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THE EXCRETION OF FREE α-AMINO ACIDS IN CHILDREN

BY

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The determination of the total content of free α-amino acids in urine has become routine in paediatric practice in numerous diseases. Interest in aminoaciduria has greatly increased since it became known that many congenital metabolic disorders are accompanied by an increased excretion of free α-amino acids in the urine (Paine, 1960; Woolf, 1961).

The excretion of free α-amino-N is usually expressed quantitatively in one of the following ways: (a) mg. α-amino-N excreted per 24 hours (Huisman, 1957; Ghadimi and Shwachman, 1960); (b) mg. α-amino-N per kg. body weight per 24 hours (Huisman, 1957; Childs, 1952; Berger, 1959; Ghadimi and Shwachman, 1960); (c) mg. α-amino-N as percentage of the total nitrogen excretion in mg. per 24 hours (Huisman, 1957; Berger, 1959; Ghadimi and Shwachman, 1960); and (d) mg. α-amino-N in relation to the creatinine excretion in mg. per 24 hours (Ghadimi and Shwachman, 1960; Fowler, Norton, Cheung, and Pratt, 1957).

The absolute 24-hour excretion of amino acids is strongly dependent on age, making this way of expression inapplicable for comparison of aminoaciduria in children of various ages. The other three methods are all in use.

We have made a comparative investigation of the various ways in which aminoaciduria is expressed, because so little seems to have been published on this subject.

Material and Methods

The excretion of α-amino acids in the urine was determined in the following groups of persons.

Group I: 29 normal children on a free diet. Aminoaciduria was determined in 24-hour urine samples and expressed as mg. α-amino-N/kg./24 hr. and $\frac{\text{α-amino-N}}{\text{total N}} \times 100$.

Group II: 13 normal adults on a free diet. The same determinations were done, and because they had not been weighed accurately, aminoaciduria was expressed as $\frac{\text{α-amino-N}}{\text{total N}} \times 100$ only.

Group III: 47 children with various diseases, including diseases with hyperaminoaciduria. The α-amino acid excretion was determined in 24-hour urine samples and expressed as α-amino-N/kg./24 hr., $\frac{\text{α-amino-N}}{\text{total N}} \times 100$ and $\frac{\text{α-amino-N}}{\text{creatinine}}$.

Group IV: 19 children, including 13 in Group I. Aminoaciduria, expressed as $\frac{\text{α-amino-N}}{\text{total N}} \times 100$, was determined in 24-hour urine samples as well as in morning urine samples voided after awakening.

Group V: 15 children, including 13 in Group III. Aminoaciduria, expressed as $\frac{\text{α-amino-N}}{\text{total N}} \times 100$, was determined on two occasions for each child. The interval between the first and second determination varied from one month to two years. Many of the second determinations were done on morning urine samples.

Children under 2 years of age and patients with known liver or glomerular disorders were excluded from this study.

In Group III aminoaciduria was determined twice in two boys on different occasions. In Group III there were also two girls with vitamin D resistant rickets, and during treatment with high doses of vitamin D, aminoaciduria was determined six and seven times respectively on different occasions. Therefore, the number of 24-hour urine samples in the 47 children of Group III was 60.

Where immediate analysis was not possible all urine samples were stored at −4° C. The determination of free α-amino-N in urine was done according to the titrimetric ninhydrin-CO_2 method (Van Slyke, Dillon, MacFadyen and Hamilton, 1941). The determinations of total nitrogen and of creatinine in urine were carried out according to the micro-Kjeldahl method and the method of de Vries and Daatselaar (Gorter and de Graaff, 1956), respectively.

TABLE 1
AGE DISTRIBUTION OF THE CHILDREN STUDIED

Age (yr.)	Groups I	III	IV	V
2–7	12	11	11	4
8–12	11	21	4	6
13–17	6	15	4	5
Total	29	47	19	15

TABLE 2
α-AMINO-N EXCRETION IN NORMAL CHILDREN

Author	mg. α-amino-N/kg./24 hr.	$\frac{\text{α-amino-N}}{\text{total N}} \times 100$	No. of Children
Childs (1952)	2·6 (1·6–4·4)	———	34
Huisman (1957)	2·45 ± 1·00	1·05 ± 0·30	72
Berger (1959)	3·0 (1·0–5·0)	1·2 (0·4–2·0)	40
This study	2·94 ± 1·00		29
This study		1·22 ± 0·32	42

