THE RELIABILITY OF ESTIMATING ∝ -AMINO ACID NITROGEN/CREATININE RATIO IN RANDOM URINE SPECIMENS

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SYNOPSIS

The diurnal variations of urinary ∝ -amino acid nitrogen and creatinine were studied in two adults and one child. The results show that amino N/creatinine ratios of part day urine specimens do not accurately reflect the 24 hour value. Thin layer chromatography of urinary amino acids was also carried out to ascertain whether there were diurnal changes in the excretion of individual amino acids.

INTRODUCTION

Until recently, creatinine excretion has been assumed to be constant and widely accepted as a measure of the completeness of a 24 hour urine collection. For this reason, amino acid excretion has been expressed as creatinine ratios. For young children and infants, where a 24 hour specimen is difficult, a random specimen is often used instead and the results expressed on the basis of creatinine excretion. This is because it is generally considered that a value so determined is comparable to that obtained from a 24 hour urine collection[7].

However, it has been observed that ∝ -amino acid N/creatinine ratios of random urine of patients sent to this laboratory, especially those of young infants, are exceedingly variable. For the same patient, there can be a 12 fold difference between the highest and the lowest value determined for different samples sent on the same day (Table I). The difficulty of interpreting these results prompted this study to (i) investigate whether there is a variability in amino N and creatinine excretion in part day urine collections of normal subjects and (ii) re-examine whether amino N/creatinine ratio of random urine is a valid index of amino acid excretion.

METHODS

Amino N and creatinine were studied in three healthy subjects, a male and a female adult and a three year old female child. Timed samples of urine were collected periodically for 24 hours from all the subjects. Urine specimens were stored at $-10°$ and analysed within 48 hours from the start of the collection.

Amino N in urine was determined by the method of Khachadurian *et al*[2] and urinary creatinine by the Technicon Autoanalyser Method N-116[8]. The coefficients of variation for 10 determinations carried out by different technicians, on different days, were found to be 5% for amino N and 7% for creatinine assay.

Thin-layer chromatography of amino acids was carried out on cellulose coated thin-layer plates. Urine aliquots applied to the plates were chromatographed in one dimension in the solvent system, butanol:acetone:acetic acid:water, 35:35:10:20 (v/v). After development, the plates were dried and redeveloped in the same solvent system containing 0.5 g% (w/v) of ninhydrin. The amino acids were visualised by heating the plate at 100° for 15 minutes.

TABLE I

AMINO N/CREATININE RATIOS OF MULTIPLE SAMPLES OF PATIENTS' URINE, COLLECTED RANDOMLY ON THE SAME DAY

Patient	Age	Observed amino N/creatinine * (mg/mg)
N. L.	1 month	0.07, 0.10, 0.29, 0.30, 0.41, 0.80
L. W. C.	1 month	0.02, 0.06, 0.16
G. S.	6 months	0.03, 0.30

*Amino N/creatinine ratios (mg/mg) of random urine from normal children aged 0-1 year has a value of 0.13 ± 0.11 (95% range, n = 11).

RESULTS

Table I is a list of some of the patients' amino N/creatinine values demonstrating the wide discrepancies between multiple samples of the same patient's urine samples that were sent on the same day. The values of 11 controls were also included in the table. The wide dispersion of the values of patients N.L. and G.S. illustrates the difficulty of making a diagnosis based on a comparison with controls.

Table II shows that the 24 hour urinary excretion of amino N and creatinine of the three subjects are within the observed normal range. In this connec-

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TABLE II

NORMAL 24 HOUR AMINO NITROGEN AND CREATININE EXCRETION IN CHILDREN AND ADULTS. ALSO INCLUDED IN THE TABLE ARE THE VALUES OF THE THREE SUBJECTS THAT WERE SELECTED IN THIS STUDY

Age groups	Urinary amino N (mg/24 h)			Urinary creatinine (mg/24 h)		
	n	mean	observed range	n	mean	observed range
2-5 years	9	23	3—59	—	—	—
adult (♀)	11	81	53—150	11	852	659—1,289
adult (♂)	28	102	49—161	19	1,432	844—1,950
3 year old subject		28			256	
adult (♀) subject		73			1,062	
adult (♂) subject		74			1,594	

