QUANTITATIVE STUDIES ON HUMAN URINARY METABOLITES OF D-, DL-, ACETYL-L-, AND ACETYL-D-TRYPTOPHAN

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The metabolism of D-tryptophan in man was recently studied by Langner and Berg (1), who found that about one-third to one-half of a 2 gm. dose was excreted unchanged in the urine. Considerable D-kynurenine and traces of indolepyruvic acid and acetyltryptophan were also excreted by their subjects. Langner and Volkmann (2) found that acetyl-D-tryptophan was poorly absorbed and most of the compound was accounted for in the feces and urine. Only 5 to 10 per cent of acetyl-L-tryptophan was found in the stool, and a small amount was excreted in the urine. Rose, Coon, Lambert, and Howe (3) have found that acetyl-L-tryptophan was essentially as good as L-tryptophan in the maintenance of nitrogen balance in human subjects who were deprived of tryptophan. Other studies in this field have been cited in these recent publications (1-3).

Methods for the quantitative determination of several metabolites of tryptophan have recently been developed and applied in studies on the urinary metabolites of L-tryptophan in man and other species (4, 5). These determinations included kynurenine, N°-acetylkynurenine, o-aminohippuric acid, anthranilic acid, anthranilic acid glucuronide, kynurenic acid, N-methyl-2-pyridone-5-carboxamide (pyridone), and some related compounds. Similar studies have now been carried out with the urinary metabolites of D-, DL-, acetyl-L-, and acetyl-D-tryptophan in man.

Ingestion of acetyl-D-tryptophan failed to alter the urinary excretion of the metabolites studied. The total yield of metabolites from acetyl-L-tryptophan and D-tryptophan was about 50 per cent less and 50 per cent more, respectively, than the yield of metabolites from a comparable dose of L-tryptophan. The yield of metabolites from 19.6 mmoles of DL-tryptophan was close to that expected from the same amount of the amino acid administered as separate doses of the D and L isomers. This suggested that

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simultaneous metabolism of this dose of D- and L-tryptophan had little, if any, effect on the metabolism of either isomer.

**Materials and Methods**

The D-tryptophan used in these experiments had an optical rotation of $[\alpha]_D^{25} +30.5^\circ$ ($c = 0.5$ gm. per 100 ml. of solution in water). The acetyl-L-tryptophan was prepared from L-tryptophan ($[\alpha]_D^{25} -31.2^\circ$) by the method of du Vigneaud and Sealock (6), and the DL-tryptophan was supplied by Merck and Company.¹

The four subjects used were normal male laboratory personnel, three of whom were utilized in previous studies on L-tryptophan (5). The supplements were administered as a single oral dose suspended in water. The urine collections, analytical methods, and other experimental details have been described (4, 5).

Paper chromatograms were carried out on the effluents from the Dowex 50 columns which were used to separate the aromatic amines. Aliquots of the fractions (15 ml.) were concentrated to dryness in a vacuum desiccator over CaCl$_2$ and NaOH. The residue was dissolved in a few drops of 50 per cent alcohol and spotted on Whatman No. 1 filter paper. After being exposed to ammonia, the chromatograms were developed as described by Mason and Berg (7), except that the solvent contained 1 ml. of glacial acetic acid per 100 ml. (5). The papers were routinely sprayed with diazotized sulfanilic acid and Ekman’s reagent for diazotizable aromatic amines by the procedures described by Dalgliesh (8).

**Results**

The administration of acetyl-D-tryptophan failed to alter the urinary excretion of any of the metabolites studied so that the data were not included in Table I.

Ingestion of D-tryptophan resulted in a large increase in kynurenine excretion, with smaller increases in urinary kynurenic acid, N$\alpha$-acetyllykynurenine, xanthurenic acid, and pyridone (Table I). There was a negligible change in the excretion of anthranilic acid glucuronide, o-aminohippuric acid, and aromatic amine Fraction A after administration of D-tryptophan. Paper chromatograms of the aromatic amine fractions, developed under conditions which resolved DL-kynurenine (8, 9), indicated that most of the urinary kynurenine after ingestion of D-tryptophan was identical in $R_P$ value with synthetic D-kynurenine. Ingestion of D-tryptophan failed to affect the intensities of the spots corresponding to L-kynurenine or N$\alpha$-acetyllykynurenine, and no anthranilic acid was detected on the chromatograms.