TABLE 3
COMPARISON OF THREE EXPRESSIONS FOR AMINOACIDURIA (24-HOUR URINE) (FREQUENCY DISTRIBUTION)

$\frac{\text{α-amino-N}}{\text{total N}} \times 100$	mg. α-amino-N/kg./24 hr.				$\frac{\text{α-amino-N}}{\text{creatinine}}$			
	0–2	2–4	4–5	>5	<0·15	0·15–0·24	0·25–0·34	>0·35
<1·3	4	10	1	1	7	8	0	1
1·3–1·7	1	18	2	0	4	16	1	0
1·8–2·2	0	4	6	2	0	3	7	2
2·3	0	2	0	9	0	1	3	7

TABLE 4
COMPARISON OF TWO INDICES FOR (HYPER) AMINOACIDURIA

$\frac{\text{α-amino-N}}{\text{total N}} \times 100$	$\frac{\text{α-amino-N}}{\text{creatinine}}$	
	<0·25	⩾0·25
<1·8	35	2
⩾1·8	4	19

TABLE 5
REPEATED DETERMINATION OF α-AMINO-N EXCRETION IN 15 CHILDREN

Difference between First and Second Determinations $\frac{\text{α-amino-N}}{\text{total N}} \times 100$	No. of Children
0–0·1	9
0·1–0·2	3
0·2–0·3	0
0·3–0·4	1
0·4–0·5	2

Results

The results are summarized in Tables 2 to 5 and Figs. 1 and 2. Table 2 shows the normal values for α-amino-N excretion found in our laboratory, expressed as mg. α-amino-N/kg. body weight and as the index $\frac{\text{α-amino-N}}{\text{total N}} \times 100$. Because the findings in children and adults were similar as to the index $\frac{\text{α-amino-N}}{\text{total N}} \times 100$, they were combined. Index values of 1·8 and higher, approximately above the 97th percentile of the normal distribution, are considered as hyperaminoaciduria.

Table 3 gives a comparison of the three different expressions for aminoaciduria in 60 24-hour urine samples of 47 children (Group III). Age had no appreciable influence on the values.

In all three designations the excretion of α-amino-N is related to a variable. In case of obesity or low body weight, excretion per kg. body weight may deviate from the normal. The relation to total nitrogen excretion may be expected to be less reliable in children on either protein-poor or protein-rich diets. Nevertheless this approach is popular among the published papers in paediatrics. The relation of α-amino-N excretion to creatinine excretion also has its objections, because creatinine excretion is not entirely constant (Vestergaard and Leverett, 1958), and certainly not in children with muscular atrophy as is often the case in mental defectives.

It can be seen from Table 3 that there is good mutual correlation, though the upper limit of normal for aminoaciduria expressed as mg./kg./24 hr. is not clearly defined and the normal range of this measure for aminoaciduria (Table 2) is somewhat greater than for the index $\frac{\text{α-amino-N}}{\text{total N}} \times 100$.

If the two indices are compared (Table 4), it is seen that of 37 children with a normal ratio $\frac{\text{α-amino-N}}{\text{total N}} \times 100$ there are 35 (95%) with a ratio $\frac{\text{α-amino-N}}{\text{creatinine}}$ of $< 0{\cdot}25$. Of the 23 children with a increased ratio $\frac{\text{α-amino-N}}{\text{total N}} \times 100$ index (1·8), 19 also have an increased $\frac{\text{α-amino-N}}{\text{creatinine}}$ index. Thus, the normal upper limit for the $\frac{\text{α-amino-N}}{\text{creatinine}}$ ratio, according to this study, can be placed at about 0·25. Ghadimi and Shwachman give 0·11 as normal average, with a standard deviation of 0·064. Therefore, the upper limit of the normal according to their data is also 0·25.

