Variations in urinary inorganic sulphate sulphur and urea nitrogen excretion in children on a rural african diet

W. K. SIMMONS*

SUMMARY

A study was conducted to test the most suitable time of day to evaluate urinary urea nitrogen and inorganic sulprate sulphur when used as indicators of the relative recent protein intake. The investigation was conducted in Kenya, East Africa.

It was concluded from the study that anytime during the morning before lunch was a suitable time for the collection. It was also decided that either urea nitrogen or inorganic sulphate sulphur was better used as a ratio with creatinine than as calculated from a timed urine sample.

INTRODUCTION

The urinary excretion of urea and inorganic sulphate sulphur has been proposed as an indicator of the protein nutrition status or probably more precisely an indicator of the relative recent protein intake. These indicators have been used by various investigators around the world. However, there is little agreement as to whether the urea or inorganic sulphate sulphur should be used as timed samples or as ratios with creatinine. Also, the time of day the samples were collected has not been standardized. The following test should help to show (a) whether a timed sample or a ratio with creatinine is best and (b) the best time of day to collect a urine sample for the determination of both urea and inorganic sulphate sulphur as studied on children on a rural African diet.

---

* Pan American Health Organization/World Health Organization, c/o Instituto de Nutrição, Cidade Universitaria, 50.000 Recife, Brazil.
Recibido: 1-9-1971.

Experimental Techniques

The investigation was conducted in Kenya, East Africa, on children of the Wakamba tribe. Sixteen male children were studied who were all five years of age. Five adult personnel were with the children constantly during the entire study. Three of the adults spoke English, Swahili and Wakamba, so as to make sure that the children understood all instructions.

Since the diets of various tribes change throughout the year (1) the diet of the Wakamba tribe was carefully studied for that period of the year in wich the study was conducted. The night before the study began the children ate their typical diet at 7:00 p.m. The children were instructed to rise at 6:00 a.m. the next morning and discard their urine. This was carefully supervised. Urine samples were then collected at 8:00 a.m., 10:00 a.m., 12:00 noon, p.m., 4.00 p.m., and 6.00 a.m. the following morning Thus a 24 hour sample of urine was obtained. The first five collection periods (A-E) were each two-hour periods; period F constituted a 14-hour period and the results were divided by 7, so all six collection periods (A-F) would be equal. Therefore all values of both urea nitrogen and inorganic sulphate sulphur are in mg per 2 hours. The volume of urine was recorded and acidified with 1N HCl to a pH 3. The urine samples were then transported to the loboratory and frozen.

The children had breakfast at 8:00 a.m., lunch at 12:00 noon and dinner at 7:00 p.m. The diet was carefully controlled during the entire study, so as to be sure that it was typical for that season of the year. The diet can be seen in Table 1. Breakfast consisted of corn (maize) meal plus a small amount of sugar. Lunch was a mixture of 2/3 corn with 1/3 beans boiled for 2 hours. Dinner was thick maize meal (Ugalt) plus English potato soup.

Creatinine was determined by the method of Folin and Wu (2), urea nitrogen by the method of Valey (3) and inorganic sulphate sulphur by the method of Berglund and Sorbo (4). A statistical analysis was carried out by Welch's Test (5).

Results

Table 2 and Figure 1 show the average values and levels of significance of inorganic sulphate sulphur over a 24 hour period. The highest level of 41.9 mg was recorded during the fasting periods (A) (6:00 a.m. - 8:00 a.m.). The lowest value of 14.8 mg was noted just before lunch. There was also a highly significant (1% level) increase for all the values after lunch where the values rose to 25.5 mg, 27.3 mg and 24.3 mg. for periods D, E, and F, respectively.

A somewhat different picture is seen in Table 3 and Figure 2 where the same values for sulphur are arranged in a ratio with creatinine (inorganic sulphate sulphur/creatinine). Again the lowest value was recorded right before lunch which was 445.1. However, the first period of collection was not the highest as in Table 2. (Inorganic sulphate sulphur per 2 hours). There was also a highly significant increase (1% level) for each value after lunch. The values are 616.9, 677.8, and 665.8 for periods D, E, and F, respectively.

