2. Title: Study of Urinary Chemical Compositions and Mucosubstances.

Principal Investigators: Sakorn Dhanamitta, M.D.
Aree Valyasevi, M.D.

Assistant Investigators: Jaratbhan Yooktatat, B.Sc.
Potjanee Threeratana, B.Sc.

Background

Considerable attention to the importance of uromucoprotein in the urolithiasis problem has been increasing recently. Boyce et. al (1) reported that probably every urinary stone contains a matrix composed of mucoprotein derived from urine. A mucoid matrix was demonstrated in all calculi (2), and the urine was found to contain increased quantities of mucosubstances during calculous formation in both man (3) and animal (4).

Mia and Cornelius (5) reported that a significant daily increase in the 4,000 to 9,000 molecular weight fraction of salt soluble (0.58 M NaCl) urinary biocolloids was observed in the urine of sheep receiving a "calculi—provoking" diet as compared to normal sheep.

Boyce and Swanson (6) reported that the mucoprotein of Tamm and Horsfall is much increased in the urine of patients with renal calculous disease.

Previous studies from this laboratory suggested that the bladder stone disease, as seen in Thailand, is probably started very early in life and somehow related to the nutritional status of the youngster and, possibly, of the mother (7—8). Therefore, it is reasonable to investigate the mucoprotein contents of urine in infants and children resident in the hyper—endemic area (villages) and the hypo—endemic area (city).

Objectives

To investigate the mucoprotein content of urine and their fractionation in newborn and infants of hyper—and hypo—endemic area.

Descriptions

Twenty—four hour urine samples were collected from male newborn and infants whose ages ranged from 3 days to 12 months. Collections were made from the subjects residented in Ubol villages and city under constant nursing supervision. Pediatric urine collection bags were used for the collection. After each voiding, the bag was emptied into the container which was always kept at a temperature of 1° to 5°C. Repeat collections were performed on the following days if the first collection was incompleted.

Qualitative tests for pH, protein and sugar were made by Combistix paper strip ®. Microscopic examinations were performed daily on centrifuged, freshly voided urine samples within two hours of collection to determine any abnormalities.

Twenty—four urine samples were measured for volume and pH. All urine samples showed negative tests for protein, glucose, occult blood and contained normal urinary sediments.

Collections were also made from newborn and infants residented in suburb of Bangkok by using the same procedures.

The amount of the total non—dialyzable solid (TNDS) was determined by taking dry weight at 90-100°C for 1-1 1/2 hours of the non—dialyzable urine after three day cold dialysis against demineralized water. The rest of the non—dialyzable urine was lyophilized and measured for TNDS by the method according to Boyce and King (7).

Subfractionation of the urinary mucosubstances was determined by Gel Filtration as described by Gale, Cornelius and Bishop (8). The Tamm-Horsfall (T—H) mucoprotein was salted out with 0.58 M. NaCl

from an aliquot of the original urine samples and was measured at 280 mu using a Beckman DU Spectrophotometer. The results were expressed as ODU per 24 hours. The 0.58 M. NaCl-soluble fraction was dialyzed for 48 hours in 5/8 inch cellophane tubing with distilled water at 5°C and was evaporated to concentrate the protein fraction approximately 10 fold. This non-dialyzable total salt-soluble protein fraction was subsequently fractionated on 3 columns (1 × 26 cm) using Sephadex (G-25, G-50, and G-100) as previously described by Mia and Cornelius (9).

Progress

The number of subjects, mean ages, urine volume and urinary pH in different age group and location are shown in Table I.

Tables 2 and 3 show the amount of urinary total non—dialyzable solids (TNDS) excreted by newborn and infants from villages, Ubol city, Bangkok and U.S.A., as determined by heated and weighed, and by lyophilized and weighed respectively. It is shown that village newborn excreted significantly higher amount of TNDS than those by city and Bangkok groups ($P < 0.1$ and 0.01-0.02 respectively). When the village newborn is compared with the U.S., it is also demonstrated that village newborn excreted higher TNDS than the U.S. who excreted comparable to the Ubol city and Bangkok newborn. There are no significant difference in the TNDS excretion in infants, aged over 30 days, between these four groups except the Bangkok infants (1 to 6 month old) excreted significantly higher than those excreted by the village and city groups. No explanation could be given. The results from these two methods are comparable.

Table 4 shows percentage of various molecular weight groups of salt-soluble urinary proteins of male infants from Ubol village, Ubol city and Bangkok. Significantly higher excretion in the 5,000 to 10,000 and the 10,000 to 100,000 molecular weight fractions was observed in village infants as compared to the city and Bangkok. On the contrary, the excretion of 1,000 to 5,000 molecular weight fraction is markedly lower in the village than the city and Bangkok infants.

