

# THE URINARY SULFUR/CREATININE RATIO IN THE ASSESSMENT OF PROTEIN NUTRITIONAL STATUS

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## INTRODUCTION

There are several indices for the assessment of protein nutritional status in population groups. (WHO, 1963; Jelliffe, 1966). Besides the dietary, clinical and anthropometric assessment, laboratory methods seem to be a more objective and precise approach in evaluating the nutritional status. Laboratory values often do suggest marginal or acute deficiencies even though the patient appears clinically normal. Clinical signs usually occur after prolonged inadequate intake of nutrients. However, no real reliable biochemical test is available for protein malnutrition. A decrease of the serum protein level, especially serum albumin is not a particular sensitive index and not specific for clinical protein deficiency alone. (Committee on Procedures, 1970). During the past five years much attention has been paid to the decrease of different protein fractions as an index for protein malnutrition, especially prealbumin (Ingenbleek, 1972; Ingenbleek *et al.*, 1975) retinol-binding protein (RBP) and transferrin appear to be sensitive parameters. The urinary urea N/creatinine ratio and urinary sulfur/creatinine ratio are more related to the dietary status. Low ratios are reflecting merely a physiological response to a low nitrogen and sulfur intake. Low ratio of urinary urea to creatinine is found in children eating small amounts of proteins. The sulfur/creatinine ratio reflects more the dietary intake of high quality proteins, in particular those with a high sulfur content. (Committee on Procedures, 1970; Miller and Donoso, 1963). Un-

fortunately the sulfur/creatinine ratio has received little attention until now although in combination with the urea/creatinine ratio it is a good indicator for the immediate protein intake and of great value in field surveys. In this study the method for the determination of inorganic sulfur is described and the urinary sulfur/creatinine ratio of different groups in Thailand is compared with the urinary urea N/creatinine ratio.

## MATERIALS AND METHODS

Random urine samples were collected during the morning and acidified with hydrochloric acid (0.1 ml concentrated HCl in 30 ml urine). The samples were frozen until analysis.

Creatinine was measured by a modification of the procedure of Folin and Wu and urea-nitrogen was determined using a modification of the method of Marsh, Fingerhut and Miller. Both methods are described by Technicon Instruments Corporation for the Auto Analyzer. (Technicon Instruments Corp., 1972a, b).

The inorganic sulfate in urine was determined by a revised method of Klipp and Barney (1959), based on the absorption at 540 nm of chloranilate ions which are released into solution from the slightly soluble barium salt of chloranilic acid (2, 5-dichloro-3, 6 dihydroxyquinone) in the presence of inorganic sulfate. The driving force of the reaction involved is the formation of insoluble barium sulfate. Urinary cations interfering

with the colour development are removed, by a cation exchange resin. With a modification of this method by Wainer and Koch (1963) the excess of barium chloranilate is removed, then the solution is brought to pH 3 with glacial acetic acid so that the cations of the urine samples have little effect on the final pH. Procedure: On a wooden rack small Buchner funnels  $G_1$  were placed. With a Pasteur pipette a Dowex 50 $\times$ 4 suspension in distilled water was pipetted into the funnels until the resin column was about 1 cm. The resin was rinsed with 11 ml of distilled water, and placed under the funnels calibrated tubes until 10 ml. Into the tubes 1 ml of acetate buffer pH 4.63 was added. On the resin 0.1 to 0.3 ml of urine was pipetted and the resin rinsed three times with 1 ml of distilled water. Into the tubes 5 ml of 95% alcohol was added and the tubes filled up with distilled water to 10 ml. To the tubes about 30 mg of barium chloranilate was added and the tubes closed with stoppers and mixed several times for 10 minutes. Then centrifuged for 10 minutes. From the supernatant 5 ml was pipetted and 2 ml 50% acetic acid/distilled water was added. The optical density was read at 540 nm in a 1 cm cuvette in a spectrophotometer. By the same procedure a standard curve was prepared by pipetting standard solutions of ammonium sulfate into the tubes.

**Reagents:** For acetate buffer: 51 ml of 0.2 M acetic acid was mixed with 49 ml of 0.2 M sodium acetate  $3H_2O$  and 90 ml of distilled water. The pH adjusted to 4.63 with acetic acid and filled up with distilled water to 200 ml. For 50% acetic acid: 100 ml glacial acetic acid was mixed with 100 ml distilled water. For standard solutions: 1.3214 gm  $(NH_4)_2SO_4$  was dissolved in 100 ml of distilled water (100 mmol S). Diluted 2, 4, 6, 8, 10 and 12 ml aliquots to 100 ml with distilled water. The responding concentrations of the standard curve were 2, 4, 6, 8, 10 and 12 mmol S/100 ml.

The standard curve of the inorganic sulphate determination was linear. The precision for the method is good and the recovery of added sulfate was about  $100 \pm 5\%$ .

The urea N/creatinine ratio and the sulfur/creatinine ratio of the following groups were determined.

