedly increased, while crystal excretion had completely stopped (Vervaet et al., 2009). Although crystal formation in the tubular fluid appeared to have decreased at that time, histological analysis showed that the number of regenerating epithelial cells had clearly increased, suggesting that, after withdrawal of the toxic stress of EG (and its metabolites), the kidney fully unfolds its natural regenerating ability, leading to an increased presentation of dedifferentiated cells capturing nearly every crystal passing by (Vervaet et al., 2009). *Finally*, in humans, by investigating the luminal expression of HA and OPN in renal tissue obtained from renal transplant patients and preterm infants at two consecutive time points, that is, prior to and during intratubular nephrocalcinosis,

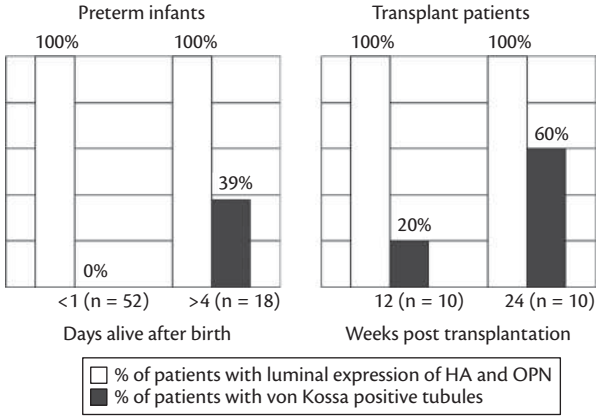


Fig. 204.7 Expression of crystal-binding molecules HA and OPN versus the development of intratubular nephrocalcinosis in preterm infants and transplant patients. Tubular luminal membrane expression of HA and OPN is consistently present in all investigated renal tissues at all time points. As the incidence of intratubular nephrocalcinosis (as assessed on Von Kossa stained renal sections) increases with time, it can be inferred that epithelial changes precede nephrocalcinosis.

Adapted with permission from Verhulst *et al. Kidney Int.* 2005 Oct; 68(4):1643–7. Preconditioning of the distal tubular epithelium of the human kidney precedes nephrocalcinosis.

it was found that these epithelial alterations precede the onset of intratubular nephrocalcinosis (Verhulst et al., 2005) (Fig. 204.7). Particularly in preterm infants, which are always born without nephrocalcinosis, it is well documented that nephrocalcinosis may develop after birth due to clinical treatment as furosemide administration and hyperalimentation favour crystal formation in the tubular fluid (Schell-Feith et al., 2000). Hence, the risk of crystals meeting and adhering to dedifferentiated epithelial cells is increased. This finding also corroborates that epithelial changes associated with nephrocalcinosis are not necessarily induced by crystal formation in the tubular fluid.

Natural defence mechanisms against intratubular nephrocalcinosis

The kidneys' priority to maintain homeostasis inevitably creates tubular fluid conditions where more of a particular substance needs to be excreted than that theoretically can be dissolved in water. In comparison to pure water, however, the tubular fluid/urine is capable of keeping considerably more substance in solution (Hodgkinson, 1980; Tiselius, 1984, 1989, 1991; Ogawa, 1993b; Streit et al., 1998). This indicates that the kidney, being evolutionary challenged to assure the survival of its host, has developed a series of mechanisms by which an increased amount of substance can be excreted per unit of volume without compromising its own function due to excessive crystallization. These 'defence' mechanisms act on the physiological, physicochemical, crystal adhesion, and post-crystal-adhesion level.

Physiological defence

Obviously, the most certain and simple way to circumvent crystallization is to decrease the crystal ion concentrations and prevent supersaturation. Physiologically, this can be achieved by either reducing the excreted ion load, by increasing the amount of water in the tubular system, or by increasing urinary acidification. An example of these mechanisms can be inferred from functional studies. First, data from transgenic mouse studies indicate that SLC26A6, one of three oxalate transporting members of the SLC26A anion exchanger family (Mount and Romero, 2004; Markovich and Aronson, 2007), seemingly increases oxalate absorption in the proximal tubules, thus reducing urinary oxalate excretion (Lohi et al., 2002; Freel et al., 2006). Although it is counterintuitive that oxalate, as a terminal metabolite, is not immediately excreted, a delicate balance between proximal reabsorption in the S1–S2 segments and secretion in the S3 segment might protect the kidney from peak oxalate concentration levels by a gradual excretion of the oxalate load (Greger et al., 1978; Knight et al., 1981; Marengo and Romani, 2008). Second, with respect to calcium, high calcium concentrations in the tubular fluid are able to reduce antidiuretic hormone-stimulated water permeability of the collecting duct (i.e. aquaporin 2 downregulation) by triggering the apical calcium sensing receptor of the epithelial cells at this nephron segment, leading to protection against crystallization by polyuria (Dillingham et al., 1987; Jones et al., 1988; Hebert, 1996; Hebert et al., 1997). Recently, Renkema et al confirmed these observations in TRPV5$^{-/-}$ mice, which display hypercalciuria due to impaired active Ca^{2+} reabsorption and concomitant hyperphosphaturia, polyuria, and increased urinary acidification, but without renal crystal deposition (Renkema et al., 2009). Third, Renkema et al. additionally revealed that, next to polyuria, activation of the calcium sensing receptor by hypercalciuria results in urinary acidification by increased activity of H^+-ATPase. This process exerts an even more pronounced protection against crystal formation/deposition, as ablation of the *Atp6v1b1* gene, coding for the collecting duct specific B1 subunit of H^+-ATPase, in TRPV5 $^{-/-}$ mice prevents the increased urinary acidification seen in these animals and evokes extensive calcium phosphate precipitation in the renal medulla, despite polyuria (Renkema et al., 2009).

Overall, as physiological restriction of the concentrations of crystal components cannot lead to the excretion of increased amounts of substance per unit of volume, these mechanisms should be considered a first line of renal defence against crystallization. Their range of action, however, is narrow and easily overruled by the dominant need of body homeostasis, and therefore may only prevent crystallization temporarily.

Physicochemical defence

Physicochemical defence against crystallization involves molecules that directly and physically interfere with the different thermodynamic processes of crystallization. These crystallization modulators can be classified according to their effect as either inhibitor or promotor and, based on their nature and size, as either inorganic low-molecular-weight or organic high-molecular-weight compounds as illustrated in Table 204.3 (Kok, 1991; Ryall, 1997; Cerini et al., 1999; Khan and Kok, 2004; Basavaraj et al., 2007; Khan and Canales, 2009). Their mode of action relies on three principles:

1. Inhibiting supersaturation by lowering the effective concentrations of circulating ions by chelation. In this process the ions are bound by an ionic partner (the chelator) to form a soluble complex, thereby preventing bonding with other ions and the subsequent formation of potentially insoluble compounds.
2. Inhibiting crystal growth and aggregation by adsorption to the crystal surface and stereologically occupying/neutralizing potential ion deposition or crystal contact sites.

Table 204.3 Urinary modulators of crystallization

Class	Promotor	Inhibitor
Low molecular weight		Citrate (Tiselius et al., 1993a, 1993b)
		Magnesium (Meyer and Smith, 1975; Hallson et al., 1982)
		Pyrophosphate (Meyer and Smith, 1975)
High molecular weight	Tamm–Horsfall protein (Rose and Sulaiman, 1984; Grover et al., 1990; Hess, 1992)	Tamm–Horsfall protein (Mo et al., 2004, 2007)
	Lipids and membrane fragments (Hackett et al., 1990; Khan et al., 2000; Fasano and Khan, 2001; Gambaro et al., 2006)	Urinary prothrombin fragment 1 (Grover et al., 1998, 1999)
		Inter-alpha inhibitor (Atmani et al., 1996)
	Albumin (Cerini et al., 1999)	Osteopontin (Shiraga et al., 1992; Worcester et al., 1992; Asplin et al., 1998; Wesson et al., 2003)
		Bikunin (Medetognon-Benissan et al., 1999)
		Calgranulin (Pillay et al., 1998)
		Human urinary trefoil factor (Thongboonkerd et al., 2008)
		Renal lithostathine (Verdier et al., 1992, 1993)
		Alpha-1-microglobulin (Atmani et al., 1993; Tardivel et al., 1999)
		Albumin (Ryall et al., 1991)
		Fibronectin (Tsujihata et al., 2000)
		Glycosaminoglycans (Bowyer et al., 1979; Angell and Resnick, 1989; Shirane et al., 1989; Suzuki and Ryall, 1996; Rodgers et al., 1994

3. Promoting crystal nucleation, thereby dividing the total precipitated mass into numerous smaller particles which are easily eliminated as compared to excretion of larger ones.

The efficiency by which the kidney modulates crystallization to secure its structure and function is valued by considering the number of crystals which can be excreted during crystalluria without apparent harm. Robertson et al. measured that up to 7200 crystals can be excreted per mL of urine (Robertson, 1969; Robertson et al., 1969). However, because of the detection limits of the method used to study the particle size distribution, a rather marked underestimation could be suggested (Robertson, 1969). Re-appraisal of these data by Kok and Khan showed that up to 24,000 crystals per mL can be expected to be present in the urine during crystalluria (Kok and Khan, 1994).

Due to the obvious importance of managing crystallization in order to maintain proper renal function, it was hypothesized that defective or imbalanced promotor and inhibitor activities could act as a causative mechanism in renal calcification. Although not consistently found, this idea is supported by the fact that urine from stone forming patients tends to be less capable of inhibiting crystal nucleation, growth and/or aggregation as compared to controls (Baumann et al., 1985; Ryall et al., 1986; Asplin et al., 2002, 2009). Therefore, many studies focused on identifying the urinary modulators and aimed at finding differences in terms of concentration and/or activity of these molecules between the healthy population and patients with nephrocalcinosis/nephrolithiasis (Worcester, 1996; Asplin et al., 1998; Medetognon-Benissan et al., 1999; Suzuki et al., 2001; Jaggi et al., 2007). However, despite their generally straightforward effects in *in vitro* crystallization experiments, none of the alleged urinary modulators, except citrate (Caudarella and Vescini, 2009; Zuckerman and Assimos, 2009), Tamm–Horsfall protein (THP) (Mo et al., 2004, 2007), and osteopontin (OPN) (Wesson et al., 2003; Mo et al., 2007), have been proven to play a role *in vivo* so far.

Overall, the vast amount of studies on these and other modulators have shown that the urinary inhibitory capacity of crystallization seems to be provided by an amalgam of compounds with redundant and overlapping activities. Whether crystallization is either actively being controlled (as would be expected in case of a biological mechanism) or merely being dealt with by inhibitors that are already present in the tubular fluid is currently not known.

Defence mechanisms against crystal adhesion

Given the putative adverse effects of crystal adhesion to the tubular epithelium, prevention of crystal–cell interactions might be an awarding strategy to counter potential tubular deterioration. As described above (see 'The molecular phenotype of crystal-binding epithelia'), an effective line of defence is provided by the fact that nephron segments, where crystal formation may regularly occur, are lined by an epithelium which, under healthy conditions, has no affinity for crystals. An alternative defence mechanism was suggested by the early investigations of Lieske et al. (1995) and Verkoelen et al. (1996) on the nature of the interaction between crystals and cells. These studies showed that crystal adhesion could be inhibited by pretreating the crystals with specific anions, some of which are natural constituents of urine, that is, nephrocalcin, uropontin, citrate, and the glycosaminoglycans (GAGs) chondroitin sulphate A or B, heparan sulphate, and hyaluronic acid (Lieske et al., 1995; Verkoelen et al., 1996). This concept of crystal coating was further corroborated by the observation that pretreatment of crystals with human urine before exposure to MDCK-cells significantly reduced crystal adhesion (Ebisuno et al., 1999). In contrast, however, Schepers et al. found that pretreatment of crystals with urine had no significant effect on the level of crystal-binding to both confluent LLC-PK1 cells of proximal origin and migrating/proliferating MDCK-I cells of distal tubule/collecting duct origin (Schepers et al., 2002). Furthermore, with respect to GAGs, Verkoelen et al. showed that relatively high concentrations of heparan sulphate, chondroitin sulphate A, and chondroitin sulphate B, not reached in urine, were required to provide some protection of the cells against the interaction with crystals (Verkoelen et al., 1996). Although GAGs contribute to the overall urinary inhibitory activity (Sallis and Lumley, 1979; Sallis, 1987), these substances do not seem to play an important role in the prevention of crystal retention. Altogether, although a contribution of urinary macromolecules cannot be fully excluded, the presentation of a non-crystal-binding epithelial phenotype still appears to be the

major mechanism of the kidney's intrinsic protection against crystal adhesion.

Post-crystal-adhesion defence mechanisms

Reports from *in vitro, in vivo*, and human renal tissue studies indicate that the tubular epithelium is not a helpless bystander undergoing progression of nephrocalcinosis, however, it is able to clear adhered crystals from the tubular lumen in two ways depending on the size of the crystals.

For crystals with diameters that are smaller than that of the epithelial cells they contact, it is well known that the tubular epithelium is capable of clearing them via endocytosis and subsequent lysosomal disintegration (Lieske et al., 1992, 1994, 1997; Ebisuno et al., 1997; Schepers et al., 2003; Grover et al., 2008). Larger crystal deposits, however, seem to be particularly cleared by a process of 'transepithelial uptake', both in animal and human (Bruijn et al., 1994, 1995; Vervaet et al., 2009) (Figs 204.8 and 204.9). Whereas De Bruijn et al. (1994, 1995) were the first to describe this process in a qualitative way and termed it 'exotubulosis', Vervaet et al. (2009) quantitatively corroborated the course of events and documented its important contribution to clearance of nephrocalcinosis. The key aspect of this process involves epithelial coverage of the crystalline deposit by proliferation and migration of epithelial cells neighbouring the crystal attachment site. After epithelial overgrowth, the basement membrane of the crystal deposition site degrades and crystals are directed to the interstitium (Fig. 204.8). By this time, the tubules are already resealed as the new epithelial cell layer, covering the crystals, matures and produces a new basement membrane adjacent to the crystals (Fig. 204.8). This translocation process is not transcellular, as crystals are not taken up and

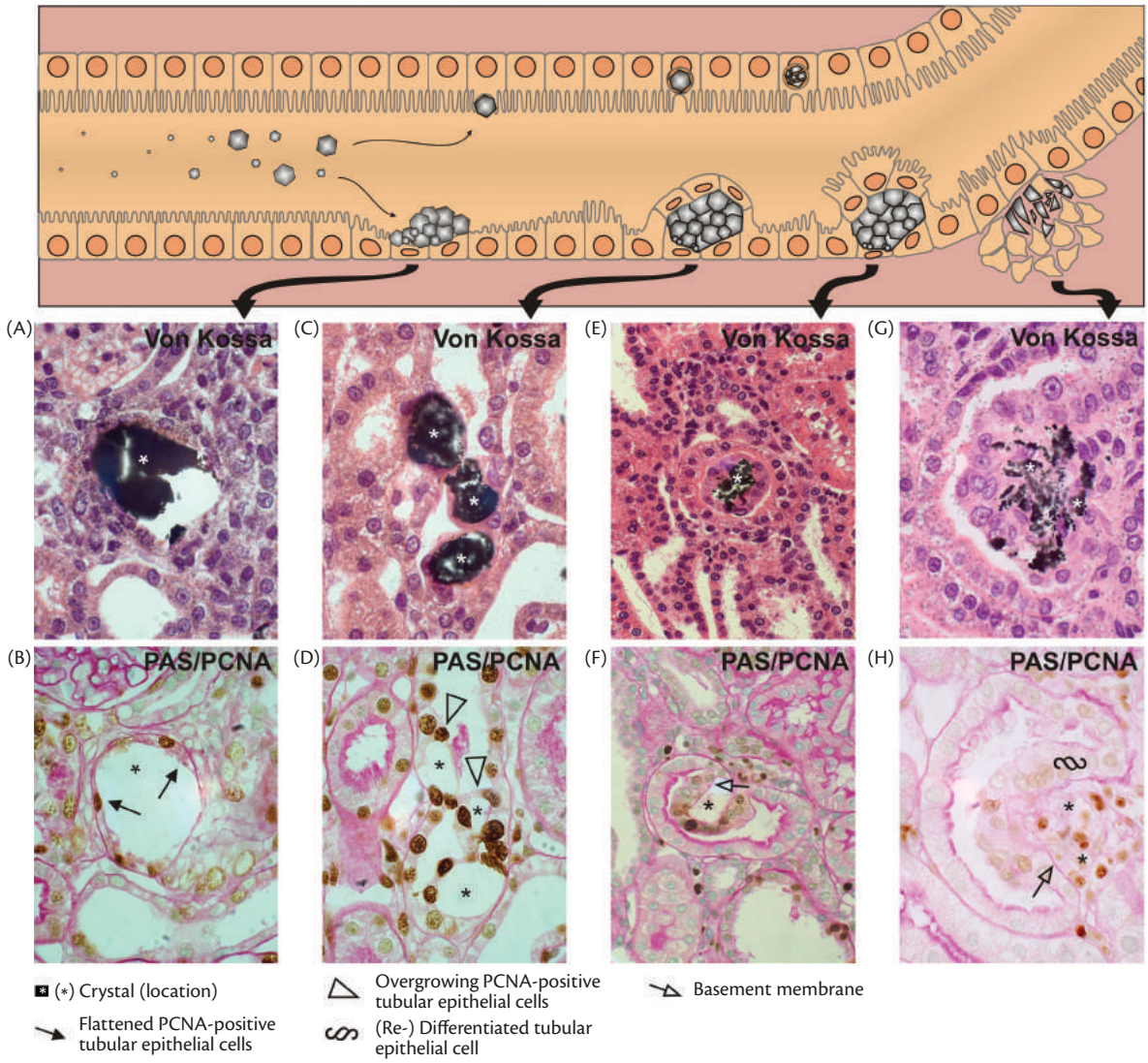
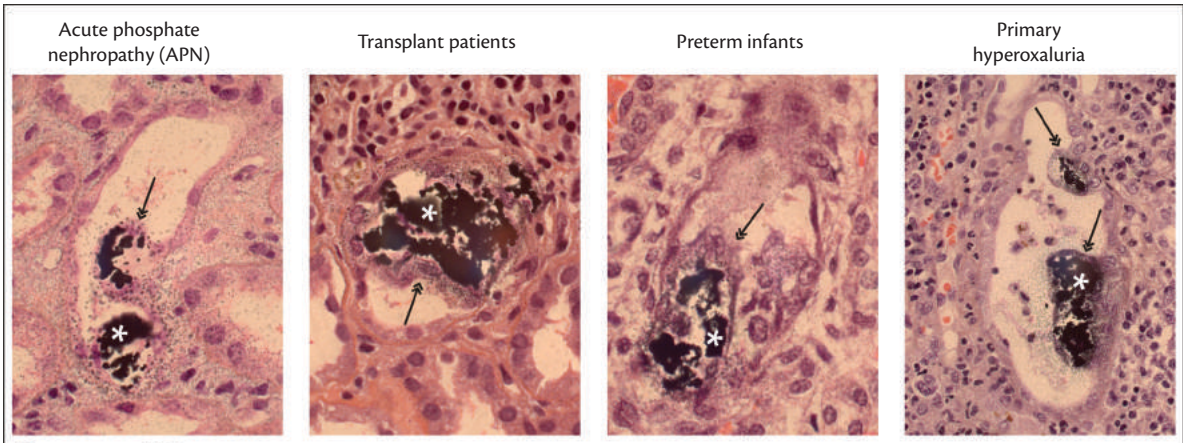


Fig. 204.8 Mechanisms of renal crystal clearance: endocytosis (upper epithelium) and transepithelial uptake (lower epithelium). Crystals form in the tubular fluid and can adhere to a dedifferentiated tubular epithelium (A) and (B). Epithelial cells neighbouring the crystal-adhesion site grow over the adhered crystals (C) and (D). This new epithelium differentiates, polarizes and deposits a new basement membrane on top of the crystals (E) and (F). Crystals are subsequently translocated to the interstitium, where they disintegrate/dissolve amidst resident and recruited inflammatory cells (G) and (H). Sections B, D, F and H are PAS/PCNA stained sections serial to the Von Kossa stained sections A, C, E and G. Histological sections are from rats treated with 1% ethylene glycol.

Adapted with permission from Vervaet *et al*. *Kidney Int*, 2009 Jan; 75(1): 41–51. An active renal crystal clearance mechanism in rat and man.



	APN	Transplants	Preterm inf.	Prim. Hyperoxal.
Number of patients	16	10	8	2
Number of biopsies	16	20	8	3
Number of crystal containing biosies	16	12	8	3
Number of crystal containing biosies showing overgrown crystals	16	6	7	3

Fig. 204.9 Frequency and examples of epithelial cells covering crystals in human kidney biopsies with nephrocalcinosis of different aetiology.
Adapted with permission from Vervaet *et al. Kidney Int*, 2009 Jan; 75(1): 41–51. An active renal crystal clearance mechanism in rat and man.

pass through the epithelial cells, nor is it truly paracellular as the crystals do not migrate between two healthy cells, but apparently are translocated together with the remains of the original attachment site. Once exposed to the interstitial environment, crystals are being degraded and dissolved amidst a limited number of resident and recruited inflammatory cells. As crystal deposits actually are conglomerates of mineral material and an organic matrix, and, in addition, can be dissolved *in vitro* in solutions with low pH, it is currently hypothesized that crystals are cleared by the combined and local action of proteases and proton secretion (Ryall et al., 2001; Fleming et al., 2003; Grover et al., 2008; Vervaet et al., 2009). Roughly 90% of adhered crystals can be cleared by 'transepithelial uptake' and the time frame from crystal adhesion up to completion of crystal clearance takes (<) 2 weeks. A very small number of translocated crystals ends up in granulomatous like structures (De Bruijn et al., 1994, 1995; Vervaet et al., 2009), which appear to last longer in the kidney.

With respect to the mechanism driving 'transepithelial uptake', it is known that calcium oxalate crystals are able to induce cellular proliferation, and hence may induce their own 'transepithelial uptake' (Lieske et al., 1992). However, it is now clear that other crystal types, such as calcium phosphate, cystine, adenine, and melamine, can be overgrown by neighbouring tubular epithelial cells as well (Vervaet et al., 2009). This indicates that not the crystal type per se but rather a conserved reaction of an injured tubular epithelium drives this process. As described above, retained crystals are generally associated with dedifferentiated tubular epithelial cells. Dedifferentiation of epithelial cells is particularly characterized by the loss of tight junctions with their neighbouring cells. As epithelial cells, by default, strive to maximize cell–cell contact, overgrowth of crystals is probably the result of the natural ability of the tubular epithelium to restore epithelial integrity and functionality.

Animal models

Unlike humans, rodents like rats and mice hardly form crystals in the tubular fluid or urine and do not spontaneously present pathologies with associated nephrocalcinosis or nephrolithiasis. Therefore, their use as *in vivo* models for human renal pathological mineralization particularly requires the deliberate induction of calcium oxalate or calcium phosphate supersaturation (and subsequent crystal formation) in the urinary system. Table 204.4 enlists the major experimental approaches for achieving urinary supersaturation and resultant nephrocalcinosis. The general principles behind these animal models are: (1) direct excess delivery of the respective ions (as a salt) to the animal body either via the drinking water, the food, injection, or subcutaneous minipump infusion; (2) physiologic interference with ion handling; (3) the use of metabolic precursors of oxalate; (4) surgical procedures; and (5) genetic modifications.

It should be noted that although the majority of these models were developed to investigate the mechanisms involved in calcium-salt nephrolithiasis, they rarely succeed in inducing the experimental correlate of clinically relevant kidney stones, however, they consistently induce intratubular nephrocalcinosis. Furthermore, although their use has provided particular valuable information in this respect, it should also be mentioned that there still is a lack of animal models mimicking many of the diseases known to cause, or at least be associated with, nephrocalcinosis in humans. Development

Table 204.4 Classification of common nephrocalcinosis models according to their approach

Direct administration	Physiologic interference	Metabolic precursor	Surgical procedure	Genetic modification
Hypercalciuria				
	Furosemide (Alon et al., 1996)			CLC-5 KO (Wang et al., 2000)
	Vitamin D administration (Sanderson, 1959)			GHS inbred rat (Bushinsky et al., 1995)
	PTH administration (Caulfield and Schrag, 1964)			
Hyperphosphaturia				
Inorganic phosphate (Bushinsky et al., 2000)				CLC-5 KO (Wang et al., 2000) NaPi-2 KO (Chau et al., 2003)
Hyperoxaluria				
NaOx (Khan et al., 1982), KOx (Marengo et al., 2004), NH_4Ox (Kumar et al., 1991)	Vitamin B_6 deficiency (Di Tommaso et al., 2002)	Ethylene glycol (Boeve et al., 1993)	Bowel resection (Worcester et al., 2006)	AGT-1 KO (Salido et al., 2006)
	Magnesium deficiency (Grimm et al., 1990)	Glyoxylate (Okada et al., 2007)		
	High protein diet (Amanzadeh et al., 2003)	Hydroxyproline (Khan et al., 2006)		

AGT-1, alanine-glyoxylate aminotransferase 1; Ca, calcium; CLC-5, chloride channel 5; GHS, genetic hypercalciuric stone forming rat; KO, knockout; KOx, potassium oxalate; NaOx, sodium oxalate; NaPi-2, sodium phosphate cotransporter 2; NH_4Ox, ammonium oxalate.

of such models might prove useful in revealing and understanding new aetiological and mechanistic aspects of renal crystallization.

Clinical features of intratubular nephrocalcinosis

Intratubular nephrocalcinosis is as harmful to renal function as the number of tubules it functionally impairs. Whereas the mechanism of tubular impairment and its consequences are straightforward for *obstruction*, it is more difficult to ascribe any direct deleterious effect to mere *crystal adhesion*. Since both processes differ in their nature, different ways of affecting renal function are to be expected. While obstruction presents itself rather acutely, adhesion might have chronic effects adding to the severity of an already underlying pathology or condition.

Tubular obstruction acutely impairs tubular function by *mechanical blockage* of tubular fluid flow, followed by tubular atrophy, interstitial inflammation, and interstitial fibrosis, hence acute kidney injury and even chronic renal damage/insufficiency (Chevalier, 2006; Markowitz et al., 2005; Gonlusen et al., 2006; Bani-Hani et al., 2008). Histological evidence was found in pathologies with acute and/or excessive forms of crystal formation and subsequent intratubular retention such as acute phosphate nephropathy (Markowitz et al., 2004), primary hyperoxaluria, jejuno-ileal bypass-induced enteric hyperoxaluria (Evan et al., 2003; Worcester et al., 2006), several drug-induced crystal nephropathies (methotrexate, aciclovir) (Perazella, 1999; Yarlagadda and Perazella, 2008) and recently melamine (Brown et al., 2007; Evan et al., 2006; Puschner et al., 2007; Bhalla et al., 2009; Hau et al., 2009). Since the histopathology in these different types of crystal deposition shows important parallels with classical (ureteral) obstructive nephropathy, the bulk of the associated tubulointerstitial changes most likely results from obstruction itself rather than of a *chemical (nephrotoxic) effect* of the retained crystals. With respect to nephrolithiasis, it has been observed that in hyperparathyroid, brushite, and cystine stone formers and patients with distal renal tubular acidosis, *tubular obstruction* presents itself as calcium phosphate (cystine in cystine stone formers) crystal plugging of the ducts of Bellini with crystals protruding out of the papillary slits/mouths into the pelvic lumen (Evan et al., 2005, 2006, 2007b, 2008b). It is hypothesized that these crystal plugs, besides inducing fibrosis, tubular atrophy, and even glomerular pathology (Evan et al., 2006), can form the nidus or platform for stone formation in these kidneys (Williams et al., 2006; Evan et al., 2007a; Miller et al., 2009).

In less severe/acute forms of nephrocalcinosis, as found in transplant patients and preterm infants, the effect of mere *crystal adhesion* to the tubular epithelium might be more straightforward. It can be logically inferred that the physical presence of adhered crystals may affect normal tubular fluid flow and proper cellular/tubular function. Furthermore, one may assume that crystals may hamper the redifferentiation/regeneration of the cells to which they are attached, thereby impeding restoration of a sufficient amount of functioning tubules after an insult. In extremis, once adhered, crystals may enlarge by growth and aggregation with other crystals, which in turn may lead to obstructive tubulopathy. Numerous *in vitro* studies investigating the epithelial reaction to calcium phosphate and calcium oxalate crystal contact (either apical or basolateral), report production of inflammatory mediators (MCP-1, PGE-2) and reactive oxygen species (ROS) and release of lactate dehydrogenase (LDH), all being indicative for injury (Khan et al., 1992; de Water et al., 2001; Umekawa et al., 2003, 2006; Schepers et al., 2005a; Escobar et al., 2008). Moreover, several *in vivo* experiments and observations in renal necropsy tissue of patients with oxalosis demonstrated an association between renal crystal

deposition and a mild inflammatory reaction, with macrophages and multinucleated giant cells as the main inflammatory cells in the vicinity of the crystals (Lieske et al., 1992; de Water et al., 1996, 1999, 2000). However, despite these reactions and observations *in vitro* and *in vivo*, up to now no long lasting clinical detrimental effect of crystal–cell contact or adhesion has been unequivocally proven. In kidney transplant patients, Pinheiro et al. reported a 12-year allograft survival of 75% in the absence of nephrocalcinosis, whereas in the presence of nephrocalcinosis allograft survival decreased to 48% (Pinheiro et al., 2005). Although these data suggest an association between nephrocalcinosis and an increased risk of allograft failure, it should be noted that half of the allografts survive despite the presence of nephrocalcinosis. Even so, Habbig et al. reported that intratubular calcification in allografts of paediatric patients was not correlated to a worse graft outcome (Habbig et al., 2009). Likewise, Bagnasco et al. observed that renal function at 2 years post transplant did not differ between control grafts and grafts with nephrocalcinosis (Bagnasco et al., 2009). However, at 1 year post transplant, renal function of the latter grafts was found to be significantly lower than controls, suggesting only a short-term and transient detrimental effect of crystal deposition (Bagnasco et al., 2009).

In distal tubular acidosis, a disorder known to be associated with an increased incidence of nephrocalcinosis and deterioration of renal function, cases have been reported in which renal function remained intact despite the presence of renal crystal deposits (Bajpai et al., 2005; Evan et al., 2007b) or in which nephrocalcinosis had no apparent effect on the development of renal failure (Caruana and Buckalew, 1988). Furthermore, in several prospective and retrospective studies, in which preterm neonates with nephrocalcinosis were compared with birth-weight and postnatal (or gestational) age-matched controls without nephrocalcinosis, no clear evidence for an association between neonatal nephrocalcinosis and renal dysfunction in the long term was found (Jones et al., 1997; Saarela et al., 1999a; Abitbol et al., 2003; Porter et al., 2006; Kist-Van Holthe et al., 2007). Remarkably, several preterm follow-up studies even describe resolution of renal calcification (in up to 75% of patients) with age and discontinuation or adaptation of neonate diuretic therapy, corroborating the existence of crystal clearing mechanisms (Hufnagle et al., 1982; Downing et al., 1991; Alon et al., 1994; Saarela et al., 1999a, 1999b; Hoppe et al., 2002; Schell-Feith et al., 2003, 2010; Porter et al., 2006; Kist-Van Holthe et al., 2007; Lee et al., 2007). Finally, the controversial effect of crystal deposition on renal function is further supported by an interesting observation in patients who underwent combined liver–kidney transplantation for treatment of primary hyperoxaluria. After transplantation, early failure of the renal graft frequently occurs and is generally attributed to new calcium oxalate crystal deposition as the body gets rid of its stored oxalate overload (Worcester et al., 1994). By histological examination of protocol biopsies, Noel et al. observed that, despite progressive renal failure, the amount of renal oxalate deposits decreased, suggesting that crystal deposition does not continuously contribute to renal deterioration (Noel et al., 1992). These observations, however, do not exclude the possibility that retained crystals may have initiated the process. Nonetheless, whereas deterioration of renal allografts eventually always occurs, nephrocalcinosis is not consistently found in these kidneys, making its contribution to the functional course of the allograft hard to assess.

Overall, it is likely that the individual renal outcome depends on numerous factors, such as the severity of the underlying disorder and the extent, rate, and duration of crystal formation/adhesion on the one hand and the activity of renal crystal clearing mechanisms on the other hand (Lieske et al., 1994, 1997; Grover et al., 2008; Vervaet et al., 2008).

Interstitial nephrocalcinosis

Mechanism of interstitial nephrocalcinosis

The presence of crystals (calcium oxalate or calcium phosphate) in the renal interstitium is defined as interstitial nephrocalcinosis. Two independent mechanisms may explain the appearance of these crystals in the interstitium, translocation of intratubular crystals and *de novo* interstitial crystal formation.

It has been hypothesized that translocation of crystals can be established via transcytosis, a process during which small intraluminal crystals are internalized within apical vesicles (either or not receptor mediated) and translocated transcellularly to the basolateral side where the crystals are released into the interstitial extracellular environment (Lieske et al., 1997). Although apical endocytosis of small crystals has been described (Lieske et al., 1994, 1997; Schepers et al., 2003), there is no evidence today supporting the basolateral release of crystals into the interstitium. Instead, these crystals are likely to disintegrate in lysosomes (Lieske et al., 1997; Grover et al., 2008). Alternatively, De Bruyn et al. and Vervaet et al. demonstrated *in vivo* transepithelial crystal uptake with subsequent interstitial degradation/dissolution (De Bruijn et al., 1994, 1995; Vervaet et al., 2009) (see 'Post-crystal-adhesion defence mechanisms' and Fig. 204.8). Both endocytosis and transepithelial crystal uptake do not seem to be pathologic processes, and therefore are to be considered defence mechanisms against renal calcification since crystals disappear from the healthy kidney either in intracellular vesicles (lysosomes) or via the extracellular interstitium (Lieske et al., 1992, 1997; Grover et al., 2008; Vervaet et al., 2009). Deficiency or saturation of these clearance mechanisms, however, would reasonably result in tubular and/or interstitial crystal accumulation. In addition, it is currently unknown, what happens to the ionic constituents of the crystals after their dissolution. Are they merely washed out of the kidney or do they accumulate and, at a later stage, contribute to interstitial supersaturation and *de novo* crystal formation?