A similar pattern is seen in Table 4 and Figure 3 when the urea nitrogen was compared to the inorganic sulphate sulphur excretion (Table 2, Figure 1). Again, the highest value (797.2 mg) was recorded from the fasting sample (period A). The lowest value was noted just before lunch (330.6 mg), but a highly significant increase (1% level) was anly recorded in the 2:00 p.m. - 4:00 p.m. (period E) (548.2 mg) and the 4:00 p.m. - 6:00 a.m. (period F) (476.5). Only 355.2 mg of urea nitrogen was excreted in period D (12:00 noon - 2:00 p.m.).

In Table 5 and Figure 4 the urea nitrogen values are given in a ratio with creatinine (urea nitrogen/creatinine). There was a steady decline in values until the lowest point was reached in the collection after lunch. The value was 8.8 (period D). There was only a highly significant (1% level) increase when the 12:00 noon - 2:00 p.m. (period D) was compared to the final collection period (4:00 p.m. - 6:00 a.m.) (period F). The value for the final collection period being 13.2.

Discussion

A similar study was conduct by Lee and Arroyave (6) in which the urea nitrogen/creatinine and N-methyl nicotinamide was measured under similar conditions. However, Lee and Arroyave (6) used adult volunteers. Since protein calorie malnutrition is more prominent in pre-school age children it seemed advisable to conduct the present study on children of that age group. Also, since the excretion of urea (7-13) and inorganic sulphate sulphur (12-16) have been studied as an indicator of the protein nutrition status in different parts of the world these two metabolites were considered the best for the present study.

The fasting levels (collection period A) of both inorganic sulphate sulphur and urea nitrogen were the highest for both metabolites. These results are confusing and are in contrast to those of Lee and Arroyave (6). Their results showed urea nitrogen to be lowest during the fasting period. Our results suggest that at 6:00 a.m. the discard was not complete. If so this could indicate the inexactness of timed urine samples collected from children because as stated earlier stringent measures were taken to validade the accuracy of the study. However, after the initial collection period neither urea nitrogen nor the inorganic sulphate suphur were elevated after the breakfast of corn (maize) meal. There was a decrease until after lunch when both metabolites increased significantly. This is in agreement with Lee and Arroyave's (6) observation.

However, when both inorganic sulphate sulphur and urea nitrogen were used in ratios with creatinine the highest values were not noted during the fasting (collection period A). There was also a decreased in both ratios until lunch. Then both ratios increased after lunch.

It is interesting to note that the breakfast of corn meal did nor the inorganic sulphate sulphur were elevated after the sulphate sulphur whereas the nitrogen intake of lunch was ample to raise the excretion of both metabolites. This supports previous evidence that the level of excretion of both metabolites in mid-morning specimens is determined by the previous sustained level of protein intake.

It therefore seems justified to conclude from the study that if one uses either inorganic sulphate sulphur or urea nitrogen to study the protein nutrition status, either of the metabolites are better used in a ratio with creatinine than as a timed urine sample. This is because (a) timed urine samples are difficult to collect in the field and (b) the use of creatinine helps to compensate for the high values of early morning urine samples.

Also the study indicates that during the morning any time before lunch is the best time to conduct such a study involving either the excretion of urea nitrogen or inorganic sulphate sulphur if either metabolite is used in a ratio with creatinine.

