

4. Title: Effect of 4-Hydroxy-L-proline and Orthophosphate Supplementations on crystalluria

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#### OBJECTIVES

To find the possible sources of oxalic acid and the possible role of phosphate on oxalate metabolism.

#### DESCRIPTION

Fifteen male infants ranging in age from 6 to 12 months who lived in three villages (Nong Jarn, Tung Kun Noi and Nong Kae) in Ubol Province were studied. These three villages are located within 15 km of Ubol city. There have been histories of bladder stone disease among the inhabitants of the villages.

4-Hydroxy-L-proline, 1.0 gm per day, was given orally for 10 days after a control period and for 7 days after an orthophosphate supplementation period. The orthophosphate, approximately 500 mg P in 5 ml solution (containing 1.75 g  $\text{Na}_2\text{HPO}_4$  and 0.27 g  $\text{KH}_2\text{PO}_4$  to provide an approximately neutral pH), was administered daily for a period of 7 days. All mothers were instructed to follow their routine feedings, including breast milk, premasticated glutinous rice and water. Water was normally given to infants while feeding glutinous rice. In the present study this was considered the control feeding. All subjects received the sequence of hydroxy-L-proline, orthophosphate and 4-hydroxy-L-proline after the control regimen, except five infants from Nong Kae who received only one period of 4-hydroxy-proline supplementation.

Twenty-four hour urine collections were made during the last day of giving each supplement, and freshly voided early morning urine samples were collected each day by utilizing pediatric urine collection bags. Qualitative tests for pH, protein and sugar, and microscopic examination were performed daily as previously described. (1)

#### PROGRESS

Table 1 shows number of subjects, mean ages and sequence of the supplementation. Qualitative tests for urinary protein and sugar were normal. The number of leucocytes and erythrocytes in the centrifuged urine samples was found to be less than five per high power field.

The occurrence and clumping of oxalate crystals are shown in Table 2. During the control period, 20 occurrences and 4 clumpings out of 60 examinations were found. When the oral hydroxyproline was given, 93 occurrences and 47 clumpings among the 150 examinations were demonstrated. These increases are statistically significant.

The occurrence of crystalluria was remarkably reduced after the phosphate supplementation; 21 occurrences and 5 clumpings were found among the 150 examinations. However, when the hydroxyproline was subsequently given, the number of occurrences and clumpings were again significantly increased (46 occurrences and 34 clumpings out of 70 examinations). These differences are statistically significant.

The number of infants who showed crystalluria and clumping was similar during the control and phosphate supplementation periods (10 and 11 showed crystalluria and among these, 3 to 5 demonstrated clumping). However all infants demonstrated oxalate crystalluria with clumping when hydroxyproline was given.

Fig. 1 shows that the oxalate crystals formed during the control period were mostly in the octahedral form. During the hydroxy-proline period, clumping of crystals was demonstrated, as shown in Fig. 2. The crystals were bigger and mostly in dumbbell and ring forms. When these infants received the phosphate supplementation, clumping disappeared within three days and the number of oxalate crystals was remarkably reduced. Few crystals, in either dumbbell or ring forms, were still found, but these crystals were partially broken, as shown in Fig. 3.

From the data presented, it appears that the oral administration of hydroxyproline increased oxalate crystalluria and also induced clumping of these crystals. The mechanism(s) by which hydroxyproline acts is not yet known. However, hydroxyproline is known to be a precursor of glyoxalate and oxalate. Urinary oxalate excretion may be increased during hydroxyproline administration, which in turn could induce more oxalate crystalluria. Studies are underway to determine the oxalate excretion values.

It is of special interest to note that oral hydroxyproline administration induced clumping of oxalate crystals. It is likely that urinary hydroxyproline excretion and concentration were increased after the supplementation which may be a factor in the clumping. Data from previous studies showed that village infants excreted more hydroxyproline than city infants of the same age group when expressed on the basis of 24-hour excretion or per gram of creatinine (2). There may be many other factors involved in clumping which are not known at the present time.

Hydroxyproline is derived almost entirely from collagen. In rats, it is estimated that about one third of the total urinary hydroxyproline is derived from soluble collagen (3). Increased urinary hydroxyproline during growth depends on an increase in soluble collagen in the tissues (4).

High levels of urinary hydroxyproline have been demonstrated in patients with bone diseases and some patients with hyperparathyroidism (5). It is intriguing to speculate that many conditions which lead to stone formation, such as immobilization, bone diseases, or hyperparathyroidism could be related partially to effects on hydroxyproline excretion rather than only to calcium excretion as has been generally suggested.

