SEATO CLINICAL RESEARCH STUDY ON BLADDER STONE*

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GENERAL INFORMATION

From previous epidemiologic study of bladder stone[1], significant differences were demonstrated in prevalence of the disease among persons in the Central, North and Northeast areas of Thailand. The highest prevalence rates were observed in Chiengmai, Kohnkaen and Ubol Provinces (23.3, 18.6, and 12.0 per 1000 respectively), an intermediate rate in Korat (8.0 per 1000) and the lowest rates in Saraburi, Pratoomtanee and Smutprakarn Provinces (2.3, 1.2, and 1.7 per 1000 respectively).

Study of infant feeding practice revealed a striking correlation of early rice feedings with bladder stone endemicity. When bladder stone prevalence in villagers living within one region were compared as to the time of starting rice supplements, a singificant correlation ($p<0.01$) between early rice feeding and a higher attack rate was obtained.

From previous work it was known that citrate calculi can be produced in rats fed with low case in diet (15% by weight) and prevented by increasing the protein level to 30% with either case in or soybean protein. Gelatin, however is ineffective[2]. Supplementation of methionine and/or lysine monohydrochloride can also reduce or prevent stone formation in these rats[3]. Attempts have been made to evaluate the possible role of methionine, cystine and lysine in the pathogenesis of bladder stone.

Urolithiasis has been found to occur in patients with hypercalcuria[4] and hyperoxaluria [5, 6]. Stone can also be produced in rats fed with low phosphorus diet[7]. Recurrent attacks of renal stone can be reduced or prevented by giving high magnesium[8] and phosphorus[9] diet. Previous study of the urinary constituents in children 2 to 10 years old, living in Nong Kohn village (endemic area) and Ubol city (hypo-endemic area), showed no significant differences in urinary magnesium, phosphate, oxalate and uric acid excretion. These findings do not support the possible low intake of magnesium, phosphate and vitamin B_6 as being a factor in the pathogenesis of this disease among the studied subjects. Aurinary metabolites study in infants under 1 year old was carried out during the period covered by this report.

The study of organic matrices and the search for specific urinary mucosubstances (biocolloids) important in the binding of ions and necessary for induction of crystal growth, are currently receiving greater attention in the research concerning urolithiasis. A mucoid matrix was demonstrated in al calculi[10], and the urine was found to contain increased quantities of mucosubstances during calculous formation in both man[11] and animal[12]. The calcium binding properties ot urinary mucosubstances were demonstrating by Boyce et al[13] and Maclagan[14]. Interrelation of matrix and crystalline components suggested that the former served as an architectonic function in calculous growth[15, 16]. Study of urinary mucosubstances of subjects living in an endemic area is in progress. Preliminary results are included in the report.

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STUDY REPORTS

1. Title "Study of Urinary Mucosubstances and Organic Matrix of Stone"

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Objectives

To study the relationships of urinary mucosubstances (mucoprotein and mucopolysaccharides) as well as the composition of stone matrices, in vessical lithiasis in Thailand.

Description

Twenty-four-hour urine samples were collected from male newborns and infants whose ages ranged from 6 days to 12 months. Urine collection bag was used to assist in the collection. After each voiding, the bag was emptied into the container which was always kept at a temperature of $1^0$to 5^0C.

In the villages, collections were made at school where the infants and mothers stayed until the collections were completed. All were under supervision of nurses at all times. If the mothers refused to come to the schools, supervision and instructions for collection were given at their homes. Repeated collections were performed on the following days if the first collection was incomplete. Only cooperative mothers were included in the study.

The number of urine samples and mean age of subjects in the 3 studied areas are shown in Table I. The infant feeding habits in different areas are shown in Table II. Four (out of six) studied villages were of average size with population from 438 to 642. Their chief occupation is farming and general econmical status is poor. the remaining two villages were larger with populations of 1529 and 2022. These latter two villages were located near a big pond and were better off economically. The dietary habits are the same. Glutinous rice and fermented fish are the main staple food for adults and children. Practically all infants are breast-fed and over half of them were supplemented with glutinous rice within the first months of life. There has been history of bladder stone in these 6 villages.

The urine samples collected from Ubol city and Bangkok infants were used for control since the prevalence of disease is very low in Ubol city and practically zero in Bangkok. The inhabitants of these two areas better off economically and nutritionally than the Ubol villages. General diets of adults include regular rice with a good amount of meat, fish, egg, vegetables and fruits. Infants are either breast-fed or supplemented with commercial formula. Solid foods, regular boiled rice with egg or chopped pork and banana, are usually started from about 3 to 4 months of age.

The amount of the total non-dialyzable solid (TNDS) was determined according to Boyce and King (J. Clin. Invest. 37:315, 1958). After a three-day cold dialysis, the dialysand was lyophilized and recorded as TNDS. This determination has been completed on 30 samples from villages, 32 samples from Ubol city and 34 samples from Bangkok.

