Urol Res (1998) 26:83–88

ORIGINAL PAPER

Masayuki Yuzawa · Kazuhiko Tozuka · Akihiko Tokue

Effect of citrate and pyrophosphate on the stability of calcium oxalate dihydrate

Received: 4 March 1997 / Accepted: 13 October 1997

Abstract The effect of citrate and pyrophosphate on the stability of calcium oxalate dihydrate (COD) was studied in an aqueous solution over 7 days. COD was precipitated from 1.0×10^{-2} mol/l calcium oxalate solution with the addition of citrate and/or pyrophosphate and separated by centrifugation immediately, 48 h and 7 days after the precipitation. The percentage of COD in the precipitate, determined by X-ray diffraction, increased with a rise in citrate or pyrophosphate concentration and reached ≥90% at a citrate concentration of 1.0×10^{-3} mol/l or a pyrophosphate concentration of 2.0×10^{-4} mol/l. The resulting COD was completely transformed into calcium oxalate monohydrate within 48 h when the precipitate was composed of <90% COD. Nearly pure COD produced with pyrophosphate was stable over 7 days while that with citrate underwent partial transformation within 48 h. An additive effect of citrate and pyrophosphate was found on the stability of COD. It was concluded that a pyrophosphate concentration above a critical point was sufficient to prevent solution-mediated transformation of COD, and this critical point might be lowered to the physiological range with the presence of citrate.

Key words Calcium oxalate dihydrate · Citrate · Pyrophosphate · Stability

Introduction

Two hydrates of calcium oxalate – calcium oxalate monohydrate (COM) and calcium oxalate dihydrate (COD) – are found in urinary calculi. When calcium oxalate stones are divided into COM stones and COD stones, COM stones are less easily disintegrated than COD stones with extracorporeal shock wave lithotripsy (ESWL) because of their size and consistency. Although COM stones account for the majority of calcium oxalate stones, the ratio of COM stones to COD stones tends to increase with an increase in stone size and with an older age [5]. Furthermore, there are many COM stones that have a structure resembling COD stones [12]. These findings indicate the possibility that not a few COD stones have changed into COM stones.

Both COM and COD crystals are formed in human urine, although urine prefers the formation of COD. However, COM crystals are almost always the sole product in a simple supersaturated solution of calcium oxalate. COD crystals can be formed in vitro by several methods [1, 3, 4, 6, 8, 9], but these crystals rapidly change into COM in aqueous solutions, which is quite different from COD crystals grown in urine. Citrate and pyrophosphate are shown to be potent substances that inhibit the transformation of COD into COM [9]. With the use of citrate and pyrophosphate, we investigated the stability of COD in an aqueous solution over 7 days.

Materials and methods

All solutions were prepared with reagent-grade chemicals and distilled water. Calcium oxalate was precipitated from a solution with the same initial concentration, 1.0×10^{-2} mol/l, of calcium and oxalate at room temperature (25°C) and separated by centrifugation either immediately, or 48 h or 7 days after the precipitation. For each experiment, 10 ml of 4.0×10^{-2} mol/l calcium chloride solution was rapidly added to 30 ml of 1.3×10^{-2} mol/l sodium oxalate solution in a 50-ml flask. Sodium citrate and/or sodium pyrophosphate was added to sodium oxalate solution beforehand, as the initial concentration of citrate ranged from 1.5×10^{-4} to 2.0×10^{-2} mol/l and that of pyrophosphate from 1.5×10^{-5} to 5.0×10^{-4} mol/l. A 10 ml aliquot was withdrawn from the slurry of calcium oxalate after mixing the flask and centrifuged at 2000 r.p.m. for 10 min. The deposit was then suspended in 10 ml of distilled water and collected again by centrifugation.

M. Yuzawa (✉) · A. Tokue
Department of Urology, Jichi Medical School,
Minamikawachi-machi, Kawachi-gun,
Tochigi-ken 329-0498, Japan
Tel: +81-0285-44-2111 (ext 3423), fax: +81-0285-40-6595

K. Tozuka
Department of Urology, Jichi Omiya Medical Center,
Saitama, Japan

After this procedure was repeated, the deposit was suspended in 5 ml of 99.5% ethanol, collected by centrifugation and driven with vacuum for 1 h. The resultant powder sample was stored at room temperature.

Each powder sample was characterized by X-ray diffraction within 24 h. A clay film was made of powder sample and ethanol drops on a glass slide, dried at room temperature and scanned with an X-ray diffraction device using Cu Kα radiation (Rigaku Geigerflex 2013 X-ray diffractometer) in the range of 40° to 3° (2θ).

