THE UTILIZATION OF D-AMINO ACIDS BY MAN*

VIII. TRYPtopHAN AND ACETYLTRYPtoPHAN

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In a previous publication (1) we expressed the opinion that, with the exception of a 5 per cent urinary loss, all of the orally administered acetyl-DL-tryptophan may be available to man. Subsequently Luck, Boyer, and Hall (2) found that 70 to 83 per cent of intravenously administered acetyl-DL-tryptophan was excreted unchanged in the urine within 6 hours of the injection. These findings led them to the conclusion that intravenously administered acetyl-L- and acetyl-D-tryptophan are not utilized by man.

The divergent conclusions reached in the two studies may be the result of (a) differences in chemical characteristics of the assay methods employed or (b) differences arising from the routes of administration of the test substances. The wide-spread practical and fundamental implications of the latter possibility prompted us to reinvestigate the fate of orally administered acetyltryptophan by measurements of urinary metabolites in the adult and nitrogen balance and body weight changes in the infant. Our original contention that orally administered acetyl-DL-tryptophan may be completely utilized by man received additional support from the failure to find by the colorimetric methods of Luck and associates any increase of tryptophan values in the urine of subjects fed acetyl-L- or acetyl-DL-tryptophan. Furthermore no trace of acetyltryptophan could be detected by application of their isolation procedures to these specimens directly or to 5-fold concentrates of the urines. Feeding tryptophan-deficient diets supplemented in turn for 4 day periods with L- or DL-tryptophan and acetyl-L- or acetyl-DL-tryptophan to infants disclosed that subnormal growth and N retention were induced in all three subjects only by the diet reinforced with DL-tryptophan. This additional evidence of the utilization of acetyl-L- and acetyl DL-tryptophan indicates beyond a doubt that the metabolic fate of these substances may be drastically affected by the manner of administration.

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Commercially available L- and DL-tryptophan (Merck), containing 13.5 and 13.6 per cent N respectively, were employed in these experiments. The necessary acetyl derivatives of the compounds were obtained in good yields by the method of du Vigneaud and Sealock (3) and the nitrogen content and specific rotation of the products found to be in good accord with the accepted values.

**Excretion Experiments**—As in the previous studies adult subjects were given 0.01 mole (2.46 gm.) of acetyl-L- or acetyl-DL-tryptophan in 240 cc. of water. The urines were collected and pooled for the succeeding 24 hours and analyzed subsequent to adjustment of the pH to 3 with dilute HCl. It is apparent from the typical data shown in Table I that the ingestion of either isomer of acetyltryptophan did not increase the titer of aromatic metabolites of the urines above the control levels as measured by the procedures described by Luck and his associates. This finding and the fact that acetyltryptophan could not be isolated from the urines by the method described by these authors would seem to support the view that orally administered acetyl-L- or acetyl-DL-tryptophan may be utilized by man. The analyses of these urines for free phenols by the method of Marenzi (4) and \( N^1 \)-methylnicotinamide by the procedure of Huff and Perlzweig (5) indicate that neither isomer of acetyltryptophan is converted to these substances. This is of interest in view of the ready metabolic conversion of L-tryptophan to pyridine derivatives reported by others (7).
Nitrogen Retention and Growth Experiments—In a previous report (8) we have shown that the infant can be maintained in normal growth and nitrogen retention on a complete diet which supplies a minimum of 40 mg. of L-tryptophan per kilo of body weight per day. With this information on hand, we felt that the availability of the derivatives for the support of these body functions at normal levels could be determined by supplementing the tryptophan-deficient diet with equivalent amounts of acetyl-L- or acetyl-DL-tryptophan. The biological value of the D component of racemic tryptophan was also tested by this technique.

The observations reported here were made on three normal healthy male infants who were given the synthetic diets in five feedings daily at the rate of about 100 calories per kilo of body weight. They were also given 50 mg. of ascorbic acid and 15 drops of oleum percomorphum daily. The diet periods were of 4 days duration and were consecutive. The subjects were immobilized by the use of abdominal restraints and 24 hour specimens were collected by means of adapters in bottles containing 10 cc. of 15 percent (by volume) HCl and 1 cc. of 10 per cent alcoholic thymol. The feces were collected in 19 cm. porcelain evaporating dishes held in place by especially constructed mattresses and accumulated under refrigeration for each period in jars containing 200 cc. of 70 per cent alcohol. The infants were weighed daily during the course of the experiment.

