

4. Title: "Sulfate Sulfur Excretion in Infants under 1 year old"

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Objectives:

To evaluate the excretion of inorganic sulfate by newborn and infant living in hyper-and hypo-endemic areas of Thailand.

Low levels of sulfate excretion might be interpreted as an indication of either low intake of methionine/cystine or some aberration in the normal oxidative pathways of organic sulfur to inorganic sulfate.

Description:

The urine samples, collected for the urinary mucosubstances study, were determined for sulfate sulfur excretion. Aliquots of the urine samples were analyzed for inorganic sulfate by the benzidine sulfate method as described by Kleeman, Taborsky and Epstein⁽¹⁾, and by Natelson⁽²⁾. Determinations for sulfate were made both before and after mild hydrolysis with hydrochloric acid in order to estimate free inorganic sulfate and total inorganic sulfate, respectively. Analyses were performed in quadruplicate. Each day two levels of a standard sodium sulfate solution were assayed in triplicate and the levels of sulfate in the urine samples estimated from these values. Many of the urine samples showed sulfate considerably lower than anticipated, therefore, recovery studies were performed to ascertain whether there was some interference with the precipitation of benzidine sulfate. Urine samples were assayed alone and in the presence of added standard sodium sulfate. Recoveries from 85-105% were obtained suggesting the complete precipitation of benzidine sulfate and the validity of the low sulfate values in the urine samples.

Determinations were made for creatinine and uric acid by automated procedures⁽³⁾

Progress

As indicated previously, urine samples were collected from three geographic areas; villages, Ubol City, and Bangkok. Although collections were made in several villages where vesical lithiasis was commonly observed, all data from the villages were grouped together. For the purposes of presentation the subjects have been divided into three groups according to age: one to thirty days old, one to six months old, and seven to twelve months old. The mean ages of the newborn and infants from the three geographic areas are shown in Table I. It can be seen that the mean ages of the subjects in the age groups of one to six months and seven to twelve months were very similar for the villages, Ubol, and Bangkok. Unfortunately, in the age group of one to thirty days the mean age of the population from Bangkok was considerably lower than from the villages and from Ubol City. This could not be foreseen and depended upon the availability of subjects and other factors.

The data for each age group are presented as total sulfate sulfur, free sulfate sulfur, and the difference between the two assay values which is reported as ethereal sulfate sulfur. Also, values are reported on the basis of concentration of sulfate in the urine, the quantity excreted in 24 hours, and in terms of sulfate excreted per gram of creatinine.

The sulfate sulfur excreted by boys one to thirty days of age is reported in Table 2. There was a progressive increase in the mean urinary concentration of total sulfate sulfur going from the villages, to Ubol, to Bangkok. The Bangkok value was significantly higher than the village value ($0.02 < P < 0.05$) although there was no significant difference between the villages and Ubol nor between Ubol city and Bangkok. In terms of free sulfate sulfur in the urine, the mean Ubol city value was higher than the village value ($0.05 < P < 0.10$) as was the Bangkok value ($P < 0.01$). It is of some interest that the percent of free sulfate in the urine in respect to total sulfate averaged only 63.4 for the village newborn whereas the Bangkok value approximated the accepted value in the United States of about 90%. When expressed on the basis of total sulfate sulfur excreted in 24 hours, the mean Bangkok value is higher than the village value but was not significantly higher. The quantity of free sulfate excreted per day was less in the village boys than in those from Ubol city or Bangkok but only the Bangkok value was significantly higher ($0.02 < P < 0.05$). It should be noted that the mean age of the Bangkok subjects was somewhat less than that of the subjects from the villages and from Ubol city. For this reason the data is actually weighted against the Bangkok infants.