Selected powder samples of COD were evaporation-coated with gold and examined within 2 weeks with a scanning electron microscope (Hitachi S-2500). The mean diameter of COD crystals was determined in 32 samples obtained immediately after the precipitation and composed of ≥95% COD. Thirty well-defined crystals of COD were chosen at random and the length along the *a*-axis measured indirectly on a scanning electron micrograph. The crystal size was also evaluated after 7 days in seven of these samples where relatively large crystals of COD had been expected to remain unchanged.

Pure crystals of COM and COD, both obtained by the above method and verified by X-ray analysis, were mixed on an agate mortar in various proportions to make a reference curve for X-ray diffraction. Pure COD crystals obtained immediately after the precipitation were prepared in seven different experiments and mixed with pure COM crystals respectively. Two characteristic peaks, $d = 6.18$ for COD and $d = 5.93$ for COM, were selected and the height of these peaks measured by the base-line method on the X-ray diffraction chart [17]. The ratio of COD peak height to the total of COD and COM peak heights was compared with the actual content of COD in the mixture.

Results

X-ray diffraction study failed to detect any substances other than COM and COD in the precipitates. COD without a trace of COM, shown by X-ray analysis and scanning electron microscopy, was successfully produced by the addition of citrate or pyrophosphate and was stable in the dry condition at room temperature for more than 2 weeks. When a known mixture of COM and COD (5–95% COD) was studied by X-ray diffraction, a linear relationship was found between the ratio of the COD peak height to the total of the COM and COD peak heights and the actual content of COD in the mixture (Fig. 1).

COM was usually the sole product in a solution of 1.0×10^{-2} mol/l calcium chloride and 1.0×10^{-2} mol/l sodium oxalate without any additives. However, more than 5% COD was found in three of ten powder samples produced without any additives and separated immediately after precipitation. The percentage of COD in powder samples obtained immediately after precipitation increased in logarithmic fashion with a rise in the concentration of citrate or pyrophosphate. The production of ≥90% COD was observed at a citrate concentration of 1.0×10^{-3} mol/l or above and at a pyrophosphate concentration of 2.0×10^{-4} mol/l or above (Figs. 2, 3). When citrate and pyrophosphate were added simultaneously, the additive effect of citrate and pyrophosphate on the production of ≥90% COD was found (Fig. 4).

COD produced with citrate began to transform into COM in an aqueous solution and almost completely within 48 h when the citrate concentration was below 1.0×10^{-3} mol/l. However, COD formed above this concentration was only partially transformed after 48 h and often incompletely after 7 days (Fig. 2). COD produced with pyrophosphate was much more stable than that produced with citrate and remained unchanged in an aqueous solution over 7 days when the pyrophosphate concentration was equal to 2.0×10^{-4} mol/l or above. However, it was completely transformed into

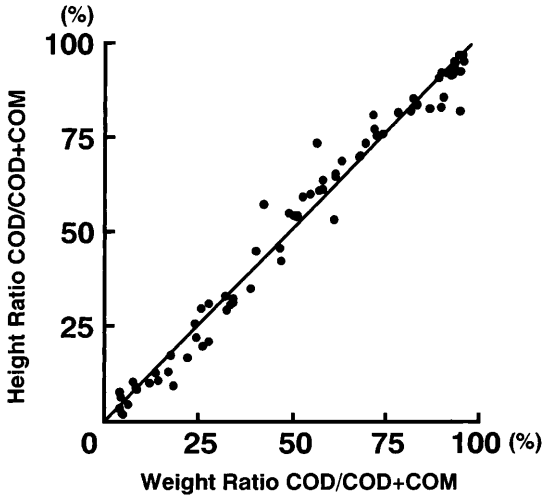

Fig. 1 Correlation between the ratio of calcium oxalate dihydrate (COD) peak height to the total of COD and calcium oxalate monohydrate (COM) and the actual content of COD ($y = 1.02x - 0.12$, $r = 0.994$)

