STUDIES ON THE METABOLISM OF NICOTINIC ACID IN RABBITS

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Several compounds have been described as metabolites of nicotinic acid and nicotinamide in mammals. These are nicotinic acid (1), nicotinamide (2), nicotinuric acid (2), trigonelline (2), N\(^1\)-methylnicotinamide (NMN) (3-6), and 1-methyl-3-carboxylamide-6-pyridone (7, 8). According to the observations of various workers mammals may be divided into two groups in so far as the metabolism of nicotinic acid is concerned: mammals which aminate nicotinic acid, such as man, the dog, cat, and rat, and mammals which deaminate nicotinamide to nicotinic acid, such as the rabbit and guinea pig. The mechanism of amination and methylation of nicotinic acid and the sites where these reactions take place have been elucidated by Perlzweig, Bernheim, and Bernheim (9) and by Ellinger (10, 11). Ellinger and Abdel Kader (12) have observed that rabbits normally eliminate only nicotinic acid. Extra dietary nicotinamide and diethylnicotinamide have been found to be deaminated to nicotinic acid, which is subsequently methylated to trigonelline. An increased excretion of trigonelline by rabbits after administration of nicotinic acid has also been observed by these workers. Komori and Sendju (1), however, showed that after feeding nicotinic acid to rabbits nicotinic acid and nicotinuric acid were excreted in the urine, but no trigonelline was found. Huff and Perlzweig (4) also showed that rabbits were unable to methylate nicotinic acid. Perlzweig, Rosen, Pearson, Peck, and Parks (13) observed that rabbits fed a supplement of nicotinamide excreted nicotinuric acid. Ellinger and Abdel Kader (12), however, did not find any nicotinuric acid in rabbit urine either before or after administration of nicotinic acid. They observed that rabbits did not eliminate NMN in the urine, even after injection of a large dose of nicotinic acid. Handler (14) could not detect NMN in the urine of rabbits fed nicotinic acid. Holman and de Lange (15) are of opinion that, although nicotinic acid is not methylated directly in the body, methylated nicotinic acid (trigonelline) can be oxidized to a pyridone. Perlzweig et al. (13) observed that rabbits excreted less than 4 per cent of a total dose of nicotinamide as NMN and its pyridone. This indicated that NMN is not a normal metabolite of nicotinic acid in rabbits. From the résumé cited

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above it will be seen that contradictory views exist about the metabolism of nicotinic acid in rabbits. The present investigation, therefore, was undertaken to study the metabolism of nicotinic acid in rabbits by modified methods with a view to furnishing further evidence on the subject.

In the estimation of trigonelline by the method of Sarett (16) it has been observed by us that the size of the test-tube influences considerably the intensity of the color produced by benzidine hydrochloride with hydrolyzed trigonelline. It has also been observed that it is necessary to neutralize the solution carefully so that it does not become acid in any case.

We have observed that NMN is very unstable in alkali. As rabbit urine is highly alkaline, it is probable that NMN which is formed in the body is destroyed in the urinary tubules before it is excreted. Urinary excretion of NMN by rabbits was studied, therefore, after feeding mandelic acid, which lowers the pH of the urine. In the estimation of NMN in urine by the method of Carpenter and Kodicek (17) it has been found that riboflavin, which also gives a yellowish green fluorescence, interferes in the estimation of NMN. The method has been modified, therefore, for the estimation of NMN in urine in the presence of riboflavin.

EXPERIMENTAL

Excretion of Metabolites of Nicotinic Acid after Injection of 50 Mg. of Nicotinic Acid—Four rabbits, weighing from 900 to 1100 gm., were placed in individual metabolism cages. They were fed germinated gram (Cicer arietinum), a leguminous seed, ad libitum. The amount of the diet consumed was noted. Trigonelline, nicotinic acid, nicotinuric acid, NMN, and 1-methyl-3-carboxylamide-6-pyridone values of the gram were estimated. NMN and 6-pyridone were absent from the gram. The 24 hour urine was collected in a bottle containing 2 cc. of concentrated hydrochloric acid. Aliquots of the filtered urine were taken for the estimation of different metabolites of nicotinic acid. After estimating the daily normal excretion of these metabolites, each of the animals was injected with 50 mg. of nicotinic acid intraperitoneally, and the metabolites excreted in the urine were estimated daily till the excretions reached the normal level. In calculating these values the average amounts of nicotinic acid derivatives normally excreted were deducted and the excretion of the metabolites was expressed as the percentage of the nicotinic acid injected. The results are given in Table I.