TABLE 1

CONTENT OF MEALS

Breakfast	8:00 a.m.	Porriage - maize (corn) meal plus sugar (small amount)
Lunch	12:00 noon	2/3 maize plus 1/3 beans
Dinner	7:00 p.m.	Ugalt (thick maize meal) plus English potato soup (in boiled water)

TABLE 2

AVERAGE VALUES AND LEVELS OF SIGNIFICANCE OF URINARY SULPHUR (mg) OF AFRICAN CHILDREN

Period	Average	Collection Periods Compared	V	C	Level of Significance
A 6 a.m. to 8 a.m.	41.9	A & B A & C	1.57 2.11	0.04 0.01	N.S. 5 %
B 8 a.m. to 10 a.m.	21.4	A & D A & E	1.25 1.12	0.05 0.03	N.S. N.S.
C 10 a.m. to 12 noon	14.8	A & F B & C	1.35 2.41	0.03 0.14	N.S. 5 %
D 12 noon to 2 p.m.	25.5	C & D C & E	3.47 4.81	0.12 0.17	1 % 1 %
E 2 p.m. to 4 p.m.	27.3	C & F	3.68	0.17	1 %
F* 4 p.m. to 6 a.m.	24.3				

*Average 2 hour period

$$S_1^2 = \frac{\Sigma x^2 - (\Sigma x)^2/n_1}{(n_1 - 1)} \qquad C = \frac{S_1^2 / n_1}{S_1^2/n_1 + S_2^2/n_2}$$

$$V = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}}$$

TABLE 3

AVERAGE VALUES AND LEVELS OF SIGNIFICANCE OF URINARY INORGANIC SULPHATE SULPHUR/CREATININE (mg/mg) RATIOS OF AFRICAN CHILDREN

Period	Average	Collection Periods Compared	V	C	Level of Significance
A 6 a.m. to 8 a.m.	530.9	A & B	0.26	0.53	N.S.
B 8 a.m. to 10 a.m.	547.2	A & C	1.71	0.25	5 %
C 10 a.m. to 12 noon	445.1	A & D	1.82	0.14	5 %
D 12 noon to 2 p.m.	616.9	A & E	2.98	0.23	1 %
E 2 p.m. to 4 p.m.	677.8	A & F	2.55	0.34	1 %
F* 4 p.m. to 6 a.m.	665.8	B & C	1.95	0.23	5 %
		C & D	5.62	0.34	1 %
*Average 2 hour period		C & E	6.80	0.47	1 %
		C & F	5.58	0.61	1 %

TABLE 4

AVERAGE VALUES AND LEVELS OF SIGNIFICANCE OF URINARY UREA NITROGEN (*mg*) OF AFRICAN CHILDREN

Period	Average	Collection Periods Compared	V	C	Level of Significance
A 6 a.m. to 8 a.m.	797.2	A & B	1.60	0.08	N.S.
B 8 a.m. to 10 a.m.	493.3	A & C	2.54	0.01	5 %
C 10 a.m. to 12 noon	330.6	A & D	2.38	0.04	5 %
D 12 noon to 2 p.m.	355.2	A & E	1.82	0.05	5 %
E 2 p.m. to 4 p.m.	458.2	A & F	1.70	0.06	N.S.
F* 4 p.m. to 6 a.m.	476.5	B & C	2.86	0.14	1 %
		C & D	0.60	0.27	N.S.
*Average 2 hour period		C & E,	2.79	0.22	1 %
		C & F	2.80	0.17	1 %

TABLE 5

AVERAGE VALUES AND LEVELS OF SIGNIFICANCE OF URINARY UREA NITROGEN/CREATININE (mg/mg) RATIOS OF AFRICAN CHILDREN

Period	Average	Collection Periods Compared	V	C	Level of Significance
A 6 a.m. to 8 a.m.	11.8	A & B	0.80	0.36	N.S.
B 8 a.m. to 10 a.m.	10.8	A & C	1.00	0.19	N.S.
C 10 a.m. to 12 noon	10.1	A & D	1.87	0.04	5 %
D 12 noon to 2 p.m.	8.8	A & E	0.13	0.11	N.S.
E 2 p.m. to 4 p.m.	11.6	A & F	0.80	0.20	N.S.
F* 4 p.m. to 6 a.m.	13.2	B & C	2.39	0.29	N.S.
		C & D	1.54	0.17	N.S.
		C & E	1.62	0.36	N.S.
		C & F	2.91	0.48	1 %

