Lack of a Tryptophan-Niacin Relationship
in Corn and Tobacco*

L. M. Henderson,† Jacqueline F. Someroski, D. R. Rao, Pei-Hsing Lin Wu, T. Griffith, and
Richard U. Byerrum

From the Department of Chemistry and Chemical Engineering, University of Illinois, Urbana, Illinois, and
the Kedzie Chemical Laboratory, Michigan State University, East Lansing, Michigan

(Received for publication, July 29, 1958)

Although the biosynthesis of niacin in Neurospora and mammals has been elucidated in a general way, the pathway by which this vitamin is formed in plants has remained obscure. An increase in the niacin content of cereals during germination has been observed (1). Winterstein and Trier (2) suggested that niacin is derived from proline, whereas Guggenheim (3) proposed ornithine as a precursor.

Following the observation of Kreil et al. (4) that tryptophan could replace niacin in the diet of the rat, evidence of a tryptophan-niacin relationship in plants has been sought (5-13). Several of these efforts have led to suggestive evidence that tryptophan or various of its metabolites are precursors of niacin. In general, the evidence has been based on observed small increases in the niacin content of plants grown in the presence of suspected precursors.

Recently, isotopic evidence has conclusively excluded the niacin-precursor role of tryptophan in some plants and bacteria (14). The failure of ring-labeled tryptophan to serve as a precursor of nicotine formed by root cultures of tobacco (15) coupled with the evidence that the pyridine ring of nicotine arises directly from nicotinic acid (16) constituted strong evidence against the conversion of tryptophan to niacin in this plant. Leete (17) and Grimshaw and Marion (18) have confirmed the failure of tryptophan and its metabolites to serve as a precursor of nicotine using intact Nicotiana glauca.

EXPERIMENTAL

Germination of Corn Plants—Two hybrid varieties of corn were used, Crow's Hybrid WF9 X MI4 and Tennessee Inbred No. 13.² The seeds were washed repeatedly with distilled water to remove fungicide. They were sterilized by soaking for 3 to 6 minutes in a solution of 0.2 per cent mercuric chloride in 50 per cent ethanol, then rinsed 3 times with 30 ml. portions of sterile, distilled water. The seeds were then soaked in a minimum volume of sterile, distilled water for 14 to 24 hours at which time they were transferred to the sterile 1-l. Erlenmeyer flasks used for germination. 15 soaked seeds were placed in each of 6 flasks containing 15 ml. of a solution of the compound under test. CO₂-free filtered air was drawn through each flask at a slow rate. In some cases the CO₂ produced was collected in a solution of NaOH. Sterile water was added as needed, usually 10 ml. to each flask on the fourth day. Germination and subsequent growth proceeded in the dark for 7 to 9 days at 28.5°C. The tritium labeled compounds used were added after 4 to 5 days of growth rather than at the beginning of germination. 3-Hydroxyanthranilate was sterilized by filtration and the tryptophan by autoclaving.

Growth of Tobacco Plants—Nicotiana rustica L., employed in this investigation, was grown in a greenhouse for approximately 3 months until the plants were between 10 and 15 cm. in height. A method of administering tryptophan to the tobacco plants was used which resulted in over 95 per cent absorption in 3 to 4 hours and therefore essentially eliminated bacterial destruction of the amino acid. To accomplish this the plants of the desired size and age were taken from the greenhouse and the roots removed with scissors. New roots were then allowed to regenerate for about 2 weeks in 125-ml. Erlenmeyer flasks containing a nutrient medium (19). At the end of that time, the nutrient medium in each flask was replaced with 3 ml. of medium containing 1 mg of DL-tryptophan-7a-C14 (0.7 μc.). This solution was taken up by the plant roots in 3 to 4 hours. 3 ml. of nutrient medium were then added to each flask and were rapidly absorbed. 50 ml. more of the nutrient medium were added to each flask and the plants were allowed to metabolize the radioactive amino acid for 10 days. Radioactivity was detected in the foliar parts of the plant 12 hours after administration of the amino acid. No adverse effect of the amino acid on plant growth or metabolism was noted.

Test Compounds—Tryptophan-7a-C14 was synthesized from aniline-1-C14 (20) and had a specific activity of 153 μc. per millimole. The tritium labeled 3-hydroxyantranilic acid and tryptophan were prepared according to the method of Wilsbach (21). The specific activities were 1660 and 3700 μc. per millimole, respectively.

Isolation of Compounds from Corn—At the end of the experimental periods the solutions remaining in the flasks were pooled and the unabsorbed compound was estimated by isotope analysis as well as by the microbioassay for tryptophan and by the fluorometric method for 3-hydroxyantranilic acid.