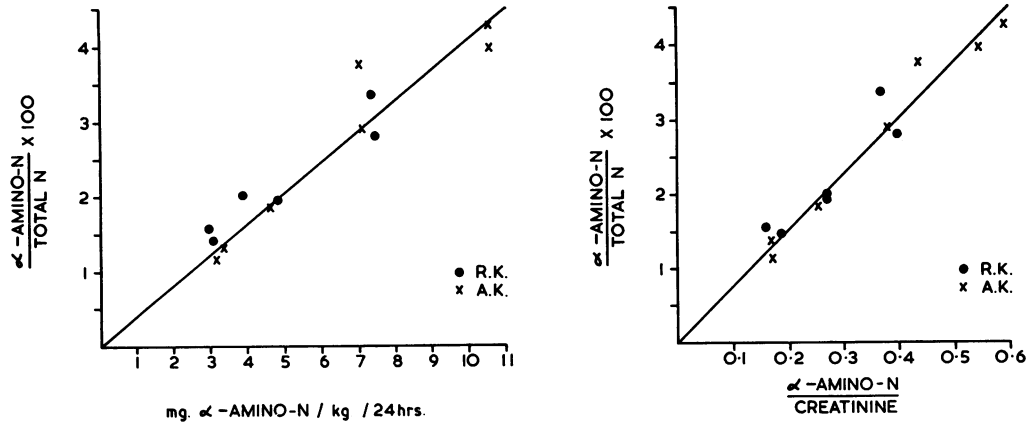

FIG. 1.—Hyperaminoaciduria in two sisters with resistant rickets during treatment.

The opinion, held by Ghadimi and Shwachman, that these indices are insufficiently mutually correlated and of limited applicability is based on study of a large group of patients, none of whom, however, showed hyperaminoaciduria. Their conclusion is, therefore, not valid for deciding for or against the presence of hyperaminoaciduria.

Fig. 1 shows the relation between the two indices for aminoaciduria in two sisters with vitamin D resistant rickets which showed gradual improvement during therapy with high doses of vitamin D. The correlation holds good also in severe hyperaminoaciduria.

The index $\frac{\alpha\text{-amino-N}}{\text{total N}} \times 100$ has been used most frequently in paediatrics. It is also the index which has provided normal values with good mutual agreement (Table 1). Since, in addition, from the age of 1 year on, neither age nor sex influences this index (Huisman, 1957; Berger, 1959), we think there is no reason to abandon this designation. Berger found a slight rise during puberty, but this was not confirmed by our data. Only in the case of very unusual diets and, of course, in severe functional disorders of the glomeruli, are abnormal values for this index obtained depending on abnormal urea excretion. This index, however, has the great advantage that in cases where no 24-hour urine is obtainable, reliable values are obtained with morning samples of urine, as can be seen from Fig. 2 and from Jagenburg's (1959) study. In 19 children (Group IV) morning samples of urine gave values that lay on the average 0·12 lower than in 24-hour urine samples, with the very small standard deviation of 0·02.

Within wide limits, the index $\frac{\alpha\text{-amino-N}}{\text{total N}} \times 100$ varies only slightly with diet (Berger, 1959). This means that the reproducibility is good (Table 5). In this table, repeated determinations are compared in 15 children (Group V) on a free diet; the interval between the first and second determination varied from one month to two years. The mean of the first determination in these 15 children (including patients with hyperaminoaciduria) was 1·66; and the mean of the second determination was 1·71. The differences between the first and second determination were not significant (Wilcoxon pairs test) and the reproducibility thus appears very good.

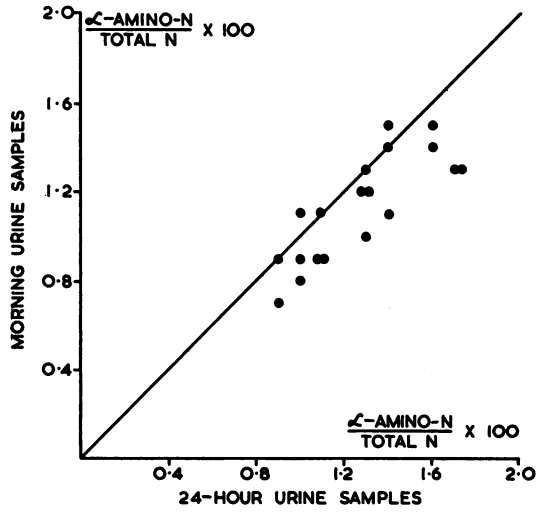

FIG. 2.—Correlation between values for aminoaciduria in 24-hour urine samples and early morning samples.

Summary

The three ways of expressing aminoaciduria in common use have been mutually compared in 60 urine samples from 49 children with normal and with increased aminoaciduria. The index $\frac{\alpha\text{-amino-N}}{\text{total N}} \times 100$ has proved itself equal to other measurements. Reproducibility on a free diet is good. Of special advantage is the fact that when using this way of expressing aminoaciduria a 24-hour urine sample is not necessary.

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