The results of studies on infants one to six months old are presented in Table 3. It was found that both total sulfate and free sulfate concentrations in the urine samples from the villages were significantly lower than in samples from Ubol city and from Bangkok ($P < 0.01$). Also, the percent of free sulfate in the urine showed a difference at the same level of significance. There was no significant difference between the Ubol city and Bangkok values for total sulfate and free sulfate concentrations although in both cases the Bangkok values were higher. A comparison of the three areas on the basis of daily excretion indicates that the village infants excreted the smallest quantity of both total sulfate and of free sulfate, the city infants showed an intermediate value, and the Bangkok infants the highest value of the three. The differences in free sulfate excretion were highly significant ($P < 0.01$) when comparisons are made between villages and city and between villages and Bangkok.

The findings concerning young boys six to twelve months of age are presented in Table 4. Approximately the same trends were observed in this age group as in the one to six month age group with the village infants excreting less total sulfate and less free sulfate than the subjects from Ubol city and Bangkok.

Many investigators prefer to express excretion data in terms of the creatinine levels in the urine rather than on the basis of concentrations of a constituent which may be influenced by water output, or on the basis of 24 hour excretion which may be in error because of incomplete collections. The data from the present study have been calculated on the basis of the creatinine concentration in each sample and the results presented in Table 5. In the age group of one to thirty days, the mean creatinine concentration in the urine samples of the villagers was a little less than either the city subjects (not significant) or the newborn from Bangkok ($0.05 < P < 0.10$). The mean total sulfate sulfur excretion per gram of creatinine was approximately the same in the newborn from the three geographic areas. In the age group of one to six months, the mean creatinine concentrations were not significantly different in the three areas but the mean sulfate/creatinine ratio of the village urine samples was significantly less than either the city samples ($0.01 < P < 0.02$) or the Bangkok samples ($P < 0.01$). In the age group of seven to twelve months the creatinine concentrations were not significantly different in the urine samples from the three areas. In this latter age group, however, the mean sulfate/creatinine ratio of the village urine samples was less than either the Ubol city or Bangkok samples at the same level of significance ($P < 0.01$).

Our data are in agreement with the infant feeding study which revealed that all village infants were breast fed and supplemented with glutinous rice in a very early life. None of the village infants had egg, meat and fish during their first year of life, except one infant who occasionally had some fish. However, further study regarding possible aberration in the normal oxidative pathways of organic sulfur to inorganic sulfate is required before any conclusion can be drawn.

- (1) Kleeman, C.R., Taborsky, E. and Epstein, F.H.: Proc. Soc. Exper. Biol. Med. 91:480, 1956
- (2) Natelson, S.: Microtechniques of Clinical Chemistry, Second Edition Charles C. Thomas Publish, Springfield, Illinois. pp. 402
- (3) Technicon Autoanalyzer, Chauncey, N.Y., U.S.A.

Table I
Mean Ages of Subjects

	0-30 days	1-6 months	7-12 months
Villages	23.3	3.3	9.6
Ubol city	20.4	3.8	10.0
Bangkok	7.8	3.5	10.5

Table II
Sulfate Sulfur Excretion in Boys 1 to 30 days old

Location	No.	Total Sulfate Sulfur	Free Sulfate Sulfur	Ethereal Sulfate Sulfur	% Free Sulfate Sulfur
Villages					
mg/100 ml	7	5.8 ± 0.8 ^y	3.5 ± 0.9 ^{d,w}	2.3 ± 0.4 ^{c,y}	63.4 ± 6.5 ^{b,w}
mg/24 hours		16.2 ± 3.5	9.1 ± 3.0 ^y	7.1 ± 2.4 ^{c,z}	
Ubol city					
mg/100 ml	8	7.3 ± 1.2	6.4 ± 1.1	0.9 ± 0.3	84.7 ± 4.4
mg/24		17.0 ± 3.4	15.8 ± 4.2	1.2 ± 0.6	
Bangkok					
mg/100 ml	9	9.8 ± 1.5	9.8 ± 1.5	1.0 ± 0.3	90.3 ± 3.7
mg/24 hours		23.2 ± 5.8	21.1 ± 4.4	2.1 ± 1.7	