¹ Generously supplied to us by the Medical Division of Merck and Company, Inc., Rahway, New Jersey.
grams either before or after supplementation with the unnatural amino acid. Furthermore, the determinations of kynurenine as steam-volatile diazotizable amine in the kynurenine fractions (5) were in good agreement with the determinations based on direct diazotization of the kynurenine fractions, while the yields of volatile amine from the acetylkynurenine fractions were too low to account for the increases in aromatic amine found in

**Table I**

**Urinary Excretion of Metabolites before and after Administration of D-, DL-, and Acetyl-L-tryptophan to Human Subjects**

The data have been expressed as micromoles excreted per 24 hours and have been corrected for recoveries of added standards. Four subjects were used for each supplement and the average results have been presented. The supplement was administered upon completion of the 2nd day of urine collection.

<table>
<thead>
<tr>
<th>Supplement</th>
<th>Dose, nmol</th>
<th>Day No.</th>
<th>AAG†</th>
<th>oAH</th>
<th>AcK</th>
<th>K</th>
<th>KA</th>
<th>XA</th>
<th>MPCA</th>
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<td>28</td>
<td>14</td>
<td>14</td>
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<td>124</td>
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<td>2</td>
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<td>6</td>
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<td>14</td>
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<td>87</td>
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<td>Acetyl-L-tryptophan</td>
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<td>5</td>
<td>26</td>
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<td>15</td>
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<td>28</td>
<td>16</td>
<td>28</td>
<td>17</td>
<td>71</td>
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</table>

* The following abbreviations are used: A, aromatic amine Fraction A; AAG, anthranilic acid glucuronide; oAH, o-aminohippuric acid; AcK, acetylkynurenine; K, kynurenine; KA, kynurenic acid; XA, xanthurenic acid; MPCA, N-methyl-2-pyridone-5-carboxamide.
† Calculated as anthranilic acid.

these fractions on direct diazotization. Since the paper chromatograms indicated the presence of large amounts of tryptophan in both the kynurenine and acetylkynurenine fractions, tryptophan was suspected as the main source of the increases in the diazotizable amine in the acetylkynurenine fractions. The addition of 1.0 gm. of D-tryptophan to normal 24 hour basal urines resulted in increases in the diazotizable aromatic amine in the acetylkynurenine fractions of magnitudes similar to those observed after the ingestion of 2.0 gm. of D-tryptophan. The added D-tryptophan

increased the diazotizable amine in the kynurenine fractions to an extent which would account for less than 10 per cent of the increase in diazotizable aromatic amine in the kynurenine fractions after ingestion of the supple-

**Table II**

*Comparison of Increased Urinary Excretion of Metabolites Following Administration of Various Tryptophan Supplements*

The data were calculated by subtracting the average basal excretion of the metabolite from the values obtained for the first 2 days after supplementation. Column 6 (the sum of Columns 1 and 2) may be regarded as the "expected yield" of metabolites from 19.6 mmoles of DL-tryptophan, while Column 5 represents the "observed yield." The data for the 9.8 and 19.6 m mole doses of L-tryptophan were taken from Brown and Price (5), but the calculations included 2 days before and 2 days after supplementation.

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>μmoles increase of urinary metabolites after administration of</th>
<th>Sum of Columns 1 and 2</th>
</tr>
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<tr>
<td></td>
<td>9.8 mmoles</td>
<td>19.6 mmoles</td>
</tr>
<tr>
<td>Amine Fraction A</td>
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<td>-4</td>
</tr>
<tr>
<td>Anthranilic acid glucuronide</td>
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</tr>
<tr>
<td>o-Aminohippuric acid</td>
<td>7</td>
<td>23</td>
</tr>
<tr>
<td>Acetylkynurenine</td>
<td>19*</td>
<td>4</td>
</tr>
<tr>
<td>Kynurenic acid</td>
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</tr>
<tr>
<td>Kynurenic acid</td>
<td>14</td>
<td>50</td>
</tr>
<tr>
<td>Xanthurenic acid</td>
<td>45</td>
<td>11</td>
</tr>
<tr>
<td>Pyridone</td>
<td>70</td>
<td>103</td>
</tr>
<tr>
<td>Total†</td>
<td>331</td>
<td>212</td>
</tr>
</tbody>
</table>