*Average 2 hour period

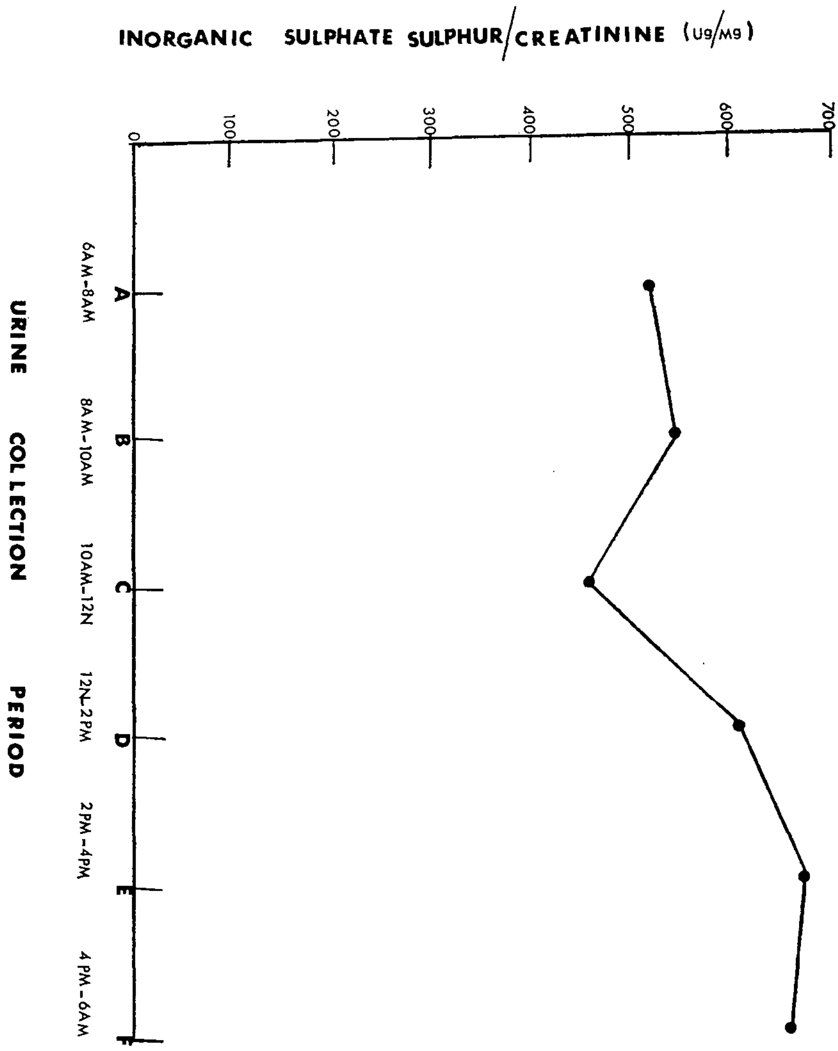

Figure 2.—Urinary Excretion of Inorganic Sulphate Sulphur/Creatinine Ratio (ug/mg) over a 24-Hour Period.