Excretion of NMN after Feeding Mandelic Acid—Four rabbits (800 to 900 gm.) were placed in individual metabolism cages and urine was collected with 2 cc. of concentrated hydrochloric acid. NMN excreted in the urine was determined both before and during the 3 day period of feeding mandelic acid (60 mg. per kilo per day), with and without the intraperi-
toneal injection of 50 mg. of nicotinamide in each case. The results are
given in Table II. To study the reaction of urine of rabbits before and
after feeding mandelic acid, urine was collected under toluene and the pH
determined. The pH of the urine varied between 8.8 and 9 before and
between 7.5 and 8 after the administration of mandelic acid. The stability
of a dilute solution of NMN (1 mg. per cent) in 0.3 N sodium hydroxide for
varying periods was also studied. The results are presented in Table III.

**Table I**

72 Hour Urinary Excretion of Nicotinic Acid and Its Derivatives by Rabbits Injected
with 50 Mg. of Nicotinic Acid

The results represent the percentage of injected nicotinic acid excreted as dif-
ferent metabolites of nicotinic acid.

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Nicotinic acid and amide</th>
<th>Nicotinuric acid</th>
<th>NMN</th>
<th>6-Pyridone</th>
<th>Trigonelline</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.4</td>
<td>3.8</td>
<td>0.20</td>
<td>56.0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>3.0</td>
<td>3.2</td>
<td>0.15</td>
<td>56.8</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>7.9</td>
<td>2.4</td>
<td>0.18</td>
<td>57.5</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>8.0</td>
<td>3.4</td>
<td>0.17</td>
<td>55.4</td>
<td>0</td>
</tr>
</tbody>
</table>

**Table II**

24 Hour Urinary Excretion of NMN by Rabbits before and after Injections of 60 Mg.
of Nicotinamide with and without Mandelic Acid (60 Mg. per Kilo)

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Without injection of nicotinamide</th>
<th>After injection of nicotinamide</th>
<th>Without injection of mandelic acid</th>
<th>After injection of mandelic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>γ</td>
<td>26</td>
<td>γ</td>
<td>14</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>40</td>
<td>14</td>
<td>33</td>
</tr>
<tr>
<td>7</td>
<td>9</td>
<td>42</td>
<td>26</td>
<td>66</td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td>13</td>
<td>25</td>
<td>105</td>
</tr>
</tbody>
</table>

Excretion of Trigonelline and Nicotinic Acid by Fasted Rabbits after Injec-
tion of Trigonelline Sulfate—Rabbits (1200 to 1600 gm.) were fasted for 3
days. They were allowed to drink water only during the fasting period.
Urinary trigonelline and nicotinic acid were estimated before and after the
intraperitoneal injection of 50 mg. of trigonelline sulfate. The results are
given in Table IV.

Estimation of Nicotinic Acid—Nicotinic acid was estimated according to
the method of Banerjee, Ghosh, and Bhattacharya (18) as modified by
Nandi and Banerjee (19).
Estimation of Nicotinuric Acid—Nicotinuric acid was estimated by the method of Perlzweig, Levy, and Sarett (20), slightly modified. The modification consists in the treatment of the 5 N acid hydrolysate of the sample with saturated solution of potassium permanganate; then the procedure for the estimation of nicotinic acid, as described by Nandi and Banerjee (19), is followed. In this case, however, more permanganate was required to decolorize the sample, necessitating a larger amount of disodium hydrogen phosphate. In case permanganate was in excess, a few crystals of oxalic acid were used to decolorize the solution.

