THE DIGESTION AND ABSORPTION OF ACETYLTRYPTOPHAN

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(Received for publication, April 27, 1956)

In a recent paper (1) data were presented to show that acetyl-d-tryptophan was excreted in the feces, whereas acetyl-L-tryptophan was preferentially absorbed and utilized. Three possibilities were presented to explain the absorption of the L isomer: (a) It is deacetylated when passing through the gastrointestinal tract, (b) it is absorbed as acetyl-L-tryptophan and then deacetylated, and (c) both (a) and (b) may occur. This paper presents experimental data taken in an attempt to learn more about the mechanism of digestion and absorption of acetyltryptophan.

Methods and Results

Acetyl-L-tryptophan was prepared as before (1). Acetyl-DL-tryptophan was synthesized by the acetylation of DL-tryptophan (2). In order to determine whether gut mucosa contains an enzyme which will deacetylate acetyltryptophan (1, 3), a section of fresh dog duodenum and jejunum was flushed with water and split longitudinally. All visible parasites were removed, and the mucosa was then separated as directed by Hawk, Oser, and Summerson (4).

5 ml. of the aqueous extract of the mucosa were incubated at 37°, pH 6.0 to 8.5, with 2 mg. of acetyl-L-tryptophan for 24 hours. Chromatographs of the incubation sample indicated that no hydrolysis had occurred. All chromatographs were resolved on Whatman No. 1 paper in a solvent system of methanol-butanol-benzene-H₂O (2:1:1:1) (5) and developed with p-dimethylaminobenzaldehyde. In this system L- and n-tryptophan give slightly different Rp values, whereas DL-tryptophan yields an elongated spot. From this evidence it was concluded that the digestive enzymes secreted by the gut mucosa do not hydrolyze acetyltryptophan.

The structure of acetyltryptophan suggests that carboxypeptidase would attack the molecule. Using acetyl-DL-tryptophan as the substrate, Putnam and Neurath (6) showed that 17.9 per cent hydrolysis occurred within 105 minutes of incubation with carboxypeptidase crystallized either five or seven times. Gilbert et al. (7) reported a rate of hydrolysis of only 6 μmoles for acetyl-DL-tryptophan per hour per mg. of enzyme N.

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Carboxypeptidase, crystallized three times, obtained from the Worthington Biochemical Corporation, was diluted to contain 0.083 mg. of N per ml. with 0.02 M phosphate buffer, pH 7.7, containing 0.46 mg. per cent of MgCl₂. Aliquots of this solution were incubated in a Dubnoff metabolic shaker at 37° with an equal volume of a 0.05 mM solution of acetyl-D- or acetyl-L-tryptophan in buffer or a 0.10 mM solution of acetyl-DL-tryptophan in buffer. At 0, 1, 2, 4, and 24 hours, aliquots of the incubation mixture were withdrawn. The enzymatic action was stopped by boiling for 5 minutes, and the coagulated protein was removed by centrifugation. The filtrate was chromatographed with 20 µl. applications. Incubation samples containing acetyl-D-tryptophan as substrate did not yield a spot for D-tryptophan, indicating that no hydrolysis had occurred. Samples with acetyl-L- or acetyl-DL-tryptophan as substrate yielded a spot for L-tryptophan after 1 hour incubation. At subsequent time intervals the spot became more pronounced (Fig. 1).

Quantitative results from microbiological assays supported the chromatographic evidence that hydrolysis of the acetyl-L and acetyl-DL analogue
had occurred. Essentially, the assay method for determining L-tryptophan with *Lactobacillus plantarum*, ATCC 8014, was similar to that described by other workers (8, 9). Both the basal medium described by Stokes et al. (10) and the tryptophan assay medium from the Difco Laboratories were found satisfactory. Incubation was carried out at 31° for 18 hours, and the growth response of the test organism was measured turbidimetrically in a Klett-Summerson colorimeter at 660 m. In preliminary investigations it was found that *L. plantarum* did not utilize the acetylated isomers of tryptophan. Quantitative measurements of L-tryptophan could be obtained in the presence of the acetylated analogue at the concentration

<table>
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<th>Table I</th>
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<td>Hydrolysis of Acetyltryptophan by Carboxypeptidase*</td>
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<table>
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<tr>
<th>Substrate in assay aliquot</th>
<th>Time of incubation at 37.5°</th>
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<tr>
<td></td>
<td>1 hr.</td>
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<td>γ per cent</td>
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<td>Acetyl-L-tryptophan</td>
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<td>4.31</td>
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<td>Acetyl-DL-tryptophan</td>
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* Measured by microbiological assay with *L. plantarum* 8014 and calculated from a standard curve.

ratios expected in the assay of the carboxypeptidase-acetyltryptophan incubation mixture.