* The presence of large amounts of D-tryptophan in these fractions interfered with the determination of acetylkynurenine which resulted in high readings. Paper chromatograms of these fractions indicated that D-tryptophan did not give rise to acetylkynurenine.
† Does not include amine Fraction A.

ments of D-tryptophan. Thus the apparent increases in urinary acetylkynurenine after administration of D-tryptophan (Tables I and II) were not attributable to acetylkynurenine (or anthranilic acid), but were probably due to interference in the determinations by the D-tryptophan (1), while the D-tryptophan in the urine had a negligible effect on the determination of the relatively high levels of kynurenine found.

Acetyl-L-tryptophan ingestion was followed by slight increases in urinary
levels of all the metabolites studied (Table I). The largest increases were in the excretion of the pyridone, \( o \)-aminohippuric acid, kynurenic acid, \( N^\alpha \)-acetylkynurenine, and the diazotizable amine in Fraction A. Supplementation with acetyl-L-tryptophan had almost no effect on kynurenine excretion. Paper chromatography of the fractions of these urine samples did not reveal additional information.

The ingestion of 19.6 mmoles of \( \text{DL} \)-tryptophan was followed by the excretion of a pattern of urinary metabolites expected from the data obtained after giving 9.8 mmoles of each isomer separately (Tables I and II). Kynurenine was the chief urinary metabolite of \( \text{DL} \)-tryptophan and paper chromatograms indicated that most of the increase was in \( \text{D} \)-kynurenine. The increase in the excretion of the various metabolites of \( \text{DL} \)-tryptophan resulted in a pattern of metabolites quite different from that observed after administration of 19.6 mmoles of L-tryptophan (Table II).

Paper chromatography of the various aromatic amine fractions revealed only questionable traces of anthranilic acid, and anthranilic acid glucuronide usually was not detectable. The paper chromatograms of Fraction A usually were unsatisfactory, and no clearly defined spots were seen on inspection with ultraviolet light or after spraying the paper with the reagents.

A comparison of the levels of urinary excretion of the various metabolites prior to the administration of each of the three supplements (Table I) indicated that there was little variation in the basal levels of excretion of these metabolites.

**DISCUSSION**

Rose et al. (3) found that acetyl-\( \text{D} \)-tryptophan was not utilized by adult humans. Langner and Volkmann (2) showed that acetyl-\( \text{D} \)-tryptophan was poorly absorbed and most of an oral dose was eliminated unchanged in the urine and stool. Since acetyl-\( \text{D} \)-tryptophan did not alter the urinary excretion of any of the metabolites determined in the present studies, it would appear that it is metabolically inert in man.

Acetyl-\( \text{L} \)-tryptophan was found by Rose et al. (3) to be as good as the free amino acid for maintenance of nitrogen balance on a diet deficient in tryptophan, and Langner and Volkmann (2) found only 5 to 10 per cent of an oral dose of 10.0 mmoles of acetyl-\( \text{L} \)-tryptophan in the stool. The present studies indicated that acetyl-\( \text{L} \)-tryptophan was partially converted to nicotinic acid, but to a lesser extent than an equivalent dose of free \( \text{L} \)-tryptophan. The fact that acetyl-\( \text{L} \)-tryptophan evidently was largely absorbed, but yielded low levels of most of the metabolites studied, supports the hypothesis of Langner and Volkmann (2) that there may be an enzymatic mechanism for the absorption of the acetylated amino acid. If the amino acid was deacetylated prior to or during absorption, one would expect it to
be metabolized like the free amino acid. The fact that acetyl-L-tryptophan was the only supplement studied which yielded more acetyl-kynurenine than kynurenine (Table II) may be regarded as further support for the hypothesis of Langner and Volkmann (2). However, as Langner and Volkmann have indicated, other explanations of these observations must still be considered.