The embryos were separated from their endosperms and the root and shoot length determined. After drying at 80°C for 8 hours the plants were ground in a mortar and extracted for 1 hr.

* Supported in part by grants A-801 and RG-4700 from the United States Public Health Service.
† Present address, Department of Biochemistry, Oklahoma State University, Stillwater, Oklahoma.
² Kindly supplied by Dr. R. H. Hageman of the Department of Agronomy, University of Illinois, Urbana, Illinois.
³ Kindly supplied by Dr. H. C. Kincer, Tennessee Agricultural Experiment Station, Knoxville, Tennessee.
with 60 per cent boiling ethanol. The extract was evaporated to dryness and the residue hydrolyzed in 10 ml. of 1.0 N hydrochloric acid at 120° for 1 hr. in an autoclave. The hydrolysate was filtered, diluted to volume, and an aliquot removed for micro-bioassay for niacin.

To the remaining hydrolysate, 50.0 mg. each of trigonelline and niacin were added and after neutralization to pH 7.0 the bioassay for niacin was filtered, diluted to volume, and an aliquot removed for micro-bioassay for niacin.

Just adjusting to pH 7.8 this eluate was passed through a Dowex 1 column in the hydrogen phase. After washing the column with water both compounds were eluted with 0.3 N hydrochloric acid at 120° for 1 hr. in an autoclave. The hydrolysate was sublimed at 105° at 20-30 mm. Hg. The sublimate was considered a positive test for trigonelline.

The trigonelline was isolated as the crystalline picrate which was purified by recrystallization from absolute ethanol. Niacin was sublimed at 105° at 20-30 mm. Hg. The sublimate was recrystallized from absolute ethanol.

Isolation of Nicotine from Tobacco—At the end of the experimental feeding period the plants were removed from the flasks in which they had been growing, cut into small pieces with scissors, and rapidly dried at 80 to 90° under infrared lamps. Nicotine was then isolated from the dry plant material as the dipicrate as described previously (19).

Isotope Determination—The C¹⁴ in the experiments with corn was determined as C⁴O₂ after wet combustion (23) with a vibrating reed electrometer. Tritium was also determined by gas counting using the procedure of Wilzbach et al. (24).

In the experiments with tobacco, radioactive tryptophan and nicotine dipicrate were counted as such with a Tracerlab, Inc., proportional flow counter and a Nuclear Chicago Corporation model 192x scaler.

**RESULTS AND DISCUSSION**

**Experiments with Corn**—A preliminary experiment, in which nonisotopic tryptophan was used, failed to show any marked effect of tryptophan on the niacin content of the corn embryos. Nason (7) reported a greater than normal synthesis of niacin by excised corn embryos when tryptophan or 3-hydroxyanthranilic acid was added to the medium. In the preliminary experiments with the whole seed of one of the strains of corn used by Nason, tryptophan added at the beginning of germination inhibited root and shoot development slightly at 0.1 mg. per plant and by almost 50 per cent at 1 mg. per plant. Table I summarizes the results of niacin analysis on these corn embryos and the remaining endosperm. Whereas the niacin content per unit of dry weight of embryos was greater when tryptophan was added to the medium, the increase appeared to result from growth suppression. The niacin content per embryo was not greatly changed by tryptophan. These data obtained with whole kernels showed somewhat less consistent increases in niacin in response to tryptophan than were recorded by Nason with excised embryos. Nevertheless, niacin synthesis increased the amount of this vitamin from 16.7 µg. per plant in the germinated embryos to approximately 25 µg. per plant in the germinated embryos. The results here could be interpreted as resulting from slight stimulation of niacin synthesis by tryptophan. Subsequent experi-

**TABLE I**

<table>
<thead>
<tr>
<th>Effect of tryptophan addition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean shoot length</td>
</tr>
<tr>
<td>mg</td>
</tr>
<tr>
<td>Whole seed ungerminated</td>
</tr>
<tr>
<td>Germinated seeds</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>100 µg. DL-trypotphan per plant</td>
</tr>
<tr>
<td>1000 µg. DL-trypotphan per plant</td>
</tr>
</tbody>
</table>