Besides translocation, crystals can also be formed *de novo* in the interstitium. Interestingly, although various crystal types can be found in the interstitium (e.g. calcium phosphate, calcium oxalate and adenine (Evan et al., 2003; Vervaet et al., 2009)), up to now, *de novo* crystal formation has only been observed for calcium phosphate crystals (Evan et al., 2005, 2006, 2008b; Coe et al., 2010a). By detailed histopathological and ultrastructural analysis of papillary biopsies of idiopathic calcium oxalate stone formers, Evan and co-workers were able to identify the basement membrane of the thin loops of Henle as the initial site of *de novo* interstitial crystal formation, characterized by scattered microscopic apatite deposits (Evan et al., 2003) (Fig. 204.10). These authors further illustrated that growth of these crystals is likely to cause the initial deposits to coalesce and extend into the medullary interstitium, outgrowing towards the papillary surface where they form a plaque either lying beneath or protruding into the urothelium. The presence of these suburothelial apatite plaques in stone formers was recognized

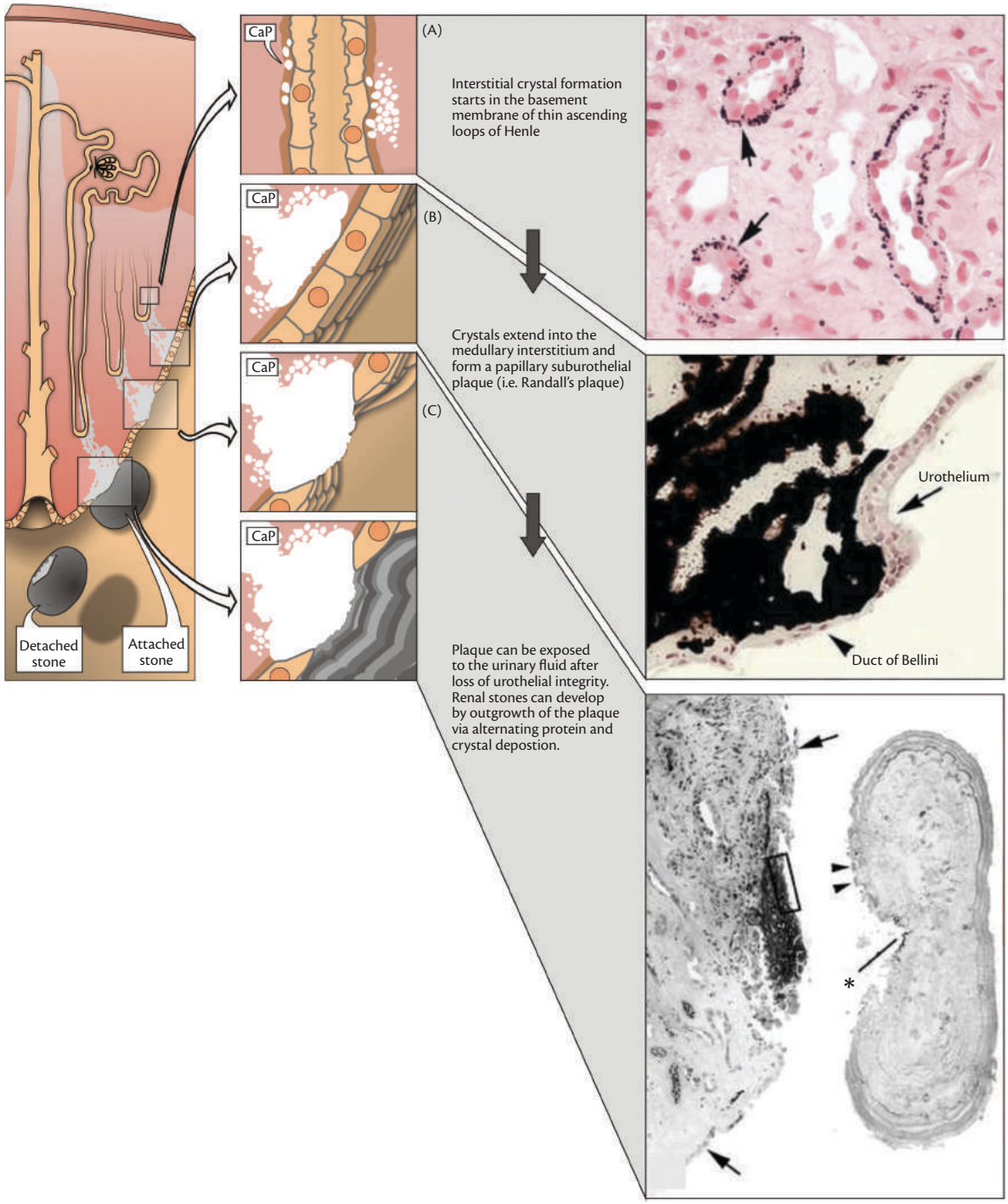


Fig. 204.10 Histopathological course of *de novo* interstitial nephrocalcinosis, known as Randall plaque formation, which may result in nephrolithiasis. (A), (B), and (C) medullary biopsy sections of patients with idiopathic calcium oxalate nephrolithiasis. Crystal deposits are stained black by the Yasue method. (A) Identification of the initial site and size of calcium phosphate deposition in and beneath the basement membrane of thin loops of Henle. (B) Regions of interstitial plaque located near the papillary tip with crystal accumulation beneath the urothelium and around the distal ends of ducts of Bellini. (C) Biopsy section of an endoscopically confirmed attached kidney stone. During the sectioning process the stone detached, so the underlying renal tissue (left side) and the stone (right side) have been approximated in this figure. A large base of interstitial plaque (black) is completely devoid of its normal urothelial lining cells. The detached stone also shows small darkly stained plaque remnants (*) consistent with what can be expected of prior attachment. Arrow and arrowheads show intact epithelial cells.

Adapted with permission from Vervaet *et al. Nephrol Dial Transplant*, 2009 Jul; 24(7):2030–5. Nephrocalcinosis: new insights into mechanisms and consequences. Evan *et al. J Clin Invest*, 2003 Mar; 111(5):607–16. Randall's plaque of patients with nephrolithiasis begins in basement membranes of thin loops of Henle. Coe *et al. Urol Res*, 2010 Jun; 38(3):147–60. Three pathways for human kidney stone formation.

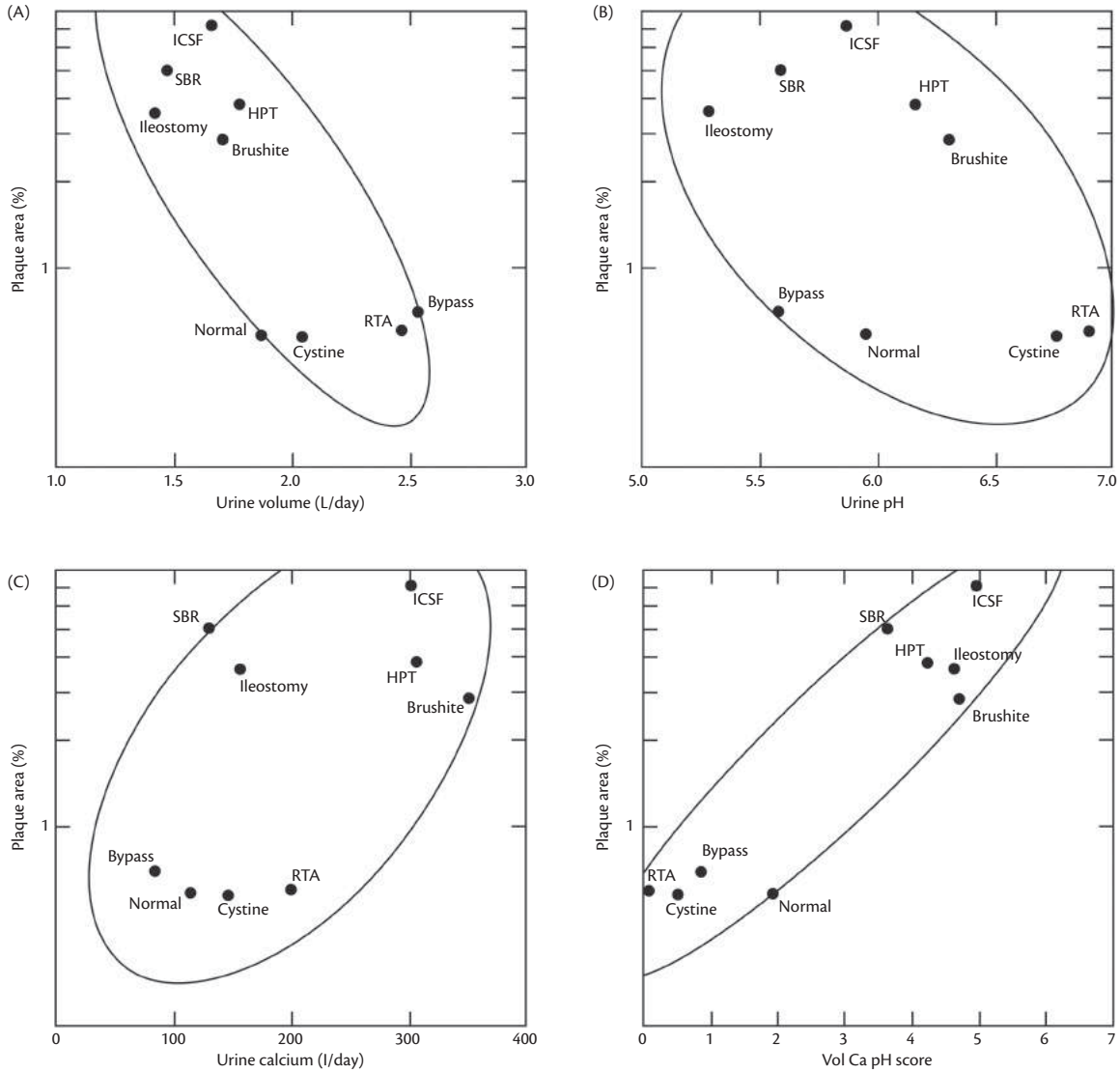


Fig. 204.11 Relationship between papillary Randall's plaque surface area and urine chemistry. Ellipses are 68% nonparametric containment estimates. Plaque area versus urinary volume (A), pH (B), and calcium (C). (D) Plaque area versus multivariate combined effects of urine volume, pH and calcium excretion. Graphs show normals and stone formers of different aetiology. ICSF, idiopathic calcium oxalate stone formers; SBR, Small bowel resection; HPT, hyperparathyroidism; Brushite: brushite stone formers; Bypass, intestinal bypass; RTA, renal tubular acidosis; Cystine, cystine stone formers.

Adapted with permission from Coe *et al. Urol Res*, 2010; 38:239–47. Plaque and deposits in nine human stone diseases.

in the 1930s by Alexander Randall, hence their name, 'Randall's plaques' (Randall, 1937, 1940). As stones attached to the papilla in idiopathic calcium oxalate stone formers consistently are found attached to Randall's plaques, it is thought that the calcium oxalate stones of this patient group actually initiate and grow on plaques exposed to the pelvic urine (Williams et al., 2006; Evan et al., 2007a; Miller et al., 2009).

With respect to the mechanism underlying *de novo* interstitial crystal formation, it has been hypothesized that epithelial and/or interstitial osteoblast-like cells might actively be involved. However, up to now, no *in vivo* evidence of an osteoblast-like phenotype has been reported in calcified kidneys (Evan and Bledsoe, 2008). Alternatively, based on multivariate analysis on urinary volume, urinary calcium, urinary pH, and papillary plaque coverage in several stone forming pathologies and controls, Coe and colleagues observed a strong correspondence between renal physiology (i.e. urine calcium molarity and pH) and plaque formation (Kuo et al., 2003; Coe et al., 2010a) (Fig. 204.11). This indicates that interstitial crystal formation (starting in and beneath the basement membrane of the epithelium of the thin loops of Henle) most likely is the pathologic result of a chemically driven supersaturation inherent to the structural and functional organization of the kidney. The key question, as posed by Halperin et al. (2006), here is 'What factors lead to high enough concentrations of calcium and phosphate in the inner medullary interstitial compartment so that their ion product exceeds their solubility product constant and calcium phosphate crystals form?' Although the mechanism of interstitial crystal formation is far from being understood, several proposed mechanisms might shed light on two important aspects in the answer to this question: (1) 'How do calcium and phosphate reach

the interstitial compartment?' and (2) 'How do they obtain their active form, that is, ionized calcium and trivalent phosphate, allowing them to form crystals?'

With respect to (increased) calcium delivery, a 'vas washdown' mechanism (Coe et al., 2010a) could be proposed. The thick ascending limb, being impermeable to water, reabsorbs calcium (~20% of filtered load) into the outer medullary interstitial fluid, thereby enriching the blood in the vasa recta with calcium. Given the countercurrent flow organization of the kidney, the calcium-enriched blood travels down, making exposure of the basolateral side of the thin loop epithelium to a calcium enriched interstitial fluid likely. The slow intratubular and intravascular fluid flow (~10% of the cortical flow) in this renal region, which is important in building up the osmotic gradient responsible for the concentrating ability of the kidney, is likely to contribute significantly to this process. Likewise, it can be hypothesized that phosphate, of which 10% of the filtered load is reabsorbed in the distal tubules, may also be washed down into the medullary interstitium via the peritubular capillaries. This might explain why pathological conditions either increasing the filtered load of calcium and phosphate or affecting proper tubular ion handling may lead to increased interstitial delivery of calcium and phosphate. It should be mentioned, however, that these hypotheses need verification by quantitative data on the actual concentrations and dynamics of calcium and phosphate in the renal interstitium, both in health and disease.

Next to increased delivery of calcium and phosphate, the concentrations of their active forms are critical regarding crystal formation. With respect to calcium, both the reabsorbed amount and changes in ionic strength may modify the concentration of ionized calcium in the medullary interstitial compartment. However, in particular with respect to phosphate, interstitial pH appears to be a critically important factor as variations in pH determine the concentration of trivalent phosphate. A high pH (alkaline environment) increases the concentration of trivalent phosphate (and vice versa). In the kidney, the two sites where addition of alkali ($HCO3^-$) into the medullary interstitium can be most important are the loop of Henle (via reabsorption of $NaHCO_3$) and the medullary collecting duct (via H^+/K^+-ATPase-mediated K^+ reabsorption) (Halperin et al., 2006; Kamel et al., 2007). Increased alkalinization in this renal region may therefore substantially add to the risk of calcium phosphate precipitation. As for calcium and phosphate, quantitative measurements of interstitial pH in the vicinity of thin loop basement membranes, in health and disease, are currently lacking. The lack of clear data on this matter probably reflects the technical challenges this kind of research encompasses.

Clinical features of interstitial nephrocalcinosis

Currently, no evidence has been provided showing that mere interstitial nephrocalcinosis, whether *de novo* or acquired via translocation, impairs renal function. Even the most minute calcium phosphate particles (as predecessors of Randall's plaques) are formed *de novo* in basement membranes of morphologically normal epithelial cells of Henle's loop, are not associated with inflammation, and do not appear to damage epithelial cells. Even when these interstitial particles grow into the vicinity of collecting ducts and ducts of Bellini, these epithelia show no morphological abnormalities (Evan et al., 2003). Only when calcification completely surrounds the thin loops of Henle, has an association with epithelial injury has been found (Evan et al., 2003). In addition, extensive calcification might present itself as a physical interstitial barrier impairing proper medullary/papillary function. However, this might only be a local effect as, based on the observed correlation between low urinary volume (and high urinary calcium) and papillary plaque coverage (Kuo et al., 2003), the overall concentrating ability seems not to be influenced by interstitial calcification. Whether in the context of Randall's plaque formation the absence of morphological epithelial injury and interstitial inflammation/fibrosis is due to the crystal type (consistently being calcium phosphate) or to the site of origin is currently not known.

Currently, the primary known clinically important feature of Randall's plaques is their proposed anchor function for stones found attached to the papillae of stone formers (Sallis, 1987; Evan et al., 2003). This concept was first proposed by Alexander Randall in the late 1930s (Randall, 1937, 1940). He observed calcium phosphate lesions (plaques) on the papillary surface and noticed that renal stones were intimately attached to them. The incidence of these papillary plaques, however, was higher than that of (attached) clinical renal stones. In addition, Randall noticed that the mineral type of the stones was generally different from that of the plaques, the latter being consistently made up of calcium phosphate. Based on these observations, he concluded that renal stones originated as a slow deposition/crystallization of urinary salts (calcium oxalate, calcium phosphate or uric acid) upon a lesion of the renal papilla. Recent endoscopic, surgical, and histopathological studies confirm the presence of Randall's plaques in a variety of different types of stone formers (Evan et al., 2003, 2005, 2006, 2008a, 2008b, 2009; Coe et al., 2010a) (Table 204.1). However, attachment of stones to Randall's plaques so far has only been established for idiopathic calcium oxalate stone formers (Evan et al., 2003, 2007a; Miller et al., 2009). Ultrastructural studies of the interface between calcium phosphate plaque and calcium oxalate stones in these patients indicate that stones develop on a plaque by progressive alternation of successive protein matrix deposition and crystal nucleation (Evan et al., 2007a). Importantly, these stones may not stay attached indefinitely, however, and may detach and be excreted or grow further in the pelvis, ureter, or bladder. Consistent with this view is the observation of calcium phosphate (apatite) remnants either on their surface for recently detached stones or within the stone body for older stones (Fig. 204.12) (Miller et al., 2010).

In contrast to Randall's plaques, initially appearing innocuous, it is known that interstitial calcium oxalate crystals can be associated with inflammatory cells (De Water et al., 1999, 2000; Vervaet et al., 2008). Whether this is due to a higher reactivity of calcium oxalate is not known. Although it has been shown that fibroblasts can produce inflammatory mediators upon contact with oxalate ions and calcium oxalate crystals (Umekawa et al., 2006), it is of interest that interstitial crystals and associated inflammation are always associated with intraluminal crystal formation. The recruitment of inflammatory cells may therefore already be initiated by prior interactions between crystals and the apical (luminal) membrane of epithelial cells known to result in the production of inflammatory mediators by the latter (Khan et al., 1992; Umekawa et al., 2003, 2006; Schepers et al., 2005a; Escobar et al., 2008). Altogether, it can be suggested that interstitial inflammation associated with interstitial crystal deposition can only be found in disorders presenting with intraluminal crystal formation and/or handling.

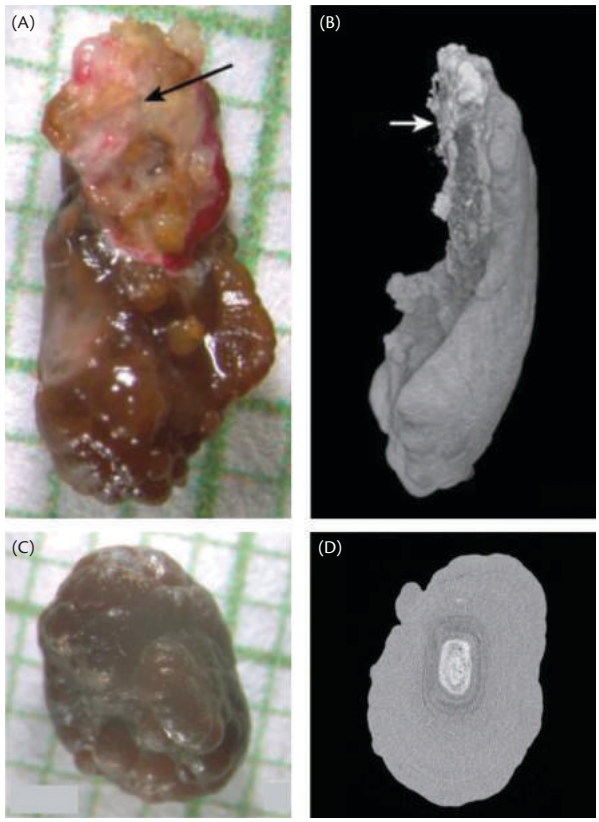


Fig. 204.12 Two examples of stones found loose in idiopathic calcium oxalate stone formers. (A) View of stone immediately after removal, showing region (*) that appeared to be covered with a mixture of tissue and mucus. (B) Surface image of μCT reconstruction of stone in (A); closest to the viewer is the right edge of view (A). Arrow indicates surface corresponding to marked region in (A). This region showed material at the surface that was x-ray bright, indicating the presence of calcium phosphate (CaP), most likely in the form of apatite. (C) View of stone showing no evidence of having been recently attached to tissue. (D) μCT section through stone in (C) shows significant region of interior CaP (white region), surrounded by calcium oxalate monohydrate. Background grid in (A) and (C) shows 1 mm squares.

Adapted with permission from Miller *et al. BJU Int*, 2010 Jan; 105(2):242–5. In idiopathic calcium oxalate stone-formers, unattached stones show evidence of having originated as attached stones on Randall's plaque.

Animal models

Whereas intratubular nephrocalcinosis can be induced in animal models rather easily, inducing interstitial nephrocalcinosis appears to be a challenging task. Up to now, *de novo* formation of interstitial calcium phosphate crystals has only been observed in two mutant mice models, the sodium–hydrogen exchanger regulatory factor (NHERF-1)$^{-/-}$ and THP$^{-/-}$ mice (Evan et al., 2010; Liu et al., 2010).

NHERF-1 has recently been identified to regulate both transcription and membrane insertion of the type IIa sodium-dependent phosphate cotransporter Npt2a (Khundmiri et al., 2008). Since Npt2a is responsible for the reabsorption of about 80% of the phosphate filtered at the glomerulus, NHERF-1 knockouts, not surprisingly, present a mineral disorder phenotype. In NHERF-1 null mice, Shenolikar et al demonstrated decreased brush-border membrane Npt2a expression and increased expression of Npt2a in intracellular vesicles of proximal renal tubules (Shenolikar et al., 2002). These molecular changes were accompanied by hyperphosphaturia, hypercalciuria (as a result of increased active vitamin D synthesis in the hypophosphataemic animal), hyperuricosuria, decreased bone mineral content, and failure to adapt to phosphate deprivation, hence a pathophysiologic state favouring renal calcification (Shenolikar et al., 2002; Cunningham et al., 2005). Histological investigation of renal tissue of these mice by Weinman et al. (2006) and Evan et al. (2010) showed interstitial calcium salt deposition confined to the renal medulla, and particularly in animals aged 1 year or older. Although the deposits are found in the interstitium, there are two important differences compared to Randall plaque formation as observed in idiopathic calcium oxalate stone formers (ICSF) (Evan et al., 2003). First, interstitial crystal deposition in ICSF is much more abundant than in NHERF-1 knockouts and second, the initial location of the deposits in the NHERF-1 null mice was not limited to the basement membranes of the thin loops of Henle (as was shown for ICFS (Evan et al., 2003)) but was equally present in the basement membranes of the inner medullary collecting ducts and thin loops of Henle.

THP, or uromodulin, is a kidney-specific, glycosylated protein, specifically expressed by the epithelial cells of the thick ascending limb of Henle's loop and mainly located at the apical plasma membrane of those cells (Bachmann et al., 1985). Under physiological conditions, THP is the most abundant protein in urine (Kumar and Muchmore, 1990). Although its precise biological function remains rather elusive, it has been hypothesized to play a role in water/electrolyte handling in the thick ascending limb (Wiggins, 1987; Ying and Sanders, 1998) and suspected to act as a defence protein against urinary tract infection and renal stone formation (Raffi et al., 2006; Mo et al., 2004, 2007; Kumar and Lieske, 2006). With respect to the latter, a THP knockout model has been generated in which medullary crystal deposition in the kidney already occurs at age 2–3 months (Liu et al., 2010). In accordance with the NHERF-1$^{-/-}$ model, these deposits are mainly located in the interstitial space and are surrounding both the thin loops of Henle and the inner medullary collecting ducts. In contrast to NHERF-1$^{-/-}$ mice, however, THP$^{-/-}$ mice showed intratubular crystal deposition as well, particularly during the first 8 months of life (Liu et al., 2010). It should be mentioned here that another THP knockout model generated by Raffi et al. (2006), even at follow up until 3 years, did not develop any type of renal crystals. Possibly, variations in genetic background and knockout targeting strategy are responsible for this marked difference (Liu et al., 2010).

Concluding paragraph

In the last decade, it became clear that from a cell biological and clinical perspective nephrocalcinosis and nephrolithiasis are to be considered two independent manifestations of a wide variety of underlying clinicopathological conditions. Histopathologic differentiation between intratubular and interstitial nephrocalcinosis has proven useful in developing an understanding of how and under which conditions intratubular or interstitial nephrocalcinosis might progress to nephrolithiasis.

With respect to their aetiological mechanisms, it can be stated that intratubular nephrocalcinosis particularly develops as a consequence of phenotypical changes of the tubular epithelium, which, due to prior injury, gains crystal-binding properties. These findings therefore suggest that diseased kidneys, presenting dedifferentiated epithelia, have a considerably increased risk of developing

intratubular nephrocalcinosis when crystal formation occurs. Considering interstitial nephrocalcinosis it can be stated that *de novo* interstitial crystal formation, rather than being caused by some prior epithelial injury, is the result of (patho)physiological disturbances in proper ion and/or acid–base handling.

As to the question 'Why do some patients develop renal insufficiency attributed to nephrocalcinosis, whereas others do not?', it can be stated that 'Kidneys undergoing crystal deposition at an extent, duration, or rate above a certain threshold might deteriorate, whilst below this threshold the kidney may (continuously) clear the adhered crystal deposits and secure renal function'.

References

Abid, M. R., Razzaque, M. S., and Taguchi, T. (2005). Oxidant stress in renal pathophysiology. *Contrib Nephrol*, 148, 135–53.

Abitbol, C. L., Bauer, C. R., Montane, B., *et al.* (2003). Long-term follow-up of extremely low birth weight infants with neonatal renal failure. *Pediatr Nephrol*, 18, 887–93.

Aihara, K., Byer, K. J., and Khan, S. R. (2003). Calcium phosphate-induced renal epithelial injury and stone formation: involvement of reactive oxygen species. *Kidney Int*, 64, 1283–91.

Alon, U. S. (1997). Nephrocalcinosis. *Curr Opin Pediatr*, 9, 160–5.

Alon, U. S., Kaplan, R. A., Gratny, L. L., *et al.* (1996). Histological long-term outcome of furosemide-induced nephrocalcinosis in the young rat. *Pediatr Nephrol*, 10, 191–4.

Alon, U. S., Scagliotti, D., and Garola, R. E. (1994). Nephrocalcinosis and nephrolithiasis in infants with congestive heart failure treated with furosemide. *J Pediatr*, 125, 149–51.

Amanzadeh, J., Gitomer, W. L., Zerwekh, J. E., *et al.* (2003). Effect of high protein diet on stone-forming propensity and bone loss in rats. *Kidney Int*, 64, 2142–9.

Andreoli, S. P. (1991). Reactive oxygen molecules, oxidant injury and renal disease. *Pediatr Nephrol*, 5, 733–42.

Angell, A. H. and Resnick, M. I. (1989). Surface interaction between glycosaminoglycans and calcium oxalate. *J Urol*, 141, 1255–8.

Antus, B., Exton, M. S., and Rosivall, L. (2001). Angiotensin II: a regulator of inflammation during renal disease? *Int J Immunopathol Pharmacol*, 14, 25–30.

Asplin, J. R., Arsenault, D., Parks, J. H., *et al.* (1998). Contribution of human uropontin to inhibition of calcium oxalate crystallization. *Kidney Int*, 53, 194–9.

Asplin, J. R., Mandel, N. S., and Coe, F. L. (1996). Evidence of calcium phosphate supersaturation in the loop of Henle. *Am J Physiol*, 270, F604–13.

Asplin, J. R., Parks, J. H., Chen, M. S., *et al.* (1999). Reduced crystallization inhibition by urine from men with nephrolithiasis. *Kidney Int*, 56, 1505–16.

Asplin, J. R., Parks, J. H., and Coe, F. L. (1997). Dependence of upper limit of metastability on supersaturation in nephrolithiasis. *Kidney Int*, 52, 1602–8.

Asplin, J. R., Parks, J. H., Nakagawa, Y., *et al.* (2002). Reduced crystallization inhibition by urine from women with nephrolithiasis. *Kidney Int*, 61, 1821–9.

Asselman, M., Verhulst, A., De Broe, M. E., *et al.* (2003). Calcium oxalate crystal adherence to hyaluronan-, osteopontin-, and CD44-expressing injured/regenerating tubular epithelial cells in rat kidneys. *J Am Soc Nephrol* 14, 3155–66.

Asselman, M., Verhulst, A., Van Ballegooijen, E. S., *et al.* (2005). Hyaluronan is apically secreted and expressed by proliferating or regenerating renal tubular cells. *Kidney Int*, 68, 71–83.

Atmani, F., Lacour, B., Strecker, G., *et al.* (1993). Molecular characteristics of uronic-acid-rich protein, a strong inhibitor of calcium oxalate crystallization in vitro. *Biochem Biophys Res Commun*, 191, 1158–65.

Atmani, F., Mizon, J., and Khan, S. R. (1996). Inter-alpha-inhibitor: a protein family involved in the inhibition of calcium oxalate crystallization. *Scanning Microsc*, 10, 425–33.

Bachmann, S., Koeppen-Hagemann, I., and Kriz, W. (1985). Ultrastructural localization of Tamm-Horsfall glycoprotein (THP) in rat kidney as revealed by protein A-gold immunocytochemistry. *Histochemistry*, 83, 531–8.

Bagnasco, S. M., Mohammed, B. S., Mani, H., *et al.* (2009). Oxalate deposits in biopsies from native and transplanted kidneys, and impact on graft function. *Nephrol Dial Transplant*, 24, 1319–25.

Bajpai, A., Bagga, A., Hari, P., *et al.* (2005). Long-term outcome in children with primary distal renal tubular acidosis. *Indian Pediatr*, 42, 321–8.

Bani-Hani, A. H., Campbell, M. T., Meldrum, D. R., *et al.* (2008). Cytokines in epithelial-mesenchymal transition: a new insight into obstructive nephropathy. *J Urol*, 180, 461–8.

Basavaraj, D. R., Biyani, C. S., Browning, A. J., *et al.* (2007). The role of urinary kidney stone inhibitors and promoters in the pathogenesis of calcium containing renal stones. *EAU-EBU Update Series*, 5, 126–36.

Baud, L. and Ardaillou, R. (1986). Reactive oxygen species: production and role in the kidney. *Am J Physiol*, 251, F765–76.

Baud, L. and Ardaillou, R. (1993). Involvement of reactive oxygen species in kidney damage. *Br Med Bull*, 49, 621–9.

Baumann, J. M., Lauber, K., Lustenberger, F. X., *et al.* (1985). Crystallization conditions in urine of patients with idiopathic recurrent calcium nephrolithiasis and with hyperparathyroidism. *Urol Res*, 13, 169–74.

Belliveau, J. and Griffin, H. (2001). The solubility of calcium oxalate in tissue culture media. *Anal Biochem*, 291, 69–73.

Bhalla, V., Grimm, P. C., Chertow, G. M., *et al.* (2009). Melamine nephrotoxicity: an emerging epidemic in an era of globalization. *Kidney Int*, 75, 774–9.

Bigelow, M. W., Wiessner, J. H., Kleinman, J. G., *et al.* (1997). Surface exposure of phosphatidylserine increases calcium oxalate crystal attachment to IMCD cells. *Am J Physiol*, 272, F55–62.

Bigelow, M. W., Wiessner, J. H., Kleinman, J. G., *et al.* (1998). Calcium oxalate crystal attachment to cultured kidney epithelial cell lines. *J Urol*, 160, 1528–32.

Boeve, E. R., Ketelaars, G. A., Vermeij, M., *et al.* (1993). An ultrastructural study of experimentally induced microliths in rat proximal and distal tubules. *J Urol*, 149, 893–9.

Borghi, L., Meschi, T., Amato, F., *et al.* (1996). Urinary volume, water and recurrences in idiopathic calcium nephrolithiasis: a 5-year randomized prospective study. *J Urol*, 155, 839–43.

Bowyer, R. C., Brockis, J. G., and McCulloch, R. K. (1979). Glycosaminoglycans as inhibitors of calcium oxalate crystal growth and aggregation. *Clin Chim Acta*, 95, 23–8.

Brown, C. A., Jeong, K. S., Poppenga, R. H., *et al.* (2007). Outbreaks of renal failure associated with melamine and cyanuric acid in dogs and cats in 2004 and 2007. *J Vet Diagn Invest*, 19, 525–31.

Bushinsky, D. A., Grynpas, M. D., Nilsson, E. L., *et al.* (1995). Stone formation in genetic hypercalciuric rats. *Kidney Int*, 48, 1705–13.

Bushinsky, D. A., Parker, W. R., and Asplin, J. R. (2000). Calcium phosphate supersaturation regulates stone formation in genetic hypercalciuric stone-forming rats. *Kidney Int*, 57, 550–60.

Cao, L. C., Jonassen, J., Honeyman, T. W., *et al.* (2001). Oxalate-induced redistribution of phosphatidylserine in renal epithelial cells: implications for kidney stone disease. *Am J Nephrol*, 21, 69–77.

Carr, G., Simmons, N. L., and Sayer, J. A. (2006). Disruption of clc-5 leads to a redistribution of annexin A2 and promotes calcium crystal agglomeration in collecting duct epithelial cells. *Cell Mol Life Sci*, 63, 367–77.

Carruthers, B. M., Copp, D. H., and McIntosh, H. W. (1964). Diurnal variation in urinary excretion of calcium and phosphate and its relation to blood levels. *J Lab Clin Med*, 63, 959–68.

Caruana, R. J. and Buckalew, V. M., Jr. (1988). The syndrome of distal (type 1) renal tubular acidosis. Clinical and laboratory findings in 58 cases. *Medicine (Baltimore)*, 67, 84–99.

Caudarella, R. and Vescini, F. (2009). Urinary citrate and renal stone disease: the preventive role of alkali citrate treatment. *Arch Ital Urol Androl*, 81, 182–7.

Caulfield, J. B. and Schrag, P. E. (1964). Electron microscopic study of renal calcification. *Am J Pathol*, 44, 365–81.

Cerini, C., Geider, S., Dussol, B., *et al.* (1999). Nucleation of calcium oxalate crystals by albumin: involvement in the prevention of stone formation. *Kidney Int*, 55, 1776–86.

Chau, H., El Maadawy, S., McKee, M. D., *et al.* (2003). Renal calcification in mice homozygous for the disrupted type IIa Na/Pi cotransporter gene Npt2. *J Bone Miner Res*, 18, 644–57.

