**Note**

**Fate of Nicotinamide Differs Due to an Intake of Nicotinamide**

Katsumi SHIBATA, Hisako SHIMADA, and Hiroshi TAGUCHI*

Department of Human Health Science, Faculty of Human Sciences, Osaka International University for Women, Moriguchi, Osaka 570, Japan

*Laboratory of Biological Chemistry, Faculty of Bioresources, Mie University, Tsu, Mie 514, Japan

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We found that the catabolism of nicotinamide (Nam) differs due to an intake of Nam itself in rats. When rats were fed with a Nam-free, tryptophan-limiting diet, the major catabolite of niacin was N'-methyl-4-pyridone-3-carboxamide (4-Py). However, its percentage was changed with increasing the intake of Nam. The major metabolite was N'-methylnicotinamide (MINA) in the diet containing 0.006% Nam, or 0.1% Nam. The toxicity of excess Nam was observed when rats were fed with a 0.5% Nam-containing diet. In this diet, the major metabolite was Nam N-oxide and it was noted that the urinary excretion of nicotinic acid and its metabolite nicotinuric acid was observed. Therefore, these acids might be detected only when the toxicity of Nam appears.

**Key words:** N'-methylnicotinamide; N'-methyl-2-pyridone-5-carboxamide; N'-methyl-4-pyrdione-3-carboxamide; nicotinuric acid; nicotinamide N-oxide

The fates of Nam and NiA are exactly the same in rats fed a normal diet (for example, 20% casein diet) containing a physiological range of Nam or NiA, but the fates differ when rats are fed a diet containing an excess of Nam or NiA. In this study, we found that the fate of Nam differs due to the intake of Nam itself.

Nam and NiA were obtained from Wako Pure Chemical Industries Ltd. MNA chloride was obtained from Tokyo Kasei Kogyo, 2-Py and 4-Py were synthesized by the methods of Pullman and Colowick and of Shibata et al. respectively. Nam N-oxide and NuA were obtained from Aldrich Chemical Co., Inc. All other chemicals used were of the highest purity available from commercial sources.

The animal room temperature was maintained at around 22 C and about 60% humidity, and a 12-h light/12-h dark cycle was maintained. Body weight and food intake were measured daily around 09:00 a.m., and food and water were renewed daily.

Weanling male rats of the Wistar strain (3 weeks old) were obtained from Clea Japan (Tokyo, Japan) and immediately placed in individual metabolic cages (CT-10; Clea Japan). They were then divided into four groups, and fed *ad libitum* for 17 days, one group with a Nam-free, Trp-limiting diet, the same diet +0.006% Nam (normal amount), the same diet +0.1% Nam (large amount), or the same diet +0.5% Nam (extra-large amount). The urine samples were collected in amber bottles with 1 ml of 1 M HCl and stored at ~25 C until needed. The rats were killed by decapitation after the last urine samples had been collected, and the liver of each animal was removed. The livers were stored at ~25 C until needed for measuring total Nam.

The contents of Nam, 2-Py, and 4-Py in the urine were simultaneously measured by the HPLC method of Shibata et al. while the content of MNA in the urine was measured by HPLC method of Shibata. The content of Nam N-oxide, NiA, and NuA were measured by the HPLC method of Shibata. The content of total Nam (total Nam = free Nam + NAD+ + NADP+) in the liver was measured by the HPLC method of Shibata.

The changes in the body weights of the four groups are shown in Fig. A. The final body weight gains of all the groups fed with the Nam added diets were higher than that of the group fed with the Nam-free diet. However, the body weight gain of the rats fed with the 0.5% Nam diet was lower than that fed with the Nam-free diet.

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**Fig.** Effects of Dietary Nam Levels on the Body Weight Gain (A) and Food Intake (B).

○ 0% Nam; ● 0.006% Nam; □ 0.1% Nam; ■ 0.5% Nam. Each point and bar represent the mean ± SEM for five rats.