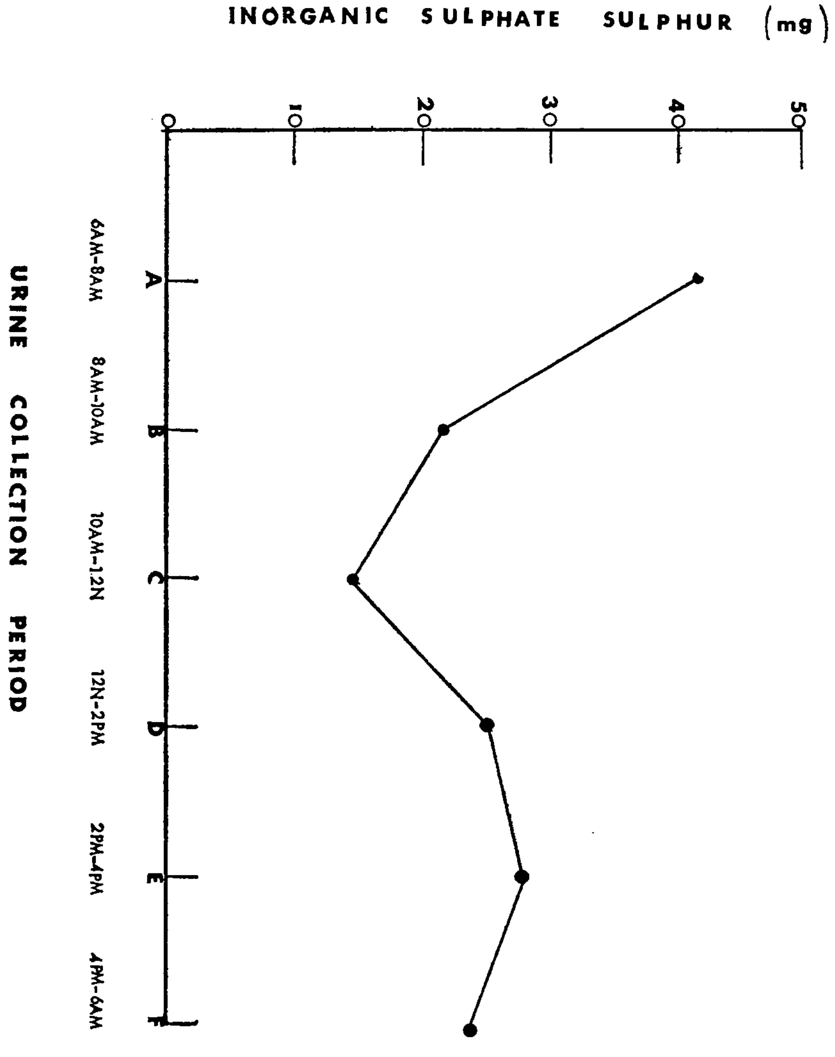

Figure 1.—Urinary Excretion of Inorganic Sulphate Sulphur (mg) over a 24-Hour Period.

