N-Methyl-4-pyridone-5-carboxamide as a Metabolite of Nicotinic Acid in Man and Monkey*

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In previous experiments reported from this laboratory (1) N-methyl-4-pyridone-5-carboxamide was isolated from rat urine and identified as the major urinary metabolite of the pyridine nucleotides. Recently Wieland et al. (2) synthesized this 4-pyridone and have found their synthetic product to be identical with the compound isolated from rat urine. Experiments have now been carried out to determine whether the pyridine nucleotides are similarly metabolized by the monkey and the human.

In the case of the monkey, C14-nicotinic acid was injected into the animal, whereas the isotope dilution method was used to study the excretion of N-methyl-4-pyridone-5-carboxamide and N-methyl-2-pyridone-5-carboxamide by the human.

EXPERIMENTAL PROCEDURE

Monkey Experiment

A 4-year-old 18-pound male Rhesus monkey was used for the study. A dose of 3.5 mg (127.5 µc) of C14-nicotinic acid plus 17.2 mg of nonlabeled nicotinic acid was injected intramuscularly. The monkey was confined in a metabolism cage, and food and water were given ad libitum. Urine samples were collected at 24-hour intervals for 8 days, and after each collection the cage was washed with water. Labeled metabolites of nicotinic acid were separated and identified as described in previous studies (1, 3).

Each 24-hour urine collection was analyzed by paper chromatography, with n-butanol, acetone, and H2O (45:5:50) (4). For the first 4 days, the radioautograms showed six bands which were identified as follows (listed in order of increasing Rf value): Band 1, N-methylnicotinamide; Band 2, nicotinuric acid; Band 3, nicotinic acid; Band 4, 4-PY; Band 5, 2-PY; and Band 6, nicotinamide. However, only four compounds were found on the 6th and 8th days, namely, Bands 1, 4, 5, and 6. These four metabolites have also been found in rat urine as persisting metabolites (1). The distribution of radioactivity among the metabolites is shown in Table I. The percentage of radioactivity excreted as 2-PY increased with time after injection, so that at 6 days it accounted for 73%, whereas only 7% was present as the 4-PY. Thus, in monkey urine 2-PY was the major metabolite of the stored form of nicotinic acid (i.e. of the pyridine nucleotides), rather than 4-PY, which had preponderated in the experiment with rats. The difference presumably is due to species.

Price et al. (5) in 1955 stated that N-methyl-2-pyridone-5-carboxylic acid was excreted in normal human urine at the rate of 3 to 6 mg daily, and in 1956 (6) they reported that its glycine conjugate was also excreted in human urine at the rate of 3 to 12 mg daily. However, they did not find an increased excretion of either compound after the administration of 202 mg of nicotinic acid.

In order to determine whether the monkey excretes these compounds, a portion of the second-day urine collection was divided into three equal aliquots, each containing 365,000 c.p.m. To the first portion were added 13 mg of nonlabeled N-methyl-2-pyridone-5-carboxylic acid; to the second were added 10 mg of nonlabeled N-methyl-2-pyridone-5-carboxamidocarboxylic acid; and to the third portion nothing was added. The addition of known compounds to the urine samples enabled these compounds to be located on chromatograms by scanning with ultraviolet light.3 The three aliquots were applied as bands on separate sheets of Whatman No. 3MM filter paper together with their respective reference compounds, and the chromatograms were developed in 80% aqueous propanol. In the case of the third (control) aliquot, no band corresponding to either added compound was detectable under ultraviolet light. The identified bands from the first two aliquots were cut and eluted with water. The elution was considered complete when the absorption at 258 mμ was essentially zero. After the eluates were evaporated to a minimal volume under reduced pressure (all subsequent evaporations were also carried out in this manner), the volume was measured, 100 µl of this aliquot were plated on a planchet, and the radioactivity was determined with a Q gas counter. The total radioactivity in the N-methyl-2-pyridone-5-carboxylic acid band was only 14 c.p.m. and the total in the glycine conjugate band was only 76 c.p.m. These traces of radioactivity are insignificant percentages of the 765,000 c.p.m. put on the paper (and found in the bands earlier identified), and it can therefore be concluded that N-methyl-2-pyridone-5-carboxylic acid and its glycine conjugate are not metabolites of nicotinic acid in the monkey.

