Variation in Excretion of Certain Amino Acids With Age

R. Stambaugh, D. T. Davidson, Jr., and J. R. Elkinton

By relating amino acid excretion to creatinine excretion in a specimen of urine from fasting patients, the normal variation of cystine, glutamine, glycine, and alanine excretion in relation to creatinine excretion and the age of the individual has been defined. No amino acid excretion differences between the two sexes were detectable. In general, the excretion levels for these four amino acids were found to be highest in infancy and decreased markedly with age. However, this decrease is not a uniform or gradual one. The greatest decrease occurs between the 3-5-year and 6-8-year groups, and the excretion levels again increase slightly between the 9-11-year and 12-14-year groups.

The assessment of pathologic patterns of excretion of several amino acids requires an extensive quantitative knowledge of the normal patterns in human subjects without known metabolic disease. To date, this knowledge is inadequate both as to total amounts of each amino acid excreted per unit time and as to variation with sex, age, and size.

In 1949 Herman et al. (1) measured many of the amino acids in specimens of urine collected over periods of 24 hr. Because of the obvious difficulty in obtaining such accurate collections, other investigators have attempted to relate the excretion rates of amino acids to that of total nitrogen (2-4). Total nitrogen excretion varies greatly, and amino acid excretion but slightly, with daily variation in protein intake (4, 5); exceptions to this are histidine and 1-methylhistidine (4). For these reasons the excretion rate of creatinine, which is more closely related to the size of the total metabolizing lean body mass (6),

From the Laboratory of Research Biochemistry, Elwyn School, Elwyn, Pa., and the Department of Pediatrics and the Chemical Section of the Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pa.

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would appear to be a better reference point for quantitation of the rate of excretion of the several amino acids. This was done in 1957 by Fowler et al. (4), who concluded that no significant changes took place after the age of 10. However, more data were needed to define the variation of amino acid excretion with age and sex.

In this investigation we have attempted to define more accurately the relationships of rates of excretion of four of the major amino acids per unit of creatinine excreted to age and sex. Enough human subjects were used to determine the distribution of normal values in a series of age groups up to 30 years of age and in both sexes. In general, the excretion levels for these four amino acids were found to be the same in both sexes and to be highest in infancy and to decrease markedly with age.

**Experimental Material and Methods**

**Subjects**

The experimental subjects were 120 healthy males and 40 healthy females, the majority of whom were mentally retarded students of the Elwyn School between infancy and 30 years of age; no clinical or biochemical evidence was present of any of the known inborn errors of metabolism.

**Collection and Preparation of Specimens**

Fasting urine specimens were collected in the morning before breakfast and before any appreciable amount of physical activity. The specimens were preserved for analysis by refrigeration and by a layer of toluene on the surface of the specimen. Amino acid analyses were carried out within 7 days after collection. Ten-milliliter samples were centrifuged to remove the sediment. Six milliliters of the supernatant solution was transferred to a RSCo Model A-1930 electric desalting apparatus. Sufficient voltage was applied to give an initial current of 0.6 amp. When the current decreased to 0.5 amp, the specimen was pipetted off and recentrifuged. The supernatant solution was used for the creatinine and amino acid analyses. The creatinine content of the desalted specimen was determined photometrically by its color reaction with picric acid in alkaline solution (7, 8).

**Chromatography of the Amino Acids**

A specimen of the desalted fasting urine of a volume to contain 65–130 μg. of creatinine was applied to Whatman No. 1 chromatography
paper (18⅓ × 22½ in.) for two-dimensional paper chromatography. The air-dried spot was then wet once with 30% hydrogen peroxide, which converts the cystine to cysteic acid and the methionine to methionine sulfoxide. When the spot had dried the amino acids were separated by the method of Levy and Chung (9), using n-butanol:acetic acid:H₂O (4:1:5, v/v/v) and m-cresol:phenol:borate buffer, 0.114 M, pH 9.3 (25:25:7, w/w/v), for development of the two-dimensional paper chromatogram. The 0.114 M borate buffer represents a modification of their original method, which used a 0.057 M borate buffer.

Quantitation of the Amino Acids on the Paper Chromatogram

The dried papers were developed by dipping in a modified ninhydrin solvent consisting of 200 mg of ninhydrin in acetone:acetic acid:collidine (100:10:1, v/v/v) and heating in an oven for one hour at 60°. The spots were immediately cut out and eluted for 1½ hr. at room temperature with 4.0 ml. of a solution containing 0.5% Cu(NO₃)₂:10% nitric acid:95% ethanol (5:1:14, v/v/v).

At the end of 1½ hr., the solutions were filtered into 1.0-cm. cuvettes, and the absorbance of the solution was determined at 515 mµ in a Beckman DU spectrophotometer using a reagent blank.

The concentration of each amino acid was calculated from the absorbance and from a standard curve for each amino acid. These standard curves were prepared by chromatographing known quantities of each amino acid with the same techniques that were used for the urine specimens. Repeated determinations of known quantities of these four amino acids demonstrated an average error of ± 7.3% for alanine, ± 6.2% for glutamine, ± 10.3% for glycine, and ± 10.2% for cystine.

Results

The results are presented in Table 1 and in Fig. 1-4.

The excretion of all four amino acids relative to that of creatinine fell with increasing age. For each amino acid the greatest decrement occurred between the 3-5-year group and the 6-8-year group (P < 0.01), and there was a slight rise or leveling off between the 9-11-year and the 12-14-year groups. The widest variations occurred with glycine.

