

STUDY REPORTS:

1. Title: Effect of orthophosphate and non-fat dry milk supplementations on urine composition.

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Objectives:

To determine the influence of a variety of dietary supplements upon the urinary excretions of total phosphate, pyrophosphate, calcium, magnesium, oxalic acid, uric acid, sulfate sulfur, creatinine, sodium, potassium and chloride.

Description:

Eighteen male infants ranging in age from 6 to 18 months who lived in 3 villages (Nong Kae, Nong Jarn and Tong Kun Yai) in Ubol Province were included in this study. There were histories of bladder stone disease among inhabitants of these villages.

A cross-over (multiple latin square) design was employed so that each subject received supplements of orthophosphate, milk phosphate and a control regimen. Each supplement provided approximately 600 mg of phosphorus daily. The orthophosphate, having an approximately neutral pH, was administered in 6.6 ml of solution containing 2.32 g Na_2HPO_4 and 0.36 g KH_2PO_4 . The milk phosphate was supplied in the form of non-fat dry milk. Fifty-five grams of milk were mixed in 150 ml distilled water and fed to each subject once daily. Distilled water was given as a placebo. All infants except baby B₉ were on breast-feeding and pre-masticated glutinous rice supplementation. Baby B₉ was fed with sweetened condensed milk and glutinous rice. All infants were continued on their routine feedings in addition to the supplements.

Each supplement was given for 6 days before changing to a different supplement. Twenty-four hour urine samples were collected beginning immediately after giving the 6th dose by utilizing pediatric urine collecting bags as previously described (1). The administration of the supplements and the urine collections were carried out by nurses trained in field-clinical studies.

Aliquots of the 24-hour urine samples were analyzed for uric acid, total phosphate, creatinine, calcium, sodium, potassium and chloride by automated techniques. Urinary magnesium was determined by an atomic absorption spectrophotometer (Perkin-Elmer). Urinary oxalic acid was determined by the fluorometric method described by Zarembski and Hodgkinson (2) and urinary pyrophosphate was analyzed by the method described by Fleisch et al. (3). Citric acid was determined by the method of Stern (4) and inorganic sulfate by an isotope dilution technic.

Freshly voided early morning urine samples were collected daily and examined qualitatively for pH, protein, and sugar by COMBISTIX[®] paper strip. Microscopic examinations were performed on centrifuged specimens within two to three hours after collection. Urinary creatinine, oxalic acid and pyrophosphate were also determined on the samples collected on the first, second, and fourth day of orthophosphate supplementation.

Five infants, age ranging from 6 to 12 months, in Bangkok were also given orthophosphate supplementation. Collection of urine was made before and on the sixth day of phosphate and placebo administrations. The same determinations were also carried out on these urine samples.

Progress:

The age, weight and height of each village subject, sequences of the supplementation and names of the villages are shown in Table 1. No changes in the amounts of foods consumed were observed during the orthophosphate supplementation, but some reduction in rice intake was noted during the milk supplementation. Three subjects (B₈, B₁₅, B₁₇) developed mild diarrhea (5 to 7 loose stools daily) during the orthophosphate administration.

Table 2 which was reported previously by Dhanamitta (5), shows the occurrence of oxalate and uric crystalluria following supplementation with a variety of substances. It can be seen that when the infants received orthophosphate, they demonstrated no oxalcrystalluria and very seldom, uric acid crystalluria. Disappearance of the oxalate crystalluria usually occurred within 24 hours after the supplementation of phosphate. It was observed that orthophosphate supplementation resulted in an increase in the pH of the urine samples. The mean pHs during control, placebo, and milk periods were 5.8, 5.9, and 5.6, respectively. Following orthophosphate administration, the mean urinary pH rose to 7.2. To determine whether this change in pH was responsible for the disappearance of the oxalcrystalluria, sodium bicarbonate (2-3 g/day) was administered to five children for 6 days. The mean urinary pH rose to 7.8 in these infants, but oxalcrystalluria continued to occur. Thus, it appears that pH alone is not a critical factor in the phosphate effect.