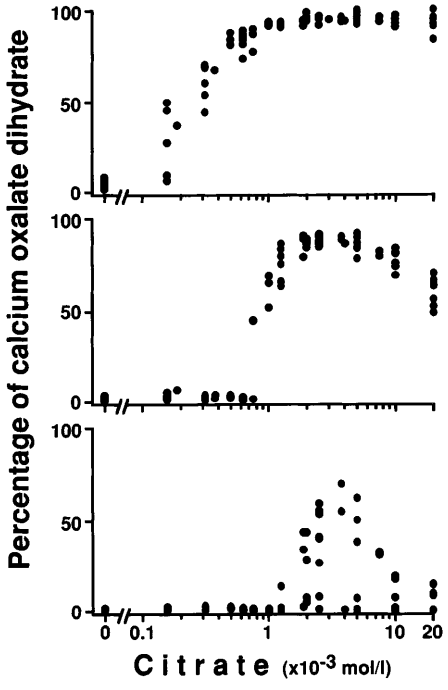

Fig. 2 Percentage of COD in the precipitate separated immediately (*top*), 48 h (*centre*) and 7 days (*bottom*) after precipitation by the addition of citrate

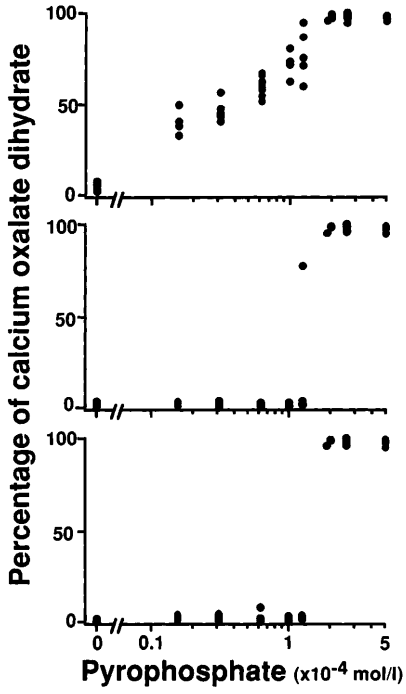

Fig. 3 Percentage of COD in the precipitate separated immediately (*top*), 48 h (*centre*) and 7 days (*bottom*) after precipitation by the addition of pyrophosphate

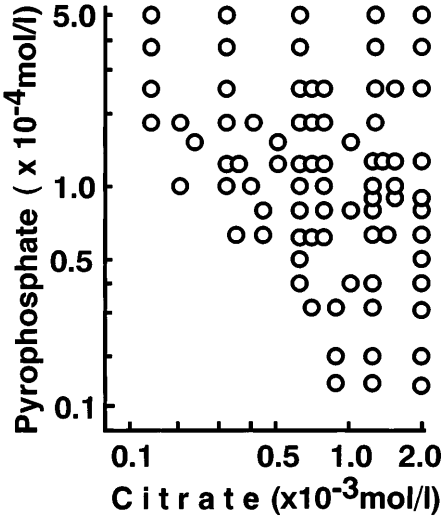

Fig. 4 Additive effect of pyrophosphate and citrate on the production of COD; ≥90% COD was obtained immediately after the precipitation (*open circles*)

COM within 48 h below this concentration of pyrophosphate (Fig. 3). An additive effect of citrate and pyrophosphate on the stability of COD was observed when the citrate concentration was between 5.0×10^{-4} and 5.0×10^{-3} mol/l (Fig. 5). In this range of citrate concentration the precipitate containing ≥90% COD after 48 h or 7 days was found below a pyrophosphate concentration of 2.0×10^{-4} mol/l with a minimum requirement for pyrophosphate at a citrate concentration of 2.5×10^{-3} mol/l. However, the stability of COD seemed to be reduced above a citrate concentration of 5.0×10^{-3} mol/l.

Only bipyramidal crystals of COD were observed on scanning electron microscopy in 32 powder samples obtained immediately after the precipitation and composed of ≥95% COD. The mean diameter of 30 COD crystals ranged from 0.9 ± 0.2 μm to 4.6 ± 1.1 μm (mean ± standard deviation) in these samples and the crystal size at a given concentration of the additives is shown in Fig. 6. The crystal size depended mainly on the citrate concentration and increased markedly at a citrate concentration of 1.0×10^{-2} mol/l or above. These relatively large crystals of COD were also unstable without pyrophosphate and began to transform into COM within 48 h (Fig. 2). The crystal size was also measured after 7 days in seven samples obtained at a citrate concentration of 1.0×10^{-2} mol/l or above and a pyro-