Our results for glycine and alanine excretion agree well with the few values obtained by Fowler et al. (4). Our values for cystine excretion appear to be slightly higher than theirs, and they did not determine the level of glutamine excretion in their patients.
Table 1. Rates of Excretion of Glycine, Alanine, Cystine, and Glutamine

<table>
<thead>
<tr>
<th>Age group (yr.)</th>
<th>Glycine</th>
<th>Alanine</th>
<th>Cystine</th>
<th>Glutamine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± s.d.</td>
<td>s.d.</td>
<td>n</td>
<td>Mean ± s.d.</td>
</tr>
<tr>
<td>0-2</td>
<td>2,597 ± 807</td>
<td>9</td>
<td>829 ± 296</td>
<td>9</td>
</tr>
<tr>
<td>3-5</td>
<td>2,436 ± 843</td>
<td>13</td>
<td>886 ± 352</td>
<td>13</td>
</tr>
<tr>
<td>6-8</td>
<td>1,251 ± 737</td>
<td>11</td>
<td>492 ± 151</td>
<td>10</td>
</tr>
<tr>
<td>9-11</td>
<td>993 ± 688</td>
<td>33</td>
<td>450 ± 178</td>
<td>31</td>
</tr>
<tr>
<td>12-14</td>
<td>1,084 ± 518</td>
<td>40</td>
<td>478 ± 140</td>
<td>37</td>
</tr>
<tr>
<td>15-19</td>
<td>1,147 ± 654</td>
<td>42</td>
<td>—</td>
<td>145 ± 69</td>
</tr>
<tr>
<td>20-29</td>
<td>1,119 ± 597</td>
<td>11</td>
<td>—</td>
<td>133 ± 37</td>
</tr>
</tbody>
</table>

*Standard deviation.
†Number of subjects.
‡Not amenable to statistical analysis since the concentration in the specimens occasionally fell below the minimum detectable level of 130 μM/gm. creatinine.

There appeared to be no difference in the excretion levels of these amino acids between the two sexes.

Discussion

The excessive excretion of amino acids in the urine is a manifestation of aberrant cellular function. It may result from deranged intermediary amino acid metabolism in various organs and from impaired renal tubular reabsorption. For the clinician, patterns of urinary amino acid excretion may provide essential clues in diagnosis, and in genetic studies the excretion patterns provide valuable information for the elucidation of metabolic defects occurring in obscure syndromes. Our interest is primarily in the latter area, which includes inborn errors of metabolism, especially those associated with mental retardation.

However, the normal ranges of amino acid excretion have never been accurately defined, and one is faced with the problem of identifying pathologic excretion patterns without a sufficient knowledge of the normal values. This is especially true when one tries to evaluate the excretion levels of the individual amino acids—that is, a "specific" aminoaciduria. In this study we have accumulated sufficient data for determining the range of normal variation of amino acid excretion in different age groups for four of the amino acids. Except for cystine, our results are in good agreement with the data of Fowler et al. (4). Our cystine-to-creatinine ratios, shown in Fig. 3 and Table 1, appear to
be slightly higher than those reported by Fowler et al. (4). This difference might be explained by the fact that they used 24-hr. urine specimens for analysis, while we used the morning specimen collected before breakfast. However, it seems more likely that their values are too

Fig. 1. Micromoles of glycine excreted per gram of creatinine. Dots represent males, and circles, females. Values reported by Fowler et al. (4) are represented by crosses.

Fig. 2. Micromoles of alanine excreted per gram of creatinine. Symbols are as in Fig. 1. Dotted line represents minimum detectable concentration of alanine by these technics.
low due to a partial loss from auto-oxidation to cysteic acid during the isolation procedure. Prior conversion of cystine to cysteic acid with hydrogen peroxide eliminates the loss due to auto-oxidation.

The importance of accurately relating the amino acid excretion to age, in addition to creatinine excretion, is evident in the excretion of cystine by an 18-year-old cystinuric, as shown in Fig. 3. If this value were found for a one-year-old child, there would be considerable doubt that the child had cystinuria; for an 18-year-old girl the abnormality is unequivocal.

The explanation for the sharp statistical decrease between the 3–5 and 6–8-year age groups, followed by a secondary slight rise in excre-

![Fig. 3. Micromoles of cystine excreted per gram of creatinine. Symbols are as in Fig. 1. Cystine excretion level of an 18-year-old female cystinuric is recorded for comparison with normal values.](image)

![Fig. 4. Micromoles of glutamine excreted per gram of creatinine. Symbols are as in Fig. 1.](image)
tion in the 12–14-year age group remains obscure. Although the data of Fowler et al. (4) were inadequate to detect such a decrease in these four amino acids, it is of interest that they observed a decreased excretion of taurine between infancy and the age of 10 years. It is well known that the skeletal musculature grows more rapidly than the body as a whole between the ages of 5 and 10 (10), and one might speculate that the decreased amino acid excretion levels result from increased utilization for muscle growth.

Whatever the explanation for the variation of the excretion rates of glycine, alanine, cystine, and glutamine, relative to creatinine, our data do provide in each instance and for each age group a "normal" population of values with which any single determination in an unknown patient may be compared. This should be of great diagnostic and research use in the assessment of these particular amino acids in "overflow" or renal aminoacidurias (5, 11).

References

7. Folin, O., J. Biol. Chem. 17, 469 (1914).