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Zinc Phosphate Identified as a Constituent of Urinary Calculi

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For several years x-ray diffraction has been in use in the physics department of this institute for the analysis of urinary calculi. During this time approximately 192 stones have been studied. The method used has been described in a comprehensive paper by Prien and Frondel (*1*) and also in a subsequent paper by Prien (*2*). In general the study of calculi which has been made here agrees well with the findings of the above workers. Several different rare patterns have been obtained, however, that have not previously been recognized as possibilities. It is the purpose of this paper to report one of these findings.

In March 1951 a calculus was received measuring 39 mm along its largest axis. The stone was analyzed as principally a mixture of carbonate-apatite and magnesium ammonium phosphate hexahydrate. A totally different pattern was obtained from several yellowish-white concentric layers and from the thin outer crust (Fig. 1). This pattern was not at the time identified.

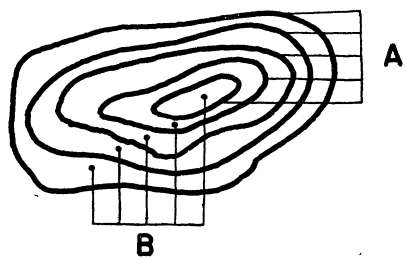

FIG. 1. Zinc phosphate in large calculus. A, zinc phosphate: B, carbonate-apatite and magnesium ammonium phosphate.

In December 1952 a 20-mm calculus was received from a different patient. The large central portion of this stone (about 80%) was yellowish white and gave a diffraction pattern identical with that of the unknown calculus pattern noted above. Surrounding the central portion was a layer of dark calcium oxalate monohydrate (Fig. 2). This dark layer was covered with a thin crust of the central material.

The fact that the relative intensities and the

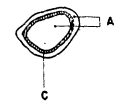

FIG. 2. Zinc phosphate in small calculus. A, zinc phosphate: C, calcium oxalate.

d-values of the diffraction lines produced by the unknowns were the same led to the belief that they were most likely produced by a single constituent. It was recognized that different states of hydration of commonly occurring compounds might account for the appearance of this unfamiliar pattern. Spectrographic analysis[1] indicated zinc and phosphorus as the elements present in major proportions. X-ray diffraction interplanar spacing data[2] for zinc phosphate were consulted. Although the available diffraction spacings for $Zn_3(PO_4)_2 \cdot 4H_2O$ contained fewer *d*-values than the calculus pattern, the given spacings agreed closely in *d*-values and relative intensities with the pattern lines.

For more complete verification the compound was prepared in the chemical laboratory by combining solutions of the soluble salts—zinc chloride and sodium orthophosphate. After the precipitate was washed well and recrystallized from orthophosphoric acid, it gave a diffraction pattern which was identical with that of the unknown calculus powder. Table 1 shows clearly the close agreement of the calculus pattern and that of the prepared $Zn_3(PO_4)_2 \cdot 4H_2O$.

No explanation for the urolithiases of the zinc phosphate calculi described in this paper has been offered.

TABLE 1

COMPARISON OF X-RAY DIFFRACTION POWDER PATTERN INTERPLANAR SPACINGS*

Only lines with relative intensities of 4 and above have been included in this table. In all, 63 lines with *d* values down to 0.905 have been checked and confirm the identification of the pattern.

Calculus*		Prepared powder†		Calculus		Prepared powder	
d‡	I/I_1§	*d*	I/I_1	*d*	I/I_1	*d*	I/I_1
9.21	9	9.17	9	2.61	4	2.61	6
5.32	4	5.32	5			2.54	5
5.08	5	5.11	5	2.53	4	2.51	5
4.86	6	4.86	6	2.27	5	2.27	6
4.59	7	4.58	8	2.10	4	2.10	5
4.42	6	4.42	7	2.01	4	2.01	6
3.99	5	3.99	6	1.94	6	1.96	7
3.88	4	3.88	4	1.83	5	1.83	6
3.47	5	3.47	6	1.57	5	1.57	6
3.39	8	3.39	8	1.53	4	1.53	5
2.86	10	2.86	10	1.51	4	1.51	5
2.65	4	2.65	5				

* Unknown calculus pattern.
† Chemically prepared $Zn_3(PO_4)_2 \cdot 4H_2O$.
‡ Values in angstrom units.
§ Relative intensity (visual estimation).

[1] This analysis was made through the courtesy of the Research Laboratories Division, General Motors Corporation.
[2] Interplanar spacing cards prepared by American Society for Testing Materials, 1950 edition.

Zinc is known to be a constituent of most foods and in small quantities is necessary for body nutrition (*3, 4*). Although small traces of zinc can occur in urine, the major portion of this element entering the body is excreted by way of the intestinal tract.

