THE AMINO ACID REQUIREMENTS OF MAN

VIII. THE METABOLIC AVAILABILITY OF THE OPTICAL ISOMERS OF ACETYLTRYPTOPHAN*

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It is generally recognized that the optical isomers of tryptophan are almost equally effective in the rat for purposes of growth (1–3), but that the corresponding acetylated compounds behave quite differently. Thus, acetyl-L-tryptophan functions readily as a component of the food in place of the free amino acid, but acetyl-D-tryptophan, whether administered orally or subcutaneously, is not utilized by the species in question (2).

In man, the metabolic deportment of the stereoisomers of tryptophan and their acetyl derivatives is not so clear. In Paper VII of this series (4), evidence was presented to the effect that m-tryptophan is incapable of replacing L-tryptophan, on an equal weight basis, as measured by the nitrogen balance technique. This was interpreted as indicating that very little m-tryptophan, if any, is inverted by human subjects. With respect to the acyl derivatives, Albanese, Frankston, and Irby (5) reported several years ago that acetyl-nn-tryptophan may be available to man except for a 5 per cent urinary loss. This conclusion was based upon indirect evidence obtained in a study of the excretion of an unidentified metabolite believed to have its origin in d-tryptophan and said to yield indigo red on treatment with iodine (6). According to the authors (5), the oral administration to human subjects of 0.01 M quantities of acetyl-dl-tryptophan failed to induce the excretion of the unidentified metabolite, in contrast to the effects produced by corresponding amounts of unsubstituted dl-tryptophan.

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In a later paper, Albanese, Davis, and Lein (7) reported the results of feeding experiments in infants in which diets deficient in tryptophan were supplemented for 4 day periods with L-tryptophan, DL-tryptophan, acetyl-L-tryptophan, and acetyl-DL-tryptophan, respectively. Subnormal growth and decreased nitrogen retention were said to have been induced only by the diet reinforced with DL-tryptophan. This was interpreted as providing additional evidence in favor of a greater utilization of acetyl-DL-tryptophan than of the free racemic amino acid. In the meantime, Luck, Boyer, and Hall (8) described three human experiments in which acetyl-DL-tryptophan was administered intravenously rather than orally. The results showed that 70 to 83 per cent of the compound was excreted unchanged, mostly within the first 6 hours after the injection. The acetyl-tryptophan isolated from the urine was racemic; consequently, no preferential utilization or retention of acetyl-L-tryptophan was indicated under the conditions employed in the tests. Albanese et al. (7) pointed out that the path of administration may have affected the outcome of the experiments, a fact which was fully appreciated and mentioned by Luck and his associates.

The nitrogen balance technique, as developed in this laboratory with amino acid diets, affords an excellent opportunity of determining definitely and directly whether acetylation of L-tryptophan increases its availability to the human organism. The problem is an interesting one, not only from the metabolic standpoint, but for practical considerations as well. The cost differential between L-tryptophan and the racemic amino acid is such that the use of the latter would represent a significant saving, particularly in human studies, provided the substance could be rendered wholly effective merely by the expedient of acetylation. Therefore, tests were undertaken for the purpose of comparing the effects of L- and DL-tryptophan and the acetyl derivatives of the optical isomers. The results are outlined below.

**EXPERIMENTAL**

Five experiments of varying duration were carried out. Two of these consisted of test periods at the end of experiments involving other amino acids, while the other three experiments were made on individuals who had not previously participated in our amino acid investigations. As is our custom, all of the subjects were young men who were majoring in biochemistry or had had sufficient biochemical training to appreciate fully the purposes of the experiments.