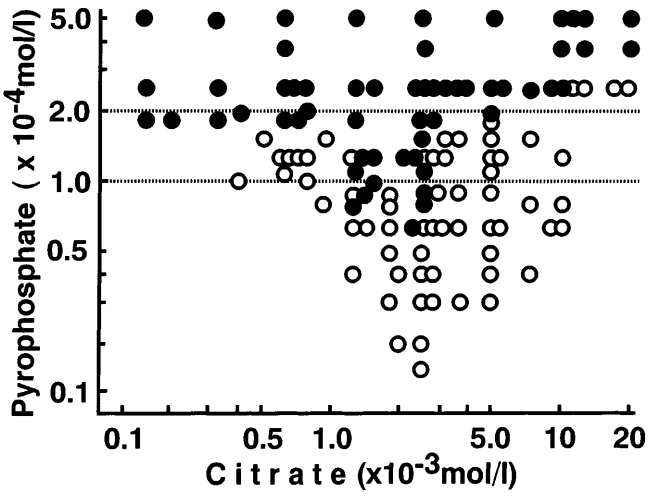

Fig. 5 Additive effect of pyrophosphate and citrate on the stability of COD; ≥90% COD was obtained after 48 h (*open circles*) and 7 days (*filled circles*)

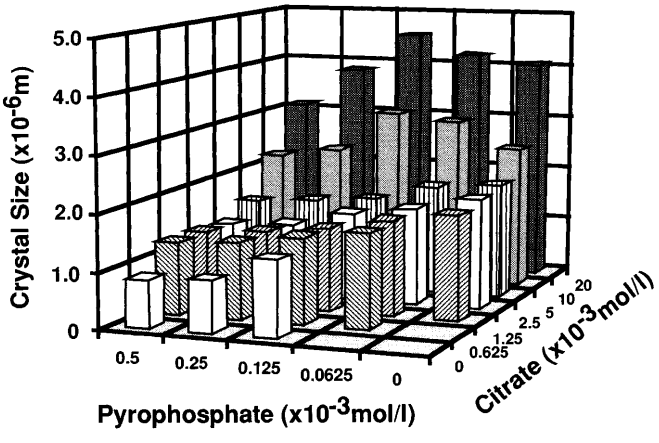

Fig. 6 The mean crystal size of pure COD obtained immediately after the precipitation at a given concentration of citrate and pyrophosphate

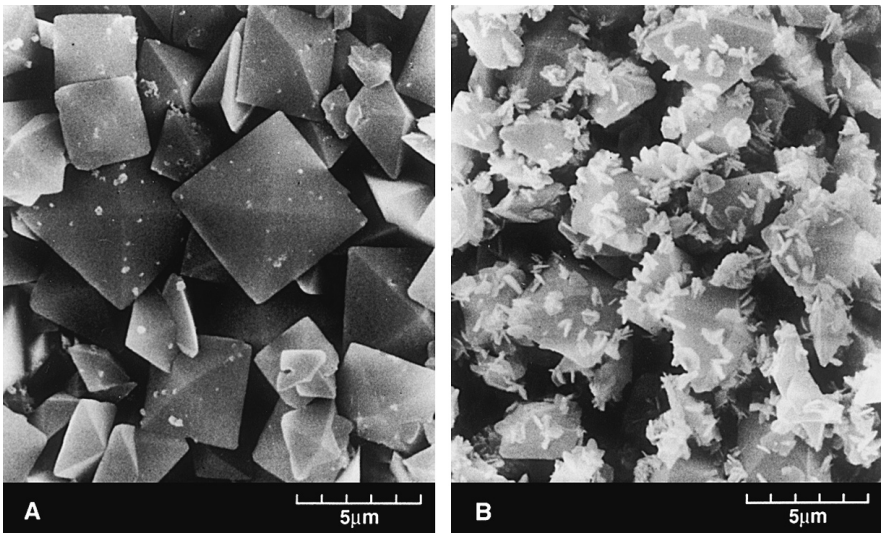

Fig. 7 Scanning electron micrograph of pure COD (**A**) and partially transformed COD (**B**) obtained immediately and 7 days after precipitation with 2.0×10^{-2} mol/l citrate and 2.5×10^{-4} mol/l pyrophosphate. Original magnification ×5000

phosphate concentration of 2.5×10^{-4} mol/l or above. The formation of COM was not confirmed in five samples, since neither a decrease in crystal size nor a decrease in COD percentage was found after 7 days. However, the surface of COD crystals was partially encrusted with indefinite tiny crystals. Distinct encrustation with COM crystals was observed in two samples and associated with a decrease of ≥5% in the COD percentage. However, a decrease in crystal size was found in only one sample, where the crystal size decreased from 4.0 ± 0.9 μm to 3.3 ± 0.9 μm and the COD percentage from 95% to 84% (Fig. 7).