The composition of the diets employed for the assay is shown in Table II. These were made to contain approximately 100 calories per 100 gm. and have the following percentile caloric distribution: protein 14, fats 36, carbohydrate 50. The protein moiety of the tryptophan deficient diet was prepared by sulfuric acid hydrolysis of casein as previously described by us (9). In order to improve the cystine-poor characteristic of this preparation, the final product was reinforced with 1 per cent L-cystine of the protein content estimated as N \times 6.25. Owing to uncertainties regarding the complete human requirements of B complex vitamins, brewers' yeast was employed instead of a mixture of the synthetically available vitamins. The amount of L-tryptophan derived from this source appears to be approximately 6 mg. per gm. (10). Thus the quantity of L-tryptophan provided by the diets per kilo of body weight can be roughly estimated (Table II). The final nitrogen content of each batch of diet was determined by micro-Kjeldahl analysis.

The nitrogen retention data were obtained from the results of micro-Kjeldahl analyses of the daily 24 hour urine collections, period pools of the feces, and daily N intake as computed from the consumption record and nitrogen content of the diet.

The data thus obtained are collected in Table III and show that only Diet B failed to maintain nitrogen retention and body weight gain at the
### TABLE II
Composition of Diets

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Diet A</th>
<th>Diet B</th>
<th>Diet C</th>
<th>Diet D</th>
<th>Diet E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid-hydrolyzed casein*</td>
<td>3.4</td>
<td>3.4</td>
<td>3.4</td>
<td>3.4</td>
<td>3.4</td>
</tr>
<tr>
<td>L-Tryptophan</td>
<td>0.053</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.034</td>
</tr>
<tr>
<td>DL-Tryptophan</td>
<td>0</td>
<td>0.040</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Acetyl-L-tryptophan</td>
<td>0</td>
<td>0</td>
<td>0.048</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Acetyl-DL-tryptophan</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.048</td>
<td>0</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>0.035</td>
<td>0.035</td>
<td>0.035</td>
<td>0.035</td>
<td>0.035</td>
</tr>
<tr>
<td>Brewers' yeast</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Olive oil</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Dextrin-maltose No. 2†</td>
<td>9.0</td>
<td>9.0</td>
<td>9.0</td>
<td>9.0</td>
<td>9.0</td>
</tr>
<tr>
<td>Arrowroot starch</td>
<td>2.3</td>
<td>2.3</td>
<td>2.3</td>
<td>2.3</td>
<td>2.3</td>
</tr>
<tr>
<td>Salt mixture‡</td>
<td>1.6</td>
<td>1.6</td>
<td>1.6</td>
<td>1.6</td>
<td>1.6</td>
</tr>
<tr>
<td>Water</td>
<td>78.0</td>
<td>78.0</td>
<td>78.0</td>
<td>78.0</td>
<td>78.0</td>
</tr>
<tr>
<td>Total</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Estimated L-tryptophan content, mg...

<table>
<thead>
<tr>
<th></th>
<th>Diet A</th>
<th>Diet B</th>
<th>Diet C</th>
<th>Diet D</th>
<th>Diet E</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>59.0</td>
<td>26.0</td>
<td>26.0</td>
<td>26.0</td>
<td>40.0</td>
</tr>
</tbody>
</table>

* N × 6.25 = gm. of protein.
† Kindly supplied by Mead Johnson and Company.
‡ The salt mixture employed had the following composition (measured in gm.): FeSO₄ 0.9, NaCl 6, calcium gluconate 48, Ca(OH)₂ 12, KH₂PO₄ 7, KCl 6, MgO 0.1.

### TABLE III
Effect of Optical Isomers of Tryptophan and Acetyltryptophan on Nitrogen Retention and Body Weight of Infant

The supplements were administered per kilo of body weight. All results given as daily averages.