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Table I. Numbers and Mean Ages of Subjects, and 24 hour Urine Volumes, Urinary Creatinine and pHs.

Age and Locality	No. of Subjects	Mean Age	Urine Vol. ml/24 hour	Creatinine mg/24 hour	Average pH
3 – 15 days					
Village	10	9 days	85±22	14.5±2.4	6.0
Ubol City	12	9 days	142±20	14.6±2.2	6.1
Bangkok	20	5 days	146±18	22.5±2.8	6.6
16 – 30 days					
Village	14	22 days	236±26	21.7±1.8	6.4
Ubol City	17	24 days	239±27	21.3±1.4	6.0
Bangkok	3	21 days	304±40	29.5±8.4	6.8
1+ – 6+ months					
Village	46	2.7 months	244±17	33.9±1.8	6.4
Ubol City	38	2.7 months	261±20	32.5±2.3	6.0
Bangkok	21	3.6 months	290±25	42.5±5.3	6.9
7 – 12 months					
Village	16	9.5 months	205±16	56.0±5.9	6.6
Ubol City	17	9.5 months	242±41	44.9±4.2	6.8
Bangkok	19	10.2 months	174±19	41.3±6.1	6.4

Table 2. Urinary Total Non–Dialyzable Solids (TNDS)*
(mg/24 hours) in Different Ages Groups and Locations (Thailand)

Area		Newborn No.	3 – 15 days	No.	16 – 30 days	Infants No.	1+ – 6+ months	No.	7 – 12 months
Ubol Villages	(V)	10	a,w 130.0±21.7	14	120.5±20.1	46	w 114.0±8.5	16	146.6±14.1
Ubol City	(C)	12	57.8±9.6	17	86.6±7.9	38	m 110.2±9.6	17	152.3±27.6
Bangkok	(B)	20	53.9±9.9	3	107.8±27.5	21	189.3±19.7	19	119.2±17.0

* Samples were heated at 95°C until constant weight were obtained.

Probability Value	V vs C	V vs B	C vs B
P 0.01	a	w	m
0.01 P 0.02	b	x	n
0.02 P 0.05	c	y	o
0.05 P 0.10	d		p

Table 3. Urinary Total Non–Dialyzable Solids (TNDS)*
(mg/24 hours) in Infants of Different Ages Groups (Thailand and U.S.)

Area		Newborn No.	3 – 15 days	No.	16 – 30 days	Infants No.	1+–6+ months	No.	7 – 12 months
Ubol Village	(V)	10	109.9±19.4 [bx]	12	103.6±17.1	44	108.4±8.3 [w]	15	131.5±13.4
Ubol City	(C)	9	55.2±7.9	16	85.6±8.3	36	102.5±8.7 [m]	16	131.5±21.3
Bangkok	(B)	16	50.8±10.8	3	85.8±15.1	19	170.4±15.6	16	127.7±20.3
U.S.A.**			(7) 87.9±26.8				(3) 124.5		

* Samples that were adequated in amount were studied (lyophilized and weighed)

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

Probability Value	V vs C	V vs B	C vs B
P 0.01	a	w	m
0.01 P 0.02	b	x	n
0.02 P 0.05	c	y	o
0.05 P 0.10	d	z	p

Table 4. Percentage of Various Molecular Weight Gruops of Salt Soluble Urinary Proteins of Male Infants from Ubol Village, Ubol City and Bangkok

Area	Number of Samples	Mean Age	Molecular Weight Groups—Percent of Total in 24 hour Urine Molecular Weight 1000 – 5000	Molecular Weight > 5000 – 10000	Molecular Weight > 10000 – 100,000	Molecular Weight > 100,000
Infant			a,w	a,w	c,w	b,z
Village	11	4.7 months	11.6±2.0	46.7±3.2	17.4±4.1	24.1±2.4
City	13	5.2 months	66.2±3.8	10.6±2.0	8.1±1.4	15.3±2.4
Bangkok	10	6.9 months	54.9±4.2	4.9±1.2	5.6±2.2	34.6±3.3
Newborn			d	c		d
Village	13	14.9 days	26.4±6.1	33.4±5.1	13.8±2.4	28.8±4.0
City	11	14.7 days	35.7±6.7	18.0±3.8	13.4±4.8	38.2±4.0

Villages compared to City and Bangkok

Probability Value			City	Bangkok
	P <	0.01	a	w
0.01	P	0.02	b	x
0.02	P	0.05	c	y
0.50	P	0.10	d	z