Chevalier, R. L. (2006). Pathogenesis of renal injury in obstructive uropathy. *Curr Opin Pediatr*, 18, 153–60.

Coe, F. L., Evan, A. P., Lingeman, J. E., *et al.* (2010a). Plaque and deposits in nine human stone diseases. *Urol Res*, 38, 239–47.

Coe, F. L., Evan, A. P., Worcester, E. M., *et al.* (2010b). Three pathways for human kidney stone formation. *Urol Res*, 38, 147–60.

Coe, F. L., Parks, J. H., Evan, A., *et al.* (2007). Pathogenesis and treatment of nephrolithiasis. In R. J. Alpern and S. C. Hebert (eds.) *Seldin and Giebisch's The Kidney*, pp. 1945–77. Burlington, MA: Academic Press.

Cunningham, R., Xiaofei, E., Steplock, D., *et al.* (2005). Defective PTH regulation of sodium-dependent phosphate transport in NHERF-1$^{-/-}$ renal proximal tubule cells and wild-type cells adapted to low-phosphate media. *Am J Physiol Renal Physiol*, 289, F933–8.

De Albuquerque, P. F. and De Paola, D. (1959). Experiments in urolithiasis: I. Histochemical studies. *J Urol*, 81(3), 345–50.

De Bruijn, W. C., Boeve, E. R., van Run, P. R., *et al.* (1994). Etiology of experimental calcium oxalate monohydrate nephrolithiasis in rats. *Scanning Microsc*, 8, 541–9.

De Bruijn, W. C., Boeve, E. R., van Run, P. R., *et al.* (1995). Etiology of calcium oxalate nephrolithiasis in rats. I. Can this be a model for human stone formation? *Scanning Microsc*, 9, 103–14.

De Bruijn, W. C., de Water, R., Boeve, E. R., *et al.* (1996). Lectin-cytochemistry of experimental rat nephrolithiasis. *Scanning Microsc*, 10, 557–76.

De Bruijn, W. C., Ketelaars, G. A., Boeve, E. R., *et al.* (1993). Electron energy-loss spectroscopical and image analysis of experimentally induced rat microliths. II. *J Urol*, 149, 900–5.

De Water, R., Boeve, E. R., van Miert, P. P., *et al.* (1996). Pathological and immunocytochemical changes in chronic calcium oxalate nephrolithiasis in the rat. *Scanning Microsc*, 10, 577–87.

De Water, R., Leenen, P. J., Noordermeer, C., *et al.* (2001). Cytokine production induced by binding and processing of calcium oxalate crystals in cultured macrophages. *Am J Kidney Dis*, 38, 331–8.

De Water, R., Noordermeer, C., Houtsmuller, A. B., *et al.* (2000). Role of macrophages in nephrolithiasis in rats: an analysis of the renal interstitium. *Am J Kidney Dis*, 36, 615–25.

De Water, R., Noordermeer, C., van der Kwast, T. H., *et al.* (1999). Calcium oxalate nephrolithiasis: effect of renal crystal deposition on the cellular composition of the renal interstitium. *Am J Kidney Dis*, 33, 761–71.

Di Tommaso, L., Tolomelli, B., Mezzini, R., *et al.* (2002). Renal calcium phosphate and oxalate deposition in prolonged vitamin B6 deficiency: studies on a rat model of urolithiasis. *BJU Int*, 89, 571–5.

Dillingham, M. A., Dixon, B. S., and Anderson, R. J. (1987). Calcium modulates vasopressin effect in rabbit cortical collecting tubule. *Am J Physiol*, 252, F115–21.

Downing, G. J., Egelhoff, J. C., Daily, D. K., *et al.* (1991). Furosemide-related renal calcifications in the premature infant. A longitudinal ultrasonographic study. *Pediatr Radiol*, 21, 563–5.

Dykstra, M. J. and Hackett, R. L. (1979). Ultrastructural events in early calcium oxalate crystal formation in rats. *Kidney Int*, 15, 640–50.

Ebisuno, S., Kohjimoto, Y., Tamura, M., *et al.* (1997). Histological observations of the adhesion and endocytosis of calcium oxalate crystals in MDCK cells and in rat and human kidney. *Urol Int*, 58, 227–31.

Ebisuno, S., Umehara, M., Kohjimoto, Y., *et al.* (1999). The effects of human urine on the adhesion of calcium oxalate crystal to Madin-Darby canine kidney cells. *BJU Int*, 84, 118–22.

Elliot, J. S. and Rabinowitz, I. N. (1980). Calcium oxalate crystalluria: crystal size in urine. *J Urol*, 123, 324–7.

Elliot, J. S., Rabinowitz, I. N., and Silvert, M. (1976). Calcium oxalate crystalluria. *J Urol*, 116, 773–5.

Erdem, A., Gundogan, N. U., Usubutun, A., *et al.* (2000). The protective effect of taurine against gentamicin-induced acute tubular necrosis in rats. *Nephrol Dial Transplant*, 15, 1175–82.

Erickson, S. B., Cooper, K., Broadus, A. E., *et al.* (1984). Oxalate absorption and postprandial urine supersaturation in an experimental human model of absorptive hypercalciuria. *Clin Sci (Lond)*, 67, 131–8.

Escobar, C., Byer, K. J., Khaskheli, H., *et al.* (2008). Apatite induced renal epithelial injury: insight into the pathogenesis of kidney stones. *J Urol*, 180, 379–87.

Evan, A. E., Lingeman, J. E., Coe, F. L., *et al.* (2008b). Histopathology and surgical anatomy of patients with primary hyperparathyroidism and calcium phosphate stones. *Kidney Int*, 74, 223–9.

Evan, A. P. (2010). Physiopathology and etiology of stone formation in the kidney and the urinary tract. *Pediatr Nephrol*, 25(5), 831–41.

Evan, A. P. and Bledsoe, S. B. (2008). Bone genes in the kidney stone former. In A. P. Evan, J. A. McAteer, J. E. Lingeman, *et al.* (eds.) *Renal Stone Disease. Proceedings of the Second International Urolithiasis Research Symposium*. American Institute of Physics, Melville, pp. 33–43.

Evan, A. P., Coe, F. L., Gillen, D., *et al.* (2008a). Renal intratubular crystals and hyaluronan staining occur in stone formers with bypass surgery but not with idiopathic calcium oxalate stones. *Anat Rec (Hoboken)*, 291, 325–34.

Evan, A. P., Coe, F. L., Lingeman, J. E., *et al.* (2006). Renal crystal deposits and histopathology in patients with cystine stones. *Kidney Int*, 69, 2227–35.

Evan, A. P., Coe, F. L., Lingeman, J. E., *et al.* (2007a). Mechanism of formation of human calcium oxalate renal stones on Randall's plaque. *Anat Rec (Hoboken)* 290, 1315–23.

Evan, A. P., Lingeman, J. E., Coe, F. L, *et al.* (2009). Intra-tubular deposits, urine and stone composition are divergent in patients with ileostomy. *Kidney Int*, 76, 1081–8.

Evan, A. P., Lingeman, J. E., Coe, F. L., *et al.* (2003). Randall's plaque of patients with nephrolithiasis begins in basement membranes of thin loops of Henle. *J Clin Invest*, 111, 607–16.

Evan, A. P., Lingeman, J. E., Coe, F. L., *et al.* (2005). Crystal-associated nephropathy in patients with brushite nephrolithiasis. *Kidney Int*, 67, 576–91.

Evan, A. P., Lingeman, J., Coe, F., *et al.* (2007b). Renal histopathology of stone-forming patients with distal renal tubular acidosis. *Kidney Int*, 71, 795–801.

Evan, A. P., Weinman, E. J., Wu, X. R., *et al.* (2010). Comparison of the pathology of interstitial plaque in human ICSF stone patients to NHERF-1 and THP-null mice. *Urol Res*, 38, 439–52.

Evenepoel, P., Lerut, E., Naesens, M., *et al.* (2009). Localization, etiology and impact of calcium phosphate deposits in renal allografts. *Am J Transplant*, 9, 2470–8.

Eylar, E. H., Madoff, M. A., Brody, O. V., *et al.* (1962). The contribution of sialic acid to the surface charge of the erythrocyte. *J Biol Chem*, 237, 1992–2000.

Fasano, J. M. and Khan, S. R. (2001). Intratubular crystallization of calcium oxalate in the presence of membrane vesicles: an in vitro study. *Kidney Int*, 59, 169–78

Finlayson, B. and Reid, F. (1978). The expectation of free and fixed particles in urinary stone disease. *Invest Urol*, 15, 442–8.

Fleming, D. E., Van, R. A., Chauvet, M. C., *et al.* (2003). Intracrystalline proteins and urolithiasis: a synchrotron X-ray diffraction study of calcium oxalate monohydrate. *J Bone Miner Res*, 18, 1282–91.

Freel, R. W., Hatch, M., Green, M., *et al.* (2006). Ileal oxalate absorption and urinary oxalate excretion are enhanced in Slc26a6 null mice. *Am J Physiol Gastrointest Liver Physiol*, 290, G719–28.

Fumagalli, M., Ramenghi, L. A., Pisoni, S., *et al.* (2011). Total body cooling: skin and renal complications. *Arch Dis Child Fetal Neonatal Ed*, 96, F377.

Gambaro, G., Valente, M. L., Zanetti, E., *et al.* (2006). Mild tubular damage induces calcium oxalate crystalluria in a model of subtle hyperoxaluria: evidence that a second hit is necessary for renal lithogenesis. *J Am Soc Nephrol*, 17, 2213–19.

Gill, W. B., Ruggiero, K., and Straus, F. H. (1979). Crystallization studies in a urothelial-lined living test tube (the catheterized female rat bladder). I. Calcium oxalate crystal adhesion to the chemically injured rat bladder. *Invest Urol*, 17, 257–61.

Glaeser, R. M. and Mel, H. C. (1964). The electrophoretic behavior of osmium tetroxide-fixed and potassium permanganate-fixed rat erythrocytes. *Biochim Biophys Acta*, 79, 606–17.

Glaeser, R. M. and Mel, H. C. (1966). Microelectrophoretic and enzymic studies concerning the carbohydrate at the surface of rat erythrocytes. *Arch Biochem Biophys*, 113, 77–82.

Gonlusen, G., Akgun, H., Ertan, A., *et al.* (2006). Renal failure and nephrocalcinosis associated with oral sodium phosphate bowel cleansing: clinical patterns and renal biopsy findings. *Arch Pathol Lab Med*, 130, 101–6.

Greger, R., Lang, F., Oberleithner, H., *et al.* (1978). Handling of oxalate by the rat kidney. *Pflugers Arch*, 374, 243–8.

Grimm, P., Nowitzki, S., and Classen, H. G. (1990). Nephrocalcinosis without functional renal impairment in rats subjected to subacute moderate magnesium deficiency, and intervention studies on the mobilization of calcium deposits. *Magnes Res*, 3, 87–92.

Grover, P. K., Moritz, R. L., Simpson, R. J., *et al.* (1998). Inhibition of growth and aggregation of calcium oxalate crystals in vitro—a comparison of four human proteins. *Eur J Biochem*, 253, 637–44.

Grover, P. K. and Ryall, R. L. (1999). Inhibition of calcium oxalate crystal growth and aggregation by prothrombin and its fragments in vitro: relationship between protein structure and inhibitory activity. *Eur J Biochem*, 263, 50–6.

Grover, P. K., Ryall, R. L., and Marshall, V. R. (1990). Does Tamm-Horsfall mucoprotein inhibit or promote calcium oxalate crystallization in human urine? *Clin Chim Acta*, 190, 223–38.

Grover, P. K., Thurgood, L. A., Fleming, D. E., *et al.* (2008). Intracrystalline urinary proteins facilitate degradation and dissolution of calcium oxalate crystals in cultured renal cells. *Am J Physiol Renal Physiol*, 294, F355–61.

Guo, C., Cenac, T. A., Li, Y., *et al.* (2007). Calcium oxalate, and not other metabolites, is responsible for the renal toxicity of ethylene glycol. *Toxicol Lett*, 173, 8–16.

Guo, C. and McMartin, K. E. (2005). The cytotoxicity of oxalate, metabolite of ethylene glycol, is due to calcium oxalate monohydrate formation. *Toxicology*, 208, 347–55.

Gwinner, W., Suppa, S., Mengel, M., *et al.* (2005). Early calcification of renal allografts detected by protocol biopsies: causes and clinical implications. *Am J Transplant*, 5, 1934–41.

Habbig, S., Beck, B. B., Feldkotter, M., *et al.* (2009). Renal allograft calcification—prevalence and etiology in pediatric patients. *Am J Nephrol*, 30, 194–200.

Hackett, R. L., Shevock, P. N., and Khan, S. R. (1990). Cell injury associated calcium oxalate crystalluria. *J Urol*, 144, 1535–8.

Haggitt, R. C. and Pitcock, J. A. (1971). Renal medullary calcifications: a light and electron microscopic study. *J Urol*, 106, 342–7.

Hallson, P. C., Rose, G. A., and Sulaiman, S. (1982). Magnesium reduces calcium oxalate crystal formation in human whole urine. *Clin Sci (Lond)*, 62, 17–19.

Halperin, M. L., Cheema, D. S., and Kamel, K. S. (2006). Physiology of acid-base balance: links with kidney stone prevention. *Semin Nephrol*, 26, 441–6.

Hammes, M. S., Lieske, J. C., Pawar, S., *et al.* (1995). Calcium oxalate monohydrate crystals stimulate gene expression in renal epithelial cells. *Kidney Int*, 48, 501–9.

Hau, A. K., Kwan, T. H., and Li, P. K. (2009). Melamine toxicity and the kidney. *J Am Soc Nephrol*, 20, 245–50.

Haydon, D. A. and Seaman, G. V. (1967). Electrokinetic studies on the ultrastructure of the human erythrocyte. I. Electrophoresis at high ionic strengths—the cell as a polyanion. *Arch Biochem Biophys*, 122, 126–36.

Hebert, S. C. (1996). Extracellular calcium-sensing receptor: implications for calcium and magnesium handling in the kidney. *Kidney Int*, 50, 2129–39.

Hebert, S. C., Brown, E. M., and Harris, H. W. (1997). Role of the Ca(2+)-sensing receptor in divalent mineral ion homeostasis. *J Exp Biol*, 200, 295–302.

Henle, F. G. (1863). Zur Anatomie der Nierre. *Goettingen Nachrichten*, 125–35.

Hess, B. (1992). Tamm-Horsfall glycoprotein—inhibitor or promoter of calcium oxalate monohydrate crystallization processes? *Urol Res*, 20, 83–6.

Hodgkinson, A. (1980). Solubility of calcium oxalate in human urine, simulated urine, and water. *Invest Urol*, 18, 123–6.

Hodgkinson, A. and Heaton, F. W. (1965). The effect of food ingestion on the urinary excretion of calcium and magnesium. *Clin Chim Acta*, 11,354–62.

Hoppe, B., Duran, I., Martin, A., *et al.* (2002). Nephrocalcinosis in preterm infants: a single center experience. *Pediatr Nephrol*, 17, 264–8.

Huang, H. S., Chen, J., Chen, C. F., *et al.* (2006). Vitamin E attenuates crystal formation in rat kidneys: roles of renal tubular cell death and crystallization inhibitors. *Kidney Int*, 70, 699–710.

Hufnagle, K. G., Khan, S. N., Penn, D., *et al.* (1982). Renal calcifications: a complication of long-term furosemide therapy in preterm infants. *Pediatrics*, 70, 360–3.

Huggins, C. B. (1933). Bone and calculi in the collecting tubules of the kidney. *Arch Surg*, 27, 203–15.

Itoh, Y., Yasui, T., Okada, A., *et al.* (2005). Preventive effects of green tea on renal stone formation and the role of oxidative stress in nephrolithiasis. *J Urol*, 173, 271–5.

Jaggi, M., Nakagawa, Y., Zipperle, L., *et al.* (2007). Tamm-Horsfall protein in recurrent calcium kidney stone formers with positive family history: abnormalities in urinary excretion, molecular structure and function. *Urol Res*, 35, 55–62.

Jones, C. A., King, S., Shaw, N. J., *et al.* (1997). Renal calcification in preterm infants: follow up at 4-5 years. *Arch Dis Child Fetal Neonatal Ed*, 76, F185–9.

Jones, S. M., Frindt, G., and Windhager, E. E. (1988). Effect of peritubular [Ca] or ionomycin on hydrosmotic response of CCTs to ADH or cAMP. *Am J Physiol*, 254, F240–53.

Kamel, K. S., Shafiee, M. A., Cheema-Dhadli, S., *et al.* (2007). Studies to identify the basis for an alkaline urine pH in patients with calcium hydrogen phosphate kidney stones. *Nephrol Dial Transplant*, 22, 424–31.

Khan, S. R. (1995). Calcium oxalate crystal interaction with renal tubular epithelium, mechanism of crystal adhesion and its impact on stone development. *Urol Res*, 23, 71–9.

Khan, S. R. (2004a). Crystal-induced inflammation of the kidneys: results from human studies, animal models, and tissue-culture studies. *Clin Exp Nephrol*, 8, 75–88.

Khan, S. R. (2004b). Role of renal epithelial cells in the initiation of calcium oxalate stones. *Nephron Exp Nephrol*, 98, e55–60.

Khan, S. R. (2005). Hyperoxaluria-induced oxidative stress and antioxidants for renal protection. *Urol Res*, 33, 349–57.

Khan, S. R. and Canales, B. K. (2009). Genetic basis of renal cellular dysfunction and the formation of kidney stones. *Urol Res*, 37(4), 169–80.

Khan, S. R., Cockrell, C. A., Finlayson, B., *et al.* (1984). Crystal retention by injured urothelium of the rat urinary bladder. *J Urol*, 132, 153–7.

Khan, S. R. and Glenton, P. A. (2008). Calcium oxalate crystal deposition in kidneys of hypercalciuric mice with disrupted type IIa sodium-phosphate cotransporter. *Am J Physiol Renal Physiol*, 294, F1109–15.

Khan, S. R., Finlayson, B., and Hackett, R. L. (1979). Histologic study of the early events in oxalate induced intranephronic calculosis. *Invest Urol*, 17, 199–202.

Khan, S. R., Finlayson, B., and Hackett, R. L. (1982). Experimental calcium oxalate nephrolithiasis in the rat. Role of the renal papilla. *Am J Pathol*, 107, 59–69.

Khan, S. R., Glenton, P. A., and Byer, K. J. (2006). Modeling of hyperoxaluric calcium oxalate nephrolithiasis: experimental induction of hyperoxaluria by hydroxy-L-proline. *Kidney Int*, 70, 914–23.

Khan, S. R. and Hackett, R. L. (1980). The use of SEM in the study of oxalate induced experimental nephrolithiasis. *Scan Electron Microsc*, 379–86.

Khan, S. R., Hackett, R. L., Finlayson, B., *et al.* (1981). Light and scanning electron microscopic studies of oxamide urolithiasis in rats. *Scan Electron Microsc*, 155–62.

Khan, S. R. and Kok, D.J. (2004). Modulators of urinary stone formation. *Front Biosci*, 9,1450–82.
Khan, S. R., Maslamani, S. A., Atmani, F., *et al.* (2000). Membranes and their constituents as promoters of calcium oxalate crystal formation in human urine. *Calcif Tissue Int*, 66, 90–6.
Khan, S. R., Shevock, P. N., and Hackett, R. L. (1992). Acute hyperoxaluria, renal injury and calcium oxalate urolithiasis. *J Urol*, 147, 226–30.
Khaskhali, M. H., Byer, K. J., and Khan, S. R. (2009). The effect of calcium on calcium oxalate monohydrate crystal-induced renal epithelial injury. *Urol Res*, 37, 1–6.
Khundmiri, S. J., Ahmad, A., Bennett, R. E., *et al.* (2008). Novel regulatory function for NHERF-1 in Npt2a transcription. *Am J Physiol Renal Physiol*, 294, F840–9.
King, J. S., Jr. (1967). Etiologic factors involved in urolithiasis: a review of recent research. *J Urol*, 97, 583–91.
Kist-Van Holthe, J. E., van Zwieten, P. H., Schell-Feith, E. A., *et al.* (2007). Is nephrocalcinosis in preterm neonates harmful for long-term blood pressure and renal function? *Pediatrics*, 119, 468–75.
Kleinman, J. G., Sorokina, E. A., and Wesson, J. A. (2010). Induction of apoptosis with cisplatin enhances calcium oxalate crystal adherence to inner medullary collecting duct cells. *Urol Res*, 38(2), 97–104.
Knight, T. F., Sansom, S. C., Senekjian, H. O., *et al.* (1981). Oxalate secretion in the rat proximal tubule. *Am J Physiol*, 240, F295–8.
Kohri, K., Kodama, M., Ishikawa, Y., *et al.* (1991). Immunofluorescent study on the interaction between collagen and calcium oxalate crystals in the renal tubules. *Eur Urol*, 19, 249–52.
Kok, D. J. (1991). *The Role of Crystallization Processes in Calcium Oxalate Urolithiasis*. Dissertation, University of Leiden.
Kok, D. J. (1996). Crystallization and stone formation inside the nephron. *Scanning Microsc*,10, 471–84.
Kok, D. J. (1997). Intratubular crystallization events. *World J Urol*, 15, 219–28.
Kok, D. J. and Khan, S. R. (1994). Calcium oxalate nephrolithiasis, a free or fixed particle disease. *Kidney Int*, 46, 847–54.
Konya, E., Umekawa, T., Iguchi, M., *et al.* (2003). The role of osteopontin on calcium oxalate crystal formation. *Eur Urol*, 43, 564–71.
Kumar, S. and Muchmore, A. (1990). Tamm-Horsfall protein—uromodulin (1950-1990). *Kidney Int*, 37, 1395–401.
Kumar, S., Sigmon, D., Miller, T., *et al.* (1991). A new model of nephrolithiasis involving tubular dysfunction/injury. *J Urol*, 146, 1384–9.
Kumar, V. and Lieske, J. C. (2006). Protein regulation of intrarenal crystallization. *Curr Opin Nephrol Hypertens*, 15, 374–80.
Kumar, V., Farell, G., Deganello, S., *et al.* (2003). Annexin II is present on renal epithelial cells and binds calcium oxalate monohydrate crystals. *J Am Soc Nephrol*, 14, 289–97.
Kummeling, M. T., de Jong, B. W., Laffeber, C., *et al.* (2007). Tubular and interstitial nephrocalcinosis. *J Urol*, 178, 1097–103.
Kuo, R. L., Lingeman, J. E., Evan, A. P., *et al.* (2003). Urine calcium and volume predict coverage of renal papilla by Randall's plaque. *Kidney Int*, 64, 2150–4.
Lee, C. T., Chen, H. C., Lai, L. W., *et al.* (2007). Effects of furosemide on renal calcium handling. *Am J Physiol Renal Physiol*, 293, F1231–7.
Li, C. Y., Deng, Y. L., and Sun, B. H. (2009). Taurine protected kidney from oxidative injury through mitochondrial-linked pathway in a rat model of nephrolithiasis. *Urol Res*, 37, 211–20.
Liang, L., Chen, J., Vittal, R., *et al.* (2006). Expression profiling of crystal-induced injury in human kidney epithelial cells. *Nephron Physiol*, 103, 53–62.
Lichtenstern, R. (1926). Mikrolithiasis. *Wien Klin Wchnschr*, 39, 1156.
Lieske, J. C., Leonard, R., and Toback, F. G. (1995). Adhesion of calcium oxalate monohydrate crystals to renal epithelial cells is inhibited by specific anions. *Am J Physiol*, 268, F604–12.
Lieske, J. C., Leonard, R., Swift, H., *et al.* (1996). Adhesion of calcium oxalate monohydrate crystals to anionic sites on the surface of renal epithelial cells. *Am J Physiol*, 270, F192–9.
Lieske, J. C., Norris, R., Swift, H., *et al.* (1997). Adhesion, internalization and metabolism of calcium oxalate monohydrate crystals by renal epithelial cells. *Kidney Int*, 52, 1291–301.
Lieske, J. C., Norris, R., and Toback, F. G. (1997). Adhesion of hydroxyapatite crystals to anionic sites on the surface of renal epithelial cells. *Am J Physiol*, 273, F224–33.
Lieske, J. C., Spargo, B. H., and Toback, F. G. (1992). Endocytosis of calcium oxalate crystals and proliferation of renal tubular epithelial cells in a patient with type 1 primary hyperoxaluria. *J Urol*, 148, 1517–9.
Lieske, J. C., Swift, H., Martin, T., *et al.* (1994). Renal epithelial cells rapidly bind and internalize calcium oxalate monohydrate crystals. *Proc Natl Acad Sci U S A*, 91, 6987–91.
Lieske, J. C., Toback, F. G., and Deganello, S. (2001). Sialic acid-containing glycoproteins on renal cells determine nucleation of calcium oxalate dihydrate crystals. *Kidney Int*, 60, 1784–91.
Lieske, J. C., Walsh-Reitz, M. M., and Toback, F. G. (1992). Calcium oxalate monohydrate crystals are endocytosed by renal epithelial cells and induce proliferation. *Am J Physiol*, 262, F622–30.
Liu, Y., Mo, L., Goldfarb, D. S., *et al.* (2010). Progressive renal papillary calcification and ureteral stone formation in mice deficient for Tamm-Horsfall protein. *Am J Physiol Renal Physiol*, 299, F469–78.
Lohi, H., Kujala, M., Makela, S., *et al.* (2002). Functional characterization of three novel tissue-specific anion exchangers SLC26A7, -A8, and -A9. *J Biol Chem*, 277, 14246–54.
Low, R. K. and Stoller, M. L. (1997). Endoscopic mapping of renal papillae for Randall's plaques in patients with urinary stone disease. *J Urol* 158, 2062–4.
Mandel, N. (1994). Crystal-membrane interaction in kidney stone disease. *J Am Soc Nephrol*, 5, S37–45.
Manna, P., Sinha, M., and Sil, P. C. (2009). Taurine plays a beneficial role against cadmium-induced oxidative renal dysfunction. *Amino Acids*, 36, 417–28.
Marengo, S. R., Chen, D. H., MacLennan, G. T., *et al.* (2004). Minipump induced hyperoxaluria and crystal deposition in rats: a model for calcium oxalate urolithiasis. *J Urol*, 171, 1304–8.
Marengo, S. R. and Romani, A. M. (2008). Oxalate in renal stone disease: the terminal metabolite that just won't go away. *Nat Clin Pract Nephrol*, 4, 368–77.
Markovich, D. and Aronson, P. S. (2007). Specificity and regulation of renal sulfate transporters. *Annu Rev Physiol*, 69,361–75.
Markowitz, G. S., Nasr, S. H., Klein, P., *et al.* (2004). Renal failure due to acute nephrocalcinosis following oral sodium phosphate bowel cleansing. *Hum Pathol*, 35, 675–84.
Markowitz, G. S., Stokes, M. B., Radhakrishnan, J., *et al.* (2005). Acute phosphate nephropathy following oral sodium phosphate bowel purgative: an underrecognized cause of chronic renal failure. *J Am Soc Nephrol*, 16, 3389–96.
Massey, L. K., Roman-Smith, H., and Sutton, R. A. (1993). Effect of dietary oxalate and calcium on urinary oxalate and risk of formation of calcium oxalate kidney stones. *J Am Diet Assoc*, 93, 901–6.
McMartin, K. E. and Wallace, K. B. (2005). Calcium oxalate monohydrate, a metabolite of ethylene glycol, is toxic for rat renal mitochondrial function. *Toxicol Sci*, 84, 195–200.
Medetognon-Benissan, J., Tardivel, S., Hennequin, C., *et al.* (1999). Inhibitory effect of bikunin on calcium oxalate crystallization in vitro and urinary bikunin decrease in renal stone formers. *Urol Res*, 27, 69–75.
Meyer, J. L. and Smith, L. H. (1975). Growth of calcium oxalate crystals. II. Inhibition by natural urinary crystal growth inhibitors. *Invest Urol*, 13, 36–9.
Miller, N. L., Gillen, D. L., Williams, J. C., Jr. *et al.* (2009). A formal test of the hypothesis that idiopathic calcium oxalate stones grow on Randall's plaque. *BJU Int*, 103(7), 966–71.
Miller, N. L., Williams, J. C., Jr., Evan, A. P., *et al.* (2010). In idiopathic calcium oxalate stone-formers, unattached stones show evidence of having originated as attached stones on Randall's plaque. *BJU Int*, 105, 242–5.
Mo, L., Huang, H. Y., Zhu, X. H, *et al.* (2004). Tamm-Horsfall protein is a critical renal defense factor protecting against calcium oxalate crystal formation. *Kidney Int*, 66, 1159–66.

Mo, L., Liaw, L., Evan, A. P., *et al.* (2007). Renal calcinosis and stone formation in mice lacking osteopontin, Tamm-Horsfall protein, or both. *Am J Physiol Renal Physiol*, 293, F1935–43.

Mo, L., Zhu, X. H., Huang, H. Y., *et al.* (2004). Ablation of the Tamm-Horsfall protein gene increases susceptibility of mice to bladder colonization by type 1-fimbriated Escherichia coli. *Am J Physiol Renal Physiol*, 286, F795–802.

Morgenroth, K., Jr., Backmann, R., and Blaschke, R. (1968). [On the forms of calcium oxalate deposits in the human kidney in oxalosis]. *Beitr Pathol Anat*, 136, 454–63.

Mount, D. B. and Romero, M. F. (2004). The SLC26 gene family of multifunctional anion exchangers. *Pflugers Arch*, 447, 710–21.

Murayama, T., Sakai, N., Yamada, T., *et al.* (2001). Role of the diurnal variation of urinary pH and urinary calcium in urolithiasis: a study in outpatients. *Int J Urol*, 8, 525–31.

Nancollas, G. H., Tsortos, A., and Zieba, A. (1996). The nucleation and growth of calcium phosphate crystals at protein and phosphatidylserine liposome surfaces. *Scanning Microsc*, 10, 499–507.

Nankivell, B. J., Borrows, R. J., Fung, C. L., *et al.* (2003). The natural history of chronic allograft nephropathy. *N Engl J Med*, 349, 2326–33.

Noel, C., Pruvot, F. R., Talaska, A., *et al.* (1992). [Transplantation for primary hyperoxaluria. Role of oxalate crystal deposits in the occurrence of kidney failure]. *Presse Med*, 21, 1997–8.

Ogawa, Y. (1993a). Circadian rhythms of urinary saturation levels of calcium oxalate and calcium phosphate in normal male individuals. *Hinyokika Kiyo*, 39, 785–9.

Ogawa, Y. (1993b). Modification of estimation of the urinary ion-activity products of calcium oxalate and calcium phosphate. *Hinyokika Kiyo*, 39, 407–11.

Okada, A., Nomura, S., Higashibata, Y., *et al.* (2007). Successful formation of calcium oxalate crystal deposition in mouse kidney by intraabdominal glyoxylate injection. *Urol Res*, 35, 89–99.

Oliver, J. (1968). *Nephrons and Kidneys*. New York: Hoeber.

Oliver, J., MacDowell, M., Whang, R., *et al.* (1966). The renal lesions of electrolyte imbalance. IV. The intranephronic calculosis of experimental magnesium depletion. *J Exp Med*, 124, 263–78.

Parks, J. H., Coe, F. L., Evan, A. P., *et al.* (2009). Urine pH in renal calcium stone formers who do and do not increase stone phosphate content with time. *Nephrol Dial Transplant*, 24, 130–6.

Perazella, M. A. (1999). Crystal-induced acute renal failure. *Am J Med*, 106, 459–65.

Pillay, S. N., Asplin, J. R., and Coe, F. L. (1998). Evidence that calgranulin is produced by kidney cells and is an inhibitor of calcium oxalate crystallization. *Am J Physiol*, 275, F255–61.

Pinheiro, H. S., Camara, N. O., Osaki, K. S., *et al.* (2005). Early presence of calcium oxalate deposition in kidney graft biopsies is associated with poor long-term graft survival. *Am J Transplant*, 5, 323–9.

Porter, E., McKie, A., Beattie, T. J., *et al.* (2006). Neonatal nephrocalcinosis: long term follow up. *Arch Dis Child Fetal Neonatal Ed*, 91, F333–6.

Puschner, B., Poppenga, R. H., Lowenstine, L. J., *et al.* (2007). Assessment of melamine and cyanuric acid toxicity in cats. *J Vet Diagn Invest*, 19, 616–24.

Raffi, H., Bates, J. M., Laszik, Z., *et al.* (2006). Tamm-Horsfall protein knockout mice do not develop medullary cystic kidney disease. *Kidney Int*, 69, 1914–15.

Randall, A. (1937). The origin and growth of renal calculi. *Ann Surg*, 105, 1009–27.

Randall, A. (1940). Papillary pathology as a precursor of primary renal calculus. *J Urol*, 44, 580–8.

Renkema, K. Y., Velic, A., Dijkman, H. B. *et al.* (2009). The calcium-sensing receptor promotes urinary acidification to prevent nephrolithiasis. *J Am Soc Nephrol*, 20, 1705–13.

Riese, R. J., Mandel, N. S., Wiessner, J. H., *et al.* (1992). Cell polarity and calcium oxalate crystal adherence to cultured collecting duct cells. *Am J Physiol*, 262, F177–84.

Riese, R. J., Riese, J. W., Kleinman, J. G., *et al.* (1988). Specificity in calcium oxalate adherence to papillary epithelial cells in cultures. *Am J Physiol*, 255, F1025–32.

Robert, M., Roux, J. O., Bourelly, F., *et al.* (1994). Circadian variations in the risk of urinary calcium oxalate stone formation. *Br J Urol*, 74, 294–7.

Robertson, W. G. (1969). A method for measuring calcium crystalluria. *Clin Chim Acta*, 26, 105–10.

Robertson, W. G. (2004). Kidney models of calcium oxalate stone formation. *Nephron Physiol*, 98, 21–30.

Robertson, W. G., Hodgkinson, A., and Marshall, D. H. (1977). Seasonal variations in the composition of urine from normal subjects: a longitudinal study. *Clin Chim Acta*, 80, 347–53.

Robertson, W. G. and Peacock, M. (1972). Calcium oxalate crystalluria and inhibitors of crystallization in recurrent renal stone-formers. *Clin Sci*, 43, 499–506.