**Abbreviation:** Trp, tryptophan; Nam, nicotinamide; NiA, nicotinic acid; NuA, nicotinuric acid; MNA, N'-methylnicotinamide; 2-Py, N'-methyl-2-pyridone-5-carboxamide; 4-Py, N'-methyl-4-pyridone-3-carboxamide; Nam N-oxide, nicotinamide N-oxide.
diet until day 9 of the experiment passed. After that day, the growth in the 0.5% Nam group was getting higher than that in the Nam-free group. The growth rate was always higher in the groups fed with the 0.006% and 0.1% Nam diets than in the Nam-free and 0.5% Nam groups. The growth retardation by Nam was observed at the 0.5% Nam diet under the conditions. Nevertheless, it is noted that body weight gain was higher in the 0.5% Nam group than in the Nam-free group.

Figure B shows the changes in a daily food. The daily food intake of the Nam-free group was around 5 g during the experiment, while those of the three Nam groups were gradually increased with growing, but, the food intake was lower in the 0.5% Nam group than that of the Nam-free group until day 4. Table I summarizes the data for 17 days of the body weight changes, food intakes, and FER.

Table II shows the liver weights and the contents of the total Nam. The liver weight was higher in the groups fed with 0.006% and 0.1% Nam than in the Nam-free group, while the liver weight in the group fed with the 0.5% Nam diet did not differ from that in the Nam-free group. When the values were expressed in terms of 100 g of body weight, these were not different.

The total Nam contents were dependent on the contents of Nam in the diets as shown in Table II. Table III shows the effects of the various amounts of Nam intake on the excretion of niacin metabolites. The major urinary excretory metabolite was 4-Py in the Nam-free group, in the 0.006% Nam group they were MNA and 4-Py, in the 0.1% Nam group they were Nam, MNA, and N-oxide, and in the 0.5% Nam group they were Nam and N-oxide. It was noted that the urinary excretion of NiA and NuA were observed only in the 0.5% Nam group.

It has generally been considered that the growth retardation caused by a large amount of Nam is simply due to a lack of methyl-donor, because the extremely increased excretion of MNA is observed when a large amount of Nam is administered. MNA is formed from Nam in the presence of S-adenosylmethionine by the catalysis of Nam methyltransferase. In this experiment, an abnormally increased urinary excretion of MNA was observed between the groups fed with the 0% and 0.006% Nam diets. Nevertheless, growth promotion was clearly observed (Fig.). Again, the abnormally increased urinary excretion of MNA was observed between the groups fed with the 0.006% and 0.1% Nam diets (Table III), however, no growth retardation was observed. These findings mean that the growth retardation of Nam is not only due to a lack of methyl-donor, but also other factors might be.

When a large amount of Nam is administered to rats, the activity of 4-Py-forming MNA oxidase greatly decreases, because it is inactivated during the catalysis. This enzyme activity is correlated with the dietary protein levels; that is, when a low protein diet is fed to rats, its activity is low. Under such conditions, the accumulation of MNA might occur even when the intake is within a physiological range, because the reaction of MNA→4-Py is extremely low. In this experiment, the diets used were low-protein diets so that the activity of 4-Py-forming MNA oxidase could be low. Therefore, the urinary excretion of MNA

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**Table I. Effects of Dietary Nam Levels on the Body Weight Gain, Food Intake, and FER**

<table>
<thead>
<tr>
<th>Nam Level (%)</th>
<th>0% Nam (%)</th>
<th>0.006% Nam (%)</th>
<th>0.1% Nam (%)</th>
<th>0.5% Nam (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (g)</td>
<td>40 ± 0.7</td>
<td>40 ± 0.7</td>
<td>40 ± 0.8</td>
<td>40 ± 0.9</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>67 ± 2.5^a</td>
<td>105 ± 2.0^a</td>
<td>98 ± 1.0^a</td>
<td>77 ± 1.7^a</td>
</tr>
<tr>
<td>Body weight gain (g; 17 days)</td>
<td>23 ± 4.4^a</td>
<td>65 ± 1.9^b</td>
<td>58 ± 2.4^b</td>
<td>37 ± 1.0^b</td>
</tr>
<tr>
<td>Food intake (g; 17 days)</td>
<td>88 ± 5.4^a</td>
<td>156 ± 3.1^b</td>
<td>150 ± 4.1^b</td>
<td>120 ± 1.8^b</td>
</tr>
<tr>
<td>FER*</td>
<td>0.258 ± 0.043^b</td>
<td>0.414 ± 0.004^b</td>
<td>0.389 ± 0.005^b</td>
<td>0.312 ± 0.008^b</td>
</tr>
</tbody>
</table>