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Effect of Ethylenediamine Tetraacetic Acid on Adenosinetriphosphatase Activity

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Calcium ion is considered indispensable for the enzymic function of myosin-adenosinetriphosphatase, the degree of activation depending on the concentration of adenosinetriphosphate (*1*). Reduction in the availability of calcium ion by citrate (*2*) or oxalate (*3*) results in decrease or complete inhibition of enzyme activity. Ethylenediamine tetraacetic acid (EDTA) is a noncolloidal, organic chelating agent that can deionize a system of its heavy metal and alkaline-earth ions through formation of stable unhydrolyzed complexes. Since EDTA has a strong affinity for calcium ions, the effect of this compound on calcium activated myosin-adenosinetriphosphatase of mouse heart homogenates was studied.

Adenosinetriphosphatase activity was estimated with the procedure of DuBois and Potter (*4*). The buffered substrate was prepared as follows: 305 mg adenosinetriphosphoric acid[1] was dissolved in 10 ml water; 50 ml 0.06 *M* Veronal buffer (pH 7.4) and 10 ml 0.06 *M* calcium chloride were added and the pH readjusted to 7.4 with *N* sodium hydroxide. The volume was made to 100 ml with distilled water and filtered. In the test, 1 ml buffered substrate was added to a 5-ml test tube containing 0.5 ml water or test solution. Five-tenths milliliter C-57 mouse heart homogenate was added and the tubes incubated for 15 min at 37.5°. The quantity of adenosinetriphosphatase that liberates 1 μg of phosphate from adenosinetriphosphoric acid in 15 min is taken as one D-P unit. In the table the activity is expressed as D-P units/milligram of fresh tissue.

A 0.04 *M* buffered stock solution of the disodium salt of ethylenediamine tetraacetic acid[2] was prepared in 0.03 *M* Veronal buffer (pH 7.4) and the pH readjusted with *N* sodium hydroxide. Lower concentrations of EDTA were obtained by diluting the stock solution with the appropriate amount of water and buffer. The solution of EDTA must be buffered to compensate for the hydrogen ions displaced by the combined calcium. In order to exclude pH variation as contributory to the effect on adenosinetriphosphatase, the pH of each complete incubation mixture was determined.

[1] Schwarz Laboratories.

[2] Obtained from Alrose Chemical Co., Providence 1, R. I., through the courtesy of H. M. Zussman.

TABLE 1

EFFECT OF ETHYLENEDIAMINE TETRAACETIC ACID ON ADENOSINETRIPHOSPHATASE ACTIVITY

Molarity EDTA in incubation mixture	Mouse No. 55			Mouse No. 73		
	pH of incubation mixture	Homogenate activity (D-P units)	Per cent original activity	pH of incubation mixture	Homogenate activity (D-P units)	Per cent original activity
0.010	7.1	2.08	8.1	7.2	4.08	13.3
0.008	7.1	4.56	17.1	7.2	5.74	18.5
0.006	7.0	5.68	21.2	7.2	8.14	26.2
0.004	7.2	9.92	37.2	7.2	9.32	30.0
0.002	7.2	41.84	156.0	7.2	50.12	161.2
0.001	7.4	34.96	130.0	7.3	41.12	132.3
0.0001	7.4	29.09	106.0	—	—	—
0.00001	7.4	26.89	100.0	—	—	—
0.0	7.4	26.88	100.0	7.4	31.02	100.0

One mole of EDTA can chelate an equivalent amount of calcium ion. Thus, since the concentration of calcium in the test mixture is 0.003 *M*, this concentration was expected to limit the activation of adenosinetriphosphatase. The results obtained are summarized in Table 1 and Fig. 1. Actually, when the level of EDTA is over 0.003 *M* the enzyme activity is diminished, and above 0.004 *M* this is linear. However, the stimulation observed between 0.001 and 0.003 *M* was not anticipated. With 0.002 *M* EDTA, the adenosinetriphosphatase was over 150% more active than in the original homogenate.

A series of tests was made with various concentrations of EDTA that had been combined with an equimolecular amount of calcium chloride, in Veronal and sufficient *N* sodium hydroxide, so that when added to the incubation mixture, the final pH was 7.3–7.4. The results are represented in Fig. 1, curve C. It is apparent that saturation of the chelation valences of EDTA before addition to the incubation mixture abolishes its effect on adenosinetriphosphatase activity. This indicates that the effects observed are due to the deionization capacity of EDTA and not to a direct toxic action of the compound on the enzyme itself.

The enzyme system utilized may contain toxic ionic species that do not permit maximum activity of adenosinetriphosphatase. EDTA, in concentrations