THE METABOLISM OF CARBOXYL-LABELED 3-HYDROXYANTHRANILIC ACID IN THE RAT*

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(Received for publication, July 17, 1956)

Since 3-hydroxyanthranilic acid was established as an intermediate in the biosynthesis of nicotinic acid in Neurospora crassa (1, 2), its role in animals (3-5) and surviving tissues (6, 7) has been studied. It is known that 3-hydroxyanthranilate may rise from 3-hydroxykynurenine by the catalytic action of kynureninase (8). Approximately 10 per cent (9) of a test dose of 3-hydroxyanthranilic acid is excreted as urinary quinolinic acid. The conversion of 3-hydroxyanthranilate to pyridine nucleotides was established by its niacin-replacing activity in rats receiving niacin-free, tryptophan-low diets (3-5). Further evidence for the role of 3-hydroxyanthranilic acid as a precursor of niacin was provided by the demonstration that rats excreted increased amounts of N\(^{\text{1}}\)-methylnicotinamide when 3-hydroxyanthranilic acid was administered (10). Proof of this relationship was provided by injecting C\(^{14}\)-carboxyl-labeled 3-hydroxyanthranilic acid and isolating labeled N\(^{\text{1}}\)-methylnicotinamide from the urine (11). The studies reported here show that the carboxyl group of 3-hydroxyanthranilic acid is rapidly disposed of as CO\(_2\) when this compound is injected into normal rats receiving a 9 per cent casein diet. Essentially all of the C\(^{14}\) in the quinolinic excreted following injection of carboxyl-labeled 3-hydroxyanthranilic acid was present in the 3-carboxyl carbon.

EXPERIMENTAL

The 3-hydroxyanthranilic acid labeled in the carboxyl group with C\(^{14}\) was the same preparation previously described (11). It was combined with appropriate quantities of unlabeled 3-hydroxyanthranilic acid in isotonic saline solution for intraperitoneal injection.

The young adult animals were taken from a group used in C\(^{14}\)-labeled tryptophan studies already described (12). Expired CO\(_2\) and urine were collected and the C\(^{14}\) content was determined by the same methods previously used (12). The N\(^{\text{1}}\)-methylnicotinamide and quinolinic acid in the urine samples were determined as before by established methods (13-16).

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Isolated, purified quinolinic acid was decarboxylated in the 2 position and the C\textsuperscript{14} content of the CO\textsubscript{2} determined (17). The nicotinic acid (5 mg.) which resulted was then decarboxylated with a mixture of 125 mg. of copper chromite (18) and 2 ml. of quinoline in a 50 ml. round bottomed flask fitted with a cold finger condenser, and immersed in a salt bath at 285\degree C for 1 hour. Nitrogen was bubbled through the reaction mixture to sweep out the CO\textsubscript{2} released. The gas stream was passed through an alcohol-dry ice trap to remove water, and then collected in a multiple-coiled trap immersed in a liquid nitrogen bath. The CO\textsubscript{2} was estimated in the Van Slyke manometric apparatus and analyzed for C\textsuperscript{14} (17). The technique for this procedure was developed by using authentic carboxyl-labeled nicotinic acid. This method was shown to give approximately 70 per cent decarboxylation.

The urea level in the urine was determined by a modified dixanthydrol urea method (19, 20). 1 ml. samples of urine were mixed with 2 ml. of 1 per cent ferric chloride and 10 ml. of 0.5 per cent sodium bicarbonate and filtered. 5 ml. of the filtrate were placed in a centrifuge combustion tube (21). The dixanthydrol derivative of urea was precipitated by the addition of a barium reagent (19) and 1 ml. of 25 per cent xanthydrol in glacial acetic acid. The precipitation was completed by mixing and cooling. The precipitate was removed by centrifuging for 5 minutes at 600 \times g, washed with methanol three times, and dried in a vacuum oven. Total carbon and C\textsuperscript{14} of the precipitate were determined by the combustion method of Van Slyke, Steele, and Plazin (17), and the urea was calculated from the total carbon.

RESULTS AND DISCUSSION

Two animals received 3.1 and 19.1 mg. of 3-hydroxyanthranilic acid in single doses. The results of the analysis of the expired CO\textsubscript{2} (Table I) indicated that hydroxyanthranilic acid is rapidly metabolized. The specific activity of the expired CO\textsubscript{2} reached a maximal value in about 40 minutes. In both experiments approximately one-third of the C\textsuperscript{14} was expired in the 1st hour, and in 3 hours approximately 60 per cent of the isotope had been disposed of as carbon dioxide.