Human Experiments

Four men and four women in this laboratory served as the subjects. Each was on a self-selected diet. The four men were


** The authors want to express their appreciation to Dr. J. M. Price for providing synthetic N-methyl-2-pyridone-5-carboxamidocarboxylic acid.

each given 100 mg of nicotinic acid orally, and the women were not given test doses. A 24-hour sample of urine collected from each subject before and after the test dose was preserved by the addition of 3 ml of glacial acetic acid, and stored at 4°C. C14-4-PY (48.8 μg) containing 14,080 c.p.m. and C14-2-PY (145.7 μg) containing 13,493 c.p.m. were added to each 24-hour sample. Since Nuchar C had been found to adsorb all the nicotinic acid derivatives, it was used to extract them from the urine samples. The acidulated urine samples were filtered, and 40 g of Nuchar C were added to each filtrate. The suspensions were stirred and filtered. The filtrates (which contained no radioactivity) were discarded. The charcoal residue of each sample was washed with 100 ml of distilled water, and the washings were discarded. The nicotinic acid metabolites were then eluted from the charcoal by 10% pyridine, each eluate was passed through a IRA-400 (OH-) column, and the column was washed with water. The combined effluent and aqueous washings from the column were evaporated to dryness to remove pyridine. The residue was extracted with methanol and the 4-PY and 2-PY were separated from N-methyl nicotinamide and nicotinamide by paper chromatography. Because the bands of 4-PY and 2-PY are so close together, these compounds had to be further purified. For this purpose the bands were cut out and eluted with water. The separation of 4-PY and 2-PY was achieved by passing each of these water eluates through a Dowex 50 (H+) column. The column was washed with water, and the eluent and washing were evaporated to dryness. The residue was used to determine the specific activity of the recovered 2-PY. Each column was then washed with 0.1 N HCl until the eluates showed no absorption at 258 μm. The column was then eluted with 1 N HCl until the eluates showed no absorption at 240 μm. The eluate was evaporated to dryness and the residue used for the determination of the specific activity of the 4-PY. The radioactivity of 2-PY and 4-PY was determined with the Q gas counter. Aliquots were taken from each sample and diluted to suitable volume for the determination of ultraviolet absorption. The ratios of observed radioactivity to optical density, determined at 258 μm for 2-PY and at 256 μm for 4-PY, were calculated as "specific activity." The specific activities of the carriers 4-PY and 2-PY previously had been similarly calculated. The amount of 4-PY or 2-PY in the urines was then calculated by isotope dilution as follows (7):

\[
\frac{a}{A} = \frac{a}{C} - 1
\]

In the first determination of the 4-PY, variable data were obtained. The variability was found to be due to the effect of acidity on the ultraviolet absorption curve (Fig. 1). The HCl remaining in the residue after the acid eluate was evaporated to dryness caused a decrease in the maximal absorption and also a shift in the absorption peak toward the shorter wave lengths. When the concentration of HCl was below 0.02 N, absorption was practically the same as in water. In order to remove HCl, the residue left after evaporation of the 1 N HCl eluate was dissolved in water and again evaporated to dryness; this procedure was repeated two or three times. The final pH for optical

<table>
<thead>
<tr>
<th>Band No.</th>
<th>Radioactivities on day</th>
<th>Percentage</th>
<th>Corresponding to position of N-Methylnicotinamide</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.2</td>
<td>10.6</td>
<td>18.8</td>
</tr>
<tr>
<td>2</td>
<td>2.9</td>
<td>1.1</td>
<td>11.5</td>
</tr>
<tr>
<td>3</td>
<td>11.0</td>
<td>8.5</td>
<td>7.0</td>
</tr>
<tr>
<td>4</td>
<td>11.0</td>
<td>8.5</td>
<td>7.0</td>
</tr>
<tr>
<td>5</td>
<td>11.0</td>
<td>8.5</td>
<td>7.0</td>
</tr>
<tr>
<td>6</td>
<td>11.0</td>
<td>8.5</td>
<td>7.0</td>
</tr>
</tbody>
</table>