<table>
<thead>
<tr>
<th>Initial weight and age of subject</th>
<th>Diet A</th>
<th>Diet B</th>
<th>Diet C</th>
<th>Diet D</th>
<th>Diet E</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. M., c, 7, mos., 5.409 kilos</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N intake, gm.</td>
<td>2.90</td>
<td>2.90</td>
<td>2.94</td>
<td>2.96</td>
<td>2.90</td>
</tr>
<tr>
<td>&quot; retention, mg. per kg.</td>
<td>160</td>
<td>96</td>
<td>132</td>
<td>136</td>
<td>120</td>
</tr>
<tr>
<td>Weight change, gm.</td>
<td>+18</td>
<td>-9</td>
<td>+16</td>
<td>+19</td>
<td>+14</td>
</tr>
<tr>
<td>D. C., c, 13, mos., 7.347 kilos</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N intake, gm.</td>
<td>3.14</td>
<td>3.00</td>
<td>3.12</td>
<td>3.10</td>
<td>3.14</td>
</tr>
<tr>
<td>&quot; retention, mg. per kg.</td>
<td>146</td>
<td>50</td>
<td>164</td>
<td>127</td>
<td>140</td>
</tr>
<tr>
<td>Weight change, gm.</td>
<td>+16</td>
<td>+4</td>
<td>+18</td>
<td>+20</td>
<td>+20</td>
</tr>
<tr>
<td>H. G., c, 10, mos., 7.418 kilos</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N intake, gm.</td>
<td>3.54</td>
<td>3.50</td>
<td>3.48</td>
<td>3.46</td>
<td>3.54</td>
</tr>
<tr>
<td>&quot; retention, mg. per kg.</td>
<td>130</td>
<td>66</td>
<td>120</td>
<td>132</td>
<td>148</td>
</tr>
<tr>
<td>Weight change, gm.</td>
<td>+15</td>
<td>+3</td>
<td>+12</td>
<td>+18</td>
<td>+23</td>
</tr>
</tbody>
</table>

levels attained on Diet A. Since the growing organism is normally in a state of high nitrogen retention, a fall from the level characteristic of the
individual must be given the same interpretation as the inducement of a
negative nitrogen balance in the adult; namely, that, unlike acetyl-\textit{L}- and
acetyl-\textit{DL}-tryptophan, the \textit{D} component of \textit{DL}-tryptophan is not utilized by
man. This inference is further corroborated by the variations in body
weight changes induced by the diets. These findings would seem to
strengthen the deductions previously made from measurements of urinary
products regarding the metabolic fate in man of \textit{D}-tryptophan and both
isomers of acetyltryptophan.

\textit{Comments}

It is evident from our previous and present observations and those of
Luck and coworkers that the amount of acetyltryptophan utilizable by man
is dependent in a large measure on the mode of administration. The poor
utilization of intravenously administered acetyltryptophan may be ascribed
to two principal causes, (\textit{a}) the parenteral route by passes some enzyme
system present in the intestinal walls which can convert both optical
varieties of the tryptophan derivative to utilizable indole substances, or
(\textit{b}) the rapid excretion of the injected compounds from the circulatory
system into the urine precludes the action of any converting mechanism
which the blood may contain. The latter possibility would seem more
plausible in view of the fact that Luck and his collaborators were able to
isolate unresolved racemic acetyltryptophan from the urine of their
subjects.

The fact that the oral administration of acetyltryptophan, unlike \textit{L}–
tryptophan (6) does not cause an increase in the output of \textit{N}^{1}\textit{methyl}ni-
cotinamide is of interest and is being further investigated.

\textbf{SUMMARY}

The earlier suggestion that orally administered acetyltryptophan is
utilized by man has been strengthened by new measurements on the ar-
omatic components of the urine. Additional support for the view is derived
from body weight and \textit{N} retention data obtained with infants maintained
on diets in which the acetyltryptophan isomers constituted the major
source of tryptophan. The \textit{D} component of \textit{DL}-tryptophan does not seem
available for these functions.

\textbf{BIBLIOGRAPHY}

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