Niacin Biosynthesis in the Developing Chick Embryo*

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In 1945, Krehl et al. (1) observed that tryptophan and niacin were incorporated into nicotinic and quinolinic acids. Since that time considerable evidence has accumulated which demonstrates the biosynthesis of niacin from tryptophan in the rat and other animals. Studies with Neurospora and mammals have demonstrated that the pathway from tryptophan goes through kynurenine, 3-hydroxykynurenine, 3-hydroxyanthranilic acid, an unstable intermediate, quinolinic acid, and hence to nicotinic acid (2).

The role of quinolinic acid as an obligatory intermediate in the conversion of tryptophan to niacin in animals and Neurospora has been questioned by some workers (3, 4). Henderson and Hirsch (5) first identified quinolinic acid as a metabolite of tryptophan in the rat. Quinolinic acid injections caused a 3-fold increase in the excretion of N-methylnicotinamide (6), and quinolinic acid will support the growth of rats on a niacin-free diet (7). One Neurospora mutant accumulates quinolinate and another mutant uses it, although somewhat less effectively than niacin (7). It has also been found that isotopically labeled quinolinic acid leads to labeled N-methylnicotinamide (8) and finally, that quinolinic acid replaced nicotinic acid for growth by Xanthomonas pruni (9).

In previous work with the chick embryo, Schweigert et al. (10) found that tryptophan injections stimulated niacin synthesis. Denton et al. (11) later confirmed this, but could find no conversion of 3-hydroxyanthranilic acid to nicotinic acid. Quagliarello and Della Pietra (12) found little conversion of 3-hydroxyanthranilic acid to niacin in vitro in homogenized chick embryo; however, their preparations failed to form nicotinic acid from quinolinic acid, although the latter was present in normal developing embryos (13). Jackson et al. (14) injected tryptophan-3-C\textsuperscript{14} into chick embryos and isolated radioactive niacin, but their data did not permit the calculation of yield and dilution.

The present study was made to establish the tryptophan-to-quinolinic pathway in the developing embryo and especially to answer the question of the role of quinolinic acid as an intermediate. It was demonstrated that radioactive isotope from aniline-1-C\textsuperscript{14} and 3-hydroxyanthranilic acid and quinolinic acids were prepared by the method of Wilzbach (16). The specific activities were 717 and 670 mu per mmole, respectively. All compounds were free of radioactive impurities separable by paper chromatography in two or more solvents.

Injection of Compounds—Tryptophan-7a-C\textsuperscript{14} was dissolved in 0.9% saline and sterilized by autoclaving for 20 minutes at a pressure of 15 pounds. Quinolinic acid was dissolved in isotonic saline, the pH adjusted to 7.0, and sterilized by autoclaving. The quinolinic acid-H\textsubscript{3} was found by microbioassay to contain less than 0.005% of nicotinic acid. Autoclaving at pH 7.0 was shown to cause no decarboxylation to form niacin. 3-Hydroxyanthranilic acid-H\textsubscript{3} was dissolved in 1 N HCl and sterilized by boiling, and the pH was adjusted under aseptic conditions to pH 7.0.

The compounds in 0.2 to 0.3 ml of solution were aseptically injected on the 10th or 12th day of incubation through a small hole drilled in the shell just below the air sac. Immediately, the opening was sealed with paraffin and the egg was placed in the incubator until the termination of the experiment.

Isolation of Compounds from Embryo—The first experiment covered the 12th to 18th days; the second study covered a period twice as long, the 10th to 22nd days. At the termination of each experiment the embryo or chick was weighed, sacrificed, and minced in a Waring Blendor, and then extracted twice with 200 ml of 80% ethanol. The extract was taken to dryness under reduced pressure and the lipid material was extracted with Skellysolve B. The dried residue was hydrolyzed with 10 volumes of 1 N NaOH for 1 hour at 120° in the autoclave. The hydrolysate was neutralized with HCl and made to volume, and aliquots were removed for microbiological assay. Nicotinic acid was determined by the Lactobacillus arabinosus assay. Quinolinic acid was determined as niacinic acid after decarboxylation for 2 hours with glacial acetic acid (5).