**Figure 1.** The effect of various concentrations of HCl on the absorption spectrum of a 0.55 mg % solution of 4-PY isolated from human urine, and the absorption spectrum in water of a solution of the same concentration of the isolated compound compared to the absorption spectrum of the synthetic compound. The synthetic compound was supplied to us by Prof. T. Wieland of the University of Frankfurt. The absorption curve for the synthetic compound was made at a concentration of 0.7 mg/100 ml of water.
TABLE II

Excretion of N-methyl-4-pyridone-5-carboxamide and N-methyl-2-pyridone-5-carboxamide in 24-hour urine collection of four normal male and four normal female subjects.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>4-PY</th>
<th>2-PY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Per kg body weight</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
</tr>
<tr>
<td>W. R. C.</td>
<td>158</td>
<td>23.96</td>
</tr>
<tr>
<td>Q. C.</td>
<td>150</td>
<td>10.2</td>
</tr>
<tr>
<td>G. K.</td>
<td>165</td>
<td>10.38</td>
</tr>
<tr>
<td>R. V. D.</td>
<td>210</td>
<td>10.70</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. Y.</td>
<td>113</td>
<td>4.66</td>
</tr>
<tr>
<td>J. J.</td>
<td>140</td>
<td>5.02</td>
</tr>
<tr>
<td>M. C.</td>
<td>120</td>
<td>6.82</td>
</tr>
<tr>
<td>C. Z.</td>
<td>120</td>
<td>6.14</td>
</tr>
</tbody>
</table>

The female subjects excreted less 4-PY than the male subjects; however, the difference appears to be merely a function of body weight. In the male group, the first subject (W. R. C.) excreted a considerably higher amount of 4-PY, a finding ascribable to a more rapid turnover of pyridine nucleotides in this subject.

The substantial increase in excretion of 2-PY, but not of 4-PY, after the 100-mg test dose of nicotinic acid in the four male subjects suggests that 2-PY is an excretion product of nicotinic acid metabolism per se, whereas 4-PY is the metabolic product of pyridine nucleotides in man, as in the rat (1).

The isotopic dilution method made it possible to study nicotinic acid metabolism in human subjects without exposing them to the radiation hazards of an administered isotope. The amount of the metabolites 2-PY and 4-PY excreted in the urine was quantitatively determined from the dilution in radioactivity following addition of the same compound labeled with radioactive carbon. During isolation, quantitative recovery is not required, since it is necessary to determine only the specific activity accurately.

From Fig. 1 it can be seen that the absorption spectrum of the 4-PY isolated from human urine is identical with that of the synthetically prepared compound, a further confirmation of the constitution of the metabolite (1).

All the data indicate that neither N-methyl-4-pyridone-5-carboxylic acid nor its glycine conjugate occurs as a metabolite of nicotinic acid excreted by the monkey, but that the principal metabolites are N-methyl-2-pyridone-5-carboxamide in the monkey and both the 2-pyridone and the 4-pyridone in the human.

SUMMARY

N-Methyl-4-pyridone-5-carboxamide (4-PY) was found in both human and monkey urine. Four normal women excreted 4.66 to 6.82 mg and four men excreted 10.2 to 23.96 mg of this compound per day. Oral administration of 100 mg of nicotinic acid caused only a slight increase in 4-PY excretion, but a marked increase in excretion of the isomeric 2-pyridone derivative (2-PY).

In a monkey treated by injection with C14-nicotinic acid, 2-PY, rather than 4-PY, was the major metabolite excreted in the urine, although 4-PY was also found. In addition, whereas N-methyl nicotinamide, nicotinuric acid, nicotinic acid, and nicotinamide were found in the first four daily urinary collections, by the sixth day only N-methyl nicotinamide, 4-PY, 2-PY, and nicotinamide were found.

N-Methyl-2-pyridone-5-carboxylic acid and its glycine conjugate were not found in the urine of the monkey treated by injection with C14-nicotinic acid.

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REFERENCES
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