**Table III**

Stability of 1 Mg. Per Cent Solution of NMN in 0.3 N Sodium Hydroxide

<table>
<thead>
<tr>
<th>Time</th>
<th>NMN present in solution</th>
<th>Per cent destruction</th>
</tr>
</thead>
<tbody>
<tr>
<td>min.</td>
<td>γ</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>40</td>
<td>62.5</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>88.7</td>
</tr>
<tr>
<td>10</td>
<td>4.5</td>
<td>100</td>
</tr>
<tr>
<td>15</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

**Table IV**

48 Hour Urinary Excretion of Trigonelline and Nicotinic Acid by Rabbits Fasted for 3 Days and Then Injected with 50 Mg. of Trigonelline Sulfate

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Trigonelline excretion</th>
<th>Nicotinic acid excretion</th>
<th>Trigonelline excretion</th>
<th>Nicotinic acid excretion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before injection of trigonelline sulfate</td>
<td>After injection of trigonelline sulfate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>400</td>
<td>24.4</td>
<td>413</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>360</td>
<td>26.5</td>
<td>360</td>
</tr>
<tr>
<td>11</td>
<td>0</td>
<td>490</td>
<td>25.8</td>
<td>419</td>
</tr>
</tbody>
</table>

Estimation of NMN—NMN was estimated by the method of Carpenter and Kodicek (17), slightly modified to avoid the interference of riboflavin. 25 cc. aliquots of urine were treated with 20 mg. of sodium hydrosulfitfe in order to destroy the fluorescence given by riboflavin. Aliquots of the treated urine were then condensed with acetone and alkali and estimated fluorometrically. Alkali was also added to the blank after the addition of acid.

Estimation of Trigonelline—Trigonelline was estimated by the method of Sarett (16), slightly modified. 2 cc. aliquots of the sample prepared as described by Sarett were hydrolyzed in Pyrex test-tubes 6 inches by \( \frac{3}{4} \).
inch. 8 cc. of 95 per cent ethanol and 2 cc. of 11 N sodium hydroxide were then added, and the tubes were well shaken and heated at 80° under a reflex condenser for 45 minutes. After cooling, 1 drop of phenolphthalein and 2 cc. of 10 N hydrochloric acid were added, drop by drop and very slowly, with constant cooling and shaking. Neutralization was then completed with N hydrochloric acid until the color of the solution became faintly pink. The volume was made up to 20 cc. and the solution filtered. 10 cc. of the filtrate were taken for the estimation of trigonelline.

6-Pyridone—6-Pyridone was estimated by the method of Rosen, Perlzweig, and Leder (21).

DISCUSSION

From data in Table I it will be seen that after injection of nicotinic acid 2.4 to 3.8 per cent of the injected nicotinic acid is excreted in the urine as nicotinuric acid. This indicates that nicotinuric acid is a normal metabolite of nicotinic acid in rabbits. Although contradictory to the findings of Ellinger and Abdel Kader (12), this result supports the observations of Perlzweig et al. (13). 55 to 57 per cent of the injected nicotinic acid is excreted as 6-pyridone of NMN and thus forms the principal metabolite of nicotinic acid. Rabbits do excrete NMN, but most of it is destroyed in the body, owing to the high alkalinity of urine, as NMN has been found to be destroyed in 0.3 N alkali within a very short time. When the pH of the urine is lowered by the administration of mandelic acid, NMN excretion by the rabbits is slightly increased. Trigonelline is not a metabolite of nicotinic acid as claimed by Ellinger and Abdel Kader (12), because no trigonelline is excreted after the administration of nicotinic acid. Rabbits excrete 50 per cent of the injected or ingested trigonelline (present in gram) in the urine. No trigonelline is excreted in the feces, and there is no increased excretion of nicotinic acid after the administration of trigonelline. It appears that rabbits metabolize trigonelline, but nicotinic acid is not formed from it. Trigonelline has no properties as a vitamin which can replace nicotinic acid.

SUMMARY

1. The metabolism of nicotinic acid has been studied in rabbits. Rabbits excrete nicotinic acid, nicotinuric acid, N'-methylnicotinamide, and 1-methyl-3-carboxylamide-6-pyridone in the urine, the latter forming the principal end-product of nicotinic acid metabolism.

2. Rabbits do not excrete trigonelline as a metabolite of nicotinic acid.

3. Rabbits excrete a negligible amount of NMN, but, after administration of mandelic acid, which lowers the pH of the urine, appreciable amounts of NMN are excreted.
4. Some modifications have been suggested for the methods of estimating trigonelline, nicotinuric acid, and NMN.

Trigonelline sulfate and 6-pyridone were kindly prepared for us by Mr. Kalidas De of the East India Pharmaceutical Works, Calcutta.

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