Discussion

The percentage of COD in a mixture of COD and COM can be estimated by X-ray, infrared and thermal analyses. It is known that X-ray diffraction has the advantage over other methods of detecting a small amount of COD in a sample. However, the quantitative estimation of COD by X-ray diffraction may be complicated by the effect of preferred orientation of calcium oxalate crystals. In this study we showed an excellent correlation between the ratio of a COD peak height to a total of COD and COM and the actual percentage of COD with an accurate estimation of 5% COD or COM.

It is possible to produce nearly pure COD from a solution of calcium chloride and sodium oxalate without any additives. It is known that COD is preferably formed at a low temperature, at a high calcium to oxalate molar ratio and at a low initial relative supersaturation. Lepage and Tawashi [8] produced uncontaminated crystals of COD successfully at 4°C by dropwise addition of a sodium oxalate solution to a calcium chloride solution. However, in addition to a low temperature, an extremely high concentration of calcium of 1.0 mol/l was used for the production of COD in their method. In the presence of citrate COD could be exclusively prepared at room temperature with a high calcium to oxalate ratio and a low relative supersaturation [3]. However, exclusive precipitation of COD has not been observed in such a calcium oxalate solution with a calcium to oxalate ratio of 1:1 and a high relative supersaturation as used in the present study. In contrast to the situation with simple solution of calcium oxalate, it is not difficult to prepare pure COD in human urine or artificial urine [1, 4, 6].

Urine contains many substances that prefer the formation of COD: citrate, pyrophosphate, magnesium, heparin, RNA, and so forth [6, 9]. These substances may inhibit the transformation of COD into COM in the urine. We noted that a small amount of COD was occasionally found in the precipitate separated immediately after the precipitation without any additives. Burns and Finlayson [2] also observed the occurrence of COD in calcium oxalate crystals precipitated from a simple solution of calcium oxalate. These indicate that COM is not the sole product in a calcium oxalate solution. There is a possibility that most of the COD crystals formed early in the precipitation have rapidly undergone the transformation in an aqueous solution without the additives. COD formed in the presence of citrate or pyrophosphate was almost completely transformed into COM within 48 h in the present experiment until the resulting COD occupied ≥90% of the precipitate. When <90% COD has been found in the precipitate separated immediately after the precipitation, the transformation of COD may be in progress at a rate determined by the concentration of citrate or pyrophosphate. This rate seems to be appreciably reduced at the lowest concentration of citrate or pyrophosphate (Figs. 2, 3).

When the precipitate was separated immediately after precipitation, the percentage of COD in the precipitate increased to ≥90% in a similar fashion with a rise in citrate and pyrophosphate concentration. There seems to be little difference in a phase-stabilizing effect between

citrate and pyrophosphate except that pyrophosphate was 5 times as potent as citrate. However, the phase-stabilizing effect of pyrophosphate contrasts with that of citrate in that nearly pure COD produced with pyrophosphate was stable over 7 days while that produced with citrate underwent partial transformation within 48 h.

Both citrate and pyrophosphate are known to be adsorbed on calcium oxalate crystals. It is indicated that pyrophosphate is adsorbed on COM crystals 6 times more than citrate; the maximum surface coverage by pyrophosphate is estimated to be 3.75×10^{-5} mol/m^2 [14]. This may be true of COD crystals [16]. In our experiment, the adsorption of pyrophosphate on COD seemed to reach a maximum at an initial concentration of 2.0×10^{-4} mol/l with the complete blockage of the transformation. Below this concentration COD was completely transformed into COM within 48 h. It is considered that the long-term stability of COD in an aqueous solution requires the nearly complete coverage of the crystal surface by the additives. Considering that the adsorption of citrate is much lower than that of pyrophosphate, the surface coverage by citrate may almost always be inadequate to prevent the transformation of COD. Thus the effect of citrate on the production and stability of COD is probably due to inhibition of COM crystallization by a reduction of calcium oxalate supersaturation [10]. Although the prolonged stability of COD was not observed with citrate alone, it is certain that the transformation of COD is appreciably delayed by the presence of citrate.

Under the present experimental conditions, the mean crystal size of COD increased at a citrate concentration of 1.0×10^{-2} mol/l or above. This increase in crystal size was accompanied by a reduction in the quantity of COD crystals precipitated in a flask, suggesting that COD crystallization was also affected by citrate. It seems that COD nucleation was more affected than crystal growth by a large amount of citrate, and a decrease in the number of COD crystals was related to an increase in crystal size. An increase in crystal size will reduce the specific surface area of COD for adsorption of pyrophosphate. However, the formation of large COD crystals that were stable over 7 days required a rather higher concentration of pyrophosphate. Since a high calcium to oxalate molar ratio favors the formation of COD, a decrease in this ratio with a large amount of citrate may promote the transformation of COD to COM and require further inhibition of COM crystallization by pyrophosphate for the prolonged stability of COD.