To the remainder of the hydrolysate were added 100.0 mg of nicotinic acid and 100.0 mg of quinolinic acid as carrier. The solution was then taken to dryness under reduced pressure and the resulting residue was extracted with two 40-ml portions of hot absolute methanol. The residue after extraction gave a negative ferrous sulfate test, indicating the absence of niacin (17). It also gave a negative ferrous sulfate test (18), showing that the quinolinic acid was completely extracted. The methanol extract was taken to dryness and the residue dissolved in 10 ml of water. After adjustment to pH 2, the solution was passed through a Dowex 50W-X8 (200 to 400 mesh) column (10 × 100 mm) in the hydrogen phase. The quinolinic acid was not adsorbed and was found in the first few fractions. The niacin was then eluted with 0.3 N HCl.
The quinolinic acid was recovered by evaporation to dryness and was crystallized twice from 2 ml of 50% ethanol. Nicotinic fractions were pooled and taken to dryness, and the nicotinic acid was sublimed at 105°C at 20 to 30 mm of Hg.

Isotope Determination—The C\textsuperscript{14} in the tryptophan experiments was determined as C\textsubscript{6}O\textsubscript{2} with a vibrating reed electrometer after wet combustion (19). Tritium was also determined by gas counting, the procedure of Wilzbach (20) being used to prepare the samples.

RESULTS AND DISCUSSION

In Table I are shown the amount of tryptophan, quinolinic acid, and nicotinic acid present in the egg and in the full term embryo. The amount of tryptophan decreased during incubation, whereas there was an increase in the amount of quinolinic (7-fold) and nicotinic (20-fold) acids. These results are in agreement with those of Denton et al. (11).

Tryptophan Experiments—In Table II are shown the results of experiments that demonstrate the incorporation of isotope from tryptophan-7a-C\textsuperscript{14} into niacin and quinolinic acid. The conversion to niacin was 0.21% in Experiment I and 0.26% in Experiment II. These values are lower than the 0.6% observed by Schweigert et al. (10) in their nonisotopic experiments with massive injections of L-tryptophan. However, if the D isomer is not utilized for niacin synthesis the conversion of isotope from L-tryptophan to niacin in Experiment II becomes 0.50%. The dilution of isotope in the conversion of tryptophan to niacin (Table II) was 262 and 100, respectively, in the two experiments.

If the major portion of the niacin synthesis occurs during the later, rapid stages of development of the embryo, the data in Table I can be used as a nonisotopic check on the isotope data for Experiment II (Table II). Approximately 0.005 mmole of niacin was synthesized during a full term incubation. During that time approximately 0.25 mmole of tryptophan disappeared. Thus, about 0.04% of the tryptophan which was lost during incubation appeared as niacin. However, for purposes of comparison with the isotope results, the conversion percentage must be based not on the tryptophan which disappeared but on the total tryptophan content. The conversion on this basis was 0.83%.

The conversion of isotope in tryptophan to quinolinic acid was 0.07% based on D-tryptophan or 0.14% based on the L isomer. Because of its suggested intermediate role and the low level of this metabolite in embryonic tissue, this small percentage of isotope in quinolinic acid was expected. Relatively low dilution of isotope (98 and 69) suggests a more rapid turnover of quinolinate than nicotinate.

3-Hydroxyanthranilic Acid Experiments—In Table III are summarized the results obtained by injecting labeled 3-hydroxyanthranilic acid into developing embryos. The conversion to niacin in the 6-day experiment was 0.21%, which was approximately the same as that observed in the first experiment with isotopic tryptophan. In the 12-day experiment this conversion percentage was increased 4-fold. The 3-hydroxyanthranilic acid injected was presumably diluted with the 3-hydroxyanthranilic acid formed from tryptophan. A major part of the 3-hydroxyanthranilic acid was probably oxidized to CO\textsubscript{2}, whereas a lesser amount was utilized for production of nicotinic acid or quinolinic acid (21, 22). In the second experiment a larger quantity of substrate was injected, but the greater percentage of conversion of the isotope to niacin probably resulted from the longer experimental period. Because of the proximity of 3-hydroxyanthranilic acid to nicotinic in the biosynthetic pathway and because of the lesser dilution with endogenous substrate, 3-hydroxyanthranilic acid might be more efficiently converted to niacin than is tryptophan. However, the difference in the percentage of incorporation of isotope into nicotinic from the two substrates was not marked. This deviation from the predicted result may, in part, arise from exchange of the tritium from label positions with the medium in the formation of niacin from 3-hydroxyanthranilic acid-H\textsuperscript{3}.