Table 3 shows the mean values of various chemical components of urine after different supplementations in both Ubol village and Bangkok infants.

Urinary phosphate, calcium, and magnesium excretions:

The total urinary phosphate excretion were low in almost all village subjects during the control period and were markedly increased after the orthophosphate supplementation. Babies 8, 15, and 17 who developed diarrhea during the orthophosphate administration did not show as pronounced a change. It is also of some interest to note that practically no change in the urinary phosphate excretion was observed in the bottle-fed baby (B₉), suggesting that his intake of phosphate was already adequate.

The mean control value for urinary phosphate of 443 ± 132 mg P/gm creatinine excretion is comparable to that previously reported by Valyasevi and Dhanamitta (1) for village infants. The values were increased to 4948 ± 1097 and 1543 ± 197 mg P/gm creatinine excretion after the orthophosphate and milk supplementations, respectively. The differences from control levels are statistically significant at the 1% and 5 to 10% levels of confidence, respectively. The mean excretion value after milk supplementation is comparable to the mean value for Ubol City infants of 1438 ± 351 mg P/gm creatinine excretion which has been reported previously (1).

The increase in phosphate excretion following oral orthophosphate supplementation occurred within one day, but the maximum effect was not observed until the 6th day (Table 4). While the phosphate excretion was increased after the orthophosphate administration, the urinary calcium excretion by village infants was markedly decreased. The mean value of urinary calcium excretion was 143 ± 31 mg/gm creatinine during the control period and was reduced to 49 ± 8 mg/gm creatinine excretion after the orthophosphate administration. This difference is statistically significant at the 1 to 2% level of confidence. The mean value in the control period is comparable to the previous report. The orthophosphate effect on calcium excretion was seen after one day of supplementation and continued during the entire 6 days of study (Table 4).

The change in urinary magnesium concentration following phosphate administration was similar to the calcium, but no statistically significant difference (P value = 0.50-0.10) could be demonstrated.

In general, the Bangkok infants excreted higher urinary phosphate, calcium and magnesium than the village subjects. An increase in urinary phosphate and a decrease in urinary calcium after the orthophosphate supplementation were demonstrated in Bangkok infants. However, the changes following supplementation were not as pronounced as in village subjects.

Urinary Pyrophosphate Excretion:

In all village subjects, the ingestion of either orthophosphate or milk induced an increase of urinary pyrophosphate excretion, ranging from 2 to 10 fold. The mean urinary pyrophosphate excretion during the control period was 3.4 ± 0.4 mg/gm creatinine excretion and was increased to 18.3 ± 1.2 and 9.5 ± 0.9 mg/gm creatinine excretion after the orthophosphate and milk administrations, respectively. The differences from control values are statistically significant (P value < 0.01). It is demonstrated that Bangkok infants excreted significantly more urinary pyrophosphate than village subjects during the control period and that the orthophosphate supplementation also induced an increase in the urinary pyrophosphate excretion. It is of interest to note that village Baby 9 who had a high urinary phosphate excretion during the control and placebo periods also excreted high pyrophosphate during these periods.

The results of serial determinations (first, second, fourth, and sixth day after the orthophosphate supplementation) of pyrophosphate in casual urine samples from village subjects are shown in Table 4. It is shown that an increase in pyrophosphate excretion occurred within 24 hours but the greatest effect was seen on the 6th day.

Urinary Oxalic Acid and Uric Acid Excretions:

It is of special interest to observe that both orthophosphate and milk supplementations induced a decrease of urinary oxalic acid excretion in all village babies except B₈, B₉, and B₁₇ who showed no differences between the placebo and phosphate supplementations. The mean oxalate value during the control period was 136 ± 12 mg/gm creatinine and was reduced to 60 ± 6 and 92 ± 9 mg/gm creatinine during the orthophosphate and milk supplementations, respectively. When compared to Bangkok infants, as shown in Table 3, village subjects when not given any supplement (control) excreted significantly more oxalate ($P = 0.01 - 0.02$) than Bangkok subjects (mean value = 99.2 ± 8.0 mg/gm creatinine).