Robertson, W. G., Peacock, M., and Nordin, B. E. (1969). Calcium crystalluria in recurrent renal-stone formers. *Lancet*, 2, 21–4.

Rodgers, A. L., Ball, D., and Harper, W. (1994). Effect of urinary macromolecules and chondroitin sulphate on calcium oxalate crystallization in urine. *Scanning Microsc*, 8, 71–7.

Rose, G. A. and Sulaiman, S. (1984). Tamm-Horsfall mucoprotein promotes calcium phosphate crystal formation in whole urine: quantitative studies. *Urol Res*, 12, 217–21.

Ryall, R. L. (1997). Urinary inhibitors of calcium oxalate crystallization and their potential role in stone formation. *World J Urol*, 15, 155–64.

Ryall, R. L. (2004). Macromolecules and urolithiasis: parallels and paradoxes. *Nephron Physiol*, 98, 37–42.

Ryall, R. L., Fleming, D. E., Doyle, I. R., *et al.* (2001). Intracrystalline proteins and the hidden ultrastructure of calcium oxalate urinary crystals: implications for kidney stone formation. *J Struct Biol*, 134, 5–14.

Ryall, R. L., Fleming, D. E., Grover, P. K., *et al.* (2000). The hole truth: intracrystalline proteins and calcium oxalate kidney stones. *Mol Urol*, 4, 391–402.

Ryall, R. L., Harnett, R. M., Hibberd, C. M., *et al.* (1991). Effects of chondroitin sulphate, human serum albumin and Tamm-Horsfall mucoprotein on calcium oxalate crystallization in undiluted human urine. *Urol Res*, 19, 181–8.

Ryall, R. L., Hibberd, C. M., Mazzachi, B. C., *et al.* (1986). Inhibitory activity of whole urine: a comparison of urines from stone formers and healthy subjects. *Clin Chim Acta*, 154, 59–67.

Saarela, T., Lanning, P., and Koivisto, M. (1999a). Prematurity-associated nephrocalcinosis and kidney function in early childhood. *Pediatr Nephrol*, 13, 886–90.

Saarela, T., Vaarala, A., Lanning, P., *et al.* (1999b). Incidence, ultrasonic patterns and resolution of nephrocalcinosis in very low birthweight infants. *Acta Paediatr*, 88, 655–60.

Sachtleben, P. and Ruhenstroth-Bauer, G. (1961). Agglutination and the electrical surface potential of red blood cells. *Nature*, 192, 982–3.

Salido, E. C., Li, X. M., Lu, Y., *et al.* (2006). Alanine-glyoxylate aminotransferase-deficient mice, a model for primary hyperoxaluria that responds to adenoviral gene transfer. *Proc Natl Acad Sci U S A*, 103, 18249–54.

Sallis, J. D. (1987). Glycosaminoglycans as inhibitors of stone formation. *Miner Electrolyte Metab*, 13, 273–7.

Sallis, J. D. and Lumley, M. F. (1979). On the possible role of glycosaminoglycans as natural inhibitors of calcium oxalate stones. *Invest Urol*, 16, 296–9.

Sanderson, P. H. (1959). Functional aspects of renal calcification in rats. *Clin Sci (Lond)*, 18, 67–79.

Sarica, K., Erbagci, A., Yagci, F., *et al.* (2004). Limitation of apoptotic changes in renal tubular cell injury induced by hyperoxaluria. *Urol Res*, 32, 271–7.

Sayer, J. A., Carr, G., and Simmons, N. L. (2004). Nephrocalcinosis: molecular insights into calcium precipitation within the kidney. *Clin Sci (Lond)*, 106, 549–61.

Schell-Feith, E. A., Kist-Van Holthe, J. E., Conneman, N., *et al.* (2000). Etiology of nephrocalcinosis in preterm neonates: association of nutritional intake and urinary parameters. *Kidney Int*, 58, 2102–10.

Schell-Feith, E. A., Kist-Van Holthe, J. E., and van der Heijden, A. J. (2010). Nephrocalcinosis in preterm neonates. *Pediatr Nephrol*, 25(2), 221–30.

Schell-Feith, E. A., Kist-Van Holthe, J. E., van Zwieten, P. H., *et al.* (2003). Preterm neonates with nephrocalcinosis: natural course and renal function. *Pediatr Nephrol*, 18, 1102–8.

Schepers, M. S., Duim, R. A., Asselman, M., *et al.* (2003). Internalization of calcium oxalate crystals by renal tubular cells: a nephron segment-specific process? *Kidney Int*, 64, 493–500.

Schepers, M. S., Van Ballegooijen, E. S., Bangma, C. H., *et al.* (2005a). Crystals cause acute necrotic cell death in renal proximal tubule cells, but not in collecting tubule cells. *Kidney Int*, 68, 1543–53.

Schepers, M. S., Van Ballegooijen, E. S., Bangma, C. H., *et al.* (2005b). Oxalate is toxic to renal tubular cells only at supraphysiologic concentrations. *Kidney Int*, 68, 1660–9.

Schepers, M. S., van der Boom, B. G., Romijn, J. C., *et al.* (2002). Urinary crystallization inhibitors do not prevent crystal binding. *J Urol*, 167, 1844–7.

Schwarz, A., Mengel, M., Gwinner, W., *et al.* (2005). Risk factors for chronic allograft nephropathy after renal transplantation: a protocol biopsy study. *Kidney Int*, 67, 341–8.

Sener, G., Sehirli, O., Ipci, Y., *et al.* (2005). Protective effects of taurine against nicotine-induced oxidative damage of rat urinary bladder and kidney. *Pharmacology*, 74, 37–44.

Shenolikar, S., Voltz, J. W., Minkoff, C. M., *et al.* (2002). Targeted disruption of the mouse NHERF-1 gene promotes internalization of proximal tubule sodium-phosphate cotransporter type IIa and renal phosphate wasting. *Proc Natl Acad Sci U S A*, 99, 11470–5.

Shiraga, H., Min, W., VanDusen, W. J., *et al.* (1992). Inhibition of calcium oxalate crystal growth in vitro by uropontin: another member of the aspartic acid-rich protein superfamily. *Proc Natl Acad Sci U S A*, 89, 426–30.

Shirane, Y., Yamamoto, A., Mizuta, K., *et al.* (1989). Effect of glycosaminoglycans on calcium oxalate crystal aggregation. *Tokushima J Exp Med*, 36, 81–5.

Sorokina, E. A. and Kleinman, J. G. (1999). Cloning and preliminary characterization of a calcium-binding protein closely related to nucleolin on the apical surface of inner medullary collecting duct cells. *J Biol Chem*, 274, 27491–6.

Sorokina, E. A., Wesson, J. A., and Kleinman, J. G. (2004). An acidic peptide sequence of nucleolin-related protein can mediate the attachment of calcium oxalate to renal tubule cells. *J Am Soc Nephrol*, 15, 2057–65.

Streit, J., Tran-Ho, L., and Königsberger, E. (1998). Solubility of the three calcium oxalate hydrates in sodium chloride solutions and urine-like liquors. *Chemical Monthly*, 129, 1225–36.

Suzuki, K. and Ryall, R. L. (1996). The effect of heparan sulphate on the crystallization of calcium oxalate in undiluted, ultrafiltered human urine. *Br J Urol*, 78, 15–21.

Suzuki, M., Kobayashi, H., Kageyama, S., *et al.* (2001). Excretion of bikunin and its fragments in the urine of patients with renal stones. *J Urol*, 166, 268–74.

Tardivel, S., Medetognon, J., Randoux, C. *et al.* (1999). Alpha-1-microglobulin: inhibitory effect on calcium oxalate crystallization in vitro and decreased urinary concentration in calcium oxalate stone formers. *Urol Res*, 27, 243–9.

Thamilselvan, S. and Khan, S. R. (1998). Oxalate and calcium oxalate crystals are injurious to renal epithelial cells: results of in vivo and in vitro studies. *J Nephrol*, 11 Suppl 1, 66–9.

Thamilselvan, S., Khan, S. R., and Menon, M. (2003). Oxalate and calcium oxalate mediated free radical toxicity in renal epithelial cells: effect of antioxidants. *Urol Res*, 31, 3–9.

Thamilselvan, S. and Menon, M. (2005). Vitamin E therapy prevents hyperoxaluria-induced calcium oxalate crystal deposition in the kidney by improving renal tissue antioxidant status. *BJU Int*, 96, 117–26.

Thongboonkerd, V., Chutipongtanate, S., Semangoen, T., *et al.* (2008). Urinary trefoil factor 1 is a novel potent inhibitor of calcium oxalate crystal growth and aggregation. *J Urol*, 179, 1615–19.

Tiselius, H. G. (1984). A simplified estimate of the ion-activity product of calcium phosphate in urine. *Eur Urol*, 10, 191–5.

Tiselius, H. G. (1989). Standardized estimate of the ion activity product of calcium oxalate in urine from renal stone formers. *Eur Urol*, 16, 48–50.

Tiselius, H. G. (1991). Aspects on estimation of the risk of calcium oxalate crystallization in urine. *Urol Int*, 47, 255–9.

Tiselius, H. G. (1997). Estimated levels of supersaturation with calcium phosphate and calcium oxalate in the distal tubule. *Urol Res*, 25, 153–9.

Tiselius, H. G., Berg, C., Fornander, A. M., *et al.* (1993a). Effects of citrate on the different phases of calcium oxalate crystallization. *Scanning Microsc*, 7, 381–9.

Tiselius, H. G., Fornander, A. M., and Nilsson, M. A. (1993b). The effects of citrate and urine on calcium oxalate crystal aggregation. *Urol Res*, 21, 363–6.

Tiselius, H. G. and Hojgaard, I. (1999). Some aspects of the intratubular precipitation of calcium salts. *J Am Soc Nephrol*, 10 Suppl 14, S371–5.

Toblli, J. E., Ferder, L., Stella, I., *et al.* (2001). Protective role of enalapril for chronic tubulointerstitial lesions of hyperoxaluria. *J Urol*, 166, 275–80.

Toblli, J. E., Ferder, L., Stella, I., *et al.* (2002). Effects of angiotensin II subtype 1 receptor blockade by losartan on tubulointerstitial lesions caused by hyperoxaluria. *J Urol*, 168, 1550–5.

Tojo, A., Unuma, K., Harada, K., *et al.* (2010). Localization and mechanism of nephrocalcinosis in pseudo-Bartter's syndrome. *Kidney Int*, 77, 9, 831–1.

Trohler, U., Bonjour, J. P., and Fleisch, H. (1976). Inorganic phosphate homeostasis. Renal adaptation to the dietary intake in intact and thyroparathyroidectomized rats. *J Clin Invest*, 57, 264–73.

Tsujihata, M. (2008). Mechanism of calcium oxalate renal stone formation and renal tubular cell injury. *Int J Urol*, 15, 115–20.

Tsujihata, M., Miyake, O., Yoshimura, K., *et al.* (2000). Fibronectin as a potent inhibitor of calcium oxalate urolithiasis. *J Urol*, 164, 1718–23.

Umekawa, T., Chegini, N., and Khan, S. R. (2002). Oxalate ions and calcium oxalate crystals stimulate MCP-1 expression by renal epithelial cells. *Kidney Int*, 61, 105–12.

Umekawa, T., Chegini, N., and Khan, S. R. (2003). Increased expression of monocyte chemoattractant protein-1 (MCP-1) by renal epithelial cells in culture on exposure to calcium oxalate, phosphate and uric acid crystals. *Nephrol Dial Transplant*, 18, 664–9.

Umekawa, T., Iguchi, M., Uemura, H., *et al.* (2006). Oxalate ions and calcium oxalate crystal-induced up-regulation of osteopontin and monocyte chemoattractant protein-1 in renal fibroblasts. *BJU Int*, 98, 656–60.

Unuma, K., Tojo, A., Harada, K., *et al.* (2009). Autopsy report on pseudo-Bartter syndrome with renal calcification induced by diuretics and diet pills. *BMJ Case Rep*, pii: 1136/bcr 12 2008. 1380.

Verdier, J. M., Dussol, B., Casanova, P., *et al.* (1992). Evidence that human kidney produces a protein similar to lithostathine, the pancreatic inhibitor of CaCO3 crystal growth. *Eur J Clin Invest*, 22, 469–74.

Verdier, J. M., Dussol, B., Casanova, P., *et al.* (1993). [Renal lithostathine: a new protein inhibitor of lithogenesis]. *Nephrologie*, 14, 261–4.

Verhulst, A., Asselman, M., De Naeyer, S., *et al.* (2005). Preconditioning of the distal tubular epithelium of the human kidney precedes nephrocalcinosis. *Kidney Int*, 68, 1643–7.

Verhulst, A., Asselman, M., Persy, V. P., *et al.* (2003). Crystal retention capacity of cells in the human nephron: involvement of CD44 and its ligands hyaluronic acid and osteopontin in the transition of a crystal binding- into a nonadherent epithelium. *J Am Soc Nephrol*, 14, 107–15.

Verkoelen, C. F., Romijn, J. C., Cao, L. C., *et al.* (1996). Crystal-cell interaction inhibition by polysaccharides. *J Urol*, 155, 749–52.

Verkoelen, C. F., Romijn, J. C., De Bruijn, W. C., *et al.* (1995). Association of calcium oxalate monohydrate crystals with MDCK cells. *Kidney Int*, 48, 129–38.

Verkoelen, C. F., Schepers, M. S., Van Ballegooijen, E. S., *et al.* (2005). Effects of luminal oxalate or calcium oxalate on renal tubular cells in culture. *Urol Res*, 33, 321–8.

Verkoelen, C. F., van der Boom, B. G., and Romijn, J. C. (2000b). Identification of hyaluronan as a crystal-binding molecule at the surface of migrating and proliferating MDCK cells. *Kidney Int*, 58, 1045–54.

Verkoelen, C. F., van der Boom, B. G., Houtsmuller, A. B., *et al.* (1998). Increased calcium oxalate monohydrate crystal binding to injured renal tubular epithelial cells in culture. *Am J Physiol*, 274, F958–65.

Verkoelen, C. F., van der Boom, B. G., Kok, D. J., *et al.* (2000a). Sialic acid and crystal binding. *Kidney Int*, 57, 1072–82.

Verkoelen, C. F., van der Boom, B. G., Kok, D. J., *et al.* (1999). Cell type-specific acquired protection from crystal adherence by renal tubule cells in culture. *Kidney Int*, 55, 1426–33.

Vermeulen, C. W. and Lyon, E. S. (1968). Mechanisms of genesis and growth of calculi. *Am J Med*, 45, 684–92.

Vermeulen, C. W., Lyon, E. S., Gill, W. B., *et al.* (1959). Prevention of phosphate stones by phytate, phosphate and hexametaphosphate: experimental urolithiasis. XV. *J Urol*, 82, 249–55.

Vervaet, B. A., D'Haese, P. C., De Broe, M. E., *et al.* (2009). Crystalluric and tubular epithelial parameters during the onset of intratubular nephrocalcinosis: illustration of the 'fixed particle' theory in vivo. *Nephrol Dial Transplant*, 24, 3659–68.

Vervaet, B. A., Verhulst, A., Dauwe, S. E., *et al.* (2008). An active renal crystal clearance mechanism in rat and man. *Kidney Int*, 75, 41–51.

Vervaet, B. A., Verhulst, A., Dauwe, S. E., *et al.* (2009). An active renal crystal clearance mechanism in rat and man. *Kidney Int*, 75, 41–51.

Vervaet, B. A., Verhulst, A., Dauwe, S. E., *et al.* (2009). An active renal crystal clearance mechanism in rat and man. *Kidney Int*, 75, 41–51.

Vervaet, B. A., Verhulst, A., De Broe, M. E., *et al.* (2010). The tubular epithelium in the initiation and course of intratubular nephrocalcinosis. *Urol Res*, 38, 249–56.

Vervaet, B. A., Verhulst, A., D'Haese, P. C., *et al.* (2009). Nephrocalcinosis: new insights into mechanisms and consequences. *Nephrol Dial Transplant*, 24, 2030–5.

Wang, S. S., Devuyst, O., Courtoy, P. J., *et al.* (2000). Mice lacking renal chloride channel, CLC-5, are a model for Dent's disease, a nephrolithiasis disorder associated with defective receptor-mediated endocytosis. *Hum Mol Genet*, 9, 2937–45.

Wassmann, S., Laufs, U., Muller, K., *et al.* (2002). Cellular antioxidant effects of atorvastatin in vitro and in vivo. *Arterioscler Thromb Vasc Biol*, 22, 300–5.

Weinman, E. J., Mohanlal, V., Stoycheff, N., *et al.* (2006). Longitudinal study of urinary excretion of phosphate, calcium, and uric acid in mutant NHERF-1 null mice. *Am J Physiol Renal Physiol*, 290, F838–43.

Wesson, J. A., Johnson, R. J., Mazzali, M., *et al.* (2003). Osteopontin is a critical inhibitor of calcium oxalate crystal formation and retention in renal tubules. *J Am Soc Nephrol*, 14, 139–47.

Wiessner, J. H., Garrett, M. R., Roman, R. J., *et al.* (2009). Dissecting the genetic basis of kidney tubule response to hyperoxaluria using chromosome substitution strains. *Am J Physiol Renal Physiol*, 297, F301–6.

Wiessner, J. H., Hasegawa, A. T., Hung, L. Y., *et al.* (2001). Mechanisms of calcium oxalate crystal attachment to injured renal collecting duct cells. *Kidney Int*, 59, 637–44.

Wiessner, J. H., Kleinman, J. G., Blumenthal, S. S., *et al.* (1987). Calcium oxalate crystal interaction with rat renal inner papillary collecting tubule cells. *J Urol*, 138, 640–3.

Wiggins, R. C. (1987). Uromucoid (Tamm-Horsfall glycoprotein) forms different polymeric arrangements on a filter surface under different physicochemical conditions. *Clin Chim Acta*, 162, 329–40.

Williams, J. C., Jr., Matlaga, B. R., Kim, S. C., *et al.* (2006). Calcium oxalate calculi found attached to the renal papilla: preliminary evidence for early mechanisms in stone formation. *J Endourol*, 20, 885–90.

Worcester, E. M. (1996). Inhibitors of stone formation. *Semin Nephrol*, 16, 474–86.

Worcester, E. M., Blumenthal, S. S., Beshensky, A. M., *et al.* (1992). The calcium oxalate crystal growth inhibitor protein produced by mouse kidney cortical cells in culture is osteopontin. *J Bone Miner Res*, 7, 1029–36.

Worcester, E., Evan, A., Bledsoe, S., *et al.* (2006). Pathophysiological correlates of two unique renal tubule lesions in rats with intestinal resection. *Am J Physiol Renal Physiol*, 291, F1061–9.

Worcester, E. M., Fellner, S. K., Nakagawa, Y., *et al.* (1994). Effect of renal transplantation on serum oxalate and urinary oxalate excretion. *Nephron*, 67, 414–18.

Xue, Y. Q., He, D. L., Chen, X F., *et al.* (2009). Shock wave induced kidney injury promotes calcium oxalate deposition. *J Urol*, 182, 762–5.

Yamate, T., Kohri, K., Umekawa, T., *et al.* (1996). The effect of osteopontin on the adhesion of calcium oxalate crystals to Madin-Darby canine kidney cells. *Eur Urol*, 30, 388–93.

Yamate, T., Kohri, K., Umekawa, T., *et al.* (1998). Osteopontin antisense oligonucleotide inhibits adhesion of calcium oxalate crystals in Madin-Darby canine kidney cell. *J Urol*, 160, 1506–12.

Yamate, T., Kohri, K., Umekawa, T., *et al.* (1999). Interaction between osteopontin on madin darby canine kidney cell membrane and calcium oxalate crystal. *Urol Int*, 62, 81–6.

Yarlagadda, S. G. and Perazella, M. A. (2008). Drug-induced crystal nephropathy: an update. *Expert Opin Drug Saf*, 7, 147–58.

Ying, W. Z. and Sanders, P. W. (1998). Dietary salt regulates expression of Tamm-Horsfall glycoprotein in rats. *Kidney Int*, 54, 1150–6.

Zuckerman, J. M. and Assimos, D. G. (2009). Hypocitraturia: pathophysiology and medical management. *Rev Urol*, 11, 134–44.

CHAPTER 205

Medical management of nephrocalcinosis and nephrolithiasis

Bridget Sinnott, Naim Maalouf, Khashayar Sakhaee, and Orson W. Moe

Introduction

The lifetime risk of nephrocalcinosis and nephrolithiasis varies tremendously in different age and ethnic groups and in different regions in the world but falls within the range of 7–15% resulting in significant morbidity as well as substantial economic costs, not only directly from medical treatment but also indirectly through time lost from work (Moe, 2006a). The yearly economic cost has been estimated to approach $5.3 billion in the United States alone (Saigal et al., 2005).The medical management of nephrocalcinosis and nephrolithiasis, which includes most importantly prevention, is of major importance in healthcare.

An important concept is that contrary to common belief, nephrolithiasis is strictly speaking not a diagnosis per se. Nephrolithiasis is a manifestation of a variety of pathophysiologic abnormalities from a wide variety of aetiologies (Bushinsky et al., 2012). Although physicians report it as a diagnosis and get reimbursed for it as such, and prescribe treatment as though it is a specific disease entity, it is no more a diagnosis than fever or oedema. A calcium oxalate (CaOx) stone can be a presenting feature of primary hyperparathyroidism from an adenoma. The diagnosis is the adenoma and the stone is the manifestation. Due to common convention and the fact that we fail to uncover specific aetiologies in most cases, nephrolithiasis has remained in the status of a diagnosis.

Although many stones have mixed mineral contents, predominantly calcareous stones constitute the largest fraction. The approximate distribution is shown in Fig. 205.1. Eighty per cent of kidney stones are primarily calcium in composition (Bushinsky et al., 2012; Moe et al., in press). Investigations uncover definitive aetiologies only in a fraction of patients. Despite that, one can usually identify empiric biochemical risk factors in urine that confer the stone forming propensity (Table 205.1). Through risk factor modification, one can lower the risk of formation (Moe et al., 2011). Therefore, the principle of managing kidney stones is somewhat analogous to that of primary hypertension. Although in the vast majority of the instances, we do not uncover the cause of, nor can we cure primary hypertension, we can lower blood pressure and reduce the risk of cardiovascular, cerebral, and renal complications. The treatment of kidney stones is also empirical in nature; lower the urinary calcium and thus the risk for stone formation (Table 205.1).

In this chapter, we will briefly review the pathophysiology and aetiology of nephrocalcinosis and three of the more common kidney stones—calcium-containing, uric acid (UA), and cystine—and focus mainly on the medical management.

Nephrocalcinosis

Pathophysiology and aetiology

Nephrocalcinosis and nephrolithiasis are two somewhat related yet distinct pathologies. Nephrocalcinosis in its broadest sense means abnormal deposition of calcium salts in renal parenchyma and as such, encompasses an extremely diverse group of disorders where any type of tissue damage can potentially result in non-specific calcification. Inclusion of all of these will render the discussion quite meaningless. In particular, cortical calcium deposition can be secondary to aetiologies as diverse as glomerulonephritis, Alport syndrome, acute cortical necrosis secondary to toxins or ischaemia, haemolytic uraemic syndrome, renal tuberculosis, acute transplant rejection or pyelonephritis, just to name a few. Medullary nephrocalcinosis however reflects a little more common thread in the underlying pathology and pathophysiology although the causes are still very diverse including medullary sponge kidney, hypercalcaemic states typically in hyperparathyroidism, distal renal tubular acidosis (dRTA), furosemide therapy (especially in infants), Bartter syndrome, ethylene glycol toxicity, vitamin D toxicity, over-aggressive phosphate therapy, and primary hyperoxaluria (PH) (Sayer et al., 2004). The common denominators appear to be an increase in calcium, oxalate, and/or phosphate load presented to the kidney and supersaturation with eventual precipitation occurring as an intratubular or interstitial event. It is most important to note that the clinician's gold standard radiologic diagnosis of nephrocalcinosis implies but should not be equated with the pathologic diagnosis of nephrocalcinosis (Cheidde et al., 2007). In fact, it is not infrequent that multiple small nephrolithiasis can masquerade as nephrocalcinosis on radiologic images.

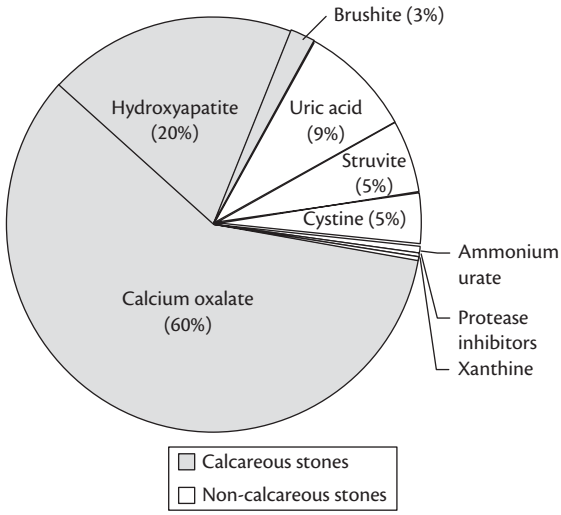


Fig. 205.1 Distribution of types of kidney stones.

Management and therapy

Treatment of nephrocalcinosis is generally directed at the underlying aetiology, with the goal of preventing further calcium deposition in the renal parenchyma and tubules and worsening of kidney function. While non-pharmacological approaches are recommended and widely used, these have not been rigorously tested. In some clinical scenarios, pharmacological agents have been shown to be effective at reducing the progression of nephrocalcinosis.

Whenever possible, the underlying cause of nephrocalcinosis should be determined and directly treated. In the case of nephrocalcinosis related to primary hyperparathyroidism, parathyroidectomy is considered the treatment of choice (Peacock, 2002). While resection of the parathyroid gland(s) has been shown to reduce hypercalciuria and the frequency of recurrent nephrolithiasis (Rejnmark et al., 2011), no studies have reported the long-term effects of parathyroidectomy on nephrocalcinosis in this setting. In nephrocalcinosis related to sarcoidosis, glucocorticoid therapy suppresses calcitriol production by the non-caseating granulomata, reducing serum and urine calcium, and prevents progression of nephrocalcinosis (Bergner et al., 2003). When nephrocalcinosis is related to PH, early institution of medical interventions aimed at reducing oxalate production and urinary CaOx saturation is critical to prevent progression of renal oxalate deposition. This includes increasing fluid intake, reducing dietary intake of oxalate precursors, using orthophosphate and potassium citrate prior to the development of significant renal insufficiency in all PH patients, and pyridoxine in PH type 1 (Hoppe et al., 2009). Definitive treatment for PH involves combined liver and renal transplantation in PH type 1 and isolated renal transplantation in PH type 2 (in whom the disease is generally less severe) (Hoppe et al., 2009). In nephrocalcinosis related to other monogenic diseases, no therapy targeting the underlying molecular defect is available and current management is primarily supportive (non-pharmacological approaches described below, typically along with thiazide diuretics and citrate therapy).

Table 205.1 Summary of pathophysiologic risk factors and interventions for kidney stones

Type	Risk	Diet therapy	Pharmacologic therapy
Calcium stones	Low urine volume	Fluid	–
	Hypercalciuria	Salt and protein restriction	Thiazide, alkali
	Hyperoxaluria	Oxalate restriction	–
	Hypocitraturia	Protein restriction	Alkali
	Hyperuricosuria	Protein restriction	Xanthine oxidase inhibitor
	High urine pH	–	–
Uric acid stones	Low urine pH	Protein restriction	Alkali
	Low urine volume	Fluid	–
	Hyperuricosuria	Protein restriction	Xanthine oxidase inhibitor
Cystine stones	Cystinuria	Methionine and salt restriction	Thiol agents
	Low urine volume	Fluid	–
	Low urine pH	Protein restriction	Alkali

Non-pharmacological approaches

Several non-pharmacological measures are advocated to reduce the urinary concentration of substances contributing to nephrocalcinosis (calcium, phosphate, or oxalate). The data behind their efficacy are extrapolated mainly from studies in nephrolithiasis, as they have not been rigorously tested in patients with nephrocalcinosis. Increasing fluid intake to achieve a urinary volume > 2 L per day is likely to be beneficial in all individuals with nephrocalcinosis as it is in kidney stone formers (Borghi et al., 1996). Reduction in urinary calcium may be achieved with restriction in dietary intake of salt (to < 150 mEq sodium daily) and animal protein (to < 1.2 g protein/kg body weight daily) (Borghi et al., 2002). Dietary oxalate reduction may also lower urinary oxalate excretion (Lieske et al., 2010). These non-pharmacological interventions have not been evaluated for nephrocalcinosis prevention and treatment in infants, in part because the of the extremely delicate balance between high intake of calcium, phosphate, vitamin D, and protein needed for tissue accretion on the one hand, and the risk of renal damage with persistent nephrocalcinosis on the other hand (Schell-Feith et al., 2010).

Pharmacological management

Citrate therapy

Citrate enhances the solubility of calcium in urine and may limit the development of nephrocalcinosis. Citrate delays progression of nephrocalcinosis and renal dysfunction in an animal model of Dent disease, even in the apparent absence of stone formation (Cebotaru et al., 2005). In terms of prevention of nephrocalcinosis in preterm neonates, sodium citrate (0.52 mmol/kg/day) from day 7 until term safely reduced urinary calcium/citrate ratio but did not significantly decrease the prevalence of nephrocalcinosis, although a positive trend was noted (Schell-Feith et al., 2006). In a small cohort of children with PH, long-term

administration of sodium citrate (0.15 g (0.5 mmol)/kg/day) (along with dietary salt and oxalate restriction) was associated with stabilization or improvement in renal function in six patients (Leumann et al., 1993). In adults with medullary sponge kidney, a renal malformation typically associated with nephrocalcinosis and recurrent calcium nephrolithiasis, potassium citrate therapy resulted in increase in urine citrate and reduction in urine calcium, and dramatic reduction in stone recurrence rates (Fabris et al., 2010).

Thiazide diuretics

In hypercalciuric children and adults, thiazides decrease urinary calcium excretion (Reusz et al., 1993; Moe et al., 2011). The effect of thiazides on the natural course of nephrocalcinosis in preterm neonates and in adults has not been studied but in a small uncontrolled series of four children with nephrocalcinosis related to metabolic bone disease (two with hypophosphatasia and two with X-linked hypophosphataemic rickets (XLH)); thiazide was associated with resolution of renal calcification (Auron and Alon, 2005). In a series of 11 children with XLH, hydrochlorothiazide (HCTZ) given over 3 years reduced urinary calcium and slowed progression of nephrocalcinosis but did not result in its resolution (Seikaly and Baum, 2001). In rats, established, nephrocalcinosis remained unaffected by thiazides, in spite of their hypocalciuric effect (Knoll and Alon, 2000). Nevertheless, thiazide diuretics would still be recommended in the management of nephrocalcinosis related to hypercalciuria. Thiazides are generally used in Dent disease to reduce hypercalciuria (Raja et al., 2002; Blanchard et al., 2008).

Calcimimetics

Calcimimetics are allosteric activators of the calcium-sensing receptors (CaSRs) (Brown, 2010). Cinacalcet, the only calcimimetic currently approved for human use, activates CaSRs in the parathyroid glands and inhibits parathyroid hormone (PTH) secretion and secondarily reduces serum calcium. In doing so, cinacalcet may reduce filtered calcium load and has therefore been touted as a potential therapy for nephrocalcinosis in some hypercalcaemic states. One should keep in mind that cinacalcet also activates CaSRs in the kidney, resulting in enhanced renal calcium excretion for any given filtered calcium load, and hence may potentially worsen nephrocalcinosis.

Furosemide-induced nephrocalcinosis: in an animal model of nephrocalcinosis, the calcimimetic agent NPS R-467 attenuated nephrocalcinosis and prevented the development of hyperparathyroidism in the furosemide-treated young rat. There are no published human studies on the use calcimimetics for the management of nephrocalcinosis related to thiazide use in preterm infants.

Nephrocalcinosis post renal transplantation: hypercalcaemia post renal transplantation has been associated with nephrocalcinosis and worsening allograft function (Egbuna et al., 2007). Although not approved for this condition, cinacalcet has been widely and successfully used in this setting (Kruse et al., 2005; Serra et al., 2005). However, some cases reports suggested that cinacalcet itself may worsen hypercalciuria, nephrocalcinosis, and renal dysfunction (Seikrit et al., 2011). Further, information on safety and efficacy of cinacalcet in post renal transplants will be gained from ongoing clinical trials.

Nephrocalcinosis in XLH: XLH is a rare inherited condition of phosphate metabolism that leads to renal phosphate wasting, bone demineralization, and growth defects. Treatment of XLH generally involves supplementation with phosphate salts and calcitriol to correct the associated skeletal abnormalities. Such treatment is sometimes complicated by nephrocalcinosis and at times of parathyroid gland hyperplasia, tertiary hyperparathyroidism, and hypercalcaemia which leads to hypercalciuria and worsens the nephrocalcinosis. In this setting, cinacalcet use has been reported to reduce serum PTH and calcium (Raeder et al., 2008), although its effects on nephrocalcinosis have yet to be described.

Calcareous stones

Pathophysiology and aetiology

The pathophysiologic mechanism(s) for calcareous stones are diverse and complex. Unlike non-calcareous stones, stone type does not designate a single physiologic derangement. These abnormalities may be present as an isolated entity or in combination (Table 205.1).

Hypercalciuria

This is the most common abnormality present in 30–60% of adult kidney stone formers (Pak et al., 1980a). Hypercalciuria is heterogeneously derived from increased intestinal calcium (Ca) absorption (Pak et al., 1974), and abnormalities in Ca transport across the kidney and bone which are in part, responsible for bone disease in this population (Sakhaee et al., 2011). Intestinal hyperabsorption of Ca may be primary and independent of serum 1,25-dihydroxyvitamin D (1,25$(OH)_2$D) as over two-thirds of hypercalciuric patients have fractional Ca absorption that does not correlate with serum 1,25$(OH)_2$D (Kaplan et al., 1977; Breslau et al., 1992). It is possible that amplified target action of normal circulating 1,25$(OH)_2$D in the gut and bone may be the result of high vitamin D receptor activity which may increase gut absorption and bone resorption (Maierhofer et al.,1983; Favus et al., 2004). Hypercalciuria is most commonly idiopathic but may occur in patients with primary hyperparathyroidism and granulomatous diseases in which increased PTH and/or calcitriol levels enhance bone resorption and/or increase intestinal Ca absorption.