**TABLE II**

**Distribution of C¹⁴ from tryptophan-7a-C¹⁴ in Crow’s hybrid corn embryos**

| Number of embryos/number of seeds | 150/200 |
| Mean shoot length, cm. | 5.5 |
| Tryptophan-7a-C¹⁴ given, mg. | 11.5 |
| Tryptophan-7a-C¹⁴ given, µg. | 5.29 |
| Tryptophan-7a-C¹⁴ not absorbed, mg. | .1 |
| C¹⁴ in embryos, µg. | 2.41 |
| C¹⁴ in endosperm, µg. | 1.11 |
| C¹⁴ in ungerminated seeds, µg. | .68 |
| C¹⁴ in respiratory CO₂, µg. | .91 |
| Total recovery of C¹⁴, µg. | 5.11 |
| Total recovery of C¹⁴, per cent. | 60 |
| C¹⁴ in niacin, µg. | 0.00014 |

**TABLE III**

**Failure of Tennessee Inbred No. 13 corn to incorporate isotope from tryptophan or 3-hydroxyanthranilic acid into niacin or trigonelline**

<table>
<thead>
<tr>
<th>Compound administered</th>
<th>No. of embryos/No. of seeds</th>
<th>Mean shoot length</th>
<th>Dry weight of embryos</th>
<th>Niacin in embryos</th>
<th>Amount of compound given</th>
<th>Amount of compound absorbed</th>
<th>Amount of C¹⁴ in niacin</th>
<th>Isotope in trigonelline</th>
</tr>
</thead>
<tbody>
<tr>
<td>DL-Tryptophan-7a-C¹⁴</td>
<td>94/150</td>
<td>10.7</td>
<td>7.45</td>
<td>210</td>
<td>30</td>
<td>22.2</td>
<td>1.4</td>
<td>trace</td>
</tr>
<tr>
<td>3-Hydroxyanthranilic acid-H²</td>
<td>94/150</td>
<td>18.0</td>
<td>9.61</td>
<td>108</td>
<td>10</td>
<td>110</td>
<td>0.3</td>
<td>0.016</td>
</tr>
<tr>
<td>DL-Tryptophan-H²</td>
<td>45/75</td>
<td>15.7</td>
<td>4.72</td>
<td>183</td>
<td>12.8</td>
<td>233</td>
<td>0.4</td>
<td>trace</td>
</tr>
</tbody>
</table>
ments reported below indicated that if tryptophan enhances niacin formation in the germinating corn seed it does not do so by serving as a precursor.

In the first isotope experiment with Crow's hybrid variety corn, 150 of 200 seeds used germinated (Table II). At the end of the experiment approximately 60 per cent of the C\(^{14}\) was accounted for, most of which was present in the growing plant. A substantial amount was collected as C\(^{15}\)O\(_2\), but only a trace appeared in niacin.

Three other experiments (Table III) provided evidence against the incorporation of tryptophan or 3-hydroxyanthranilic acid into niacin or trigonelline. Because tryptophan addition had suppressed growth in the preliminary experiment, (Table I) the last two experiments involving tritium-labeled compounds were performed somewhat differently. The germination occurred in distilled water and the test compounds were added on the fourth or fifth day. Under these circumstances the test compounds were almost quantitatively absorbed, but caused little or no suppression of growth. The niacin content of the embryos was approximately the same (15 to 20 μg. per embryo) as observed in the preliminary experiment.

**Experiments with Tobacco** When tryptophan 7α-C\(^{14}\) was fed to two groups of tobacco plants under conditions described above, no radioactive nicotine was formed in either experiment. Other substances, which have been shown to be nicotine precursors, when fed at the concentration and with the radioactivity of the tryptophan used in the present study, yielded highly radioactive nicotine (25, 26). Therefore, the results of the present work, obtained with methods which insured the absorption of tryptophan, show that the amino acid is not a precursor of the pyridine ring of nicotine in *Nicotiana rustica*, and are in agreement with the findings of Dawson (15) and Leete (17).

**Summary**

1. Isotope experiments with Crow's hybrid and Tennessee Inbred No. 13 corn indicated that tryptophan added to the germination medium was completely absorbed but did not serve as a precursor of the synthesized niacin. Likewise, the tritium of labeled 3-hydroxyanthranilic acid was not incorporated into this vitamin.

2. Tryptophan 7α-C\(^{14}\), when administered to *Nicotiana rustica* by a procedure which insured rapid absorption of the amino acid through the roots, yielded no radioactive nicotine during a metabolism period of 10 days.

3. These findings constitute strong evidence against the existence of the role of tryptophan and 3-hydroxyanthranilic acid as niacin precursors in corn and tobacco, contrary to much nonisotopic evidence which has been presented.

**References**

1. BURKHOLDER, P. R., *Science*, 97, 562 (1943).
Lack of a Tryptophan-Niacin Relationship in Corn and Tobacco