Gershoff (6) reported that ammonium acid urate and calcium oxalate monohydrate were the most common compounds found in bladder stones from Thailand. It was also demonstrated that the nuclei of 22% of the stones were a mixture of ammonium acid urate and calcium oxalate monohydrate. Thus, oxalate synthesis, excretion, and crystal formation may be important in bladder stone formation and further investigations are indicated.

#### SUMMARY

Fifteen male infants living in a hyper-endemic area of bladder stone disease (Ubol village), whose ages ranged from 6 to 12 months, were given oral supplementations of 4-hydroxy-L-proline and orthophosphate. It was found that oxalate crystalluria was markedly increased and clumping of crystals occurred after oral hydroxy-L-proline supplementations. The orthophosphate supplements reduced crystalluria and clumping. The possible role of hydroxyproline in bladder stone formation is discussed.

#### REFERENCES

1. Dhanamitta, S., A. Valyasevi, and R. Van Reen: Studies of bladder stone disease in Thailand. IX. Effect of orthophosphate and fatfree powdered milk supplementations on the occurrence of crystalluria. *Am. J. Clin. Nutr.* 20 : 1387, 1967
2. Valyasevi, A., S. Dhanamitta and P. Thuvadethakul: Study of urinary hydroxyproline excretion in infants of hyper- and hypo-endemic areas. SEATO Medical Research Laboratory and SEATO Clinical Research Center, Annual Progress Report 1966-1967, page 101.
3. Picou, D., G.A.O. Alcyne and A. Seaknis: Hydroxyproline and creatinine in infantile protein malnutrition. *Clin. Sci.* 29 : 517, 1965
4. Jasin, H.E., C.W. Fink, W. Wise and M. Ziff: Relationship between urinary hydroxyproline and growth. *J. Clin. Invest.* 41 : 1928, 1962
5. Laitinen, O., E.A. Nikkila and K. I. Kivirikko: Hydroxyproline in the serum and urine, Normal Values and Clinical Significance. *Acta. Med. Scand.* 179 : 275, 1966
6. Gershoff, N.S.: The formation of urinary stones. *Metabolism* 13 : 875, 1964.

Table 1  
Description of Subjects and Sequences of the Supplementation  
Ubol Province, Thailand

Village*	No. of Subjects	Mean Ages Months	Sequence of Supplementations
A	5	8.6	Control-HP <sub>1</sub> -PO <sub>4</sub> -HP <sub>2</sub>
B	5	9.0	Control-HP <sub>1</sub> -PO <sub>4</sub> -HP <sub>2</sub>
C	5	8.6	Control-HP <sub>1</sub> -PO <sub>4</sub>

\* A = Nong Jarn      B = Tang Kun Noi      C = Nong Kae

Control    Distilled water for 5 days

HP<sub>1</sub>        First period of 4-Hydroxy-L-proline (1 gm/day) for 10 days

PO<sub>4</sub>        Phosphate buffer (400-500 mg P/day) for 7 days

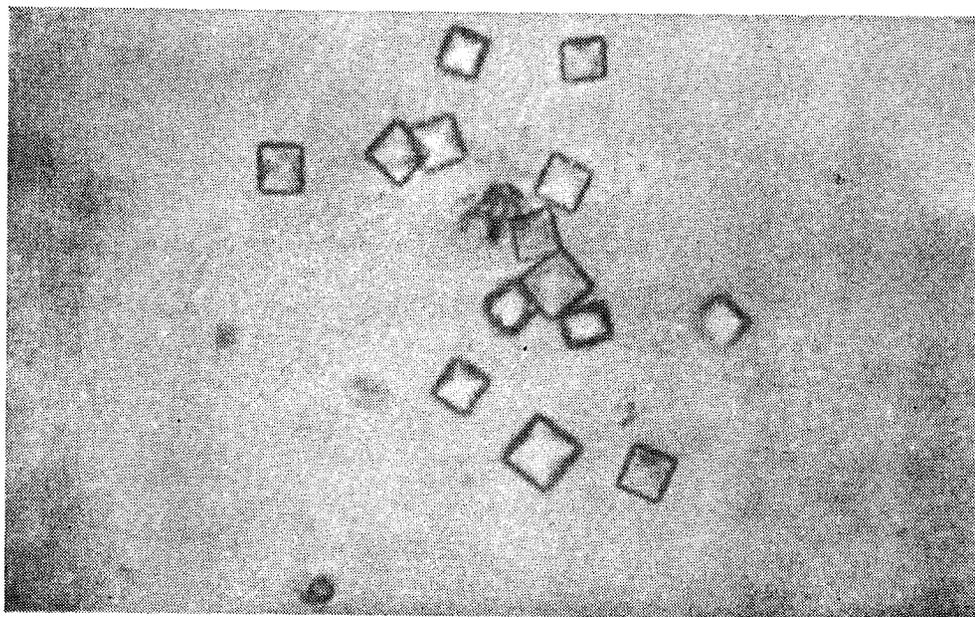
HP<sub>2</sub>        Second period of 4-Hydroxy-L-proline (1 gm/day) for 7 days