The urine samples collected during this period of intense oxidative destruction also contained substantial quantities of C\textsuperscript{14} (Table II). Rat 13 excreted approximately 9 per cent of the administered activity as quinolinic acid and 60 per cent as CO\textsubscript{2}. During the first 12 hour period, 96 mg. of urea were excreted. This urea showed 23,950 disintegrations per minute, which represented 3.06 per cent of the administered C\textsuperscript{14} and 11 per cent of the activity in the urine. Of the 27.8 per cent of the C\textsuperscript{14} administered which appeared in the urine (Table II), over half, or 16 per cent of the test dose, was present as unidentified metabolites.
Rat 2, which received 3.1 mg. of 3-hydroxyanthranilic acid, disposed of only 20 per cent of the administered C\textsuperscript{14} in the urine (Table II) in 33 hours, most of it in the 8 hour period following injection.

The quinolinic acid excreted by Rat 13 was isolated after the addition of carrier and analyzed for C\textsuperscript{14}. The results (Table II) show that the quinolinic acid formed from the injected 3-hydroxyanthranilic acid had a specific activity of 2.56 \mu c. per mmole, which is about 91 per cent of that of the injected 3-hydroxyanthranilic acid. This excreted quinolinic acid had 97.5 per cent of its C\textsuperscript{14} in the 3-carboxyl carbon and 2.5 per cent in the 2-carboxyl carbon (Fig. 1).

### Table I

**Rate of E3piration of C\textsuperscript{14}O\textsubscript{2} from Carboxyl-Labeled 3-Hydroxyanthranilic Acid**

<table>
<thead>
<tr>
<th>Rat 2</th>
<th>Rat 13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Received 3.1 mg., 0.41 \mu c., in 5 ml. isotonic NaCl solution intraperitoneally</td>
<td>Received 19.1 mg., 0.34 \mu c., in 5 ml. isotonic NaCl solution intraperitoneally</td>
</tr>
<tr>
<td><strong>Per cent administered C\textsuperscript{14} expired</strong></td>
<td><strong>Per cent administered C\textsuperscript{14} expired</strong></td>
</tr>
<tr>
<td><strong>Per min.</strong></td>
<td><strong>Cumulative total</strong></td>
</tr>
<tr>
<td>0-20</td>
<td>0.23</td>
</tr>
<tr>
<td>20-40</td>
<td>0.71</td>
</tr>
<tr>
<td>40-60</td>
<td>0.68</td>
</tr>
<tr>
<td>60-90</td>
<td>0.46</td>
</tr>
<tr>
<td>90-120</td>
<td>0.23</td>
</tr>
<tr>
<td>120-180</td>
<td>0.11</td>
</tr>
<tr>
<td>180-240</td>
<td>0.051</td>
</tr>
<tr>
<td>240-360</td>
<td>0.014</td>
</tr>
<tr>
<td>360-480</td>
<td>0.013</td>
</tr>
</tbody>
</table>

Carrier N\textsuperscript{1}-methyl nicotinamide was added to a sample of urine collected from Rat 2 during the first 8 hours, and to a mixture of equal amounts of the urines of the 8 and 25 hour period of the same rat. The results of analyses of the isolated N\textsuperscript{1}-methyl nicotinamide are shown in Table II. The N\textsuperscript{1}-methyl nicotinamide in the urine of the first 8 hour period had a specific activity equal to 60 per cent of that of the injected compound, while the N\textsuperscript{1}-methyl nicotinamide excreted during the first 33 hours had an activity equal to 22 per cent of that of the injected compound. Calculations from these data indicate that the N\textsuperscript{1}-methyl nicotinamide in the urine excreted during the second period (25 hours) was almost devoid of isotope.