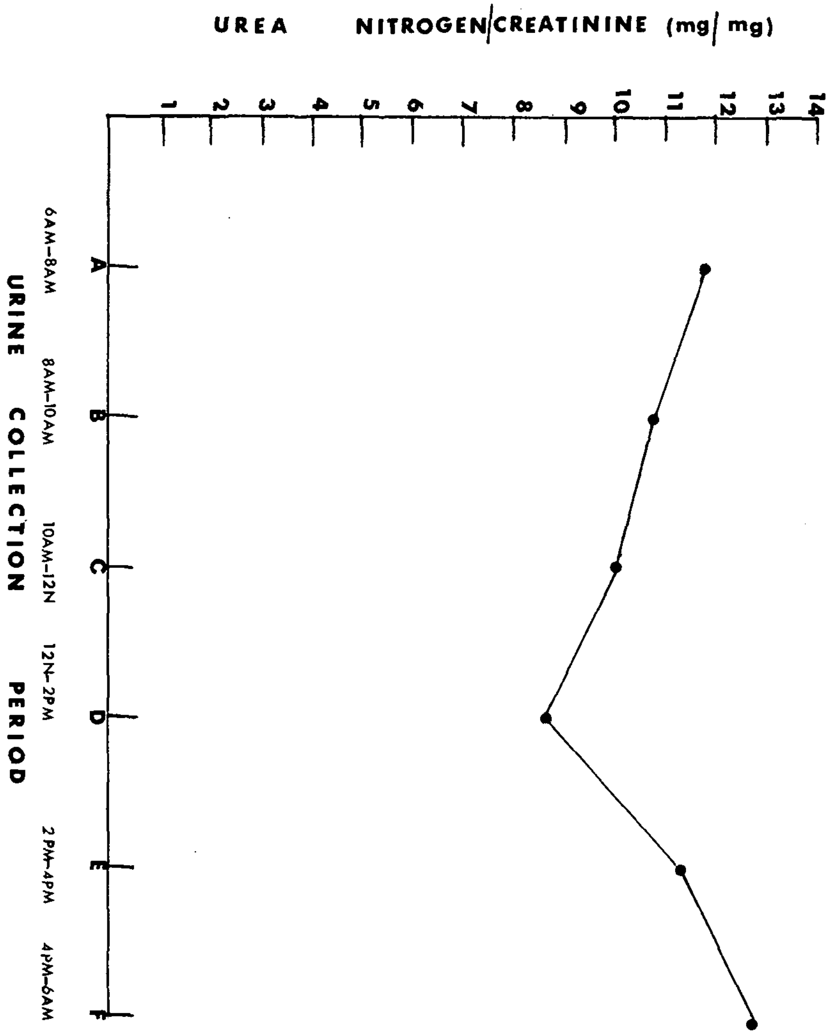

Figure 4.—Urinary Excretion of Urea Nitrogen/Creatinine Ratio (mg/mg) over a 24-Hour Period.