The incubation samples for assay were diluted so that the amount of L-tryptophan formed on hydrolysis would fall within the effective measuring range on the standard curve. Both chromatography and microbiological assay demonstrated that acetyl-DL-tryptophan was hydrolyzed to a greater extent than was acetyl-L-tryptophan (Table I).

The slow rate at which acetyltryptophan was hydrolyzed *in vitro* indicated that carboxypeptidase is not the only mechanism of deacetylation and subsequent utilization. In order to determine whether acetyltryptophan is absorbed before deacetylation, fourteen rats, fasted 48 hours, were fed 1.0 mmole (246 mg.) of acetyl-L-tryptophan as the sodium salt by stomach tube. At hourly intervals two rats were sacrificed and blood was withdrawn from the vena cava. The blood was deproteinized with phosphotungstic acid, and 50 μl. of the filtrate were chromatographed. At
each time interval acetyltryptophan was detected. Microbiological assay of the blood filtrate showed a rise in the L-tryptophan content at 6 hours. The filtrate from control rats fed an equivalent amount of L-tryptophan showed a peak at 3 hours (Fig. 2). Quantitative data on the differences of absorption of the free and acetylated tryptophan isomers will be presented in a succeeding paper.

**DISCUSSION**

Although carboxypeptidase does hydrolyze acetyl-L-tryptophan, the rate appears to be too slow to be of importance. The early appearance of acetyl-L-tryptophan in the blood of rats indicates that some of the ingested acetyltryptophan is absorbed before deacetylation can occur in the gut. It is probable that acylase, a deacetylating enzyme found in the liver and kidney of several species of mammals (1, 4), is responsible for the deacetylation and subsequent utilization of acetyl-L-tryptophan. The rise in the L-tryptophan content of the blood 6 hours after ingestion of the acetylated isomer may be the result of either deacetylation of the absorbed acetyl-

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**Fig. 2.** L-Tryptophan content in blood of rats fed equivalent amounts of L-tryptophan and acetyl-L-tryptophan. Measured by microbiological assay with *L. plantarum* 8014. Growth response of the test organism measured turbidimetrically on a Klett-Summerson colorimeter at 660 mμ.
tryptophan by acylase or the absorption of the free tryptophan formed by the action of carboxypeptidase on the acetyltryptophan remaining in the gut.

The effect of the acetyl-D-tryptophan in a racemic mixture on an enzyme such as carboxypeptidase is difficult to explain. The possibility of contamination of the acetyl DL-tryptophan was eliminated when chromatographs and a negative ninhydrin reaction showed the complete absence of free tryptophan. Consistent results were obtained with new batches of carboxypeptidase and acetyltryptophan and with a mixture of equal portions of acetyl- and acetyl-L-tryptophan. The same effect can be demonstrated in vivo. When rats fed acetyl-DL-tryptophan were subjected to the Cori technique (11) for measuring absorption and the gut filtrate was assayed for free L-tryptophan, a sharp increase in bacterial growth occurred at the 6 hour interval. Gut filtrates of rats fed acetyl-L-tryptophan showed no increase in L-tryptophan at any time interval. The increased hydrolysis of the racemic mixture shows that the D isomer does not compete for the favored position on the enzyme molecule. Further work is in progress to evaluate the effect of the acetylated D isomer in the racemic mixture on the action of carboxypeptidase.

SUMMARY

The rate of hydrolysis was considered too slow to be of importance in the absorption and utilization of acetyl-L-tryptophan. Acetyl-L-tryptophan was found in rat blood shortly after its ingestion. It was concluded that the major portion of acetyl-L-tryptophan was absorbed before deacetylation.

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