Table 2  
Effect of Oral Hydroxyproline and Phosphate Supplementation  
On Oxalate Crystalluria

Period	Supplementation	Oxalate Crystalluria*			No. of Infants		
		No. of Examination	No. of Occurrence	No. of Clumping	Total	with Crystalluria	with Clumping
1	Control 4 days	95	20	4	15	10	3
2	Hydroxyproline 10 days	150	93	47	15	15	15
3	PO <sub>4</sub> Buffer 7 days	105	21	5	15	11	5
4	Hydroxyproline 7 days	70	46	34	10	10	10

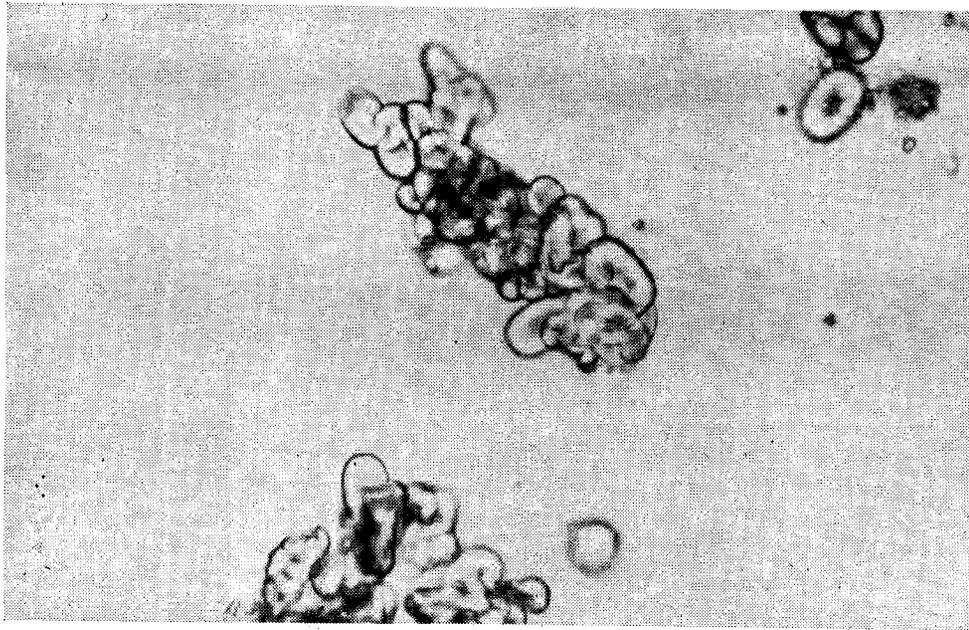
\* Statistical analysis was performed to compare the number of occurrences and clumping versus number of examination between Periods 1 : 2, 2 : 3 and 3 : 4 P-value > 0.01 (by  $\chi^2$ )

Fig. 1 Oxalate crystalluria during the Control Period.

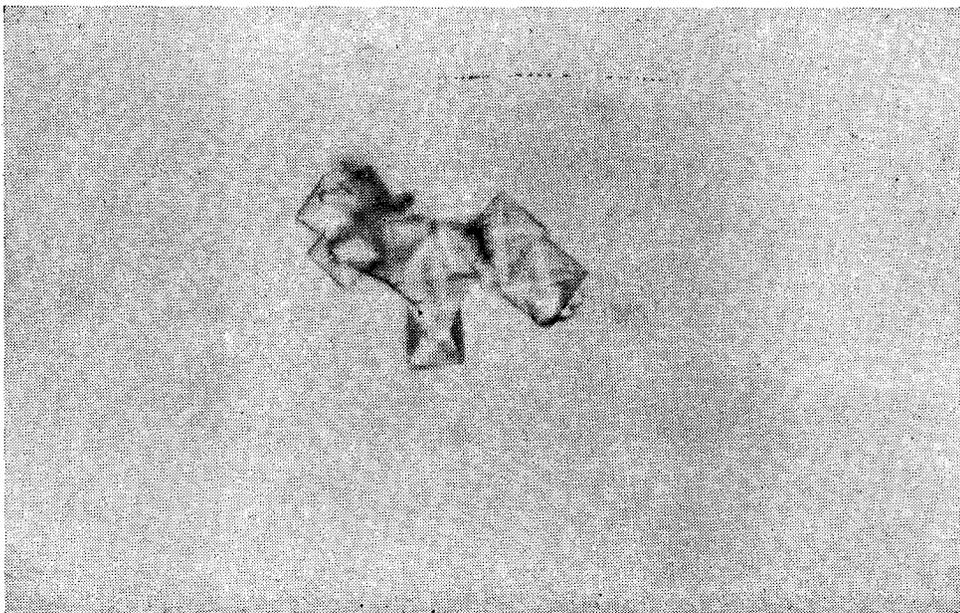


Calcium Oxalate crystals, Octahedral ( $\times 1200$ )

Fig. 2 Oxalate crystalluria during the Hydroxy-L-proline Supplementation Period.