Hyperuricosuria

As a single abnormality, hyperuricosuria is seen in 10% of Ca kidney stone formers but it is present in 40% of patients in combination with other defects (Preminger, 1992). Hyperuricosuria can be attributed to overindulgence in a high-purine diet (Coe, 1978). However, a third of patients may have endogenous UA overproduction and dietary restrictions do not lower their urinary UA excretion (Coe and Parks, 1981). Physicochemical processes involved in the formation of CaOx stones includes monosodium urate-induced heterogeneous nucleation of CaOx, epitaxial crystal growth, and altered CaOx crystallization inhibition (Coe et al., 1975; Pak and Arnold, 1975; Grover et al., 1990).

Hypocitraturia

Hypocitraturia occurs in 20–60% of Ca stone formers and is mostly reversible (Pak, 1994). Citrate is the most abundant urinary organic anion and constitutes the major urinary base. Concurrently, it is a chelator of calcium in a soluble complex and reduces urinary saturation with respect to CaOx and calcium phosphate, as indicated

in Fig 205.2 (Moe and Preisig, 2006). It inhibits the spontaneous precipitation of CaOx, agglomeration of CaOx crystals, and crystal growth (Nicar et al., 1987). Physiologic hypocitraturia occurs in acid loading or metabolic acidosis including dRTA (Backman et al., 1980), and use of carbonic anhydrase inhibitors such as acetazolamide and topiramate (Gordon and Sheps, 1957; Welch et al., 2006). Hypocitraturia may be present with various conditions exhibiting normal plasma pH (Preminger et al., 1987).

Hyperoxaluria

Hyperoxaluria is present in 10–50% of Ca stone formers (Laminski et al., 1991). The main causes of are increased oxalate production from genetic abnormalities in the oxalate synthetic pathway (rare) (Freel et al., 2006; Holmes and Assimos, 1998; Belostotsky et al., 2010), increased substrate provision from oxalate-rich foods and/or other oxalate precursors (Holmes and Kennedy, 2000), or increased intestinal oxalate absorption (Sakhaee, 2009). Oxalate absorption occurs throughout a large segment of the small bowel and, to some extent, the colon (Lindsjo et al., 1989). *Oxalobacter formigenes* in humans have been suggested to play a role in intestinal oxalate metabolism (Hoppe et al., 2006).

The most frequent clinical conditions associated with hyperoxaluria are intestinal malabsorptive disorders (Worcester, 2002; Asplin and Coe, 2007). In addition, chronic diarrhoeal state is associated with low urine volume, hypocitraturia, hypomagnesaemia, and highly acidic urine, all contributing factors to kidney stone formation.

Abnormalities in urinary pH

Low urinary pH is present in 10–30% of Ca kidney stone formers. UA may work like monosodium urate and participates in the heterogeneous nucleation of CaOx, epitaxial crystal growth, and attenuation of inhibitory urinary macromolecules of CaOx crystallization (Coe et al., 1975; Pak and Arnold, 1975). A highly alkaline urinary pH ≥ 6.7 increases the abundance of monohydrogen phosphate (pKa ~ 6.7) which, in combination with Ca, converts to thermodynamically unstable brushite ($CaHPO_4.2H_2O$) and is transformed to hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) (Fig. 205.1). Fifteen per cent of stone formers produce predominantly calcium phosphate stones, with approximately a quarter of these as brushite (Evan et al., 2005). Conditions predominantly associated with calcium phosphate stone formation include dRTA (Pak et al., 2003b), primary hyperparathyroidism (Pak et al., 2003b), use of carbonic anhydrase inhibitors (Ahlstrand and Tiselius, 1987; Kuo et al., 2002), and frequent shock wave lithotripsy (Parks et al., 2004).

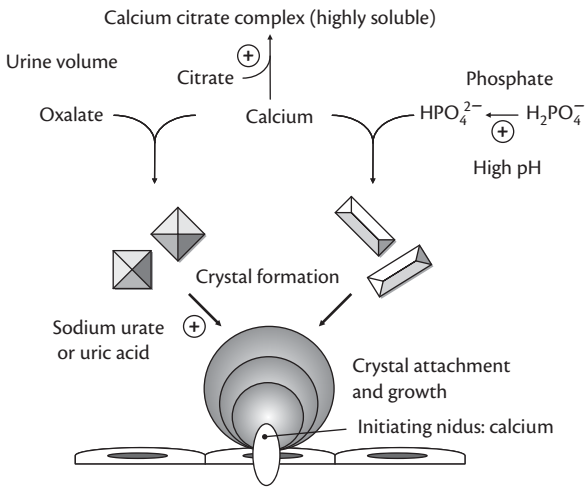


Fig. 205.2 Calcium citrate complex (highly soluble).

Therapy

Management of acute renal colic

The most important predictors of successful ureteral stone passage are stone size and location. The majority of kidney stones < 5 mm in diameter and are easily passed without surgical interventions (Hall, 2009). Stones > 10 mm are unlikely to pass spontaneously (Worcester and Coe, 2010). In the absence of complications such as renal impairment, urinary tract infection, or sepsis, smaller ureteral stones < 10mm in diameter can generally be followed with pain management and hydration. Indeed, the 2007 American Urological Association/European Association of Urology (AUA/EAU) guidelines for the management of ureteral calculi recommend medical expulsive therapy for all patients with newly diagnosed ureteral stones < 10 mm since the chance of spontaneous passage is high (Preminger et al., 2007). Spontaneous stone passage rates are higher with distal ureteral stones compared to middle or proximal ones (Hubner et al., 1993).

The acute management of kidney stones focuses primarily on pain control, hydration, and medications to assist passage. The mainstays of pain control are opioid analgesics and non-steroidal anti-inflammatory drugs (NSAIDs). NSAIDs are as effective as narcotics in the management of renal colic (Table 205.2). A high fluid intake to ensure a urine output of at least 100 mL/hour is recommended. A meta-analysis of randomized trials concluded that treatment with calcium channel blockers or alpha blockers had 65% greater chance of successful stone passage compared to the control group (Hollingsworth et al., 2006). The additional benefit of glucocorticoids with either treatment regimen was not evident (Hollingsworth et al., 2006). Another meta-analysis demonstrated a 44% greater chance of spontaneous stone passage with alpha blockers compared to no treatment (Parsons et al., 2007). The 2007 AUA/EAU guidelines for the management of ureteral calculi concluded that alpha blockers are more effective than calcium channel (Preminger et al., 2007).

Management and prevention of recurrent kidney stones

Management of recurrent kidney stones involves preventing and treating factors that promote stone formation, namely a low urine volume, high urine saturation with CaOx and calcium phosphate, and an acidic urine pH (Pak et al., 1980b). Comprehensive management also involves increasing the concentration of inhibitors of kidney stone formation such as citrate, magnesium, and potassium. Management of underlying secondary causes of hypercalciuria such as primary hyperparathyroidism or sarcoidosis involves addressing the underlying disorder for definitive treatment.

General dietary modification

When feasible, dietary modification of lithogenic factors represents the first-line strategy in the prevention of recurrent kidney stones. Recommendations for dietary modification are guided by the biochemical abnormalities evident on a 24-hour urine collection and

Table 205.2 Three meta-analyses of medications used in the management of renal colic

Medication	Number of studies	Primary outcome
Parenteral NSAIDs vs opioids vs placebo (Labrecque et al., 1994)	19 RCTs	Pain relief 20–30 min after drug administration: NSAIDs vs placebo (RR 2.34; 95% CI 1.79–3.07) NSAIDs vs opioids (RR 1.19; 95% CI 1.03–1.37)
Calcium channel blockers (CCB) or alpha blockers (AB) vs no treatment (Hollingsworth et al., 2006)	9 RCTs	CCB or AB treated groups had 65% greater chance of stone passage (pooled risk ratio 1.65; 95% CI 1.45–1.88; P < 0.0001) compared to the control group
Alpha blockers vs no treatment (Parsons et al., 2007)	11 RCTs	AB group had 44% greater stone passage (RR 1.44; 95% CI 1.31–1.59; P < 0.001) compared to the control group

CI = confidence interval; NSAIDs = non-steroidal anti-inflammatory drugs; RCTs = randomized control trials; RR = relative risk.

kidney stone composition. A comprehensive metabolic evaluation followed by successful dietary modification can control stone formation and growth.

Low fluid intake is a well-recognized risk factor for recurrent stone disease. One of the most important and universal dietary recommendations for all stone formers is the maintenance of an adequate fluid intake to ensure a dilute urine which prevents urine supersaturation with stone-forming salts (Pak et al., 1980b). It was demonstrated in an Italian randomized controlled trial (RCT) that maintaining a daily urine volume of ≥ 2 L led to a significant reduction in recurrent calcareous kidney stones among stone formers, over a 5-year period (Table 205.3) (Borghi et al., 1996).

Role of other juices

A lot of beverages have been touted to be useful in increasing urine volume (Seltzer et al., 1996; Odvina, 2006; Yilmaz et al., 2010) but not all of them are equal. Orange juice, which is mostly potassium citrate, confers an alkali load and increases citrate excretion while lemonade, which is citric acid, is not an alkali and does not cause significant citraturia (Odvina, 2006). Even epidemiologic data indicates that drinks containing caffeine and alcohol are associated with lower stone risk but those with a high content of sweeteners such as apple or grapefruit juice actually are associated with higher stone risk (Curhan et al., 1996). Part of this effect may be due to the use of fructose as sweetener (Taylor and Curhan, 2008b).

Dietary management of hypercalciuria

Calcium

Hypercalciuria is the most common metabolic abnormality found in patients who develop recurrent calcium stones. In the past, low dietary calcium intake was encouraged as it was thought to aggravate hypercalciuria. However, as our understanding of stone pathophysiology has evolved, this recommendation has fallen out of favour. Currently, normal dietary calcium intake is encouraged to prevent a net negative calcium balance, which can lead to bone loss (Heilberg and Weisinger, 2006) and an increased prevalence of fractures (Lauderdale et al., 2001). In addition, dietary calcium binds to dietary oxalate in the colon preventing its enteric absorption, subsequent excretion, and supersaturation in the urine (Taylor and Curhan, 2006). A study of Italian men with hypercalciuric CaOx stones demonstrated that a diet that is normal in calcium intake (1200 mg/day) was associated with a 50% lower risk of stone formation compared to a low-calcium diet (400 mg/day) (Borghi et al., 2002) (Table 205.3). Moreover, several additional studies have demonstrated that a higher intake of dietary calcium is associated with fewer calcium stone events in both women and men (Curhan et al., 1993, 1997).

Population-based studies have suggested that calcium supplementation rather than dietary calcium intake is not as effective in preventing recurrent stone formation and may even slightly increase the risk for stone formation (Curhan et al., 1997; Jackson et al., 2006). Conversely, other population-based studies suggest that supplemental calcium intake is not associated with kidney stone risk (Taylor et al., 2004).

Sodium and protein

Hypercalciuria is exacerbated by a high dietary sodium intake (Burtis et al., 1994). A very low sodium intake (dietary Na 50 mEq/day) has been shown to enhance proximal tubular sodium and calcium reabsorption, thereby leading to a reduction in calcium excretion (Borghi et al., 2002). A recent RCT confirmed that a low-sodium diet in calcium stone formers with hypercalciuria significantly decreased excretion of both calcium and oxalate compared to a control diet (Table 205.3) (Nouvenne et al., 2010). Benefits from dietary sodium restriction include a reduction in urinary calcium loss, an increase in urinary citrate levels and a reduction in sodium urate saturation, all of which help to reduce the precipitation of CaOx. It is recommended that recurrent calcium stone formers reduce dietary intake of sodium to 100 mEq/day.

Hypercalciuria is also exacerbated by a high animal protein intake (Hess et al., 1995). A study of Italian male hypercalciuric CaOx stone formers demonstrated that a diet low in animal protein was protective for stone recurrence (Borghi, et al., 2002). In contrast, a randomized trial of CaOx stone formers demonstrated that a low-animal-protein diet was no better than a high fluid intake in the prevention of stone recurrence (Hiatt et al., 1996). It is recommended that recurrent calcium stone formers reduce dietary intake of protein to 0.8–1 g/kg/day to prevent stone recurrence (Worcester and Coe, 2010).

Pharmacologic management of hypercalciuria

Pharmacotherapy in conjunction with lifestyle modifications may be necessary to correct kidney stone risk factors and consequent kidney stone formation. The choice of medication is directed by the metabolic abnormality identified, as well as the kidney stone composition.

Italian patients with hypercalciuria have been treated successfully with a normal calcium, low-salt, and low-animal-protein diet, therefore this is typically the first management strategy employed; whether this is applicable to the general stone forming population is unclear (Borghi et al., 2002). If diet alone is suboptimal in the prevention of stone recurrence, then a thiazide diuretic may be necessary (Table 205.4). Thiazide diuretics reduce urinary calcium excretion and promote calcium retention (Coe et al., 1988), which clinically translates into an improvement in bone density

Table 205.3 Trials of dietary interventions in the management of calcareous kidney stones

Dietary intervention	Study design	Primary outcome
High fluid intake vs usual fluid intake (Borghi et al., 1996)	RCT of 199 Italian men with history of 1 episode of a calcium stone and hypercalciuria followed for 5 years	Kidney stone recurrence rate: 12% in the treated group vs 27% in control group P = 0.008
Normal-calcium, low-salt low-protein vs low-calcium diet alone (Borghi et al., 2002)	RCT of 120 Italian men with recurrent CaOx stones and hypercalciuria followed for 5 years Comparison of a normal-calcium diet (Ca 1200 mg, Na 50 mmol/day, animal protein 52 g/day, oxalate 200 mg/day) vs low-calcium diet (Ca 400 mg/day)	Kidney stone recurrence rate: 20% in the normal-calcium diet group vs 38% in the low-calcium diet group Unadjusted RR of recurrence for study diet vs low-calcium diet 0.49 (95% CI 0.24–0.98; P = 0.04)
Low-salt diet vs control diet (Nouvenne et al., 2010)	RCT of 210 calcium stone formers with idiopathic hypercalciuria followed for 3 months	24-hour urinary calcium at 3 months: low-salt study diet (271 ± 86 mg calcium/day) compared to (361 ± 129 mg/calcium/day P < 0.001) on the controlled diet
Low-animal-protein, high-fibre, high-fluid diet vs high-fluid diet (Hiatt et al., 1996)	RCT of 99 CaOx stone formers, followed for a duration 4.5 years	Kidney stone recurrence rate: 24% in low animal protein group compared to 4% in high-fluid group

CaOx = calcium oxalate; RCT = randomized control trial; RR = relative risk.

and decrease in recurrent stone formation (Pak et al., 2003a; Moe et al., 2011).

Use of thiazide diuretics alone

Thiazides exert one of their effects by inducing mild volume depletion which leads to a compensatory increase in proximal renal tubular reabsorption of sodium and calcium (Nijenhuis et al., 2003). Use of thiazides in many RCTs have led to a significant reduction in the recurrence rates of calcium-based stones by up to 50% during a 3-year treatment period, as compared to placebo (Laerum and Larsen, 1984; Borghi et al., 1993) (Table 205.4). A meta-analysis showed that thiazide diuretics were associated with a significant reduction in kidney stone recurrence (Escribano et al., 2009). Thiazides appear to be effective in both hypercalciuric stone formers and normocalciuric stone formers (Yendt and Cohanim, 1978). Thiazide treatment of hypercalciuria, regardless of the underlying pathophysiologic mechanism (absorptive or renal), has been shown to significantly reduce stone formation rates (Pak et al., 1981).

In addition to HCTZ, several thiazide analogues including indapamide and chlorthalidone have been shown to be effective in reducing urinary calcium excretion. The optimal dose of indapamide is 2.5mg daily, chlorthalidone is 25–50 mg/daily, and HCTZ 25 mg twice daily (Reilly et al., 2010). The optimal effect of thiazide diuretics also depends upon salt intake to attenuate urinary calcium excretion and sufficient potassium repletion to avoid hypocitraturia (Nijenhuis et al., 2003; Worcester and Coe, 2010).

Thiazide-induced potassium depletion is a common side effect so potassium supplement should be prescribed with long-term thiazide treatment (Odvina et al., 2003). Potassium citrate is the potassium supplement of choice since it provides both potassium and alkali (Nicar et al., 1984). In some instances, combined treatment with thiazide and the potassium-sparing diuretic amiloride (5–10 mg/daily) may further reduce hypercalciuria as well as avoid the development of hypokalaemia and hypocitraturia (Alon et al., 1984).

Use of alkali alone

Alkali treatment alone has been shown to be effective in lowering urinary calcium excretion, raising urinary citrate, and diminishing urinary supersaturation with respect to CaOx and undissociated UA (Tables 205.4 and 205.5) (Sakhaee et al., 1983; Lemann et al., 1989). Additionally, these potassium salts have the additional benefit of increasing urinary excretion of citrate and reducing kidney stone formation (Pak et al., 1985; Whalley et al., 1996).

Dietary management of hyperuricosuria

Animal protein, in contrast to vegetable protein, is high in sulphur-containing amino acids which are metabolized to sulphuric acid leading to an acidic urine pH and hypocitraturia, therefore predisposing to calcium stone formation (Taylor and Curhan, 2006). Lowering animal protein intake reduces these lithogenic factors (Taylor and Curhan, 2006). However, the role of a low-protein diet in clinical outcome of recurrent stone event has not been clearly validated (Worcester and Coe, 2010).

Pharmacologic management of hyperuricosuric calcium stones

In hyperuricosuric CaOx stone formers, treatment with allopurinol, 300 mg daily, has been shown to be effective in reducing urinary UA and significantly reducing recurrent CaOx stones compared to placebo (P < 0.001) (Ettinger et al., 1986). However, in hyperuricosuric subjects with multiple other metabolic abnormalities, the benefit of a reduction of high urinary UA alone by allopurinol is much less effective (Ettinger, 1989). In these subjects, combined thiazide and allopurinol treatment is more effective in reducing kidney stone formation (Coe, 1977).

Dietary management of hyperoxaluria

Hyperoxaluria can be reduced by decreasing dietary oxalate intake. Foods high in oxalate, such as spinach, nuts, tofu, tea, and chocolate should be avoided in stone formers where a high urinary oxalate level is felt to be diet related. It is recommend to restrict dietary oxalate intake to < 100 mg/day and avoid excessive intake of ascorbic acid in patients with hyperoxaluria (Worcester and Coe, 2010). A population-based study has shown that high ascorbic acid consumption (> 1000 mg/day) or high fructose intake, especially in the setting of an elevated body mass index and diabetes, can increase oxalate excretion (Taylor and Curhan, 2008a). In the same study, total calcium intake (dietary and supplemental) was also inversely associated with urinary oxalate levels.

Pharmacologic management of hyperoxaluria

Hyperoxaluria contributes to calcium stone formation by increasing CaOx supersaturation. It has been demonstrated that oxalate is of equal importance to calcium in raising the level of saturation of CaOx in the urine (Pak et al., 2004). Dietary manipulation is

Table 205.4 Trials of pharmacologic interventions in the management of calcareous stones

Intervention	Study type	Treatment	Primary Outcome
Indapamide (Borghi et al., 1993)	Randomized prospective study Calcium stone formers with hypercalciuria Duration: 3 years N = 75	Diet & fluid (Gp A) Diet & fluid & indapamide 2.5 mg (Gp B) Diet & fluid & indapamide 2.5 mg & allopurinol 300 mg (Gp C)	Kidney stone recurrence rate: Gp B & C had less stone recurrence compared Gp A ($P < 0.02$) 24-hour urinary calcium excretion decreased by 50% compared to pre-treatment values in Gp B & C
Hydrochlorothiazide (Laerum and Larsen, 1984)	Double-blind RCT Recurrent stone formers Duration: 3 years N = 50	HCTZ 25 mg twice a day vs placebo	Kidney stone recurrence rate: 65% with placebo vs 25% with thiazide ($P = 0.05$)
Thiazides (Escribano et al., 2009)	Meta-analysis—5 RCTs N = 316 Duration: 5 months–3 years	4 trials compared thiazides with standard treatment; 1 study compared thiazides and K salts with standard treatment	Kidney stone recurrence rate: Statistically significant decrease in stone recurrence in thiazide Rx Gps compared to standard Rx Gp (RR 1.61 95% CI 1.33–1.96)
Potassium citrate (Pak and Peterson, 1986)	Non-randomized, non-placebo controlled trial 19 calcium oxalate stone formers with hyperuricosuria Duration: 2.35 ± 0.88 years	K citrate 60–80 mEq/day	Kidney stone recurrence rate: reduced from 1.55+/–2.7 patient/years (baseline) to 0.38+/–1.22 patient/years. No evidence of stone recurrence in 84% cases.
Allopurinol (Ettinger et al, 1986)	Double-blind RCT 60 stone formers with hyperuricosuria and normocalcaemia Duration: 3 years	Allopurinol 100 mg three times a day vs placebo	Kidney stone recurrence rate: 31% in the allopurinol Gp versus 58% in the placebo Gp ($P<0.001$)
Potassium salts (Sakhaee et al., 1991)	Non-randomized, non-placebo controlled trial 8 patients Duration: 2 weeks	80 mEq K/day as: Potassium citrate Potassium bicarbonate($KHCO_3$) Potassium chloride (KCl)	24-hour urine citrate excretion: urinary citrate increased 2.5 ± 1.6 mmol/day (baseline) to 5.1 ± 11.7 mmol/day with K citrate and to 4.5 ± 1.5 mmol/day with $KHCO_3$ ($P < 0.05$) but did not significantly increase with KCl
Potassium citrate (Barcelo et al., 1993)	Double-blind RCT 57 hypocitraturic calcium stone formers Duration: 3 years	30–60 mEq K citrate vs placebo	Kidney stone recurrence rate: 27% in the treatment group vs 80% in the placebo group.
Potassium citrate (Pak et al., 1985)	Non-randomized, non-placebo controlled trial 89 hypocitraturic calcium stone formers or uric acid stone formers	20 mEq K citrate three times a day	Kidney stone recurrence rate: Decreased in 97.8% stone formers Disease remission seen in 79.8%

usually the first management strategy, however the impact of dietary oxalate on urinary oxalate excretion is controversial (Holmes and Kennedy, 2000; Taylor and Curhan, 2008a).

Patients with inherited PH type 1 respond well to pyridoxine (vitamin B_6) and orthophosphate with a reduction in urinary oxalate levels and CaOx crystallization. However its effect on kidney stone development is unknown (Milliner et al., 1994). In contrast, patients with type 2 PH or idiopathic hyperoxaluria remain non-responsive to pyridoxine treatment.

Dietary management of hypocitraturia

Fruits and vegetables are high in potassium and alkali which results in an increased urinary citrate excretion, lowered CaOx and UA supersaturation, and therefore decreased stone formation (Meschi et al., 2004). Accordingly, a DASH (Dietary Approaches to Stop Hypertension)-style diet has been shown to increase citrate, lower supersaturation with respect to CaOx and UA salts, and significantly reducing kidney stone formation (Taylor et al., 2009, 2010). It is important to note that citrate when ingested as sodium or potassium salt is a base that will elevate urinary citrate but in the form of citric acid, it is neutral and leads to minimal elevation in urinary citrate.

As discussed above, fruit juices rich in potassium citrate (Seltzer et al., 1996; Odvina, 2006; Eisner et al., 2010) can be very effective in raising urinary pH and citrate. One caution is the high amount of calories consumed with using juices as a source of alkali.

Pharmacologic management of hypocitraturia

Citrate decreases urinary saturation of CaOx and phosphate salts via the formation of soluble calcium complexes, and indirectly by the promotion of inhibitory action against calcium salts (Tiselius et al., 1993).

Table 205.5 Major clinical trials in pharmacotherapy of non-calcareous nephrolithiasis

Stone type	Author	Treatment	N	Design	Finding
Uric acid	Pak et al. (1986)	Potassium citrate	18	Non-randomized, non-placebo controlled trial	Decreased stone events
	Pak et al. (1985)	Potassium citrate vs pretreatment	89	Non-randomized, non-placebo controlled trial	Decreased stone events
Cystine	Dahlberg et al. (1977)	D-penicillamine	89	Retrospective	Decreased stone event and dissolution of stones
	Pak et al. (1986)	Tiopronin vs conservative Rx	66	Retrospective	Both drugs equally effective in reducing stone events
	Barbey et al. (2000)	D-penicillamine or tiopronin vs conservative Rx	27	Retrospective	Decreased stone events
	Chow and Streem (1996)	D-penicillamine or tiopronin vs conservative Rx	16	Non-randomized non-placebo controlled trial	Decreased stone event

Alkalinization of the urine can be successfully achieved with potassium citrate (Sakhaee et al., 1983; Barcelo et al., 1993). A randomized double-blind trial of potassium citrate, 30–60 mEq daily, showed a lower stone recurrence rate in the treatment group compared to controls (Barcelo et al., 1993). In another study in patients with recurrent hypocitraturic calcium nephrolithiasis or UA nephrolithiasis, use of potassium citrate, 60 mEq daily, reduced stone formation in 97.8% of subjects and remission was obtained in 79.8% (Pak et al., 1985). In patients with hypocitraturia alone, potassium citrate treatment reduced the rate of total stone formation from 0.7/year to 0.13/year after treatment ($P < 0.005$). In the same study, patients with hypocitraturia with other metabolic abnormalities, there was an even more striking decline in the total stone formation rate from 1.2/year to 0.08/year following treatment with potassium citrate ($P < 0.05$) (Whalley et al., 1996).

Special consideration for calcium phosphate stones

Dietary recommendations for patients with $CaPO_4$ stones are similar to those for patients with CaOx stones, with the exception of the management of an elevated urine pH, which is invariably seen in $CaPO_4$ stone formers. This elevation in pH is often due to overt or incomplete dRTA (Pak et al., 2003b) treatment with topiramate (Welch et al., 2006), overzealous alkali treatment (Krambeck et al., 2010), and aggressive shock wave lithotripsy (Krambeck et al., 2010). Discontinuation of alkali is recommended if urine pH rises above 6.5 without a substantial decline in urinary calcium or an increase in urinary citrate. Therefore potassium alkali should be used judiciously in calcium phosphate stone formers.

Monitoring and follow-up

There are no formal evidence-based recommendations on monitoring and follow-up of stone formers, however most experts would agree that follow-up 24-hour urine and serum analysis should be performed 4–8 weeks after any dietary or pharmacologic intervention to assess efficacy and response to treatment. Periodic follow-up is warranted to monitor the patient's ongoing adherence to preventive measures for stone recurrence. Although long-term preventive treatment strategies have been shown to reduce kidney stone recurrence in patients evaluated over three decades in one university-based referral (Parks and Coe, 2009), supporting evidence has not been fully elucidated in the general stone-forming population due to limitations in adherence to long-term pharmacological and dietary interventions.

Surgical management

Surgical treatment options are not discussed in this chapter. If patients are unresponsive to medical management, shock wave lithotripsy or ureteroscopy with laser lithotripsy are both considered reasonable first-line treatment options by the AUA/EAU 2007 guidelines. Percutaneous nephrolithotomy or laparoscopic or open kidney surgeries are reserved for more complicated and intractable cases.

Non-calcareous stones

There is a wide variety of non-calcareous stones but only two types will be discussed here: UA and cystine.

Pathophysiology and aetiology

UA nephrolithiasis is primarily a disease of aciduria (Pak et al., 2001, 2002, 2005; Sakhaee et al., 2002) with some contribution from hyperuricosuria and low urine volume. UA is a weak organic acid with a pKa_1 of 5.3. Urate is much more soluble than UA so the risk of UA crystallization increases with fall in urine pH and UA crystals dissolve in an alkaline milieu rendering it quite amenable to medical therapy. The primary driver for UA stone formation is low urine pH due to higher acid production (endogenously produced or exogenously ingested) and excretion, inadequacy in urinary buffers, or both. Several defects contribute to UA stone formation. Increased acid load is seen in both type 2 diabetics and UA stone formers (Kamel et al., 2002; Cameron et al., 2006; Maalouf et al., 2010). This is due to increased endogenous acid production, intestinal alkali loss, increased absorption of organic acid from the colon, or a combination. However, increased acid load per se does not lower urine pH if the excess H^+ is adequately buffered. Another defect is that UA stone formers have a tendency not to use ammonia to buffer urinary H^+ leaving the H^+ free to react with the other buffers (Sakhaee et al., 2002; Moe, 2006b). There is a blunted ammoniagenic or excretory response to an acid load in UA stone formers (Sakhaee et al., 2002) (Fig. 205.3).

When individuals with obesity, metabolic syndrome, or type 2 diabetes get kidney stones, they have a 30–50% risk of UA stones

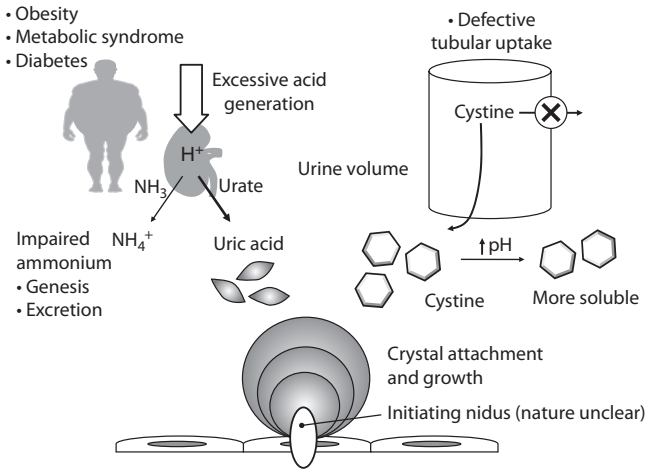


Fig. 205.3 Influences on formation of uric acid and cystine stones.

versus 5–8% in the general stone-forming population and conversely, UA stone formers commonly exhibit features of the metabolic syndrome, including impaired glucose tolerance or overt diabetes mellitus, truncal obesity, and hyperlipidaemia (Pak et al., 2003c; Ekeruo et al., 2004; Daudon et al., 2005, 2006a, 2006b; Lieske et al., 2006). A final additional factor has to be in place based on the fact that low urine pH is very common in subjects with the metabolic syndrome but most of them do not develop kidney stones (Maalouf et al., 2004). Unduly acidic urine is necessary but insufficient for UA nephrolithiasis. The last unknown factor(s) may reside in an imbalance between inhibitors and promoters and/or epithelial factors to permit adherence and crystal growth so crystalluria may progress to nephrolithiasis.

Cystine stones represent 1–2% of all cases of renal lithiasis but up to 8% of stones in children (Chillaron et al., 2010). Cystinuria is an inherited defect in renal reabsorption of cystine called system $b^{0,+}$. The functional unit is a heterodimer in rBAT (neutral and basic amino acid transport protein or *SLC3A1*) and its partner ($b^{0,+}$AT) ($b^{0,+}$ type amino acid transporter 1 or *SLC7A9*) (Chillaron, Font-Llitjos, Fort et al., 2010). Mutations in either one can cause cystinuria. Although there is generalized dibasic amino acid wasting, only cystine has low solubility and hence constitutes the only symptom. The classification of cystinuria is undergoing evolution in an attempt to unify the pre-genetic era clinical empirical classification with the classification based on molecular defects. Due to the lack of perfect genotype–phenotype correlation, there is yet to emerge a perfect system. The traditional phenotypic system of type I, II, and III (Rosenberg et al., 1966) is now shown to have little molecular basis. The current system is based on genetics (Dello Strologo et al., 2002) as type A if mutations are found in both *SLC3A1* alleles, type B if mutations are found in both *SLC7A9* alleles, and type AB if one mutation is found in each gene.

Therapy

Management of uric acid stones

UA stones generally affect adults, with the exception of rare genetic mutations in uric acid metabolism pathways characterized by massive hyperuricosuria (urinary UA concentration >1000 mg/day), significant hyperuricaemia (serum UA >10 mg/dL), aggressive stone formation, severe gout, and renal failure (Moe et al., 2002). UA stones in adults are primarily associated with the metabolic syndrome (Sakhaee et al., 2002). With increasing incidence of obesity and diabetes, there has recently been a report of UA stones in paediatric populations (Cameron et al., 1993). In adult subjects, it may present as either pure UA or mixed UA and CaOx stones (Sakhaee et al., 2002).

The diagnosis is suggested by radiolucent stones and established by stone analysis which should be followed by a complete metabolic evaluation (Cameron and Pak, 2004). Twenty-four-hour urinary profiles should consist of total volume, pH, creatinine, sodium, potassium, calcium, magnesium, oxalate, citrate, sulphate, and chloride. Urinary sulphate is a surrogate marker of high acid intake (mostly from proteins) which lowers urinary pH. Urinary dip-stick analysis may be used for the measurement of pH throughout the day to account for diurnal variations in urinary pH (Rodman, 1991; Cameron et al., 2007). Computed tomography (CT) examination shows radiolucent stones which is indicated in symptomatic patients with haematuria, pain, and abnormal renal function.

Since xanthine and 2,8-dihydroxyadenine (2,8-DHA) stones are also radiolucent, stone analysis is essential. Xanthine stones are typically encountered in inherited UA pathway disorders (Moe et al., 2002) or in patients on allopurinol treatment. 2,8-DHA stones are seen in patients with adenine phosphoribosyl transferase deficiencies (Riese and Sakhaee, 1992; Cameron et al., 1993). One important differential diagnosis frequently overlooked by practising physicians is the differentiation between hyperuricosuric CaOx nephrolithiasis and UA stones (Pak et al., 2002b; Sorensen and Chandhoke, 2002). Urinary pH and urinary UA can be used to discern these two conditions. Hyperuricosuria and urinary pH > 5.5 are typically encountered in CaOx stone formation (Pak et al., 2002), whereas acidic urine pH ≤ 5.5 and normouricosuria is predominantly detected in UA stone formers (Sakhaee et al., 2002).

Dietary treatment of uric acid stones

Patients with UA nephrolithiasis should rigidly adhere to lifestyle modifications such as sufficient fluid intake to ensure 2 L of urine per day. This includes the consideration of fluid loss caused by hot environments and from strenuous physical exercise (Sakhaee et al., 1987). Dietary protein restrictions must be encouraged mainly to reduce acid load and lower urinary UA excretion. A 24-hour urinary sulphate excretion of < 40 mEq/day confirms low animal-protein consumption. The recommended daily protein allowance is 0.8 g/kg body weight. To date, it remains unknown whether various sources of animal protein have a different effect on urinary pH.