3-Hydroxyanthranilic acid was also shown to be a precursor of quinolinic acid. The relatively small dilution of 21 when the percentage of incorporation was small, indicates that a major
portion of the substrate was metabolized further, but that approximately 5% of the quinolinic acid present at the termination of the experiment came from the 3-hydroxyanthranilic acid injected. The lesser dilution of the isotope in going from hydroxyanthranilic acid to quinoline, as compared to the dilution from tryptophan to quinolinic acid (Table II), is approximately what would be expected when the size of the tryptophan pool is taken into consideration.

Quinolinic Acid Experiments—The results of the studies involving quinolinic acid injection are shown in Table IV. Of the 1.1 mg of quinolinic acid-H3 injected on the 10th day in the second experiment, approximately 10% remained as quinolinic acid on the 22nd day. Microbioassay showed that approximately 210 pg of quinolinic acid were present when the experiment was terminated. Thus, 100 pg of quinolinic acid from endogenous sources were present, accounting for the 1.8 dilution observed. It appears that approximately 85% of the injected quinolinic acid-H3 was metabolized, since it was not present as quinolinic or nicotinate in the full-term embryo.

The specific activities of the nicotinic acid present in the embryos from these experiments were much nearer those of the compound administered than was the case with the tryptophan or 3-hydroxyanthranilic acid studies. This lesser dilution of isotope suggests a direct conversion of quinolinic to nicotinic and possibly a suppression of the formation of quinolinic from 3-hydroxyanthranilic acid.

In order to insure that the incorporation of isotope from quinolinic acid into nicotinic acid did not result from chemical dercarboxylation, a control experiment was performed. Tritium-labeled quinolinic acid was added to an extract of an embryo, and when the nicotinic acid was isolated by the described procedure it was found to be unlabeled.

The results reported here are consistent with the known pathway of tryptophan to nicotinic acid in Neurospora and nicotinic requiring mammals (2). The finding that tryptophan and 3-hydroxyanthranilic acid are precursors of quinolinic acid is in agreement with the results in the rat (23, 24) and accounts for the presence of quinolinic acid in the normal embryo (13).

The observations reported here are another indication that quinolinic acid is a true intermediate in nicotinic synthesis from tryptophan. Since the embryo is free of complicating intestinal microflora, it indicates that the nicotinic acid is being formed from quinolinic acid by the action of the enzymes of the embryo.

### Table IV

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Nicotinic acid</th>
<th>Quinolinic acid</th>
<th>Nicotinic acid</th>
<th>Quinolinic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>I*</td>
<td>21.5</td>
<td>148</td>
<td>210</td>
<td>454</td>
</tr>
<tr>
<td>H2 present (mcg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H2 incorporated (% of dose)</td>
<td>0.66</td>
<td>3.8</td>
<td>4.6</td>
<td>10.0</td>
</tr>
<tr>
<td>Specific activity before dilution (µg per mmole)</td>
<td>9.3</td>
<td>175.3</td>
<td>54.7</td>
<td>367</td>
</tr>
<tr>
<td>Dilution of isotope*</td>
<td>72</td>
<td>3.8</td>
<td>12.3</td>
<td>1.8</td>
</tr>
</tbody>
</table>

* Twelve- to 18-day embryos; 0.96 mg of quinolinic acid-H3; specific activity, 671 PC per mmole.
† Ten- to 22-day embryos; 1.10 mg of quinolinic acid-H3; specific activity, 614 PC per mmole.
‡ Based on microbioassays.

### SUMMARY

Isotope from tritium-labeled quinolinic and 3-hydroxyanthranilic acids and from tryptophan-7α-14C was incorporated into nicotinic and quinolinic acids in the developing chick embryo. In the two experiments, one of 6 days' duration and the second lasting for 12 days, the isotope from tryptophan in the nicotinic acid isolated was diluted 262- and 160-fold, respectively, and for quinolinic acid 98- and 69-fold. When the injected material was 3-hydroxyanthranilic acid the dilutions of the isotope in the nicotinic acid were 311- and 48.5-fold, and for quinolinic acid, 78- and 21-fold. When isotopic quinolinic acid was injected, dilutions of 72- and 12.3-fold were obtained for the nicotinic and 3.8- and 1.8-fold for quinolinic acid.

The findings constitute evidence that the tryptophan–nicotinic acid relationship established in many other species also exists in the chick embryo. Since quinolinic acid can lead to nicotinic acid, it appears to be involved as a true intermediate in the tryptophan–nicotinic acid sequence.

### Acknowledgments

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### REFERENCES

Niacin Biosynthesis in the Developing Chick Embryo  
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