The results of serial determinations (first, second, fourth, and sixth day after the orthophosphate supplementation) on oxalic acid content in casual urine samples from village subjects are shown in Table 4. It was observed that a slight decrease in oxalic acid excretion occurred on the second and fourth day and a remarkable drop in the excretion occurred on the 6th day after the supplementation.

No difference in the urinary oxalic acid excretion before and after vitamin B₆ (3 mg per day) administration to a separate group of village infants could be demonstrated (Table 5). Oxalate excretion has been reported to be higher than in controls in Vitamin B₆-deficient animals (6) and in human subjects fed diets deficient in pantothenic acid and vitamin B₆ (7). During the pre-supplement period, our village subjects excreted more oxalate than Bangkok infants (Table 3). Since this high oxalate excretion does not respond to vitamin B₆ supplements, it is suggestive that B₆ nutrition is adequate in our village subjects and other factors are involved in controlling oxalate excretion.

The mean value for urinary uric acid excretion by village subjects during the placebo period was 714 ± 121 mg/gm creatinine and no changes could be demonstrated during the orthophosphate and milk supplementations. This mean value is comparable to the value in Bangkok infants during the control period.

Sulfate and Citrate Excretion:

The excretion of inorganic sulfate has been used as a measure of protein nutrition since it is derived from the metabolism of the sulfur-containing amino acids. In the present study, there was little difference in the average amounts of sulfate excreted by village subjects during the control, placebo, or orthophosphate periods. The excretion was approximately 1000 mg sulfate/g creatinine. This suggests that the intakes of protein during these periods were comparable. There was an increase in the mean sulfate excretion to over 1700 mg sulfate/g creatinine during supplementation with non-fat dry milk. This was expected due to the protein content of the milk.

Citric acid excretion by village subjects had a mean value of 550 mg/g creatinine during the control period and was very similar during the placebo period. The administration of orthophosphate resulted in

increased citric acid excretion. The milk supplementation did not have as much an effect on citrate excretion as phosphate.

The effect of the phosphate supplement on citric acid excretion is thought to be related to the increase in urinary pH associated with the phosphate treatment, since alkalizing the urine is known to increase citrate excretion. Citric acid will chelate with calcium, therefore could have an influence on the formation of oxalate crystals. It was observed that when sodium bicarbonate was administered to 5 infants, the urinary pH rose to a mean of 7.8 and the excretion of citric acid concentration increased by 200 mg/g creatinine. In spite of this, oxalocrystalluria still occurred in these infants, suggesting that citric acid excretion is not a factor in the control of oxalocrystalluria in our subjects.

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

Villages ^a	Subject ^b	Ages months	Weight kg	Height cm	Supplementation
A	B ₁		10.0		PO ₄ -M-Placebo
B	B ₆		7.1		"
C	B ₇		9.1		"
B	B ₄		5.9		Placebo-PO ₄ -M
C	B _{8d}		12.9		"
C	B ₉		11.9		"
B	B ₅		7.1		M-Placebo-PO ₄
C	B ₁₈		8.9		"
A	B ₁₉		7.9		"
A	B ₁₀		8.7		PO ₄ -Placebo-M
C	B ₁₆		10.5		"
B	B ₂₀		8.4		"
A	B ₁₁		7.4		M-PO ₄ -Placebo
B	B _{15d}		6.2		"
C	B _{17d}		11.2		"
A	B ₁₂		7.7		Placebo-M-PO ₄
B	B ₁₃		7.9		"
C	B ₂₄		8.5		"
B	B _{25e}		11.7		PO ₄ -Placebo-M
A	B _{2e}		8.7		Placebo-PO ₄ -Placebo-M
A	B _{3e}		9.8		M-Placebo-PO ₄ -M

- a A = Nong Jarn, B = Tong Kun Yai, C = Nong Kae
b All subjects were on breast-feeding except B₉ who was fed with sweetened condensed milk.
c Each supplementation was given for six days.
PO₄ = Phosphate buffer (600 mg P/day)
M = Non-fat dry milk (600 mg P/day)
Placebo = Distilled water
d Diarrhea during the PO₄ supplementation.
e Only microscopic examinations of urine were studied.