- Group 1: 30 Thai recruits with an average age of 20 years checked into Phramonkut Hospital, Bangkok.
- Group 2: 115 healthy schoolchildren from the University school in Khon Kaen. This group was used as reference.
- Group 3: 88 schoolchildren from Nakhon Nayok, about 100 km northeast of Bangkok.
- Group 4: 43 schoolchildren from the resettlement area Lam-takong, about 180 km northeast of Bangkok.
- Group 5: 136 schoolchildren from the Khon Kaen irrigation and resettlement area.

The schoolchildren of Group 2, 3, 4, 5 comprised of both sexes with ages ranging from 6 to 10 years.

## RESULTS

The urinary urea N/creatinine ratio for each group is given in Table 1. Analysis of variance demonstrated a significant difference between group 2 (reference group) and group 1, 3, 4 and 5. The mean value of group 2 was the highest ( $9.67 \pm$  one SD of 2.43). The normal range for reference group 2 calculated from the mean  $\pm$  SD is  $9.67 \pm 4.86$ .

The urinary sulfur/creatinine ratio for the different groups are also shown in Table 1. The values of reference group 2 are significantly different from those of group 1, 4 and 5. The sulfur/creatinine ratios of group 2 compared to group 3 were not significantly

Table 1

Mean and SD of urinary urea N/creatinine ratio of recruits (group 1) and schoolchildren from University school Khon Kaen (reference group 2), Nakhon Nayok (group 3), resettlement Lam-takong (group 4) and Khon Kaen irrigation and resettlement area (group 5).

Group No.	No. of subjects	Urea N/creatinine		Sulfur/creatinine	
		Mean	SD	Mean	SD
1	30	3.61	1.17	0.57	0.28
2	115	9.67	2.43	0.97	0.73
3	88	5.40	2.18	0.84	0.43
4	43	5.85	2.18	0.73	0.32
5	135	5.37	1.83	0.64	0.41

Statistical significance between the groups were tested by analysis of variance. Difference between mean urea N/creatinine ratios of reference group 2 and group 1, 3, 4 and 5 was statistically significant ( $P < 0.01$ ).

Difference between mean urea N/creatinine ratios of recruits, group 1, and schoolchildren of group 2, 3, 4 and 5 was statistically significant ( $P < 0.01$ ).

Difference between mean sulfur/creatinine ratios of reference group 2 and group 1, 4 and 5 was statistically significant ( $P < 0.01$ ).

Difference between mean sulfur/creatinine ratios of schoolchildren of group 2 and 3 and recruits of group 1 was statistically significant ( $P < 0.01$  and  $0.05$ ).

different. The mean value for group 2 was also higher than the means for the other groups. The normal range of sulfur/creatinine ratio for reference group 2 is between 0.40 and 1.40. Analysis of the results shows a relationship between the urea N/creatinine ratio and the sulfur/creatinine ratio. All groups when compared with group 2 have lower urea N/creatinine ratio which corresponds to a lower sulfur/creatinine ratio.

## DISCUSSION

The urea N/creatinine ratio seems to be a good indicator for the quantity of protein intake and is more a measurement of dietary than nutritional status. The mean  $\pm$  SD of  $9.67 \pm 2.43$  as it is found in the reference group of healthy Thai schoolchildren is much lower than the value reported by Bohdal and Simmons (1969), and Simmons (1972)  $15.0 \pm 7.3$  for Asian children of 4 to 5 years. One of the reasons might be that the age of the children in the reference group is slightly older (6 to 10 years), by that having a higher

creatinine excretion. However, this does not explain the lower values of the reference group alone as reported here. The sulfur/creatinine ratio is an indicator for measuring protein intake of good quality especially those containing a high sulfur content. From this study it is clear that the sulfur/creatinine ratio is related with the urea N/creatinine ratio. A decrease in the 4 groups, compared with the reference group, of the urea N/creatinine ratio is always accompanied with a lowering in the level of the sulfur/creatinine ratio. From this can be concluded that together with the recent low dietary intake of protein also the intake of high quality protein was low.

## SUMMARY

The urinary sulfur/creatinine ratio and urea nitrogen/creatinine ratio of Thai soldiers and schoolchildren from Nakhon Nayok, 100 km northeast of Bangkok, the Lam-takong resettlement area and the Khon Kaen resettlement and irrigation area northeast of Thai-

land were determined. As reference group healthy children from the University school in Khon Kaen were selected. The urinary urea N/creatinine ratio was significantly lower for the children from Nakhon Nayok, the Lam-takong resettlement and the Khon Kaen resettlement and irrigation area when compared with the reference group. The ratio values of the soldiers was significantly lower than the ratio of every group of children. The urinary sulfur/creatinine ratio for the children in Nakhon Nayok was lower and for the children in the Lam-takong and Khon Kaen resettlements were significantly lower than the ratio of the reference group. For the reference group the normal range for urea N/creatinine ratio was between 4.8 and 14.5 and for sulfur/creatinine ratio between 0.40 and 1.40.

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