The diets varied somewhat, particularly as determined by the energy needs of the subjects. A detailed description of each ration seems unnecessary; however, this information is available for anyone who wishes it.
Invariably, the nitrogen of the food was furnished in the form of mixtures of the eight essential amino acids supplemented with glycine and urea as extra sources of this element for the synthesis of the non-essentials. Amino acid Mixture 182, which was described in Paper VII of this series (4), was employed in three of the tests. A mixture of identical composition, except for the presence of twice as much DL-threonine and 9.82 gm. daily of DL-leucine instead of half this quantity of L-leucine, was used in the other two tests. The daily allotment of each mixture was dissolved in water and flavored with 100 ml. of filtered lemon juice. Wafers IV and V, which also have been described elsewhere (9), furnished most of the energy in four of the tests. In the fifth, a similar product of slightly higher carbohydrate and fat content was used. Extra sucrose and butter fat were administered in constant daily amounts as required to adjust the diets to the exact caloric needs of the subjects. In each case, the ratio of calories derived from carbohydrate to those furnished by fat was maintained for the entire diet at approximately 2.6. The other components of the rations consisted of vitamin pills (cf. (10)) and Cellulose flour, the latter to provide a residue in the alimentary tract. One-third of the daily food allowance was consumed at each meal.

The acetyl-D, acetyl-L, and acetyl-DL derivatives of tryptophan were prepared in the laboratories of Merck and Company. These compounds, as well as the constituents of the amino acid mixtures, were recrystallized until they yielded correct analytical data. The specific rotations of the active modifications of acetyltryptophan also were in satisfactory agreement with values recorded in the literature.\footnote{The observed specific rotation of acetyl-D-tryptophan (1 per cent in water treated with 1 equivalent of sodium hydroxide) was $[\alpha]_{D}^{20} = -29.4^\circ$. Du Vigneaud et al. (2) reported $[\alpha]_{D}^{20} = -30.2^\circ$. Under identical conditions the acetyl-L-tryptophan showed a value of $[\alpha]_{D}^{20} = +29.5^\circ$. Du Vigneaud and Sealock (11) reported $[\alpha]_{D}^{20} = +29.0^\circ$.}

The results of the tests are summarized in Table I; the figures represent period averages. The findings are so unequivocal that they require little discussion. As will be observed, Subjects T. J. S. and H. Z. S. showed distinct, positive balances on diets containing 0.20 gm. of L-tryptophan daily. When, however, the tryptophan was replaced by an equivalent amount (0.24 gm.) of acetyl-D-tryptophan, each subject experienced an average daily loss of more than 1 gm. of nitrogen. Obviously, acetylation of D-tryptophan fails to improve its nutritive quality. Subject H. F. K. had for some time preceding the present test been serving in an experiment involving another amino acid and during this had received a daily intake of 0.30 gm. of L-tryptophan. When the latter was replaced by 0.24 gm. of acetyl-DL-tryptophan, a slight but unmistakable negative balance ensued. On the other hand, substitution of an equivalent amount (0.20 gm.)
of L-tryptophan for the acetyl-DL-tryptophan immediately induced a strong nitrogen retention. Evidently, this subject had a rather low tryptophan requirement which was partially satisfied by the acetyl-L-tryptophan present in the racemic compound. That acetylation does not diminish the availability of L-tryptophan is shown by the results obtained with Subjects R. L. F. and L. J. L. In both, 0.24 gm. daily of acetyl-L-tryptophan induced definite positive balances. The findings upon these subjects also demonstrate that acetyl-DL-tryptophan is not measurably superior to the free racemic amino acid in the maintenance of nitrogen equilibrium. In view of these observations, one must conclude that acetylation of D- and DL-tryptophan does not enhance their usefulness in the adult, human organism.

### SUMMARY

Comparisons have been made of the effectiveness of acetyl-D-, acetyl-L-, and acetyl-DL-tryptophan in the maintenance of nitrogen equilibrium in human subjects deprived of L-tryptophan. The results demonstrate that
acetyl-D-tryptophan, like free D-tryptophan, is not utilized to a significant extent, if at all, by adult man. Acetylation of L-tryptophan does not measurably diminish its metabolic availability, nor does acetylation of DL-tryptophan enhance its usefulness.

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