The mean crystal size may be affected by dissolution and growth of COD crystals. It remained unchanged over 7 days when a decrease in COD percentage was negligible. In fact even a significant decrease in COD percentage was not always accompanied by a decrease in crystal size. Although no evidence of dissolution of COD crystals was found, the encrustation with tiny crystals may represent the initial stage of the transformation since the surface growth of these crystals caused a significant decrease in COD percentage.

We have shown that the pyrophosphate concentration above a critical point was sufficient to prevent solution-mediated transformation of COD, and this critical point might be lowered to the physiological range with the presence of citrate. It is likely that the surface coverage by pyrophosphate is the most important process in the stabilization of COD. However, other substances may be adsorbed to stabilize COD crystals in the urine, since the physiological concentration of pyrophosphate is less than 1.0×10^{-4} mol/l [11]. Apatite spherules grown on COD crystals seem to contribute to the stabilization of COD in calcium oxalate stones, where the content of apatite tended to increase with the COD percentage [7, 13]. The close association between COD crystals and apatite spherules was also shown in the urine [15]. The entire surface of COD crystals was sometimes covered by apatite spherules. Epitaxial growth of apatite on COD crystals may prevent the transformation of COD to COM in the urine. However, further studies are needed to elucidate the exact role of apatite spherules on COD crystals.

Acknowledgement We thank Dr. O. Hiroaki (Fine Chemicals Research Laboratory, Sumitomo Chemical Company, Japan) for his help in the preparation of the electron micrograph.

References

1. Ackermann D, Brown P, Khan SR (1989) Production and application of calcium oxalate dihydrate crystal seeds. Urol Res 17: 147
2. Burns JR, Finlayson B (1980) Changes in calcium oxalate crystal morphology as a function of concentration. Invest Urol 18: 174
3. Doherty WOS, Crees OL, Senogles E (1994) The preparation of calcium oxalate dihydrate crystals. Cryst Res Technol 29: 517
4. Grases F, Millan A, Conte A (1990) Production of calcium oxalate monohydrate, dihydrate or trihydrate: a comparative study. Urol Res 18: 17
5. Hara Y (1994) Clinical features of passed calcium oxalate monohydrate and dihydrate stone. Jpn J Urol 85: 1322
6. Hesse A, Berg W, Schneider H-J, Hienzsch E (1976) A contribution to the formation mechanism of calcium oxalate urinary calculi. I. Stabilising urinary constituents in the formation of weddellite. Urol Res 4: 125
7. Konjiki T, Sudo T, Kohyama N (1980) Mineralogical notes of apatite in urinary calculi. Calcif Tissue Int 30: 101
8. Lepage L, Tawashi R (1982) Growth and characterization of calcium oxalate dihydrate crystals (weddellite). J Pharm Sci 71: 1059
9. Martin X, Smith LH, Werness PG (1984) Calcium oxalate dihydrate formation in urine. Kidney Int 25: 948
10. Meyer JL, Smith LH (1975) Growth of calcium oxalate crystals. II. Inhibition by natural urinary crystal growth inhibitors. Invest Urol 13: 36
11. Russell RGG, Fleisch H (1969) Pyrophosphate and stone formation. In: Hodgkinson A, Nordin BEC (eds) Proceedings of the renal stone research symposium. Churchill, London, p 165
12. Tozuka K, Konjiki T, Sudo T (1981) Chemical test of phosphates in urinary stones by means of the chromographic contact print method. Br J Urol 53: 216

13. Tozuka K, Konjiki T, Sudo T (1983) Study of passed stones by means of x-rays, infrared and thermal analyses. J Urol 130: 1119
14. Wagner M, Finlayson B (1978) The characteristics of adsorption of pyrophosphate and citrate onto whewellite. Invest Urol 15: 456
15. Werness PG, Bergert JH, Smith LH (1981) Crystalluria. J Crystal Growth 53: 166
16. Werness PG, Duckworth SC, Smith LH (1979) Calcium oxalate dihydrate crystal growth. Invest Urol 17: 230
17. Wright N (1941) Application of infrared spectroscopy to industrial research. Ind Eng Chem, Anal Ed 13: 1