Pharmacologic treatment of uric acid stones

The most important factor in the treatment of UA nephrolithiasis is low urine pH. Given urinary UA solubility of 96 mg/L and normal urinary UA can exceed 600mg/day, UA is always at risk of precipitation (Asplin, 1996; Sakhaee, 2009). Since UA is a weak acid with a pKa of 5.5, urine becomes supersaturated with the sparingly soluble undissociated UA at a urinary pH ≤ 5.5, increasing the risk of UA stone (Sakhaee et al., 1983, 2002). Therefore the foundation of therapy is to shift undissociated UA to the more soluble urate by raising urine pH.

Both potassium and sodium alkali treatment effectively raise urinary pH and prevent stone recurrence (Freed, 1975; Sakhaee et al., 1983; Pak et al. 1985, 1986b) (Table 205.6). In one study, potassium citrate caused a sustained increase in urinary pH, normalized urinary citrate without a significant change in urinary UA, oxalate,

Table 205.6 Differential physicochemical and therapeutic effects of potassium citrate versus sodium alkali

	Potassium citrate	Sodium alkali
Urine pH	Increase	Increase
Urine citrate	Large increase	Increase
Urine calcium	Decrease	Increase
Inhibitory activity of calcium oxalate	Increase	No change
Prevention of uric acid stones	Effective	Effective
Prevention of calcium oxalate stones	Highly effective	Effective

sodium, phosphorus, and urinary volume, and improved clinical stone event with a decline in individual stone formation rate in 98% of patients (Pak et al., 1985). The need for surgical treatment of new stone formation was abolished.

Potassium alkali is advantageous since it also reduces urinary calcium excretion and induction of CaOx crystallization from monosodium urate (Sakhaee et al., 1983; Pak, Sakhaee and Fuller, 1986b) (Table 205.6). In patients with pure UA and mixed UA/CaOx stones, treatment with potassium citrate at 30–80 mEq/day increased urinary pH and decreased undissociated UA, and reduced new stone formation from 1.20 to 0.01 stones/year (Sakhaee et al., 1983; Pak et al., 1986b). Sodium alkali is appropriate in patients with chronic kidney disease and in those with a gastrointestinal intolerance of potassium salt. The recommended daily dose for both treatments depends upon body weight and urinary pH. The suggested initial dose ranges between 30 and 40mEq/day. Urinary pH should be maintained between 6.1 and 6.7 to avoid development of calcium phosphate stones (Coe et al., 2005). In rare instances, carbonic anhydrase inhibitors (acetazolamide) can be used as an alternative alkalinizing agent. However, physicians should be aware of its side effects, such as hypocitraturia, systemic metabolic acidosis, and risk of calcium phosphate stones (Gordon and Sheps, 1957; Kuo et al., 2002; Lamb et al., 2004). Treatment with allopurinol should be considered in women with a urinary UA excretion > 600 mg/day and in men with urinary UA > 700 mg/day. Allopurinol is a first-line of treatment in patients with inherited UA metabolism disorders, primary gout, and increased tissue turnover. Although the side effects of this treatment are minimal, careful consideration must be made in patients with renal impairment (Becker et al., 2005). Febuxostate® is a related purine analogue inhibitor of xanthine oxidase. Febuxostat (Uloric®) may be used in those patients who do not tolerate allopurinol. However, its efficacy has not been tested in the kidney stone-forming population.

Management of cystine stones

Although cystine stones may affect both paediatric and adult populations, approximately two-thirds of patients develop their first kidney stone before the age of 20 (Leusmann et al., 1990; Dello Strologo et al., 2002). Cystine stones in children may be in both the kidney and the bladder. Cystine stones affect both genders, yet male subjects tend to display a more aggressive stone burden (Dello et al., 2002). The course of this disease is rather severe with a high recurrence rate, which may lead to progressive renal dysfunction (Gambaro et al., 2001). Multiple invasive and non-invasive procedures are often required due to the protracted course of illness (Pierides and Deltas, 1997; Pras, 2000).

The most common clinical manifestations include renal colic with or without stone passage, urinary tract infections, gross haematuria, and back pain (Dahlberg et al., 1977; Sakhaee, 1996). An association has also been reported between cystine stones and various systemic disorders (Scriver et al., 1970; Thier and Segal, 1972; Sakhaee and Sutton, 1996) and/or other metabolic abnormalities (Vergis and Walker, 1970; Sakhaee et al., 1989). These may concur with mixed CaOx and cystine stones (Thier and Halperin, 1980).

The most important step in the diagnosis of cystine stones is stone analysis. Cystine stones are typically radio opaque on KUB examination since the density of sulphur and calcium is similar (Thier and Halperin, 1980). Occasionally, cystine stones are large and may attain a staghorn size. Ultrasonography and CT examination can be used for the acute diagnosis of obstructive cystine stones. A quantitative cystine measurement should be made to firmly establish this diagnosis. Ion exchange chromatography can reliably detect and quantify various amino acids, including cystine, in the urine (Spackman et al., 1958). Urinary cystine excretion exceeding 250 mg/g Cr usually indicates the diagnosis of homozygous cystinuria.

Dietary treatment of cystine stones

The treatment of cystine stones is aimed at reducing urinary cystine concentration and excretion. This may be attained by fluid intake and dietary restrictions (Table 205.7). This may be achieved by drinking 3 L of fluid in children and 4–5 L of fluid in adults. Fluid intake should be homogenously distributed throughout the day and at bedtime in order to maintain consistent urinary dilution (Monnens et al., 2000; Fjellstedt et al., 2001). Fruit juices are preferred since they provide both urinary dilution and increased pH due to alkali content. Two glasses of orange juice typically increases urinary pH by 0.5 units (Pak, 1990). One should avoid drinking milk which increases urinary cystine excretion due to its high methionine content, a substrate for cystine production (Kolb et al., 1967). A low-protein diet has been recommended since meat and meat products contain methionine (Kolb et al., 1967). Limitations include poor adherence and these dietary restrictions are not suitable in growing children (Pak, 1990; Sakhaee and Sutton, 1996). Sodium intake may also affect urinary cystine excretion in both adults and children (Jaeger et al., 1986; Rodriguez et al., 1995). Reduction in dietary sodium intake of 150 mEq/day may reduce urinary cystine by 156 mg/day (650 µmol/day) (Jaeger et al., 1986).

Pharmacologic treatment of cystine stones

Alkali therapy

Since cystine solubility is pH dependent (Dent and Senior, 1955), oral alkali treatment is effective but a high dose is required due to the high pKa of cystine (8.5). The achievement of a urinary pH > 7.5 is very difficult mainly due to poor patient compliance and risk of calcium phosphate stones (Vega et al., 2007). Urinary cystine solubility is somewhat unpredictable due to the complex urinary environment consisting of electrolytes that may change the ionic strength of the whole urine (Pak and Fuller, 1983). Due to these limitations, urinary pH should be maintained between 6.5 and 7.0 and not higher. Both sodium and potassium alkali are effective in raising urinary pH. However, the preferred alkali treatment is potassium citrate since increased sodium intake poses the risk of higher urinary cystine excretion (Jaeger et al., 1986). The initial

Table 205.7 Treatment of cystine stones

Treatment type		Mode of action	Indication	Side effects
Conservative	Sufficient fluid intake	Reduces urinary cystine concentration	All patients	None
	Low-methionine diet Low-salt diet	Limitation of substrate for cystine production Reduction of cystine excretion	All patients: limited compliance	Reduced salt intake may decrease cystine solubility due to 'reduced solvent action'
Pharmacologic	Oral alkali	Increased urinary cystine solubility	All cystinuric patients (> 250 mg/day)	Urinary pH > 6.7 may increase the risk for calcium phosphate stone formation
	First-generation chelating agents (D-penicillamine)	Thiol-disulphide exchange with cysteine	Severely cystinuric patients (> 500 mg/day) or moderate cystinuric patients (250–500 mg/day) when fluid and alkali treatment are ineffective	20–69% experience dermatological, haematological, and/or renal complications
	Second-generation chelating agents (tiopronin)	Thiol-disulphide exchange with cysteine	Severely cystinuric patients (> 500 mg/day) or moderate cystinuric patients (250–500 mg/day) when fluid and alkali treatment are ineffective	14–40% experience dermatological, haematological, and/or renal complications
	Captopril	Thiol-disulphide exchange with cysteine	Unproven effectiveness	Unknown due to limited use in cystinuric patients
	Ascorbic acid	Converts cystine to cysteine	Patient intolerant of first- and second-generation chelating agents	Increases urinary oxalate which may increase the risk of calcium oxalate stones.
		Reduces cystine excretion via renal tubular competitive inhibition	Controversial: it is useful with high sodium intake	High salt increased urinary cystine

dose for adults is 15–20 mEq twice a day. Incremental adjustments are necessary to attain optimal urinary pH.

Chelating agents

Chelating agents such as D-penicillamine (di-methyl cysteine) and α-mercaptopropionylglycine must be used in subjects who are non-responsive to urinary alkalization and in those with severe cystinuria (> 500 mg/day). Both of these agents are thiol derivatives which cleave a single cystine molecule into two cysteine molecules to make a soluble disulphide compound consisting of the respective drug and cysteine molecules and lowers the excretion of sparingly soluble cystine (Crawhall et al., 1963; Lindell et al., 1995) (Fig. 205.4).

D-penicillamine (Cuprimin®, Depen®) was the first chelating agent found to lower urinary cystine (Crawhall et al., 1963) and stone incidence in cystinuric patients when added to conservative treatment (Dahlberg et al., 1977; Linari et al., 1980) (Table 205.7). D-penicillamine bears the risk of dermatological (pemiphigus), haematologic (agranulocytosis, thrombocytopenia), renal (nephrotic syndrome), and rheumatological (polymyositis) complications (Halperin et al., 1981). The dosage must be adjusted to reduce the urinary cystine concentration below its solubility limit of < 250 mg/L (1 mmol/L). Generally, the appropriate dosage in adults is approximately 1000mg/day, which reduces the urinary cystine excretion by 360 mg/day (Pak et al., 1986a). In children, the recommended dose is 20–40 mg/kg body weight/day (Fjellstedt et al., 2001). This drug should be given in divided dosages before meals for optimal absorption. Since cystine excretion is higher at night, it is also preferred that one dose is administered at bedtime (Fjellstedt et al., 2001). Vitamin B_6 deficiency may occur so supplementation (50 mg/day) is beneficial. One may initially use the lowest dose of drug with a gradual, incremental increase over a 4-week period until the optimal tolerated dosage is attained.

α-mercaptopropionylglycine (Tiopronin®) has a mechanism similar to that of D-penicillamine. Both drugs are equally effective in reducing kidney stone incidence when compared with conservative management, tiopronin is preferred because of lower incidences of side effects (Linari et al., 1980; Pak et al., 1986a) (Table 205.7). With oral dosing, one-quarter of the administered dose appeared unchanged in the urine. Mediated by a thiol-disulphide exchange mechanism, this participates in the interaction with cystine which leads to lowered urinary cystine excretion (Pak et al., 1986a). Tiopronin is effective in causing stone remission and reducing the individual stone formation rates in patients with and without prior D-penicillamine treatment (Pak et al, 1986a). In patients with stone relapse on D-penicillamine, tiopronin was shown to significantly decreased new stone formation by 71% and 61.5% of patients remained in remission. Side effects including skin reactions, oral ulcers, gastrointestinal intolerance, and nephrotic syndrome (Tasic et al., 2011) up to 43% of cystinuric patients compared to 67% on D-penicillamine (Pak et al., 1986a). Nephrotic syndrome disappears with the discontinuation of treatment.

One study in a total of 27 adult patients with cystine stones reported a significant decrease in stone episodes and urological procedures when thiol drugs (D-penicillamine and tiopronin) were added to conservative management (Barbey et al., 2000). Moreover,

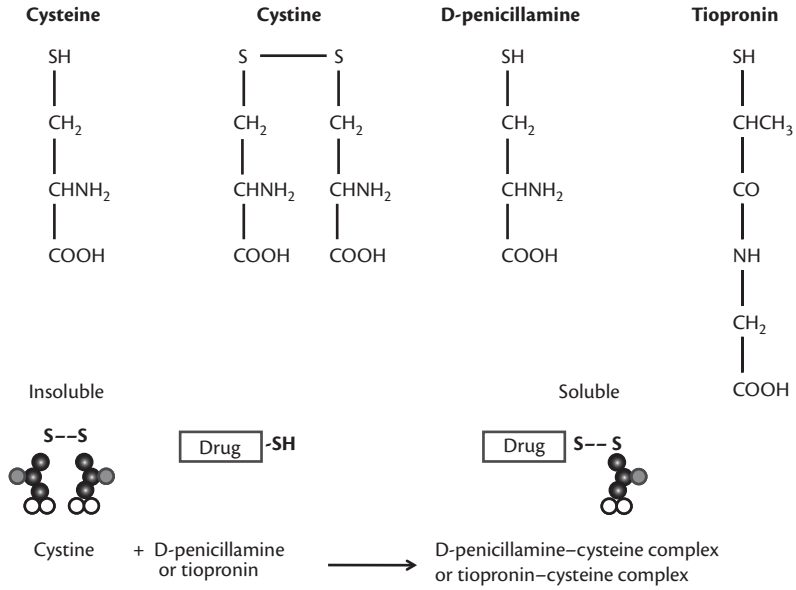


Fig. 205.4 Mechanism of action of chelating agents.

a study conducted 16 patients with cystinuria followed for 7–141 months showed the stone event per patient-year decreased significantly from 1.58 to 0.52 when D-penicillamine or tiopronin was added to those patients who were non-responsive to conservative management (Chow and Streem, 1996). This finding suggests a 65% decrease in yearly stone event rate during thiol treatment compared to conservative management.

Captopril is an angiotensin-converting enzyme inhibitor which possesses a thiol component which has been suggested to form a thiol-cysteine mixed disulphide and reduce urinary cystine excretion (Sloand and Izzo, 1987). In one report, 75–100 mg/day of captopril reduced urinary cystine excretion (Perazella and Buller, 1993). However, the effect was not confirmed by other investigators (Dahlberg and Jones, 1989; Barbey et al., 2000). The lack of efficacy of captopril was not unexpected considering that two molecules of captopril is required to bind one molecule of cystine. Therefore, due to this stoichiometry, a sufficient amount of captopril could not be safely administered to adequately lower urinary cystine excretion.

It has been suggested that ascorbic acid is an effective treatment for cystinuria. This agent is known to convert cystine to cysteine at high doses. The recommended dose is 3 g/day for children and 5 g/day for adults (Lux and May, 1983; Knoll et al., 2005). However, it is argued that its effectiveness is due to provision of alkali from the effervescent ascorbic acid tablet which contains bicarbonate (Birwe et al., 1991; Ragone, 2000). Additionally, excessive ascorbic acid ingestion may increase the risk of CaOx stone formation since this acid is one of the major precursors of oxalate formation (Traxer et al., 2003). Oral and intravenous glutamine was shown to lower urinary cystine excretion (Miyagi et al., 1979). This was suggested to be due to increased renal tubular cystine reabsorption or reduced renal tubular cystine secretion (Miyagi et al., 1979). However, the result of this study remained unverified by other investigators (Van Den Berg et al., 1980; Jaeger et al., 1986).

Conclusion

Nephrolithiasis is a systemic metabolic disease with complex underlying pathophysiology and identifiable aetiologies in fraction of instances. Unfortunately, there is still a tendency for practitioners to consider a spontaneous stone passage as a resolution and failure to do so as a surgical disease. There is a dire need for pathophysiologic investigation of kidney stones not only to unveil underlying causes but to direct therapy to normalize urinary chemistry. Empirical manipulation of urinary risk factors by dietary and pharmacologic means is very effective in prevention of recurrence and this approach should be adopted and mastered by physicians.

References

Ahlstrand, C. and Tiselius, H. G. (1987). Urine composition and stone formation during treatment with acetazolamide. *Scand J Urol Nephrol*, 21, 225–8.

Alon, U., Costanzo, L. S., and Chan, J. C. (1984). Additive hypocalciuric effects of amiloride and hydrochlorothiazide in patients treated with calcitriol. *Miner Electrolyte Metab*, 10, 379–86.

Asplin, J. R. (1996). Uric acid stones. *Semin Nephrol*, 16, 412–24.

Asplin, J. R. and Coe, F. L. (2007). Hyperoxaluria in kidney stone formers treated with modern bariatric surgery. *J Urol*, 177, 565–9.

Auron, A. and Alon, U. S. (2005). Resolution of medullary nephrocalcinosis in children with metabolic bone disorders. *Pediatr Nephrol*, 20, 1143–5.

Backman, U., Danielson, B. G., Johansson, G., *et al.* (1980). Incidence and clinical importance of renal tubular defects in recurrent renal stone formers. *Nephron*, 25, 96–101.

Barbey, F., Joly, D., Rieu, P., *et al.* (2000). Medical treatment of cystinuria: critical reappraisal of long-term results. *J Urol*, 163, 1419–23.

Barcelo, P., Wuhl, O., Servitge, E., *et al.* (1993). Randomized double-blind study of potassium citrate in idiopathic hypocitraturic calcium nephrolithiasis. *J Urol*, 150, 1761–4.

Becker, M. A., Schumacher, H. R., Jr., Wortmann, R. L., *et al.* (2005). Febuxostat compared with allopurinol in patients with hyperuricemia and gout. *N Engl J Med*, 353, 2450–61.

Belostotsky, R., Seboun, E., Idelson, G. H., *et al.* (2010). Mutations in DHDPSL are responsible for primary hyperoxaluria type iii. *Am J Hum Genet*, 87, 392–9.

Bergner, R., Hoffmann, M., Waldherr, R., *et al.* (2003). Frequency of kidney disease in chronic sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis*, 20, 126–32.

Birwe, H., Schneeberger, W., and Hesse, A. (1991). Investigations of the efficacy of ascorbic acid therapy in cystinuria. *Urol Res*, 19, 199–201.

Blanchard, A., Vargas-Poussou, R., Peyrard, S., *et al.* (2008). Effect of hydrochlorothiazide on urinary calcium excretion in dent disease: an uncontrolled trial. *Am J Kidney Dis*, 52, 1084–95.
Borghi, L., Meschi, T., Amato, F., *et al.* (1996). Urinary volume, water and recurrences in idiopathic calcium nephrolithiasis: A 5-year randomized prospective study. *J Urol*, 155, 839–43.
Borghi, L., Meschi, T., Guerra, A., *et al.* (1993). Randomized prospective study of a nonthiazide diuretic, indapamide, in preventing calcium stone recurrences. *J Cardiovasc Pharmacol*, 22 Suppl 6, S78–86.
Borghi, L., Schianchi, T., Meschi, T., *et al.* (2002). Comparison of two diets for the prevention of recurrent stones in idiopathic hypercalciuria. *N Engl J Med*, 346, 77–84.
Breslau, N. A., Preminger, G. M., Adams, B. V., *et al.* (1992). Use of ketoconazole to probe the pathogenetic importance of 1,25-dihydroxyvitamin d in absorptive hypercalciuria. *J Clin Endocrinol Metab*, 75, 1446–52.
Brown, E. M. (2010). Clinical utility of calcimimetics targeting the extracellular calcium-sensing receptor (CASR). *Biochem Pharmacol*, 80, 297–307.
Burtis, W. J., Gay, L., Insogna, K. L., *et al.* (1994). Dietary hypercalciuria in patients with calcium oxalate kidney stones. *Am J Clin Nutr*, 60, 424–9.
Bushinsky, D., Coe, F. L., and Moe, O. W. (2012). Nephrolithiasis. In M. W. Taal, G. M. Chertow, P. A. Marsden, *et al.* (eds.) *Brenner & Rector's The Kidney* (9th ed.), pp. 1455–507. Philadelphia, PA: Saunders.
Cameron, J. S., Moro, F., and Simmonds, H. A. (1993). Gout, uric acid and purine metabolism in paediatric nephrology. *Pediatr Nephrol*, 7, 105–18.
Cameron, M. A., Baker, L. A., Maalouf, N. M., *et al.* (2007). Circadian variation in urine ph and uric acid nephrolithiasis risk. *Nephrol Dial Transplant*, 22, 2375–8.
Cameron, M. A., Maalouf, N. M., Adams-Huet, B., *et al.* (2006). Urine composition in type 2 diabetes: Predisposition to uric acid nephrolithiasis. *J Am Soc Nephrol*, 17, 1422–8.
Cameron, M. A., and Pak, C. Y. (2004). Approach to the patient with the first episode of nephrolithiasis. *Clin Rev Bone Miner Metab*, 2, 265–78.
Cebotaru, V., Kaul, S., Devuyst, O., *et al.* (2005). High citrate diet delays progression of renal insufficiency in the clc-5 knockout mouse model of Dent's disease. *Kidney Int*, 68, 642–52.
Cheidde, L., Ajzen, S. A., Tamer Langen, C. H., *et al.* (2007). A critical appraisal of the radiological evaluation of nephrocalcinosis. *Nephron Clin Pract*, 106, c119–24.
Chillaron, J., Font-Llitjos, M., Fort, J., *et al.* (2010). Pathophysiology and treatment of cystinuria. *Nat Rev Nephrol*, 6, 424–34.
Chow, G. K. and Streem, S. B. (1996). Medical treatment of cystinuria: Results of contemporary clinical practice. *J Urol*, 156, 1576–8.
Coe, F. L. (1977). Treated and untreated recurrent calcium nephrolithiasis in patients with idiopathic hypercalciuria, hyperuricosuria, or no metabolic disorder. *Ann Intern Med*, 87, 404–10.
Coe, F. L. (1978). Hyperuricosuric calcium oxalate nephrolithiasis. *Kidney Int*, 13, 418–26.
Coe, F. L., Evan, A., and Worcester, E. (2005). Kidney stone disease. *J Clin Invest*, 115, 2598–608.
Coe, F. L., Lawton, R. L., Goldstein, R. B., *et al.* (1975). Sodium urate accelerates precipitation of calcium oxalate in vitro. *Proc Soc Exp Biol Med*, 149, 926–9.
Coe, F. L. and Parks, J. H. (1981). Hyperuricosuria and calcium nephrolithiasis. *Urol Clin North Am*, 8, 227–44.
Coe, F. L., Parks, J. H., Bushinsky, D. A., *et al.* (1988). Chlorthalidone promotes mineral retention in patients with idiopathic hypercalciuria. *Kidney Int*, 33, 1140–6.
Crawhall, J. C., Scowen, E. F., and Watts, R. W. (1963). Effect of penicillamine on cystinuria. *Br Med J*, 1, 588–90.
Curhan, G. C., Willett, W. C., Rimm, E. B., *et al.* (1996). Prospective study of beverage use and the risk of kidney stones. *Am J Epidemiol*, 143, 240–7.
Curhan, G. C., Willett, W. C., Rimm, E. B., *et al.* (1993). A prospective study of dietary calcium and other nutrients and the risk of symptomatic kidney stones. *N Engl J Med*, 328, 833–8.
Curhan, G. C., Willett, W. C., Speizer, F. E., *et al.* (1997). Comparison of dietary calcium with supplemental calcium and other nutrients as factors affecting the risk for kidney stones in women. *Ann Intern Med*, 126, 497–504.
Dahlberg, P. J. and Jones, J. D. (1989). Cystinuria: failure of captopril to reduce cystine excretion. *Arch Intern Med*, 149, 713, 717.
Dahlberg, P. J., Van Den, B., Kurtz, S. B., *et al.* (1977). Clinical features and management of cystinuria. *Mayo Clin Proc*, 52, 533–42.
Daudon, M., Lacour, B., and Jungers, P. (2005). High prevalence of uric acid calculi in diabetic stone formers. *Nephrol Dial Transplant*, 20, 468–9.
Daudon, M., Lacour, B., and Jungers, P. (2006a). Influence of body size on urinary stone composition in men and women. *Urol Res*, 34, 193–9.
Daudon, M., Traxer, O., Conort, P., *et al.* (2006b). Type 2 diabetes increases the risk for uric acid stones. *J Am Soc Nephrol*, 17, 2026–33.
Dello Strologo, L., Pras, E., Pontesilli, C., *et al.* (2002). Comparison between SLC3A1 and SLC7A9 cystinuria patients and carriers: a need for a new classification. *J Am Soc Nephrol*, 13, 2547–53.
Dent, C. E. and Senior, B. (1955). Studies on the treatment of cystinuria. *Br J Urol*, 27, 317–32.
Egbuna, O. I., Taylor, J. G., Bushinsky, D. A., *et al.* (2007). Elevated calcium phosphate product after renal transplantation is a risk factor for graft failure. *Clin Transplant*, 21, 558–66.
Eisner, B. H., Asplin, J. R., Goldfarb, D. S., *et al.* (2010). Citrate, malate and alkali content in commonly consumed diet sodas: implications for nephrolithiasis treatment. *J Urol*, 183, 2419–23.
Ekeruo, W. O., Tan, Y. H., Young, M. D., *et al.* (2004). Metabolic risk factors and the impact of medical therapy on the management of nephrolithiasis in obese patients. *J Urol*, 172, 159–63.
Escribano, J., Balaguer, A., Pagone, F., *et al.* (2009). Pharmacological interventions for preventing complications in idiopathic hypercalciuria. *Cochrane Database Syst Rev*, 1, CD004754.
Ettinger, B. (1989). Does hyperuricosuria play a role in calcium oxalate lithiasis? *J Urol*, 141, 738–41.
Ettinger, B., Tang, A., Citron, J. T., *et al.* (1986). Randomized trial of allopurinol in the prevention of calcium oxalate calculi. *N Engl J Med*, 315, 1386–9.
Evan, A. P., Lingeman, J. E., Coe, F. L., *et al.* (2005). Crystal-associated nephropathy in patients with brushite nephrolithiasis. *Kidney Int*, 67, 576–91.
Fabris, A., Lupo, A., Bernich, P., *et al.* (2010). Long-term treatment with potassium citrate and renal stones in medullary sponge kidney. *Clin J Am Soc Nephrol*, 5, 1663–8.
Favus, M. J., Karnauskas, A. J., Parks, J. H., *et al.* (2004). Peripheral blood monocyte vitamin d receptor levels are elevated in patients with idiopathic hypercalciuria. *J Clin Endocrinol Metab*, 89, 4937–43.
Fjellstedt, E., Denneberg, T., Jeppsson, J. O., *et al.* (2001). Cystine analyses of separate day and night urine as a basis for the management of patients with homozygous cystinuria. *Urol Res*, 29, 303–10.
Freed, S. Z. (1975). The alternating use of an alkalizing salt and acetazolamide in the management of cystine and uric acid stones. *J Urol*, 113, 96–9.
Freel, R. W., Hatch, M., Green, M., *et al.* (2006). Ileal oxalate absorption and urinary oxalate excretion are enhanced in slc26a6 null mice. *Am J Physiol Gastrointest Liver Physiol*, 290, G719–28.
Gambaro, G., Favaro, S., and D'Angelo, A. (2001). Risk for renal failure in nephrolithiasis. *Am J Kidney Dis*, 37, 233–43.
Gordon, E. E. and Sheps, S. G. (1957). Effect of acetazolamide on citrate excretion and formation of renal calculi. *N Engl J Med*, 256, 1215–19.
Grover, P. K., Ryall, R. L., and Marshall, V. R. (1990). Effect of urate on calcium oxalate crystallization in human urine: evidence for a promotory role of hyperuricosuria in urolithiasis. *Clin Sci (Lond)*, 79, 9–15.
Hall, P. M. (2009). Nephrolithiasis: treatment, causes, and prevention. *Cleve Clin J Med*, 76, 583–91.
Halperin, E. C., Thier, S. O., and Rosenberg, L. E. (1981). The use of D-penicillamine in cystinuria: efficacy and untoward reactions. *Yale J Biol Med*, 54, 439–46.
Heilberg, I. P. and Weisinger, J. R. (2006). Bone disease in idiopathic hypercalciuria. *Curr Opin Nephrol Hypertens*, 15, 394–402.
Hess, B., Ackermann, D., Essig, M., *et al.* (1995). Renal mass and serum calcitriol in male idiopathic calcium renal stone formers: role of protein intake. *J Clin Endocrinol Metab*, 80, 1916–21.

Hiatt, R. A., Ettinger, B., Caan, B., *et al.* (1996). Randomized controlled trial of a low animal protein, high fiber diet in the prevention of recurrent calcium oxalate kidney stones. *Am J Epidemiol*, 144, 25–33.
Hollingsworth, J. M., Rogers, M. A., Kaufman, S. R., *et al.* (2006). Medical therapy to facilitate urinary stone passage: A meta-analysis. *Lancet*, 368, 1171–9.
Holmes, R. P. and Assimos, D. G. (1998). Glyoxylate synthesis, and its modulation and influence on oxalate synthesis. *J Urol*, 160, 1617–24.
Holmes, R. P. and Kennedy, M. (2000). Estimation of the oxalate content of foods and daily oxalate intake. *Kidney Int*, 57, 1662–7.
Hoppe, B., Beck, B., Gatter, N., *et al.* (2006). Oxalobacter formigenes: a potential tool for the treatment of primary hyperoxaluria type 1. *Kidney Int*, 70, 1305–11.
Hoppe, B., Beck, B. B., and Milliner, D. S. (2009). The primary hyperoxalurias. *Kidney Int*, 75, 1264–71.
Hubner, W. A., Irby, P., and Stoller, M. L. (1993). Natural history and current concepts for the treatment of small ureteral calculi. *Eur Urol*, 24, 172–6.
Jackson, R. D., Lacroix, A. Z., Gass, M., *et al.* (2006). Calcium plus vitamin d supplementation and the risk of fractures. *N Engl J Med*, 354, 669–83.
Jaeger, P., Portmann, L., Saunders, A., *et al.* (1986). Anticystinuric effects of glutamine and of dietary sodium restriction. *N Engl J Med*, 315, 1120–3.
Kamel, K. S., Cheema-Dhadli, S., and Halperin, M. L. (2002). Studies on the pathophysiology of the low urine ph in patients with uric acid stones. *Kidney Int*, 61, 988–94.
Kaplan, R. A., Haussler, M. R., Deftos, L. J., *et al.* (1977). The role of 1 alpha, 25-dihydroxyvitamin d in the mediation of intestinal hyperabsorption of calcium in primary hyperparathyroidism and absorptive hypercalciuria. *J Clin Invest*, 59, 756–60.
Knoll, S. and Alon, U. S. (2000). Effect of thiazide on established furosemide-induced nephrocalcinosis in the young rat. *Pediatr Nephrol*, 14, 32–5.
Knoll, T., Zollner, A., Wendt-Nordahl, G., *et al.* (2005). Cystinuria in childhood and adolescence: Recommendations for diagnosis, treatment, and follow-up. *Pediatr Nephrol*, 20, 19–24.
Kolb, F. O., Earll, J. M., and Harper, H. A. (1967). 'Disappearance' of cystinuria in a patient treated with prolonged low methionine diet. *Metabolism*, 16, 378–81.
Krambeck, A. E., Handa, S. E., Evan, A. P., *et al.* (2010). Brushite stone disease as a consequence of lithotripsy? *Urol Res*, 38, 293–9.
Kruse, A. E., Eisenberger, U., Frey, F. J., *et al.* (2005). The calcimimetic cinacalcet normalizes serum calcium in renal transplant patients with persistent hyperparathyroidism. *Nephrol Dial Transplant*, 20, 1311–14.
Kuo, R. L., Moran, M. E., Kim, D. H., *et al.* (2002). Topiramate-induced nephrolithiasis. *J Endourol*, 16, 229–31.
Labrecque, M., Dostaler, L. P., Rousselle, R., *et al.* (1994). Efficacy of nonsteroidal anti-inflammatory drugs in the treatment of acute renal colic. A meta-analysis. *Arch Intern Med*, 154, 1381–7.
Laerum, E. and Larsen, S. (1984). Thiazide prophylaxis of urolithiasis. A double-blind study in general practice. *Acta Med Scand*, 215, 383–9.
Lamb, E. J., Stevens, P. E., and Nashef, L. (2004). Topiramate increases biochemical risk of nephrolithiasis. *Ann Clin Biochem*, 41, 166–9.
Laminski, N. A., Meyers, A. M., Kruger, M., *et al.* (1991). Hyperoxaluria in patients with recurrent calcium oxalate calculi: dietary and other risk factors. *Br J Urol*, 68, 454–8.
Lauderdale, D. S., Thisted, R. A., Wen, M., *et al.* (2001). Bone mineral density and fracture among prevalent kidney stone cases in the third national health and nutrition examination survey. *J Bone Miner Res*, 16, 1893–8.
Lemann, J., Jr., Gray, R. W., and Pleuss, J. A. (1989). Potassium bicarbonate, but not sodium bicarbonate, reduces urinary calcium excretion and improves calcium balance in healthy men. *Kidney Int*, 35, 688–95.
Leumann, E., Hoppe, B., and Neuhaus, T. (1993). Management of primary hyperoxaluria: Efficacy of oral citrate administration. *Pediatr Nephrol*, 7, 207–11.
Leusmann, D. B., Blaschke, R., and Schmandt, W. (1990). Results of 5,035 stone analyses: a contribution to epidemiology of urinary stone disease. *Scand J Urol Nephrol*, 24, 205–10.
Lieske, J. C., De La Vega, L. S., Gettman, M. T., *et al.* (2006). Diabetes mellitus and the risk of urinary tract stones: a population-based case-control study. *Am J Kidney Dis*, 48, 897–904.
Lieske, J. C., Tremaine, W. J., De Simone, C., *et al.* (2010). Diet, but not oral probiotics, effectively reduces urinary oxalate excretion and calcium oxalate supersaturation. *Kidney Int*, 78, 1178–85.
Linari, F., Marangella, M., Fruttero, B., *et al.* (1980). The natural history of cystinuria. In L. H. Smith, W. G. Robertson, and B. Finlayson (eds.) *Urolithiasis: Clinical and Basic Research*, pp. 145–58. New York, NY: Plenum Press.
Lindell, A., Denneberg, T., and Jeppsson, J. O. (1995). Urinary excretion of free cystine and the tiopronin-cysteine-mixed disulfide during long term tiopronin treatment of cystinuria. *Nephron*, 71, 328–42.
Lindsjo, M., Danielson, B. G., Fellstrom, B., *et al.* (1989). Intestinal oxalate and calcium absorption in recurrent renal stone formers and healthy subjects. *Scand J Urol Nephrol*, 23, 55–9.
Lux, B. and May, P. (1983). Long-term observation of young cystinuric patients under ascorbic acid therapy. *Urol Int*, 38, 91–4.
Maalouf, N. M., Cameron, M. A., Moe, O. W., *et al.* (2010). Metabolic basis for low urine ph in type 2 diabetes. *Clin J Am Soc Nephrol*, 5, 1277–81.
Maalouf, N. M., Sakhaee, K., Parks, J. H., *et al.* (2004). Association of urinary ph with body weight in nephrolithiasis. *Kidney Int*, 65, 1422–5.
Maierhofer, W. J., Gray, R. W., Cheung, H. S., *et al.* (1983). Bone resorption stimulated by elevated serum 1,25-(OH)2-vitamin d concentrations in healthy men. *Kidney Int*, 24, 555–60.
Meschi, T., Maggiore, U., Fiaccadori, E., *et al.* (2004). The effect of fruits and vegetables on urinary stone risk factors. *Kidney Int*, 66, 2402–10.
Milliner, D. S., Eickholt, J. T., Bergstralh, E. J., *et al.* (1994). Results of long-term treatment with orthophosphate and pyridoxine in patients with primary hyperoxaluria. *N Engl J Med*, 331, 1553–8.
Miyagi, K., Nakada, F., and Ohshiro, S. (1979). Effect of glutamine on cystine excretion in a patient with cystinuria. *N Engl J Med*, 301, 196–8.
Moe, O. W. (2006a). Kidney stones: pathophysiology and medical management. *Lancet*, 367, 333–44.
Moe, O. W. (2006b). Uric acid nephrolithiasis: proton titration of an essential molecule? *Curr Opin Nephrol Hypertens*, 15, 366–73.
Moe, O. W., Abate, N., and Sakhaee, K. (2002). Pathophysiology of uric acid nephrolithiasis. *Endocrinol Metab Clin North Am*, 31, 895–914.
Moe, O. W., Maalouf, N. M., and Sakhaee, K. (in press). Nephrolithiasis. In J. G. Fitz (ed.) *Textbook of Medicine*. Philadelphia, PA: American College of Physicians.
Moe, O. W., Pearle, M. S., and Sakhaee, K. (2011). Pharmacotherapy of urolithiasis: evidence from clinical trials. *Kidney Int*, 79, 385–92.
Moe, O. W. and Preisig, P. A. (2006). Dual role of citrate in mammalian urine. *Curr Opin Nephrol Hypertens*, 15, 419–24.
Monnens, L. A., Noordam, K., and Trijbels, F. (2000). Necessary practical treatment of cystinuria at night. *Pediatr Nephrol*, 14, 1148–9.
Nicar, M. J., Hill, K., and Pak, C. Y. (1987). Inhibition by citrate of spontaneous precipitation of calcium oxalate in vitro. *J Bone Miner Res*, 2, 215–20.
Nicar, M. J., Peterson, R., and Pak, C. Y. (1984). Use of potassium citrate as potassium supplement during thiazide therapy of calcium nephrolithiasis. *J Urol*, 131, 430–3.
Nijenhuis, T., Hoenderop, J. G., Loffing, J., *et al.* (2003). Thiazide-induced hypocalciuria is accompanied by a decreased expression of Ca^{2+} transport proteins in kidney. *Kidney Int*, 64, 555–64.
Nouvenne, A., Meschi, T., Prati, B., *et al.* (2010). Effects of a low-salt diet on idiopathic hypercalciuria in calcium-oxalate stone formers: a 3-mo randomized controlled trial. *Am J Clin Nutr*, 91, 565–70.
Odvina, C. V. (2006). Comparative value of orange juice versus lemonade in reducing stone-forming risk. *Clin J Am Soc Nephrol*, 1, 1269–74.
Odvina, C. V., Preminger, G. M., Lindberg, J. S., *et al.* (2003). Long-term combined treatment with thiazide and potassium citrate in nephrolithiasis does not lead to hypokalemia or hypochloremic metabolic alkalosis. *Kidney Int*, 63, 240–7.