Table 2. Occurrence of Crystalluria in Village Infants Following
Supplementation with a Variety of Substances

Supplement and type of crystalluria	No. of Infants	CRYSTALLURIA		
		No. of occurrences	No. of examinations	No. of infants with crystalluria
Placebo				
Oxalate	17 ^a	25	93	17
Uric acid	15 ^b	22	83	15
Orthophosphate				
Oxalate	17	0	83	3
Uric acid	15	6	73	0
Milk				
Oxalate	17	7	87	5
Uric acid	15	10	77	5

There were 17 of the original 21 infants who showed oxalate crystalluria at some time during the study.

b There were 15 of the original 21 infants who showed uric acid crystalluria at some time during the study.

Table 3. Effect of Oral Phosphate Administration on the Excretion of Chemical Components of Urine by Ubol Village and Bangkok Infants^e, Thailand, 1966-67

Component (mg/gm creatinine)	Supplementation Periods			
	Control	Placebo	Orthophosphate	Non-fat dry milk
Phosphate (P)				
V	443 ± 132 ^a	784 ± 324	4,948 ± 1,097 ^m	1,543 ± 197 ^P
B	3409 ± 367	3289 ± 658	5,155 ± 607	
Calcium				
V	143 ± 31 ^c	115 ± 24	49 ± 8 ⁿ	144 ± 40.0
B	215 ± 61	194 ± 45	156 ± 18	
Magnesium				
V	142 ± 10 ^c	122 ± 11	99 ± 10	125 ± 10
B	280 ± 60	297 ± 20	295 ± 76	—
Pyrophosphate (P)				
V	3.4 ± 0.4 ^a	4.5 ± 1.2	18.3 ± 1.2 ^m	9.5 ± 0.9 ^m
B	27.1 ± 6.0	33.9 ± 9.4	48.8 ± 5.7	—
Oxalic acid				
V	136 ± 12 ^c	125 ± 14	60 ± 6 ^m	92 ± 9 ^P
B ^f	92 ± 15	102 ± 22	107 ± 14	
Uric acid				
V	—	714 ± 121	934 ± 106	752 ± 104
B	—	787 ± 116	965 ± 259	—
Citric acid				
V	550 ± 82	496 ± 64	878 ± 137 ⁿ	728 ± 31 ^m
Sulfate				
V	1,072 ± 91	1,111 ± 106	923 ± 153	1,755 ± 186 ^m

Probability Value	Village and Bangkok	PO ₄ with Placebo or Milk with Placebo
< 0.01	a	m
0.01 < p < 0.02	b	n
0.02 < d < 0.05	c	o
0.05 < p < 0.10	d	p

e 18 village infants, 5 Bangkok infants.

f Additional oxalate determinations were performed on 22 Bangkok infants. A mean value of 99.2 ± 8.0 mg/gm creatinine (P. value = b) was found.

Table 4. Sequential Effect of Oral Orthophosphate Administration (600 mg P daily) on the Urinary Excretion of Oxalic Acid, Pyrophosphate, Calcium, and Phosphorus in Ubol Village Infants (6 to 18 months old).

mg/gm creatinine	No. of Subjects	Before PO ₄ ^a Supplement	1 day ^b	Days of PO ₄ 2 days ^b	Supplementation 4 days ^b	6 days ^a
Phosphorus	18	1,116±358	1,759±523	2,173±476	1,866±333	
Pyrophosphate	18	6.4±1.2	13.5±1.9	14.9±2.0	11.3±1.4	
Calcium	18	118±29	27±7	33±9	72±19	
Oxalic Acid	18	119±18	119±23	110±17	108±14	

a 24 hours urine sample
b Casual fresh morning urine sample

Table 5. Urinary Excretion of Oxalate by Male Infants from Ubol Village—Vitamin B₆ Supplement

	No.	Mean Age months	Oxalate Excretion in 24 hour urine mg/gm creatinine
Control	18	9.2	129±9
B ₆	18	9.2	128±16