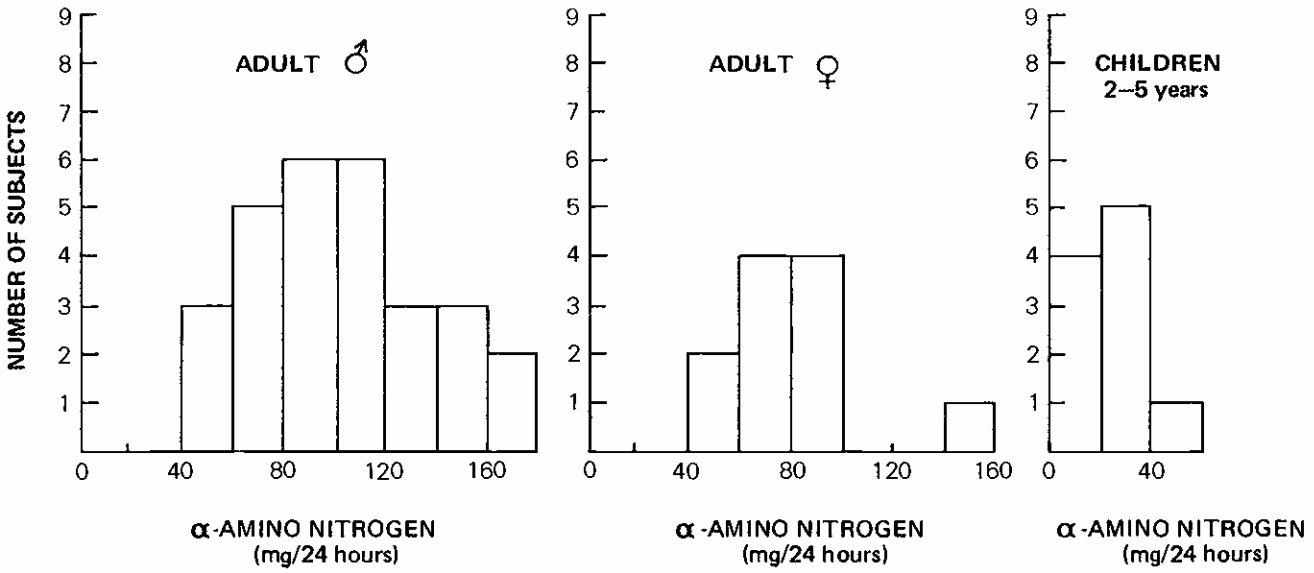

Fig. 1. Frequency distribution of α -amino nitrogen excretion per 24 hours of three groups of normal subjects, 28 male adults, 11 female adults and 9 children aged 2-5 years.

tion, the frequency distributions of urinary amino N excretion in 3 groups of normal subjects are also shown (Fig. 1).

Figs. 2-4 depict the percent deviations about the mean of daily excretion rate of amino N and creatinine in the 3 selected subjects. Changes in amino N/creatinine ratios are expressed as percent of the mean 24 hour value.

In all the subjects, the diurnal variations of a relatively wide amplitude were found for amino N excretion. Peak excretion took place during the afternoon or evening. Low values were consistently recorded during sleep in the early morning hours. However, very low values were also recorded in the day, incompatible with the hypothesis of a simple circadian rhythm. This is especially evident in the case of the child whose lowest value was immediately followed by the highest, during the period from 1530 hours to 1940 hours (Fig. 4).

Variations in creatinine excretion over the same 24 hours were relatively smaller. Nevertheless, it is important to note that changes in creatinine excretion did not parallel that of amino N excretion to the same degree and 2 rhythms of excretion were sometimes found to be out of phase. Consequently, amino N/creatinine values show a wide diurnal variation and seldom approximate to the mean 24 hour value. Moreover, the percent deviations from the mean 24 hour value even exceeded that of amino N determinations on occasions.