Pak, C. Y. (1990). Cystine lithiasis. In L. M. Resnick and C. Y. Pak (eds.) *Urolithiasis: A Medical and Surgical Reference*, pp. 133–43. Philadelphia, PA: Saunders.

Pak, C. Y. (1994). Citrate and renal calculi: an update. *Miner Electrolyte Metab*, 20, 371–7.

Pak, C. Y., Adams-Huet, B., Poindexter, J. R., *et al.* (2004). Rapid communication: relative effect of urinary calcium and oxalate on saturation of calcium oxalate. *Kidney Int*, 66, 2032–7.

Pak, C. Y. and Arnold, L. H. (1975). Heterogeneous nucleation of calcium oxalate by seeds of monosodium urate. *Proc Soc Exp Biol Med*, 149, 930–2.

Pak, C. Y., Britton, F., Peterson, R., *et al.* (1980a). Ambulatory evaluation of nephrolithiasis. Classification, clinical presentation and diagnostic criteria. *Am J Med*, 69, 19–30.

Pak, C. Y., Fuller, C., Sakhaee, K., *et al.* (1985). Long-term treatment of calcium nephrolithiasis with potassium citrate. *J Urol*, 134, 11–9.

Pak, C. Y., Fuller, C., Sakhaee, K., *et al.* (1986a). Management of cystine nephrolithiasis with alpha-mercaptopropionylglycine. *J Urol*, 136, 1003–8.

Pak, C. Y. and Fuller, C. J. (1983). Assessment of cystine solubility in urine and of heterogeneous nucleation. *J Urol*, 129, 1066–70.

Pak, C. Y., Heller, H. J., Pearle, M. S., *et al.* (2003). Prevention of stone formation and bone loss in absorptive hypercalciuria by combined dietary and pharmacological interventions. *J Urol*, 169, 465–9.

Pak, C. Y., Moe, O. W., Sakhaee, K., *et al.* (2005). Physicochemical metabolic characteristics for calcium oxalate stone formation in patients with gouty diathesis. *J Urol*, 173, 1606–9.

Pak, C. Y., Oata, M., Lawrence, E. C., *et al.* (1974). The hypercalciurias. Causes, parathyroid functions, and diagnostic criteria. *J Clin Invest*, 54, 387–400.

Pak, C. Y., Peters, P., Hurt, G., *et al.* (1981). Is selective therapy of recurrent nephrolithiasis possible? *Am J Med*, 71, 615–22.

Pak, C. Y. and Peterson, R. (1986). Successful treatment of hyperuricosuric calcium oxalate nephrolithiasis with potassium citrate. *Arch Intern Med*, 146, 863–7.

Pak, C. Y., Poindexter, J. R., Adams-Huet, B., *et al.* (2003). Predictive value of kidney stone composition in the detection of metabolic abnormalities. *Am J Med*, 115, 26–32.

Pak, C. Y., Poindexter, J. R., Peterson, R. D., *et al.* (2002). Biochemical distinction between hyperuricosuric calcium urolithiasis and gouty diathesis. *Urology*, 60, 789–94.

Pak, C. Y., Sakhaee, K., Crowther, C., *et al.* (1980b). Evidence justifying a high fluid intake in treatment of nephrolithiasis. *Ann Intern Med*, 93, 36–9.

Pak, C. Y., Sakhaee, K., and Fuller, C. (1986). Successful management of uric acid nephrolithiasis with potassium citrate. *Kidney Int*, 30, 422–8.

Pak, C. Y., Sakhaee, K., Moe, O., *et al.* (2003). Biochemical profile of stone-forming patients with diabetes mellitus. *Urology*, 61, 523–7.

Pak, C. Y., Sakhaee, K., Peterson, R. D., *et al.* (2001). Biochemical profile of idiopathic uric acid nephrolithiasis. *Kidney Int*, 60, 757–61.

Parks, J. H. and Coe, F. L. (2009). Evidence for durable kidney stone prevention over several decades. *BJU Int*, 103(9), 1238–46.

Parks, J. H., Worcester, E. M., Coe, F. L., *et al.* (2004). Clinical implications of abundant calcium phosphate in routinely analyzed kidney stones. *Kidney Int*, 66, 777–85.

Parsons, J. K., Hergan, L. A., Sakamoto, K., *et al.* (2007). Efficacy of alpha-blockers for the treatment of ureteral stones. *J Urol*, 177, 983–7; discussion 987.

Peacock, M. (2002). Primary hyperparathyroidism and the kidney: biochemical and clinical spectrum. *J Bone Miner Res*, 17 Suppl 2, N87–94.

Perazella, M. A. and Buller, G. K. (1993). Successful treatment of cystinuria with captopril. *Am J Kidney Dis*, 21, 504–7.

Pierides, A. M. and Deltas, C. C. (1997). Clinical aspects of cystinuria. *Contrib Nephrol*, 122, 167–72.

Pras, E. (2000). Cystinuria at the turn of the millennium: clinical aspects and new molecular developments. *Mol Urol*, 4, 409–14.

Preminger, G. M. (1992). Renal calculi: pathogenesis, diagnosis, and medical therapy. *Semin Nephrol*, 12, 200–16.

Preminger, G. M., Sakhaee, K., and Pak, C. Y. (1987). Hypercalciuria and altered intestinal calcium absorption occurring independently of vitamin d in incomplete distal renal tubular acidosis. *Metabolism*, 36, 176–9.

Preminger, G. M., Tiselius, H. G., Assimos, D. G., *et al.* (2007). 2007 guideline for the management of ureteral calculi. *J Urol*, 178, 2418–34.

Raeder, H., Shaw, N., Netelenbos, C., *et al.* (2008). A case of x-linked hypophosphatemic rickets: complications and the therapeutic use of cinacalcet. *Eur J Endocrinol*, 159 Suppl 1, S101–5.

Ragone, R. (2000). Medical treatment of cystinuria with vitamin c. *Am J Kidney Dis*, 35, 1020.

Raja, K. A., Schurman, S., D'Mello R, G., *et al.* (2002). Responsiveness of hypercalciuria to thiazide in Dent's disease. *J Am Soc Nephrol*, 13, 2938–44.

Reilly, R. F., Peixoto, A. J., and Desir, G. V. (2010). The evidence-based use of thiazide diuretics in hypertension and nephrolithiasis. *Clin J Am Soc Nephrol*, 5, 1893–903.

Rejnmark, L., Vestergaard, P., and Mosekilde, L. (2011). Nephrolithiasis and renal calcifications in primary hyperparathyroidism. *J Clin Endocrinol Metab*, 96, 2377–85.

Reusz, G. S., Dobos, M., Tulassay, T., *et al.* (1993). Hydrochlorothiazide treatment of children with hypercalciuria: effects and side effects. *Pediatr Nephrol*, 7, 699–702.

Riese, R. J. and Sakhaee, K. (1992). Uric acid nephrolithiasis: pathogenesis and treatment. *J Urol*, 148, 765–71.

Rodman, J. S. (1991). Prophylaxis of uric acid stones with alternate day doses of alkaline potassium salts. *J Urol*, 145, 97–9.

Rodriguez, L. M., Santos, F., Malaga, S., *et al.* (1995). Effect of a low sodium diet on urinary elimination of cystine in cystinuric children. *Nephron*, 71, 416–8.

Rosenberg, L. E., Downing, S., Durant, J. L., *et al.* (1966). Cystinuria: Biochemical evidence for three genetically distinct diseases. *J Clin Invest*, 45, 365–71.

Saigal, C. S., Joyce, G., and Timilsina, A. R. (2005). Direct and indirect costs of nephrolithiasis in an employed population: Opportunity for disease management? *Kidney Int*, 68, 1808–14.

Sakhaee, K. (1996). Pathogenesis and medical management of cystinuria. *Semin Nephrol*, 16, 435–47.

Sakhaee, K. (2009). Recent advances in the pathophysiology of nephrolithiasis. *Kidney Int*, 75, 585–95.

Sakhaee, K., Adams-Huet, B., Moe, O. W., *et al.* (2002). Pathophysiologic basis for normouricosuric uric acid nephrolithiasis. *Kidney Int*, 62, 971–9.

Sakhaee, K., Alpern, R., Jacobson, H. R., *et al.* (1991). Contrasting effects of various potassium salts on renal citrate excretion. *J Clin Endocrinol Metab*, 72, 396–400.

Sakhaee, K., Maalouf, N. M., Kumar, R., *et al.* (2011). Nephrolithiasis-associated bone disease: pathogenesis and treatment options. *Kidney Int*, 79, 393–403.

Sakhaee, K., Nicar, M., Hill, K., *et al.* (1983). Contrasting effects of potassium citrate and sodium citrate therapies on urinary chemistries and crystallization of stone-forming salts. *Kidney Int*, 24, 348–52.

Sakhaee, K., Nigam, S., Snell, P., *et al.* (1987). Assessment of the pathogenetic role of physical exercise in renal stone formation. *J Clin Endocrinol Metab*, 65, 974–9.

Sakhaee, K., Poindexter, J. R., and Pak, C. Y. (1989). The spectrum of metabolic abnormalities in patients with cystine nephrolithiasis. *J Urol*, 141, 819–21.

Sakhaee, K. and Sutton, R. A. L. (1996). Pathogenesis and medical management of cystinuria. In F. L. Coe, M. J. Favus, C. Y. Pak, *et al.* (eds.) *Kidney Stones: Medical and Surgical Management*, pp. 1007–1017. Philadelphia, PA: Lippincott-Raven.

Sayer, J. A., Carr, G., and Simmons, N. L. (2004). Nephrocalcinosis: molecular insights into calcium precipitation within the kidney. *Clin Sci (Lond)*, 106, 549–61.

Schell-Feith, E., Moerdijk, A., Van Zwieten, P., *et al.* (2006). Does citrate prevent nephrocalcinosis in preterm neonates? *Pediatric Nephrology*, 21, 1830–6.

Schell-Feith, E. A., Kist-Van Holthe, J. E., and Van Der Heijden, A. J. (2010). Nephrocalcinosis in preterm neonates. *Pediatr Nephrol*, 25, 221–30.

Scriver, C. R., Whelan, D. T., Clow, C. L., *et al.* (1970). Cystinuria: Increased prevalence in patients with mental disease. *N Engl J Med*, 283, 783–6.

Seikaly, M. G. and Baum, M. (2001). Thiazide diuretics arrest the progression of nephrocalcinosis in children with X-linked hypophosphatemia. *Pediatrics*, 108, E6.

Seikrit, C., Muhlfeld, A., Groene, H. J., *et al.* (2011). Renal allograft failure in a hyperparathyroid patient following initiation of a calcimimetic. *Nat Rev Nephrol*, 7, 237–41.

Seltzer, M. A., Low, R. K., Mcdonald, M., *et al.* (1996). Dietary manipulation with lemonade to treat hypocitraturic calcium nephrolithiasis. *J Urol*, 156, 907–9.

Serra, A. L., Schwarz, A. A., Wick, F. H., *et al.* (2005). Successful treatment of hypercalcemia with cinacalcet in renal transplant recipients with persistent hyperparathyroidism. *Nephrol Dial Transplant*, 20, 1315–9.

Sloand, J. A. and Izzo, J. L., Jr. (1987). Captopril reduces urinary cystine excretion in cystinuria. *Arch Intern Med*, 147, 1409–12.

Sorensen, C. M. and Chandhoke, P. S. (2002). Hyperuricosuric calcium nephrolithiasis. *Endocrinol Metab Clin North Am*, 31, 915–25.

Spackman, D. H., Stein, W. H., and Moore, S. (1958). Automatic recording apparatus for use in chromatography of amino acids. *Ann Chem*, 30, 1190–206.

Tasic, V., Lozanovski, V. J., Ristoska-Bojkovska, N., *et al.* (2011). Nephrotic syndrome occurring during tiopronin treatment for cystinuria. *Eur J Pediatr*, 170, 247–9.

Taylor, E. N. and Curhan, G. C. (2006). Diet and fluid prescription in stone disease. *Kidney Int*, 70, 835–9.

Taylor, E. N. and Curhan, G. C. (2008a). Determinants of 24-hour urinary oxalate excretion. *Clin J Am Soc Nephrol*, 3, 1453–60.

Taylor, E. N. and Curhan, G. C. (2008b). Fructose consumption and the risk of kidney stones. *Kidney Int*, 73, 207–12.

Taylor, E. N., Fung, T. T., and Curhan, G. C. (2009). Dash-style diet associates with reduced risk for kidney stones. *J Am Soc Nephrol*, 20, 2253–9.

Taylor, E. N., Stampfer, M. J., and Curhan, G. C. (2004). Dietary factors and the risk of incident kidney stones in men: new insights after 14 years of follow-up. *J Am Soc Nephrol*, 15, 3225–32.

Taylor, E. N., Stampfer, M. J., Mount, D. B., *et al.* (2010). Dash-style diet and 24-hour urine composition. *Clin J Am Soc Nephrol*, 5, 2315–22.

Thier, S. O. and Halperin, E. C. (1980). Cystinuria. In F. L. Coe (ed.) *Nephrolithiasis*, pp. 208–30. New York: Churchill Livingstone.

Thier, S. O. and Segal, S. (1972). Cystinuria. In J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson (eds.) *Metabolic Basis of Inherited Disease*, pp. 1504–19. pp. New York: McGraw Hill.

Tiselius, H. G., Berg, C., Fornander, A. M., *et al.* (1993). Effects of citrate on the different phases of calcium oxalate crystallization. *Scanning Microsc*, 7, 381–9; discussion 389–90.

Traxer, O., Huet, B., Poindexter, J., *et al.* (2003). Effect of ascorbic acid consumption on urinary stone risk factors. *J Urol*, 170, 397–401.

Van Den Berg, C. J., Jones, J. D., Wilson, D. M., *et al.* (1980). Glutamine therapy of cystinuria. *Invest Urol*, 18, 155–7.

Vega, D., Maalouf, N. M., and Sakhaee, K. (2007). Increased propensity for calcium phosphate kidney stones with topiramate use. *Expert Opin Drug Saf*, 6, 547–57.

Vergis, J. G. and Walker, B. R. (1970). Cystinuria, hyperuricemia and uric acid nephrolithiasis. Case report. *Nephron*, 7, 577–9.

Welch, B. J., Graybeal, D., Moe, O. W., *et al.* (2006). Biochemical and stone-risk profiles with topiramate treatment. *Am J Kidney Dis*, 48, 555–63.

Whalley, N. A., Meyers, A. M., Martins, M., *et al.* (1996). Long-term effects of potassium citrate therapy on the formation of new stones in groups of recurrent stone formers with hypocitraturia. *Br J Urol*, 78, 10–14.

Worcester, E. M. (2002). Stones from bowel disease. *Endocrinol Metab Clin North Am*, 31, 979–99.

Worcester, E. M. and Coe, F. L. (2010). Clinical practice. Calcium kidney stones. *N Engl J Med*, 363, 954–63.

Yendt, E. R. and Cohanim, M. (1978). Prevention of calcium stones with thiazides. *Kidney Int*, 13, 397–409.

Yilmaz, E., Batislam, E., Kacmaz, M., *et al.* (2010). Citrate, oxalate, sodium, and magnesium levels in fresh juices of three different types of tomatoes: evaluation in the light of the results of studies on orange and lemon juices. *Int J Food Sci Nutr*, 61, 339–45.

CHAPTER 206

Imaging and interventional treatment: urolithiasis from the surgeon's point of view

Gauthier Raynal and Olivier Traxer

Introduction

Urolithiasis holds a position of great importance in the urologist's practice:

- It is a frequently encountered disease which represents a great part of a urologist's activity.
- From a historical point of view, stone disease is linked to the beginning of urology as an individual medical specialty. Hippocrates had indeed in his oaths forbidden his students to perform bladder lithotomy: 'I will not use the knife, not even on sufferers from stone, but will withdraw in favor of such men as are engaged in this work' (Bloom, 1997).
- Stone disease is linked to many technical improvements in urology, from the beginning of endoscopy up to extracorporeal shockwave lithotripsy (ESWL) and modern endourology.

Imaging

When examining a patient for a probable stone, some questions have to be answered by imaging procedures.

Questions for the examination

Stone diagnosis and differential diagnosis

Patients mainly present with renal colic, or alternatively with sole haematuria or infection, or atypical pain. Renal colic is just a clinical syndrome and not a disease. Some diseases may cause or mimic renal colic, such as:

- Aortic aneurysm
- Non-obstructive pyelonephritis
- Non-stone-related ureteropelvic junction obstruction (and upper urinary tract tumours)
- Retroperitoneal fibrosis.

That is why it is important to rule out life-threatening conditions such as an aortic aneurysm, and to consider alternative diagnoses before linking one calcification to the clinical presentation. Up to one-third of computed tomography (CT) scans performed for acute flank pain examination lead to another diagnosis than a stone (Katz et al., 2000).

Stone size, location, and density (prognosis)

Treatment outcomes and probability of spontaneous expulsion depend on the size of the calculus and its position. The smaller and the further away from the bladder the calculus (or fragments after a procedure), the better is the prognosis of spontaneous expulsion. Stone hardness, which is correlated to stone density as seen in a KUB (kidney, ureter, bladder) plain radiograph or measured in Hounsfield units (HU) in a CT scanner, impacts the efficacy of an interventional treatment (Joseph et al., 2002; Gupta et al., 2005; Pareek et al., 2005; Weld et al., 2007).

Some additional parameters are noteworthy for prognosis of ESWL results: body mass index and skin-to-stone distance (Pareek et al., 2005).

Renal involvement

Upper urinary tract rupture is a common finding after a renal obstruction and is not necessarily an imperative indication for drainage but, for extensive urinoma or severe dilation, drainage should be considered. Evaluation of parenchyma should also be considered before stone treatment planning. Because of possible contralateral renal hypertrophia and a normal creatinine clearance, it may not be necessary to treat stone calculi which are not responsible for true symptoms in a non-functioning kidney. Function is best evaluated with nuclear imaging, if needed.

Preoperative planning and anatomical assessment

Some underlying anatomical conditions can predispose to stone formation. They also could represent a true surgical challenge and need to be known before interventions, for example:

- uretero-pelvic junction obstruction
- caliceal diverticulum
- medullary sponge kidneys (Lenarduzzi–Cacchi–Ricci disease)
- horseshoe kidneys.

Ureteric duplication can be a surgical trap if ignored: no stone is seen in one of the systems, whereas there is a true stone in the second system.

Different modalities for stone imaging

KUB plain radiograph

KUB is the simplest modality for stone imaging but has a low accuracy (Levine et al., 1997; Chan et al., 2008). Some important items

are to be evaluated: the two last ribs, the pubic symphysis, the kidneys, and the psoas shadows must be seen. Performing KUB in the lying down position can diminish bowel gas.

Calculi are seen as a calcification in the kidney area or in the theoretical path of the ureter. They have to be differentiated from phleboliths which appear as calcifications which are rounder, smoother, and clear in the centre (Fig. 206.1).

Ultrasonography

Ultrasound (US) alone does not have a better accuracy than KUB for the diagnosis of a calculus. Ureters are not visible if not dilated or if the bladder is empty, for the distal segment. When they are seen, calculi appear as a hyperechogenic area followed by a cone-shaped shadow. A 'twinkling' effect can be seen when using the Doppler mode and could help in appreciating stone composition that affects shockwave resistance (Hassani et al., 2012).

US can appreciate renal involvement and the urinary tract dilation, and is probably useful for the differential diagnosis, in the context of an acute abdominal pain.

The combined accuracy of KUB and US is clearly better than for each examination alone (Yilmaz et al., 1998). Moreover, this combination has been a gold standard for a long time, since it permits the diagnosis of calculi and assesses the differential diagnoses and the upstream impact of the obstruction.

Unenhanced computed tomography

Unenhanced CT now has a well-established evidence base for the best accuracy for urolithiasis imaging, as well as for calculi diagnosis rather than for the differential diagnosis (Fielding et al., 1997; Meagher et al., 2001). It has a very good prognostic value for spontaneous expulsion, and for active treatments results (Joseph et al., 2002; Favela et al., 2005; Gupta et al., 2005). It is economically efficient since it avoids unnecessary hospital admissions and diagnostic uncertainty (Patel et al., 2000), but there is a true concern regarding radiation exposure for stone diagnosis and treatment (John et al., 2008; Manohar and McCahy, 2011). That is why 'low-dose' procedures have emerged as a new standard for CT in urolithiasis with few or no impact on its accuracy (Liu et al., 2000; Meagher et al., 2001; Heneghan et al., 2003; Tack et al., 2003; Mulkens et al., 2007; Poletti et al., 2007; Ciaschini et al., 2009).

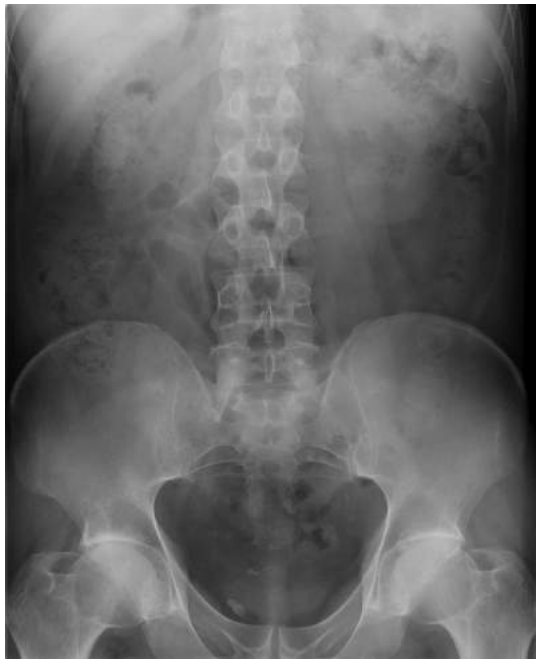


Fig. 206.1 Two right calculi located in the ureterovesical junction, above a typical phlebolith.

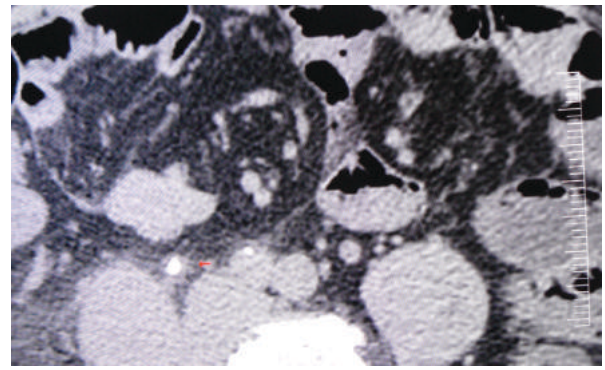


Fig. 206.2 Ureteral calculus with a rim sign.

Size, precise location, and density of calculi have to be noted for treatment planning. The biggest size has to be measured and this is not always seen in the axial view. Some authors advocate for the use of volumetric measures (Bandi et al., 2009). Ureteral calculi can be easily distinguished from phleboliths using the 'rim sign' (Fig. 206.2): a grey rim is seen around the calculus and corresponds to the ureteral oedema whereas there is no rim around a phlebolith but only a following 'comet tail' (Fig. 206.3). It can be useful to switch to bone windows that permit calculi to be better described (Fig. 206.4) (Eisner et al., 2009). One limitation, however, is that it is often difficult to discriminate different but near stones (Raynal et al., 2010). It is possible to see indirect signs of obstruction: pyelocaliceal and ureteral dilation, perinephric oedema that appears as 'blurring' of the sinus fat, or perirenal 'stranding' which makes the kidney seem unsmooth (Sourtzis et al., 1999) (Fig. 206.5).

In conclusion: different examinations for different times

For *emergency diagnosis* in the context of renal colic, unenhanced CT is the examination of choice. It is fast, efficient, accurate, and is sure to rule out alternative diagnoses. It has a good prognostic value for spontaneous expulsion, as well as for stone hardness. It has to

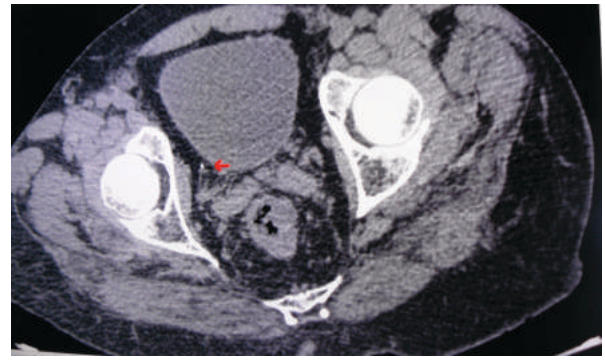


Fig. 206.3 A pelvic phlebolith with a 'comet tail'.

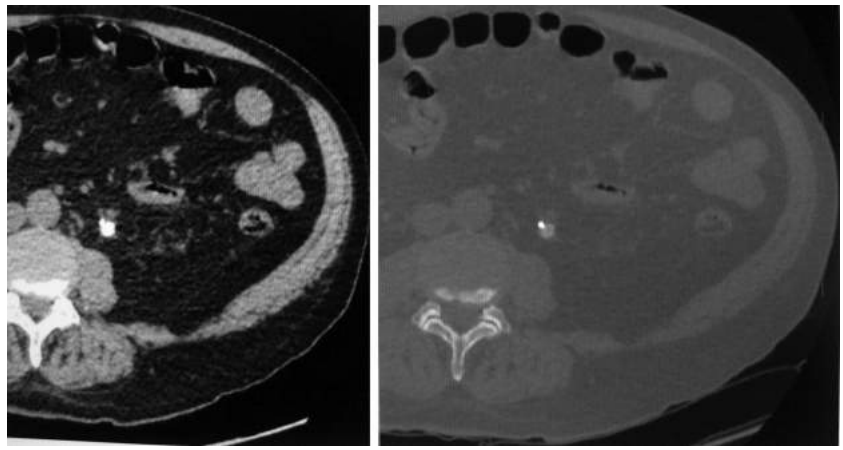


Fig. 206.4 A low-density lumbar calculus better differentiated from a double-J stent using bone window.

be performed with 'low-dose' radiation and is to be avoided in possibly pregnant women.

But in the *assessment of a stone patient and before operative planning*, an examination with contrast medium (enhanced CT or intravenous pyelography) is useful to rule out pre-existing anatomical risk factors for stone formation and not to ignore upper urinary tract anatomy before performing endoscopy or percutaneous nephrolithotomy (PCNL). However it has been assumed that unenhanced CT is sufficient before an ESWL session (Greenstein et al., 2003).

For *follow-up after treatment*, unenhanced CT is known to be the best modality for searching for all the residual fragments, but there is a concern about repeated CT radiation, and searching for very small residual fragments is questionable (Raynal et al., 2009). The KUB/US combination is probably sufficient, not KUB alone, since small invisible fragments can lead to an asymptomatic obstruction only visible with US.

Renal colic management and expulsive medical therapy

Colic management

Colicky pain results from the acute distension of the upper urinary tract, mainly due to a calculus obstruction. The flank pain is severe, intermittent, and usually presents as an acute onset of pain with radiations up to the groin or genitalia. The physiopathology of the pain is now better known and depends mainly on a cascade of prostaglandins (Ankem et al., 2005) which are responsible for maintaining abnormal ureteric contractility. Contractility of the ureter, which normally conducts urine collected in the renal pelvis at a low pressure up to the bladder at a higher pressure, is totally disorganized during an obstruction. Obstruction is painful in the acute phase, but becomes indolent after an adaptation time in such a way that it is not possible to rule out obstruction, based on the clinical presentation. This needs to be remembered in the management of the calculi, after interventional treatment, for example.

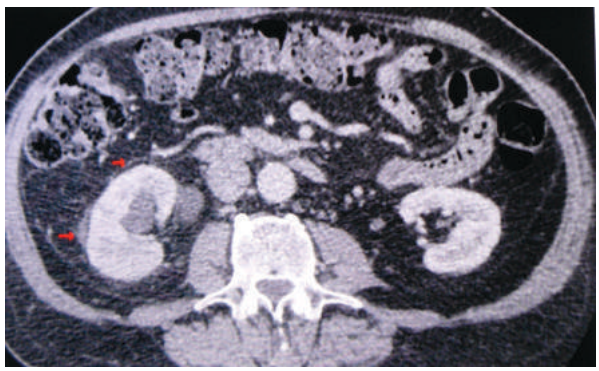


Fig. 206.5 Indirect signs of obstruction in the kidney: perirenal striations and oedema ('stranding'). Notice the difference with the left kidney which is absolutely 'smooth'.

Because non-steroidal anti-inflammatory drugs (NSAIDs) inhibit the synthesis and the pro-contractile effects of prostaglandins, they are used in colic. Holmlund and Sjödin (1978) showed in a placebo controlled trial that indomethacin can relieve pain in colic obstruction.

NSAIDs act as analgesics, prostaglandin inhibitors, and diuresis inhibitors. Inhibiting the diuresis is one way of relieving pain, but is not sufficient. Lopes et al. have shown that the antidiuretic hormone inhibitor desmopressin was not better, alone or in combination, than diclofenac for pain relief (Lopes et al., 2001). In any case, it is not logical to inhibit diuresis, whereas hyperdiuresis is useful against crystallogenesis. In meta-analyses, NSAIDs have been shown to be up to two times more efficient than analgesics for relieving renal colic pain, with the parenteral route permitting quicker efficacy. Other analgesics can be added but are clearly not at the forefront of colic management. Spasmolytic agents have no proven benefit.

Whether it is useful to maintain NSAID treatment after pain resolution is unclear, but NSAIDs can be considered as a part of medical expulsive therapy (MET).

Alpha-blocking agents, such as tamsulosin which is frequently used against prostatic enlargement symptoms but is also the most studied in the ureteral calculi context, could help to spare analgesics use. Alpha-blocking agents and NSAIDs are probably complementary, since NSAIDs induce a decrease in abnormal ureteric contractions frequency, whereas alpha-blockers induce a decrease in contraction amplitudes (Davenport et al., 2007).

Medical expulsive therapy

Some tables are available to appreciate the likelihood of ureteral calculi spontaneous expulsion (Miller and Kane, 1999; Coll et al.,

2002). The smaller and the nearer from the bladder is the calculus, the greater is the expulsion rate.