The 24-hour urine volume in different age groups is shown in Table III. The volume is comparable between the newborns and infants living in Nong Kohn village and Ubol city.

Table IV. showed that village newborns excreted significantly higher amount of TNDS than those excreted by city newborns, however, infants (between 1 to 12 months old) from both city and villages show no significant difference of TNDS excretion. When they are compared with the Bangkok and the U.S.

infants (Table V), it is shown that the Bangkok newborns excreted TNDS comparable to those excreted by Ubol city and the U.S. However, the Bangkok and Ubol city newborns excreted significant lower amount of TNDS than those excreted by the newborns from Ubol villages ($P=0.05$ and < 0.2 respectively). On the other hand, there were no significant difference between the TNDS excreted by the infants from these three groups.

Since only a small number of newborns was tested, an attempt was made at a second collection of 24-hour urine samples from a larger number of newborns from Ubol city and vlilages in February 1966. Fifty-three urine specimens from each area (Ubol city and Ubol villages) were collected; 30 were from male newborns (1 to 45 days old) and 23 from male infants (11/2-12 months). The procedure for urine collection was the same as previously described.

Attempt has been made to study the fractionation of the TNDS. A lyophylizer, which is required for the fractionation procedure, is presently on order.

Table I

Mean Age and Number of Subjects

Location	0-30 days old		1-6 months old		7-12 months old	
	No. of subj	Mean Age in days	No. of Subj	Mean Age in months	No. of subj	Mean Age in months
Ubol Villages	7	23.3	16	3.3	13	9.6
Ubol City	8	20.4	12	3.8	12	10.0
Bangkok	9	7.8	15	3.5	13	10.5

Table II

Infant Feedings History

Feedings	No. of subjects		
	Ubol villages	Ubol city	Bangkok
No. of subjects	36	32	37
A. Milk			
Breast	36	13	23
Breast & formula	0	16	9
Formula	0	3	5
B. Supplementation			
I) Newborn (under 1 month old)			
None	3	8	9
Glutinous rice	4	0	0
II) Infants (1 to 6 month old)			
None	0	5	7
Glutinous rice	16	1	0
ordinary rice	0	6	5
egg, meat & fish	1	4	9
III) Infants (7 to 12 months old)			
None	0	0	2
glutinous rice	13	3	0
ordinary rice	0	11	9
egg, meat & fish	1	7	8

Table III

24-Hour Urine Volume

	Nong Khon		Ubol City		t	P
	No.	Volume/24 hours	No.	Volume/24 hours		
Under 1 month	7	280.5 ± 43.53	8	222.3 ± 41.75	0.96	> 0.50
1+ — 3 months	10	275.5 ± 50.74	6	221.8 ± 34.43	0.87	> 0.50
3+ — 6 months	6	234.5 ± 43.06	6	230.5 ± 42.71	0.65	> 0.50
6+ — 9 months	7	206.9 ± 30.87	5	304.4 ± 130.5	0.67	> 0.50
9+ — 12 months	5	196.0 ± 30.06	7	231.14 ± 36.76	0.74	> 0.20
12+ months	5	335.0 ± 43.30	8	286.25 ± 53.36	0.709	> 0.20

Table IV

Urinary Total Non-Dialyzable Solids Excretion

	Villages		City		P
	No. of Subj	TNDS (mg/24 hr)	No. of subj	ITNDS (mg/24 hr)	
Newborn 6-30 days	6	134.8 ± 28.9	8	68.0 ± 10.2	< 0.05
Infants					
1+ — 3 months	8	103.3 ± 14.3	6	124.2 ± 24.3	< 0.50
3+ — 6 months	6	129.6 ± 17.2	6	142.4 ± 28.5	> 0.50
6+ — 9 months	9	98.3 ± 17.5	5	177.1 ± 45.9	0.10
9+ — 12 months	6	252.6 ± 119.1	7	127.0 ± 33.7	0.20

Table V

Urinary Total Non-Dialyzable Solids (TNDS)
in Different Geographic Areas

Areas	Newborn		Infants	
	No. of subj	TNDS (mg/24hr)	No. of subj	TNDS (mg/24 hr)
Ubol villages (V)	6	134.8 ± 28.9	29	138.1 ± 26.5
Ubol city (C)	8	68.0 ± 10.2	24	140.6 ± 16.1
Bangkok (BK)	9	72.5 ± 21.7	25	142.5 ± 18.0
U.S.A.*	7	87.9 ± 26.8	3	124.5

* Keutel and King: Clin. Chem. Acta II (1965)

Newborn

BK vs C P = > 0.50
BK vs V P = 0.05
C vs V P = < 0.02
BK vs US P = > 0.50
C vs US P = > 0.50
V vs US P = > 0.20

Infant

BK vs C vs V P = > 0.50