Within the limits of one dimension thin-layer chromatography, it was possible to observe that (i) excretion rates of amino acids were not constant throughout the 24 hour period (ii) peak cystine ex-

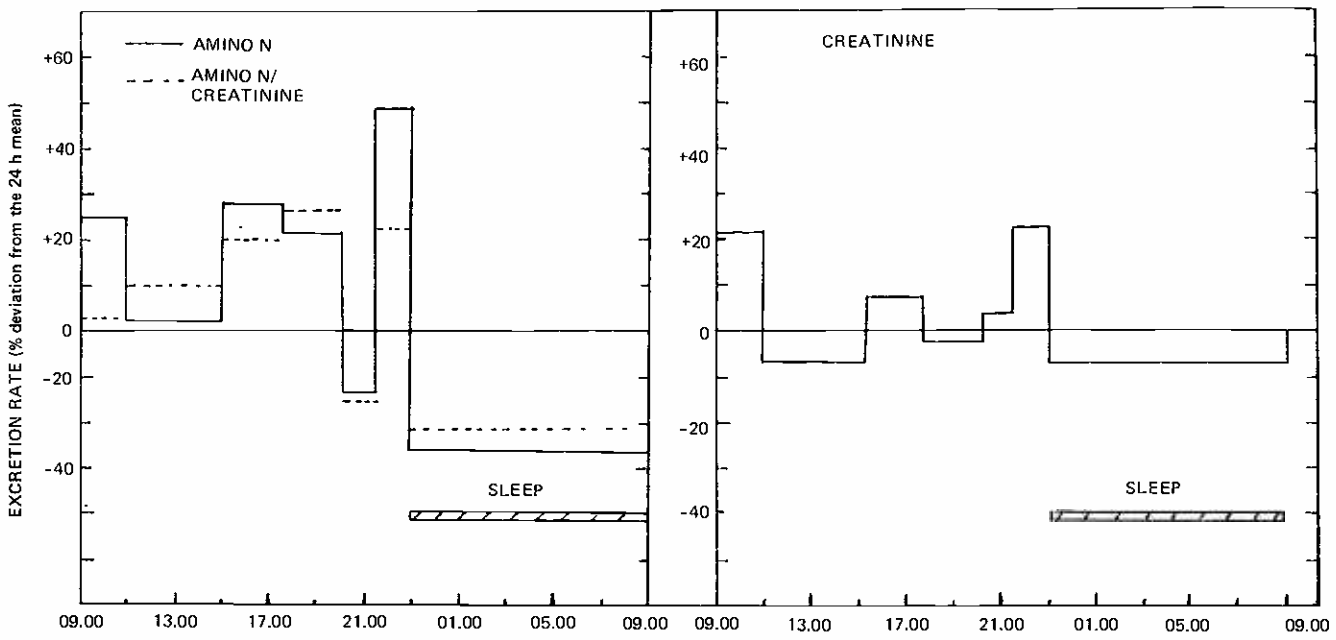

Fig. 2. Mean urinary excretion rate of amino N and creatinine in a male adult expressed as percent deviations from the mean 24 hour value. Broken lines indicate amino N/creatinine ratios.