Acetyl-L-tryptophan was the only supplement which resulted in a significant increase in the diazotizable aromatic amine in Fraction A (Table II). Attempts to identify the amine in this fraction have been unsuccessful. The other diazotizable amines detectable in human urine have been found to be tryptophan metabolites (5). It now appears possible that acetyl-L-tryptophan may be a precursor of at least part of the aromatic amine in Fraction A.

The quantity of kynurenine excreted after ingestion of 9.8 mmoles of D-tryptophan was enough to account for about 1.8 per cent of the amino acid. This was considerably less kynurenine than Langner and Berg (1) obtained from the same dose of this amino acid. In agreement with them, however, the increase in kynurenine appeared to be the result of the excretion of the D-isomer. The present studies also confirmed their findings that no detectable anthranilic acid was excreted following ingestion of D-tryptophan (1). This amino acid also had a negligible effect upon the excretion of o-aminohippuric acid and anthranilic acid glucuronide.

The addition of o-tryptophan to normal urine in amounts equal to 0.5 to 1.0 gm. per 24 hour sample had no effect on the determination of kynurenic acid. Since the spectrum of the kynurenic acid fraction was comparable to the synthetic compound, and an increase in urinary kynurenic acid was observed after each supplementation with D-tryptophan, it appears that kynurenic acid was a metabolite of the amino acid. Langner and Berg (1) found that after supplementation with D-tryptophan in man there was an increase in urinary indolepyruvic acid. This metabolite was shown to give rise to kynurenic acid in rabbits (10) and rats (11); therefore, it may be a precursor of kynurenine acid in man. The method used for xanthurenic acid determination gave somewhat variable results when low levels of the metabolite were present (5, 12), so that an apparent small increase in the excretion of this metabolite must be considered with some reservation.

It was difficult to account for the increased pyridone excretion after ingestion of D-tryptophan, each subject excreting more pyridone on both days after supplementation with the unnatural amino acid. One of the reasons for studying the urinary metabolites of DL-tryptophan was to attempt to determine whether the D-tryptophan supplement might have altered the
metabolism of dietary tryptophan. It was anticipated that the yield of pyridone might be large, but the apparent yield of pyridone from DL-tryptophan could almost be accounted for by the L-tryptophan content. It may be necessary to use isotopes to determine whether orally administered D-tryptophan is actually converted to nicotinic acid.

Apparently D- and L-tryptophan were metabolized simultaneously by man in the dosage used without significant effects of the metabolites of one isomer on the metabolic fates of metabolites of the other. However, the extreme differences in the patterns of urinary metabolites from the two isomers would make it difficult to interpret data obtained from studies in which DL-tryptophan was used.

SUMMARY

Human subjects were given single oral doses of 9.8 mmoles of D-, acetyl-L- or acetyl-D-tryptophan, and 19.6 mmoles (4 gm.) of DL-tryptophan. Aliquots of the 24 hour urine collections for 2 days before and after supplementation were analyzed quantitatively for N-methyl-2-pyridone-5-carboxamide (pyridone), xanthurenic acid, kynurenic acid, kynurenine, N'-acetyl-kynurenine, o-aminohippuric acid, anthranilic acid glucuronide, and an unidentified “aromatic amine Fraction A.” Kynurenine was the major urinary metabolite of D- or DL-tryptophan. Following ingestion of D-tryptophan there was also increased urinary excretion of kynurenic acid, xanthurenic acid, and pyridone. Acetyl-D-tryptophan failed to alter the excretion of any of the metabolites studied. The total increase of urinary metabolites from acetyl-L-tryptophan was less than half the yield of metabolites from a comparable dose of the free amino acid. Most of the kynurenine excreted upon ingestion of acetyl-L-tryptophan was acetylated. These observations indicate that acetyl-L-tryptophan was converted to nicotinic acid to a lesser extent than was L-tryptophan, and suggest that acetyl-L-tryptophan was absorbed without deacetylation.

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