Several clinical trials have shown that different treatments can promote better expulsion rates and decrease time to expulsion. Alpha-blockers (e.g. tamsulosin) and nifedipine are the most studied drugs. Expulsive effect has been confirmed in a meta-analysis of nine trials with an absolute risk reduction of 0.31. In this analysis, MET is useful not only for increasing expulsion rates and decreasing time to expulsion, but also for decreasing analgesic consumption and the number of pain onsets (Hollingsworth et al., 2006). Tamsulosin is the most studied agent but the effects of other alpha-blockers seem comparable in one trial (Yilmaz et al., 2005), suggesting that it is a class effect.

Treatment patterns differ in the trials but are often based on tamsulosin 0.4 mg a day for 2–4 weeks. Expulsion should be monitored with KUB and US.

The safety profile of alpha-blockers is excellent, since orthostatic hypotension is not such a frequent adverse effect in the stone forming population, who are often younger than the patients with prostatic symptoms. Men sometimes complain of retrograde ejaculation. Otherwise, it should be considered an off-label use.

Trials have been performed with different stone size ranges, but it can probably be assumed that MET is not useful < 5 mm (since the likelihood of expulsion is very high) and > 10 mm (since there is a very low probability of spontaneous passage but a probable need for extraction or fragmentation manoeuvres), and so is critical between 5 and 10 mm.

Tamsulosin has also shown efficacy as an adjunctive treatment after shockwave lithotripsy for promoting fragment passage in several trials (Küpeli et al., 2004; Gravina et al., 2005; Resim et al., 2005; Bhagat et al., 2007). Alpha-blockers have also shown efficacy for increasing double-pigtail stent tolerability (Wang et al., 2009; Mokhtari et al., 2011). One trial has shown that tamsulosin could be useful after ureteroscopy as an adjunctive treatment (John and Razdan, 2010).

Indications for upper urinary tract drainage

Upper urinary tract drainage is mandatory in the following indications: renal failure, obstruction with fever, or unrelieved pain after well-managed medical treatment with NSAIDs and if immediate treatment of the stone is to be delayed. The drainage is made on the first intent by a double pigtail ureteric stent ('double-J stent') and on second intent by a percutaneous nephrostomy. It is mandatory to wait 3 weeks between a pyelonephritis episode and a stone treatment. The creatininaemia has to be stable.

The benefit of double-J stenting prior to ESWL or endoscopy is unclear and still a matter of debate among urologists (see below).

Exclusive chemolysis

For radiolucent calculi (invisible with KUB and < 500 UH in the CT scanner), most of the time composed of uric acid, particularly if the dipstick pH is low, alkalinization of the urine is often sufficient and interventional treatment can be avoided for small stones. Since it takes weeks, interventional treatment can be planned for bigger stones.

Interventional management

Open or laparoscopic surgery

Surgical treatment is no longer a gold standard for stone treatment. Indeed, mean stone size at the diagnosis is smaller and far less invasive procedures have emerged. Surgery consists in a direct surgical retrieval of the stone by an iliac incision for distal stones and lumbotomy for kidney and lumbar ureteral access. Kidney calculi are treated by different approaches:

- Incision of the kidney pelvis (pyelotomy)
- Radial incisions in the parenchyma for caliceal and diverticular calculi
- Clam kidney incisions for big staghorn stones.

Incision of the parenchyma is clearly a challenge requiring good technical skills and anatomical knowledge and is very invasive, often requiring blood transfusions. In a way, kidney stone surgery has foreshadowed nephron-sparing surgery for kidney tumours. Laparoscopic techniques have led to some revival in ureteral and pelvic stone surgery since it permits a less invasive approach. Open or laparoscopic surgery has to be considered as an option for giant stones.

Palliative nephrectomy for poor functioning kidneys (i.e. scintigraphic glomerular filtration rate < 10 mL/min which could not allow avoidance of dialysis requirement if the second kidney is lost) can be considered in symptomatic patients (infection or pain).

Extracorporeal shockwave lithotripsy

Shockwave effects were initially studied in the aeronautical industry and then were studied for the treatment of urinary stones at the end of the 1970s. Different shockwave generators were developed after the first HM3 produced by Dornier, using electro-hydraulic, electro-magnetic, or piezoelectric generators.

Treatment consists in focusing shockwaves up to the rim of a calculus which is increasingly fragmented by cavitation phenomenon (micro-bubbles) and a wave of pressure. The stone is targeted with fluoroscopic and/or US guidance. Stone fragmentation depends on stone hardness and levels of applied energy. It is now clear that a slow rate (60 Hz) permits better fragmentation (Pace et al., 2005).

Some concerns have been raised regarding shockwave side effects. It is known that shockwaves produces parenchymal acute lesions and several biological alterations (Clark et al., 2009). It is contraindicated during pregnancy. It can lead to kidney haematomas and so is contraindicated in blood coagulation disorders and during antiplatelet and anticoagulant therapy. Some authors have postulated that shockwaves were in the long term responsible for a higher incidence of arterial hypertension and even diabetes mellitus (Krambeck et al., 2006; Eassa et al., 2008), but it has never been definitely demonstrated and in any case, urolithiasis and hypertension are statistically linked. Another contraindication is active urinary infection, but antibiotic prophylaxis is not necessary (Deliveliotis et al., 1997).

In obese patients, it could be less efficacious because of raised skin-to-stone distance (Patel et al., 2009). Patients with a pacemaker or cardioverter defibrillator should be monitored. Cardiac arrhythmia is a rare but described complication.

A clear advantage for ESWL is that it can be performed without any anaesthesia with the last generation lithotripters; however, it has been assumed that results are better with general anaesthesia (Sorensen et al., 2002). Without anaesthesia, better patient relaxation can be obtained thanks to listening to music (Yilmaz et al., 2003).

Good fragmentation with rapid expulsion of the stone fragments can lead to a ureteric obstruction described with the German word *steinstrasse*.

Percutaneous nephrolithotomy

PCNL (Fig. 206.6) consists of working a stone in a percutaneous tract obtained using ultrasound and/or fluoroscopic guidance. Puncture requires dilated kidney cavities, in such a way that a ureteral drain is first placed to obtain dilation by saline perfusion if needed. Stones can be grasped 'en bloc' or destroyed with ultrasound, ballistic, or laser devices.

The critical point of the technique is the puncture which is made through the kidney parenchyma, usually in an inferior and posterior calyx, since it is the site with the lowest bleeding risk according to renal vascularization anatomy, and it also presents a thin thickness of parenchyma. After puncture, a tract dilation with balloon or telescopic dilators has to be performed.

PCNL is usually performed in the prone position, for ergonomic reasons, but some authors advocate for the supine position, since it spares operative time, the patient being in the same position for ureteral stenting and percutaneous kidney puncture (De Sio et al., 2008).

It requires good skills and training and this is probably now the most important limitation of the technique. Since size of stones has become lower and lower, and endoscopy more and more performed, technical demands of PCNL limits its use. New trainees are less and less trained with the technique.

The most common complications are fever and haemorrhagic disorders, sometimes (5–10%) requiring blood transfusions or interventional transvascular embolizations (Seitz et al., 2012).

Active urinary infection and blood coagulation disorders are contraindications. Antibiotic prophylaxis is recommended. Obesity and anatomic malformations make the puncture challenging, but some assume that it is a good indication since it could permit good results in these patients.

Endoscopy

The principle of endoscopy is to get up to the stone by the natural route. It is the simplest, and probably the safest interventional approach. Rigid instruments (Fig. 206.7) (optical lenses) are usually used in the distal ureter and flexible ones (optical fibres) in the kidney. In the lumbar ureter, it depends on the surgeon's preferences. Irrigation and vision are better with rigid instruments, but flexible ureteroscopy permits working in the entire kidney with very few anatomical limitations (Figs 206.8 and 206.9). Stones can be retrieved with a grasp forceps, a basket device, or destroyed with ballistic (pneumatic) devices or with a holmium:YAG laser. It is not recommended to use ballistic devices with the flexible ureteroscopy, because it can damage the scope.

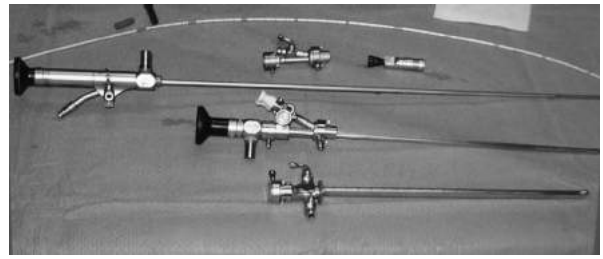


Fig. 206.7 Rigid ureteroscope (top) and cystoscope (bottom).

Because of the smaller instruments used, endoscopy takes more time than PCNL for the same stone size. It is usually recommended to stop the procedure after 2 hours, in order to avoid positioning complications, septic complications, and so on. That is why, for big stones, patients have to be informed that the endoscopic approach could require several procedures. There are few data upon post-ureteroscopy complications but they seem rare. Fever is the most common one. Haemorrhagic complications are rare and endoscopy is the safest procedure in relation to blood coagulation disorders. It can be performed without antiplatelet therapy discontinuation. Active urinary infection is a contraindication.

A natural route can lead to ureteric complications. Especially with rigid instruments, there is a rare risk of stripping of the ureter which could require ileal ureter reconstruction. Flexible ureteroscopy is helped by a ureteral access sheath which permits better irrigation and a bladder bypass that is useful to avoid iterative re-access to the ureter, and it protects the scope as well. Rigid ureteroscopy or ureteral access sheath insertion can create ureteric lesions with unclear evolution and risk of late stenosis. Some advocate for the systematic use of a safety guidewire, in order to prevent a false route (Raynal, 2011).

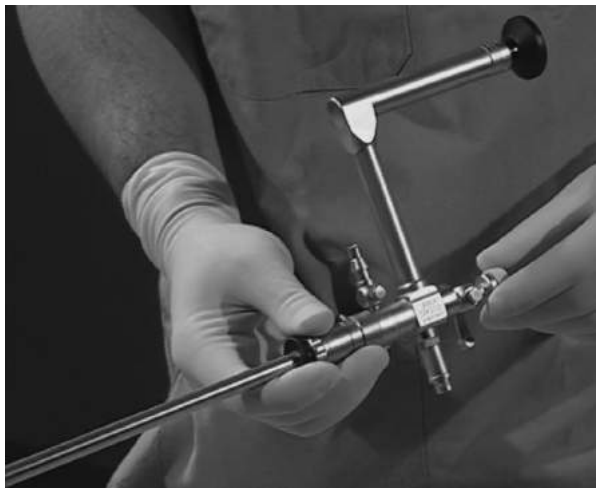


Fig. 206.6 A nephroscope.

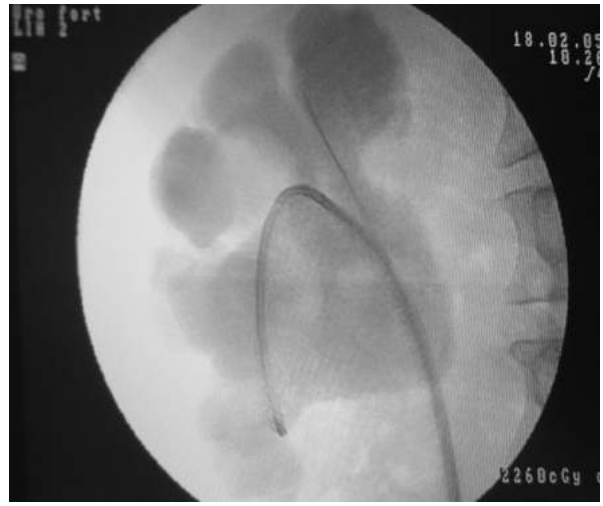


Fig. 206.8 Fluoroscopic view of a flexible ureteroscope movement in a kidney pelvis.

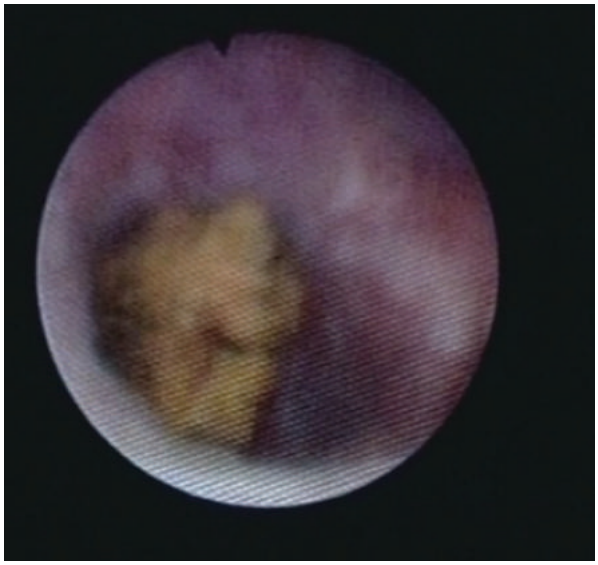


Fig. 206.9 Endoscopic view of a stone (COM type) treated by laser.

Preoperative stenting

The benefit of double-J stenting prior to ESWL or endoscopy is unclear and still a matter of debate among urologists. Stenting has been used while awaiting mobile lithotripter availability. It could prevent acute pain obstruction of *steinstrasse* after ESWL but in fact for stones > 20 mm, ESWL is not a good indication and for stones < 20 mm, the risk of *steinstrasse* is low.

In order to make ureteral access easier, it could be useful before endoscopy, and according to some publications, it could reduce operative difficulties, operative time, and increase stone free rates (Rubenstein et al., 2007; Ghoneim et al., 2010).

Quantification of results

Urolithiasis is a common condition, of which treatment accounts for $2 billion annually in the United Sates (Pearle et al., 2005). Since many techniques are available for this non-life-threatening condition, quantifying clinical results is of critical importance. At the beginning of evaluation, different items were used to quantify results until the rate of 'stone-free' (SF) patients (rate of patients without any visible fragment) became used, initially to compare lithotripter machines and thereafter to compare techniques. SF is clear, simple, and reproducible and far less subjective than the rate of 'clinically resolved cases' or 'clinically insignificant residual fragments' or other proposed items. Several studies have indeed shown that one out of four patients with residual fragments needed complementary procedures (Candau et al., 2000; Osman et al., 2005). But SF raises some questions: how should it be evaluated? Is a CT scanner, the best way to recognize fragments, mandatory or is US/KUB sufficient? When should fragments be evaluated? Stone clearance can, for example, take several months after an ESWL session.

Moreover, the SF rate has to be correlated to the number of procedures to obtain it, since retreatment or ancillary procedures such as ureteral stenting may be needed. That is why the 'efficiency quotient' was developed: SF rate (%)/(100 + retreatment rate (%) + ancillary procedure rate (%)) (Denstedt et al., 1990).

However, neither costs nor quality of life are taken into account, although stones and their treatments have a clear impact upon quality of life (Bensalah et al., 2008). Moreover, SF rate is probably a good outcome for evaluation but is not an individual therapeutic goal (Raynal et al., 2009). Searching for absolute SF may necessitate repeated treatments and can lead to overtreatment. In Germany, the reimbursement system which has changed from treatment-based reimbursement to a stone-based reimbursement (flat sum) is probably responsible for changes in the management strategy. ESWL, of which results are probably more uncertain, has declined whereas endoscopy has increasingly been used (Bergsdorf, 2007).

Indications

Thanks to different clinical guidelines, such as the European Association of Urology (EAU) and American Urological Association (AUA) guidelines, indications are clearly well defined, mainly according to size (Preminger et al., 2007).

According to stone size

In the kidney, stones < 5 mm which cause no symptoms may only require observation (Koh et al., 2012). They indeed represent a very low risk for causing symptoms and if they engage into the ureter the probability for spontaneous expulsion is high. For stones from 5 to 20 mm or smaller stones which are responsible for symptoms, ESWL is the first intention treatment, because of its simple use and the great probability of spontaneous expulsion of the fragments. For stones > 20 mm, there is such a high risk of *steinstrasse* that PCNL is the preferred method, but is concurrent with flexible ureteroscopy for which indications are clearly evolving. For staghorn stones, it has been assumed that 'sandwich' therapy (PCNL–ESWL–PCNL) is the most efficient option (Chandhoke, 1996).

Stones of the pelvic-ureteral junction or of the ureter are considered to be in an obstructive position and can damage the upper kidney. Therefore they need to be treated. Up to 10 mm, first MET with close observation for no more than 3 weeks is an option. But if expulsion has failed, ESWL is indicated. ESWL can be performed quickly after the acute onset *in situ* (Tligui et al., 2003) or after a double-J stent drainage if needed. For stones > 10 mm, ESWL can lead to *steinstrasse* and so rigid ureteroscopy is the preferred indication. For giant stones, surgery is a second-line option.

According to stone location

In the kidney, the inferior calyx is a difficult location for fragment clearance after ESWL. It has been demonstrated that the infundibulopelvic angle has a prognostic impact (Danuser et al., 2007). Some authors have proposed PCNL for inferior calyx stones > 15 mm. It is now clear that flexible ureteroscopy is a good option (Bozkurt et al., 2011).

In the ureter, most authors differentiate proximal (i.e. lumbar) ureter from distal ureter (iliac and pelvic segments). Lumbar stones can be treated by ESWL, or rigid or flexible ureteroscopy. Some authors advocate for the use of flexible ureteroscopy, if endoscopy is required, because of the risk of 'flushing back' the stone up to the kidney. With a flexible ureteroscope it is possible to treat the stone, even if it is flushed up, whereas access to the kidney is difficult with a rigid ureteroscope and impossible for inferior calyx access.

In the distal ureter, iliac location makes the fluoroscopic guidance for ESWL difficult as well as focusing the stone, because shockwaves are stopped either by the iliac bone or by the gut. ESWL is feasible

Table 206.1 Usual indications according to stone localization and size

Kidney stones			
	< 5 mm	**5–20 mm**	**> 20 mm**
Preferred option	Surveillance, if no symptom	ESWL	PCNL
Alternative option		Flexible URS, especially for multiple distant stones or inferior calyx ones	Flexible URS (up to 30 mm?) PCNL: ESWL for residual fragments PCNL: flexible URS?/ESWL:flexible URS?
Lumbar stones			
	< 5 mm	**5–10 mm**	> 10 mm
Preferred option	MET and ESWL if failed	ESWL '*in situ*'	Ureteroscopy (flexible or rigid)
Alternative option		MET	
Distal ureter stones			
	< 10 mm	**> 10 mm**	
Preferred option	MET and ESWL or rigid ureteroscopy if failed	Rigid ureteroscopy	

for pelvic stones, with a filled bladder which conducts shockwaves. But a distal location is also a good indication for rigid ureteroscopy and simple basketing or ballistic disintegration.

According to stone nature or density

Ballistic and laser devices can treat all types of calculi even if some can be harder. ESWL results are far more dependent on stone hardness. Brushite and calcium oxalate monohydrate are clearly not good indications for ESWL. But stone composition is of course not always known before treatment. The role of preoperative crystalluria is unclear, but CT-measured density has been shown to be of good prognostic value: 1000 UH could be a cut-off (Joseph et al., 2002).

Not because of their density (around 600–800 UH), but because of their elasticity, cystine stones are very difficult to treat with ESWL.

The growing role of flexible ureteroscopy for kidney stones

Because of active uncontrolled metabolic disease, such as severe cystinuria, or because of residual fragments after PCNL or ESWL, treatments may be repeated. Flexible ureteroscopy is a good option for iterative treatments for kidney stones.

Some other clinical challenges, such as obesity, multiple kidney stones too distant for focusing in a single ESWL session, coagulation disorders, or anatomical particularities, make flexible ureteroscopy a good option, even as a first-line approach (Molimard et al., 2010; Sejiny et al., 2010; Al Qahtani et al., 2012).

Synthesis: summarized indications

See Table 206.1.

References

Al-Qahtani, S. M., Gil-Diez-de-Medina, S., and Traxer, O. (2012). Predictors of clinical outcomes of flexible ureterorenoscopy with holmium laser for renal stone greater than 2 cm. *Adv Urol*, 2012, 543537.

Ankem, M. K., Jerde, T. J., Wilkinson, E. R., *et al.* (2005). Prostaglandin E(2)-3 receptor is involved in ureteral contractility in obstruction. *J Endourol*, 19(9), 1088–91.

Bandi, G., Meiners, R. J., Pickhardt, P. J., *et al.* (2009). Stone measurement by volumetric three-dimensional computed tomography for predicting the outcome after extracorporeal shock wave lithotripsy. *BJU Int*, 103(4), 524–8.

Bensalah, K., Tuncel, A., Gupta, A., *et al.* (2008). Determinants of quality of life for patients with kidney stones. *J Urol*, 179(6), 2238–43.

Bergsdorf, T., Chaussy, C., Thüroff, S. (2007). *Possible Effect of Economic Aspects on Treatment Strategies—Analysis of Ureteral Stone Treatment Under Two Different Reimbursement Systems*. Presented at the European Association of Urology Annual Congress, Berlin.

Bhagat, S. K., Chacko, N. K., Kekre, N. S., *et al.* (2007). Is there a role for tamsulosin in shock wave lithotripsy for renal and ureteral calculi? *J Urol*, 177(6), 2185–8.

Bloom, D. A. (1997). Hippocrates and urology: the first surgical subspecialty. *Urology*, 50(1), 157–9.

Candau, C., Saussine, C., Lang, H., *et al.* (2000). Natural history of residual renal stone fragments after ESWL. *Eur Urol*, 37(1), 18–22.

Chan, V. O., Buckley, O., Persaud, T., *et al.* (2008). Urolithiasis: how accurate are plain radiographs? *Can Assoc Radiol J*, 59(3), 131–4.

Chandhoke, P. S. (1996). Cost-effectiveness of different treatment options for staghorn calculi. *J Urol*, 156(5), 1567–71.

Ciaschini, M. W., Remer, E. M., Baker, M. E., *et al.* (2009). Urinary calculi: radiation dose reduction of 50% and 75% at CT—effect on sensitivity. *Radiology*, 251(1), 105–11.

Clark, D. L., Connors, B. A., Evan, A. P., *et al.* (2009). Localization of renal oxidative stress and inflammatory response after lithotripsy. *BJU Int*, 103(11), 1562–8.

Coll, D. M., Varanelli, M. J., and Smith, R. C. (2002). Relationship of spontaneous passage of ureteral calculi to stone size and location as revealed by unenhanced helical CT. *AJR Am J Roentgenol*, 178(1), 101–3.

Danuser, H., Müller, R., Descoeudres, B., *et al.* (2007). Extracorporeal shock wave lithotripsy of lower calyx calculi: how much is treatment outcome influenced by the anatomy of the collecting system? *Eur Urol*, 52(2), 539–46.

Davenport, K., Timoney, A. G., and Keeley, F. X., Jr. (2007). Effect of smooth muscle relaxant drugs on proximal human ureteric activity in vivo: a pilot study. *Urol Res*, 35(4), 207–13.

De Sio, M., Autorino, R., Quarto, G., *et al.* (2008). Modified supine versus prone position in percutaneous nephrolithotomy for renal stones treatable with a single percutaneous access: a prospective randomized trial. *Eur Urol*, 54(1), 196–202.

Deliveliotis, C., Giftopoulos, A., Koutsokalis, G., *et al.* (1997). The necessity of prophylactic antibiotics during extracorporeal shock wave lithotripsy. *Int Urol Nephrol*, 29(5), 517–21.

Denstedt, J. D., Clayman, R. V., and Preminger, G. M. (1990). Efficiency quotient as a means of comparing lithotripters. *J Urol*, 143, 376

Eassa, W. A., Sheir, K. Z., Gad, H. M., *et al.* (2008). Prospective study of the long-term effects of shock wave lithotripsy on renal function and blood pressure. *J Urol*, 179(3), 964–8.

Eisner, B. H., Kambadakone, A., Monga, M., *et al.* (2009). Computerized tomography magnified bone windows are superior to standard soft tissue windows for accurate measurement of stone size: an in vitro and clinical study. *J Urol*, 181(4), 1710–15.

Favela, R., Gutierrez, J., Bustos, J., *et al.* (2005). CT attenuation value and shockwave fragmentation. *J Endourol*, 19(1), 5–10.

Fielding, J. R., Steele, G., Fox, L.A., *et al.* (1997). Spiral computerized tomography in the evaluation of acute flank pain: a replacement for excretory urography. *J Urol*, 157(6), 2071–3.

Ghoneim, I. A., El-Ghoneimy, M. N., El-Naggar, A. E., *et al.* (2010). Extracorporeal shock wave lithotripsy in impacted upper ureteral stones: a prospective randomized comparison between stented and non-stented techniques. *Urology*, 75(1), 45–50.

Greenstein, A., Beri, A., Sofer, M., *et al.* (2003). Is intravenous urography a prerequisite for renal shockwave lithotripsy? *J Endourol*, 17(10), 835–9.

Gupta, N. P., Ansari, M. S., Kesarvani, P., *et al.* (2005). Role of computed tomography with no contrast medium enhancement in predicting the outcome of extracorporeal shock wave lithotripsy for urinary calculi. *BJU Int*, 95(9), 1285–8.

Hassani, H., Raynal, G., Spie, R., *et al.* (2012). Imaging-based assessment of the mineral composition of urinary stones: an in vitro study of the combination of Hounsfield unit measurement in noncontrast helical computerized tomography and the twinkling artifact in color Doppler ultrasound. *Ultrasound Med Biol*, 38(5), 803–10.

Heneghan, J. P., McGuire, K. A., Leder, R. A., *et al.* (2003). Helical CT for nephrolithiasis and ureterolithiasis: comparison of conventional and reduced radiation-dose techniques. *Radiology*, 229(2), 575–80.

Hollingsworth, J. M., Rogers, M. A., Kaufman, S. R., *et al.* (2006). Medical therapy to facilitate urinary stone passage: a meta-analysis. *Lancet*, 368(9542), 1171–9.

Holmlund, D. and Sjödin, J. G. (1978). Treatment of ureteral colic with intravenous indomethacin. *J Urol*, 120(6), 676–7.

John, B. S., Patel, U., and Anson, K. (2008). What radiation exposure can a patient expect during a single stone episode? *J Endourol*, 22(3), 419–22.

John, T. T. and Razdan, S. (2010). Adjunctive tamsulosin improves stone free rate after ureteroscopic lithotripsy of large renal and ureteric calculi: a prospective randomized study. *Urology*, 75(5), 1040–2.

Joseph, P., Mandal, A. K., Singh, S. K., *et al.* (2002). Computerized tomography attenuation value of renal calculus: can it predict successful fragmentation of the calculus by extracorporeal shock wave lithotripsy? A preliminary study. *J Urol*, 167(5), 1968–71.

Katz, D., Scheer, M., Lumerman, J., *et al.* (2000). Alternative or additional diagnoses on unenhanced helical computed tomography for suspected renal colic: experience with 1000 consecutive examinations. *Urology*, 56, 53–57.

Koh, L. T., Ng, F. C., and Ng, K. K. (2012). Outcomes of long-term follow-up of patients with conservative management of asymptomatic renal calculi. *BJU Int*, 109(4), 622–5.

Krambeck, A. E., Gettman, M. T., Rohlinger, A. L., *et al.* (2006). Diabetes mellitus and hypertension associated with shock wave lithotripsy of renal and proximal ureteral stones at 19 years of follow up. *J Urol*, 175(5), 1742–7.

Küpeli, B., Irkilata, L., Gürocak, S., *et al.* (2004). Does tamsulosin enhance lower ureteral stone clearance with or without shock wave lithotripsy? *Urology*, 64(6), 1111–15.

Levine, J. A., Neitlich, J., Verga, M., *et al.* (1997). Ureteral calculi in patients with flank pain: correlation of plain radiography with unenhanced helical CT. *Radiology*, 204(1), 27–31.

Liu, W., Esler, S. J., Kenny, B. J., *et al.* (2000). Low-dose nonenhanced helical CT of renal colic: assessment of ureteric stone detection and measurement of effective dose equivalent. *Radiology*, 215(1), 51–4.

Lopes, T., Dias, J. S., Marcelino, J., *et al.* (2001). An assessment of the clinical efficacy of intranasal desmopressin spray in the treatment of renal colic. *BJU Int*, 87(4), 322–5.

Manohar, P. and McCahy, P. (2011). Repeated radiological radiation exposure in patients undergoing surgery for urinary tract stone disease in Victoria, Australia. *BJU Int*, 108 Suppl 2, 34–7.

Meagher, T., Sukumar, V. P., Collingwood, J., *et al.* (2001). Low dose computed tomography in suspected acute renal colic. *Clin Radiol*, 56(11), 873–6.

Miller, O. F. and Kane, C. J. (1999). Time to stone passage for observed ureteral calculi: a guide for patient education. *J Urol*, 162(3 Pt 1), 688–90.

Mokhtari, G., Shakiba, M., Ghodsi, S., *et al.* (2011). Effect of terazosin on lower urinary tract symptoms and pain due to double-J stent: a double-blind placebo-controlled randomized clinical trial. *Urol Int*, 87(1), 19–22.

Molimard, B., Al-Qahtani, S., Lakmichi, A., *et al.* (2010). Flexible ureterorenoscopy with holmium laser in horseshoe kidneys. *Urology*, 76(6), 1334–7.

Mulkens, T. H., Daineffe, S., De Wijngaert, R., *et al.* (2007). Urinary stone disease: comparison of standard-dose and low-dose with 4D MDCT tube current modulation. *AJR Am J Roentgenol*, 188(2), 553–62.

Osman, M. M., Alfano, Y., Kamp, S., *et al.* (2005). 5-year-follow-up of patients with clinically insignificant residual fragments after extracorporeal shockwave lithotripsy. *Eur Urol*, 47(6), 860–4.

Pace, K. T., Ghiculete, D., Harju, M., *et al.* (2005). Shock wave lithotripsy at 60 or 120 shocks per minute: a randomized, double-blind trial. *J Urol*, 174(2), 595–9.

Pareek, G., Armenakas, N. A., Panagopoulos, G., *et al.* (2005). Extracorporeal shock wave lithotripsy success based on body mass index and Hounsfield units. *Urology*, 65(1), 33–6.

Pareek, G., Hedican, S. P., Lee, F. T., Jr., *et al.* (2005). Shock wave lithotripsy success determined by skin-to-stone distance on computed tomography. *Urology*, 66(5), 941–4.

Patel, M., Han, S. S., Vaux, K., *et al.* (2000). A protocol of early spiral computed tomography for the detection of stones in patients with renal colic has reduced the time to diagnosis and overall management costs. *Aust N Z J Surg*, 70(1), 39–42.

Patel, T., Kozakowski, K., Hruby, G., *et al.* (2009). Skin to stone distance is an independent predictor of stone-free status following shockwave lithotripsy. *J Endourol*, 23(9), 1383–5.

Pearle, M. S., Calhoun, E. A., Curhan, G. C., *et al.* (2005). Urologic diseases in America project: urolithiasis. *J Urol*, 173(3), 848–57.

Poletti, P. A., Platon, A., Rutschmann, O. T., *et al.* (2007). Low-dose versus standard-dose CT protocol in patients with clinically suspected renal colic. *AJR Am J Roentgenol*, 188(4), 927–33.

Preminger, G. M., Tiselius, H. G., Assimos, D. G., *et al.* (2007). American Urological Association Education and Research, Inc; European Association of Urology. 2007 Guideline for the management of ureteral calculi. *Eur Urol*, 52(6), 1610–31.

Raynal, G., Dugardin, F., Hassani, H., *et al.* (2010). Maladie de Cacchi-Ricci et tomodensitométrie sans injection. *Presse Med*, 91(4), 524–5.

Raynal, G., Petit, J., and Saint, F. (2009). Which efficiency index for urinary stones treatment? *Urol Res*, 37(4), 237–9.

Raynal, G. (2011). Re: is a safety wire necessary during routine flexible ureteroscopy? *J Endourol*, 25(5), 881.

Resim, S., Ekerbicer, H. C., and Ciftci, A. (2005). Role of tamsulosin in treatment of patients with steinstrasse developing after extracorporeal shock wave lithotripsy. *Urology*, 66(5), 945–8.

Rubenstein, R. A., Zhao, L. C., Loeb, S., *et al.* (2007). Prestenting improves ureteroscopic stone-free rates. *J Endourol*, 21(11), 1277–80.

Seitz, C., Desai, M., Häcker, A., *et al.* (2012). Incidence, prevention, and management of complications following percutaneous nephrolithotripsy. *Eur Urol*, 61(1), 146–58.

Sejiny, M., Al-Qahtani, S., Elhaous, A., *et al*. (2010). Efficacy of flexible ureterorenoscopy with holmium laser in the management of stone-bearing caliceal diverticula. *J Endourol*, 24(6), 961–7.

Sorensen, C., Chandhoke, P., Moore, M., *et al*. (2002). Comparison of intravenous sedation versus general anesthesia on the efficacy of the Doli 50 lithotriptor. *J Urol*, 168(1), 35–7.

Sourtzis, S., Thibeau, J. F., Damry, N., *et al*. (1999). Radiologic investigation of renal colic: unenhanced helical CT compared with excretory urography. *AJR Am J Roentgenol*, 172(6), 1491–4.

Tack, D., Sourtzis, S., Delpierre, I., *et al*. (2003). Low-dose unenhanced multidetector CT of patients with suspected renal colic. *AJR Am J Roentgenol*, 180(2), 305–11.

Tligui, M., El Khadime, M. R., Tchala, K., *et al*. (2003). Emergency extracorporeal shock wave lithotripsy (ESWL) for obstructing ureteral stones. *Eur Urol*, 43(5), 552–5.

Wang, C. J., Huang, S. W., and Chang, C. H. (2009). Effects of tamsulosin on lower urinary tract symptoms due to double-J stent: a prospective study. *Urol Int*, 83(1), 66–9.

Weld, K. J., Montiglio, C., Morris, M. S., *et al*. (2007). Shock wave lithotripsy success for renal stones based on patient and stone computed tomography characteristics. *Urology*, 70(6), 1043–6.

Yilmaz, E., Batislam, E., Basar, M. M., *et al*. (2005). The comparison and efficacy of 3 different alpha1-adrenergic blockers for distal ureteral stones. *J Urol*, 173(6), 2010–12.

Yilmaz, E., Ozcan, S., Basar, M., *et al*. (2003). Music decreases anxiety and provides sedation in extracorporeal shock wave lithotripsy. *Urology*, 61(2), 282–6.

Yilmaz, S., Sindel, T., Arslan, G., *et al*. (1998). Renal colic: comparison of spiral CT, US and IVU in the detection of ureteral calculi. *Eur Radiol*, 8(2), 212–17.