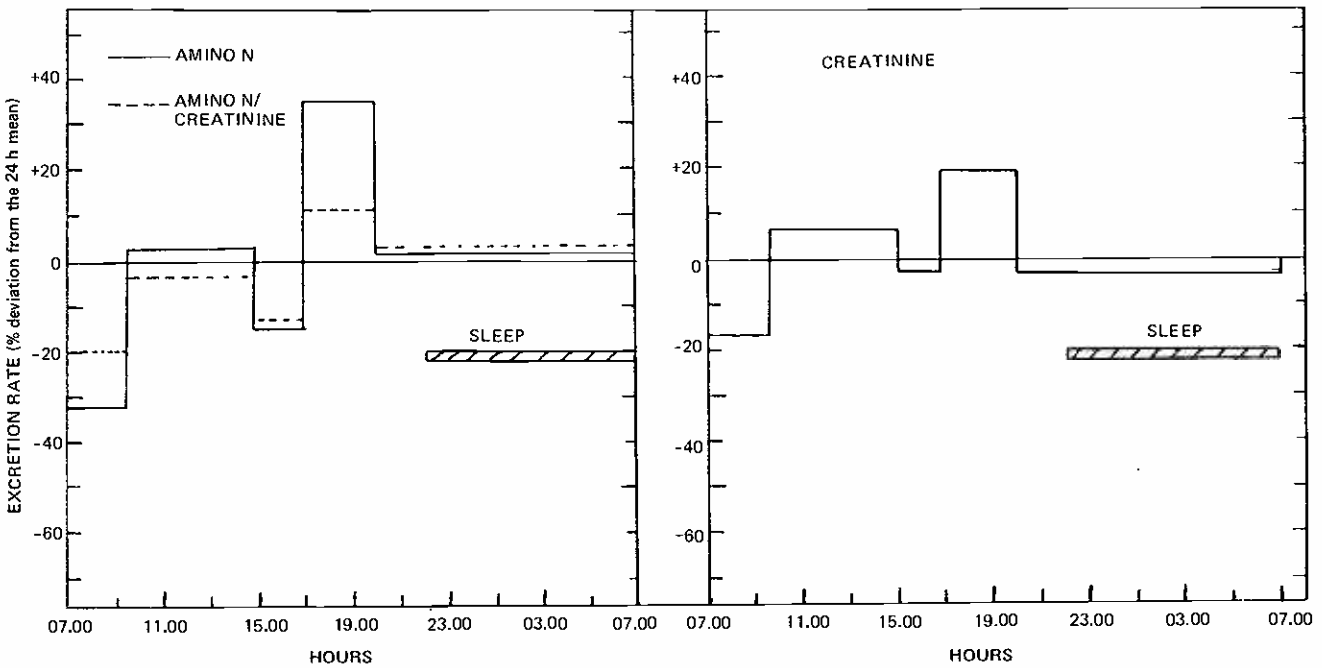

Fig. 3. Mean urinary excretion rate of amino N and creatinine in a female adult expressed as percent deviations from the mean 24 hour value. Broken lines indicate amino N/creatinine ratios.

cretion occurred in the afternoon in the adult male. Fig. 5 is a chromatogram showing the pattern of excretion rates of amino acids of the male adult over a period of 24 hours.

DISCUSSION

The results of this study show that α-amino N/creatinine ratio of random urine does not accurately reflect the 24 hour value. Actually, it introduces more errors to the inherent variations of amino N/creatinine ratios of 24 hour urine. The latter have been reported to have a coefficient of variation of 28% for adults and 23% for 3-5 year old children[6]. In this study, random urine specimens of the selected subjects were found to have deviations from the mean 24 hour value in amino N/creatinine ratios of up to 26%, 19% and 49% for the male and female adults and child respectively.

In fact, it is now generally agreed that the use of amino N/creatinine ratios, even of 24 hour urine, can compound the errors already existing in the determination of amino N excretion. This is because the amount of creatinine formed and excreted appears to be the result of various factors such as muscle mass, dietary intake, hormonal balance, mental state, physical activity and the fluid and ionic balance in the tissues[1, 5]. It is therefore more advisable to avoid expressing amino N excretion as creatinine ratios altogether.