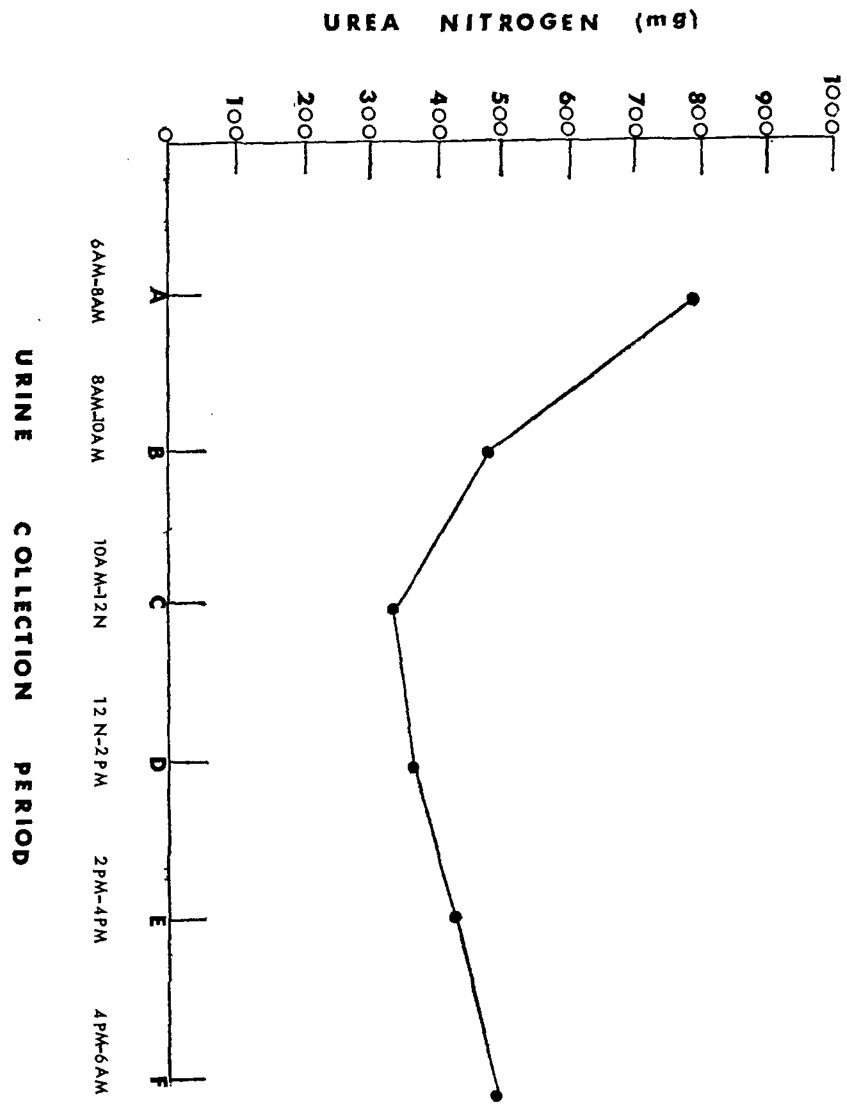

Figure 3.—Urinary Excretion of Urea Nitrogen (mg) over a 24-Hour Period.

ACKNOWLEDGMENTS

The author is indebted to Mrs. Philip Ndibo and Mr. Ronnie D'Souza for assistance with the laboratory procedures and Mr. Swinn Christensen for the statistical analysis.

BIBLIOGRAPHY

(1) Bohdal, M., Gibbs, N. E. & Simmons, W. K. Nutrition survey and campaign against malnutrition in Kenya, 1964-1968, Ministry of Health, Nairobi. 1968.

(2) Folin, O. & Wu, H. A system of blood analysis - determination of creatinine and creatine. **J. Biol. Chem.** 38, 98-106, 1919.

(3) Valey, H. Practical clinical biochemistry, 3rd ed., New York, Interscience Books. 1963.

(4) Berglund, F. & Sorbo, B. C. Turbidimetric analysis of inorganic sulphate in serum, plasma, and urine. **Scand. J. Clin. Lab. Invest.** 147, 12. 1960.

(5) Biometrika Tables for Statisticians. Volume 1. Table 11, page 136. E. S. Pearson & H. O. Hartley, Cambridge, 1962.

(6) Lee, M. & Arroyave, G. Variation in urinary excretion of urea and N-Methyl nicotinamide during the day comparison with fasting levels. **Arch. Latinoamer. Nutr.**, 16, 125-132, 1966.

(7) Arroyave, G. Biochemical evaluation of nutritional status in man. **Fed. Proc.** 20, 39, 1961.

(8) Dugdale, A. E. & Edkins, E. Urinary urea/creatinine ratio in healthy and malnourished children. **Lancet**, 1, 1062-1064, 1964.

(9) Walker, A. K. P. The nutritional state of South African child population groups as reflected by height, weight and nitrogen partition in the urine. **S. Afr. Med. J.**, 37, 400-403, 1963.

(10) Couvee, L. M. J., Nugteren, D. H. & Luyken, R. The nutritional condition of the Kapaukus in the Central Highlands of Netherlands New Guinea. **Trop Georg. Med.** 14, 27-32, 1962.

(11) Luyken, K. & Luyken-Koning, F. W. M. Studies on the physiology of nutrition in Surinam III. Urea excretion. **Trop. Georg Med.**, 12, 237-242, 1960.

(12) Bohdal, M. & Simmons, W. K. A comparison of the nutritional indices of healthy African, Asian and European children. **Bull, Wld. Hlth. Org.** 40, 166-174, 1969.

(13) Simmons, W. K. & Bohdal, M. Assessment of some biochemical parameters related to protein-calorie nutrition in children. **Bull. Wld. Hlth. Org.** 42, 897-906, 1970.

(14) Pellet, P. L. In: Evaluation of Protein Quality, pub. 1100. National Academic of Sciences - National Research Council. Report of an international conference. Committe on Protein Malnutrition, Food & Nutrition Board, Washington, D. C. 1963.

(15) Miller, D. S. & Mum Ford, P. Urinary sulphur as a measure of the protein value of diets. **Proc. Nutr. Soc.** 23, X liv.

(16) Pellet, P. L. Urinary sulphate sulphur as a measure of the protein value of diets. **Proc. Nutr. Soc.** 24, 37, 1965.