Probability Value
 P < 0.01
 0.01 < P < 0.02
 0.02 < P < 0.05
 0.05 < P < 0.10

Compared with Ubol
 a
 b
 c
 d

Compared with Bangkok
 w
 x
 y
 z

Table III

Sulfate Sulfur Excretion in Boys 1 to 6 Months Old

Location	No.	Total Sulfate Sulfur	Free Sulfate Sulfur	Ethereal Sulfate Sulfur	% Free Sulfate Sulfur
Villages					
mg/100 ml	16	5.9 ± 0.6 ^{a,w}	3.6 ± 0.4 ^{a,w}	2.3 ± 0.4 ^c	61.1 ± 5.0 ^{a,w}
mg/24 hours		13.7 ± 1.4 ^{c,w}	8.9 ± 1.3 ^{a,w}	4.8 ± 0.8 ^d	
Ubol city					
mg/100 ml	12	9.6 ± 1.1	8.3 ± 1.0	1.3 ± 0.3	85.3 ± 2.8
mg/24 hours		21.1 ± 2.6 ^y	18.4 ± 2.6	2.7 ± 0.7 ^z	
Bangkok					
mg/100 ml	15	11.2 ± 1.2	9.4 ± 1.1	1.8 ± 0.3	83.3 ± 1.9
mg/24 hours		32.8 ± 5.2	27.3 ± 4.9	5.5 ± 1.1	

Probability Value
 P < 0.01
 0.01 < P < 0.02
 0.02 < P < 0.05
 0.05 < P < 0.10

Compared with Ubol

a
 b
 c
 d

Compared with Bangkok

w
 x
 y
 z

Table IV

Sulfate Sulfur Excretion in Boys 7 to 12 Months Old

Location	No.	Total Sulfate Sulfur	Free Sulfate Sulfur	Ethereal Sulfate Sulfur	% Free Sulfate Sulfur
Villages					
mg/100 ml	11	9.3 ± 1.9 ^{d,w}	6.5 ± 1.4 ^{c,w}	2.8 ± 0.8	67.6 ± 4.9 ^{d,w}
mg/24 hours		19.1 ± 3.0 ^{c,x}	13.2 ± 2.3 ^{c,x}	5.9 ± 1.1	
Ubol city					
mg/100 ml	11	17.4 ± 3.6	14.1 ± 3.1	3.3 ± 1.0	81.1 ± 4.8
mg/24 hours		44.5 ± 10.4	36.5 ± 9.3	8.0 ± 2.2	
Bangkok					
mg/100 ml	13	19.5 ± 2.2	16.9 ± 1.9	2.6 ± 0.5	86.7 ± 2.5
mg/24 hours		44.3 ± 9.1	38.9 ± 9.0	5.4 ± 1.0	

Probability Value
 P < 0.01
 0.01 < P < 0.02
 0.02 < P < 0.05
 0.05 < P < 0.10

Compared with Ubol
 a
 b
 c
 d

Compared with Bangkok
 w
 x
 y
 z

Table V
Sulfate Sulfur Excretion in Terms of Creatinine Excretion

Location	Age of Subjects		
	0-30 days	1-6 months	7-12 months
Villages			
Total Sulfate S, mg%	5.8±0.8 ^y	5.9±0.6 ^{a,w}	9.3±1.9 ^{d,w}
Creatinine, mg%	8.0±1.0 ^z	13.0±2.8	29.8±4.3
S/C, mg/g	823±220	375±31 ^{b,w}	338±50 ^{a,w}
Ubol city			
Total Sulfate S, mg%	7.3±1.2	9.6±1.1	17.4±3.6
Creatinine, mg%	9.7±0.9	16.6±3.2	25.5±4.7
S/C, mg/g	776±106	700±123	766±110
Bangkok			
Total Sulfate S, mg%	9.8±1.5	11.2±1.2	19.5±2.2
Creatinine, mg%	12.9±2.2	15.9±4.2	20.6±3.5
S/C, mg/g	847±116	911±89	1128±215

Probability Value
P < 0.01
0.01 < P < 0.02
0.02 < P < 0.05
0.05 < P < 0.10

Compared with Ubol
a
b
c
d

Compared with Bangkok
w
x
y
z