Another objection to the assay of random urine

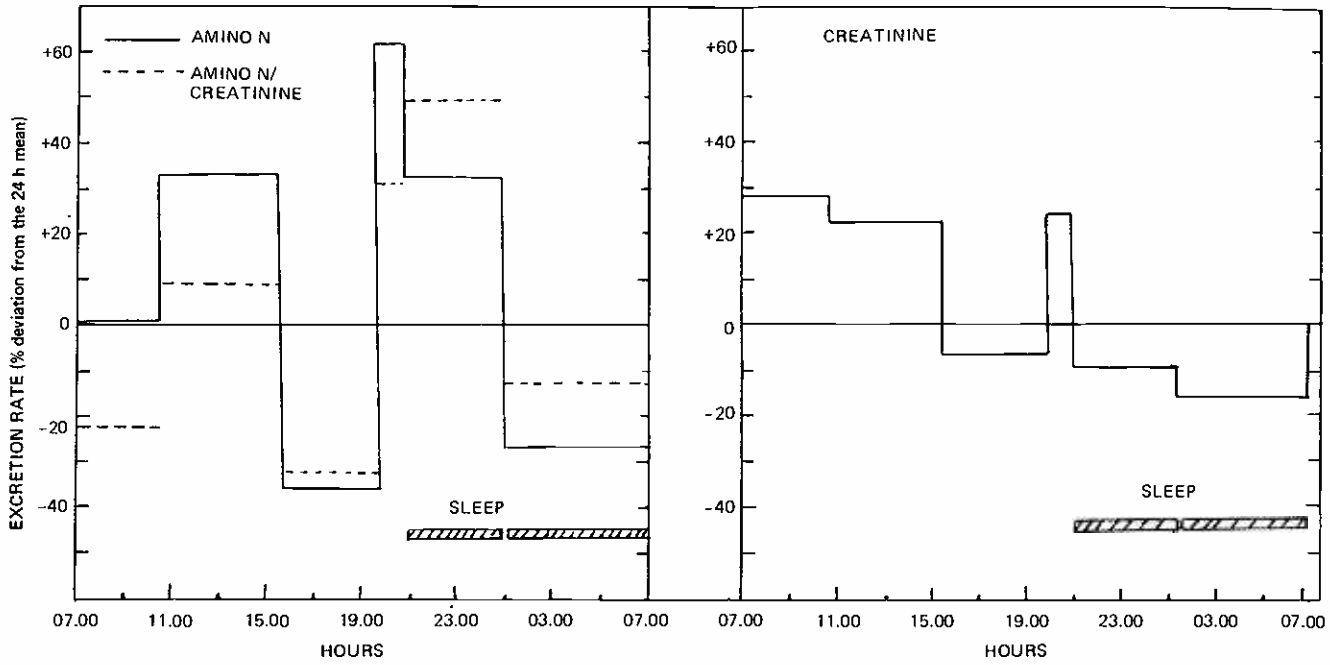

Fig. 4. Mean urinary excretion rate of amino N and creatinine in a 3 year old child expressed as percent deviations from the mean 24 hour value. Broken lines indicate amino N/creatinine ratios.

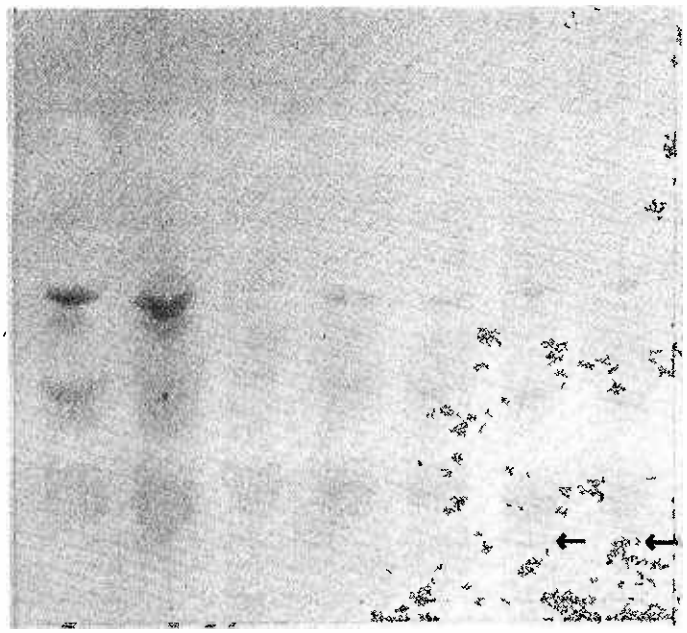

Fig. 5. Thin Layer Chromatogram of amino acids of timed urine specimens of the adult male subject. Aliquots of urine equal to the volume excreted in 0.5 second were chromatographed. Urine specimens numbered 1 to 7 were collected in the following time intervals respectively: 1930-2045 h, 2045-2220 h, 2220-0810 h, 0810-1010 h, 1010-1430 h, 1430-1700 h, 1700-1930 h.
Arrows indicate cystine spots.

is the difficulty of interpreting chromatograms of urinary amino acids. When performing chromatography of urinary amino acids, it has been the common practice to apply a urine aliquot that either contains a fixed amount of creatinine (say 10 ug.) or has been excreted in a fixed unit of time (say 1 second). The results are then compared with that obtained from normal urine which has been similarly aliquotted. It is evident that such a practice for random urine is fraught with errors since the amount of amino acids in the aliquot depends on excretion rate and amino N/creatinine ratios, both of which have been shown to fluctuate widely for part day urine collections. The chromatograms may therefore be quite unrepresentative of a 24 hour urine collection.

If only qualitative information is required, it is preferable to chromatograph an aliquot of urine that contains an amount of amino N known to be within the technical sensitivity of the method of detection. However, generalised aminoaciduria would not be recognised by such a procedure.

A further complication to the problem of assaying random urine is the finding that some amino acids are not excreted at the same rate throughout the day[3, 4]. The male adult, who was selected in this study, appeared to excrete greater quantities of cystine in the afternoon and at night; explanations for this observation requires further work. Suffice it to say that in the context of aminoacidpathies, the assay of random urine could lead to failure to detect the amino acids in question if they were excreted intermittently throughout the day.

In the light of this work, it is essential to carry out a 24 hour collection of urine if a precise evaluation of amino acid excretion is desired.

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