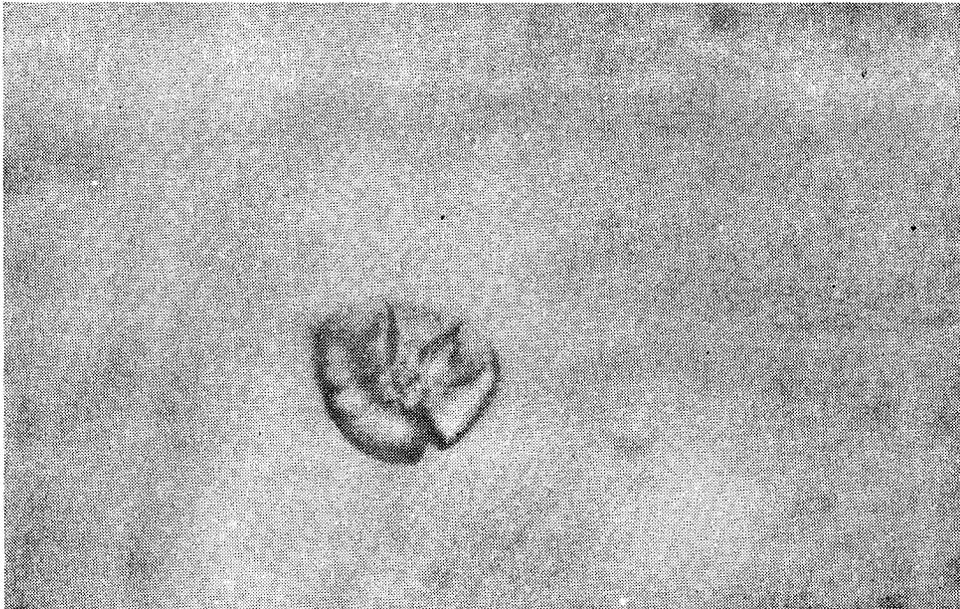


A. Clumping of Calcium Oxalate crystals, Dumbell and Ring Forms ( $\times 400$ )

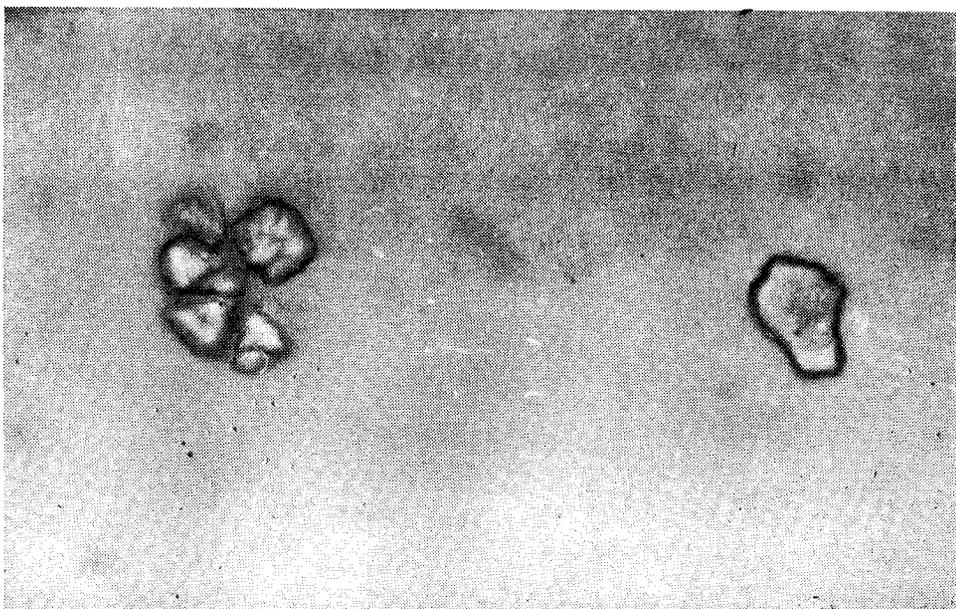


B. Clumping of calcium Oxalate crystals, Octahedral Form (x 400)

Fig. 3 Oxalate crystalluria during Phosphate Supplementation



Calcium Oxalate crystal, partially broken ( $\times 800$ )



Calcium Oxalate crystal, completely broken ( $\times 800$ )