The rapid oxidative disposal of 3-hydroxyanthranilic acid noted here provides a possible explanation for the relatively poor yields of quinolinic
TABLE II

Results of Analysis of Urine Samples for Metabolites and C\textsuperscript{14} Following 3-Hydroxyanthranilic Acid Injection

<table>
<thead>
<tr>
<th></th>
<th>Rat 2</th>
<th>Rat 13</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st 8 hr. period</td>
<td>Next 25 hr. period</td>
</tr>
<tr>
<td>N'-Methylnicotinamide</td>
<td>40.5</td>
<td>77.4</td>
</tr>
<tr>
<td>excretion, y.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quinolinic acid</td>
<td>87</td>
<td>149</td>
</tr>
<tr>
<td>excretion, ( \gamma )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>14</td>
<td>24</td>
</tr>
<tr>
<td>&quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C\textsuperscript{14}</td>
<td>19.8</td>
<td>0.1</td>
</tr>
<tr>
<td>content of urine, % test dose</td>
<td>27.8</td>
<td>0.9</td>
</tr>
<tr>
<td>3-Hydroxyanthranilic acid, specific activity, µc. per mmole</td>
<td>20.3</td>
<td>2.81</td>
</tr>
<tr>
<td>Quinolinic acid, specific activity, µc. per mmole</td>
<td></td>
<td>2.56</td>
</tr>
<tr>
<td>N'-Methylnicotinamide, specific activity, µc. per mmole</td>
<td>12.5</td>
<td>4.4*</td>
</tr>
<tr>
<td>C\textsuperscript{14}</td>
<td>97.5</td>
<td></td>
</tr>
<tr>
<td>in 2-COOH of quinolinic acid, %</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* This value represents the specific activity of N'-methylnicotinamide isolated from a sample consisting of equal parts of the urines of the 8 and 25 hour periods. Therefore, it is the activity of the urine excreted over the first 33 hours of the urine collection.

† These values were corrected to 100 per cent decarboxylation of the samples analyzed.

![Chemical Structure of 3-Hydroxyanthranilic Acid, Nicotinic Acid, N'-Methylnicotinamide, Quinolinic Acid, and Picolinic Acid]

**Fig. 1.** Fate of isotope in the metabolism of 3-hydroxyanthranilic acids
acid (10 per cent) from 3-hydroxyanthranilic acid in the intact animal in contrast to the almost quantitative conversion to quinolinic acid by rat liver preparations (22).

The relatively small yield of niacin from tryptophan in vivo is probably the result of (a) metabolism of this amino acid via other pathways, e.g. deposition in protein and excretion as kynurenine, kynurenic acid, and other metabolites, and (b) oxidation of 3-hydroxyanthranilic acid formed as an intermediate to give other products. However, tryptophan is no less effective than exogenous 3-hydroxyanthranilic acid in vivo as a source of niacin (13). This suggests that 3-hydroxyanthranilic acid formed slowly from tryptophan is less subject to oxidative destruction than that provided by injection or feeding.

It should be emphasized that the very rapid appearance of the carboxyl carbon of 3-hydroxyanthranilic acid in expired CO₂ does not mean that the remainder of the molecule is completely oxidized. Loss of this carboxyl group from the unstable product of 3-hydroxyanthranilic oxidase (6, 7) (Fig. 1) occurs in the presence of a liver enzyme (23) or a number of reagents. Evidence that this decarboxylation, followed by closure of the ring to form picolinic acid, does not account for such rapid release of the C¹⁴ as CO₂ has been obtained. After addition of carrier picolinic acid, picolinic acid was isolated from the hydrolyzed urine of a rat to which ring-labeled tryptophan had been fed. The isolated picolinic acid contained only a trace of C¹⁴ in contrast to the quinolinic acid isolated from the same urine after the addition of the same quantity of carrier.

The authors wish to thank Miss Rhoda Palter for technical assistance.

**SUMMARY**

1. Intraperitoneally administered 3-hydroxyanthranilic acid labeled with C¹⁴ in the carboxyl group was metabolized by the rat in such a manner that approximately 60 per cent of the isotope appeared as C¹⁴O₂ within 3 hours.

2. 20 to 30 per cent of the C¹⁴ appeared in the urine within 8 to 12 hours.

3. N²-Methylnicotinamide isolated from the urine collected during the first 8 hours following a small dose (3.1 mg.) of 3-hydroxyanthranilic acid had a specific activity per mole equal to 60 per cent of that of the injected hydroxyanthranilic acid.

4. Quinolinic acid isolated from the urine of the 24 hour period of a rat with high dosage (19.1 mg.) had a specific activity per mole equal to 91 per cent of the injected hydroxyanthranilic acid.

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5. The isolated quinolinic acid contained 97.5 per cent of its C\textsuperscript{14} activity in the 3-carboxyl group and the remainder of the activity in the 2-carboxyl group.

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