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**Table II. Effects of Nam Levels on the Content of Total Nam in Liver**

<table>
<thead>
<tr>
<th>Nam Level (%)</th>
<th>0% Nam (%)</th>
<th>0.006% Nam (%)</th>
<th>0.1% Nam (%)</th>
<th>0.5% Nam (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver weight (g)</td>
<td>3.43 ± 0.20^a</td>
<td>5.57 ± 0.34^b</td>
<td>5.41 ± 0.26^b</td>
<td>3.96 ± 0.20^a</td>
</tr>
<tr>
<td>Liver weight (g/100 g of body weight)</td>
<td>5.12 ± 0.31</td>
<td>5.30 ± 0.32</td>
<td>5.22 ± 0.25</td>
<td>5.14 ± 0.26</td>
</tr>
<tr>
<td>Total Nam (nmol/g)</td>
<td>1423 ± 75^a</td>
<td>1786 ± 80^b</td>
<td>2211 ± 49^b</td>
<td>3550 ± 145^a</td>
</tr>
</tbody>
</table>

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**Table III. Effects of Nam Levels on the Daily Excretion of Niacin Metabolites in Urine (in terms of nmol/g of food)**

<table>
<thead>
<tr>
<th>Nam Level (%)</th>
<th>0% Nam</th>
<th>0.006% Nam</th>
<th>0.1% Nam</th>
<th>0.5% Nam</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nam</td>
<td>N.D. ^a</td>
<td>1.8 ± 0.4</td>
<td>1.3 ± 0.1</td>
<td>10.2 ± 1.0</td>
</tr>
<tr>
<td>MNA</td>
<td>167.1 ± 2.5</td>
<td>115 ± 22.5</td>
<td>14.0 ± 1.4</td>
<td>856 ± 6.4</td>
</tr>
<tr>
<td>2-Py</td>
<td>174 ± 85.2</td>
<td>754 ± 28.4</td>
<td>132 ± 5.2</td>
<td>130 ± 5.7</td>
</tr>
<tr>
<td>4-Py</td>
<td>9585 ± 835</td>
<td>907 ± 41.1</td>
<td>892 ± 16.6</td>
<td>979 ± 9.3</td>
</tr>
<tr>
<td>N-oxide</td>
<td>186 ± 38.5</td>
<td>102 ± 32.6</td>
<td>104 ± 16.6</td>
<td>108 ± 36.8</td>
</tr>
<tr>
<td>NiA</td>
<td>440 ± 85.2</td>
<td>6826 ± 368</td>
<td>405 ± 226</td>
<td>1085 ± 226</td>
</tr>
<tr>
<td>NuA</td>
<td>75 ± 109</td>
<td>243 ± 21.3</td>
<td>2483 ± 109</td>
<td>19201 ± 1307</td>
</tr>
</tbody>
</table>

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* Sum = Nam + MNA + 2-Py + 4-Py + Nam N-oxide + NiA + NuA.
* N.D. = not detected.

Each value is expressed as nmol/g of food and is the mean ± SEM for five rats.
abnormally increased even when rats were fed the 0.006% Nam diet. In our opinion, the increased MNA is not the increased reaction of Nam→MNA but the decreased reaction of MNA→4-Py. We think that the growth retardation is not simply due to a lack of methyl-donor S-adenosylmethionine.

MNA is an inhibitor of Nam methyltransferase. Therefore, when MNA accumulates in the liver cells, the reaction of Nam→MNA is inhibited and therefore, Nam accumulates in the liver. As the result, the reaction of Nam→Nam N-oxide occurs, which is excreted into urine. Furthermore, the reaction of Nam→NiA can proceed because the concentration of Nam is over the $K_m$ value of nicotinamidase. The accumulation of NiA in the liver cells brought about the next reaction of NiA→NuA, which reaction needs CoA.

It has been said that NiA is excreted in urine, but, the urinary excretion of NiA and NuA is not observed when rats were fed with the 0.1% Nam diet. Only when the body’s capacity to methylate Nam is exhausted, the compounds NiA and NuA might appear. It might be a sign of the toxicity of Nam and also a sign of a deficiency of CoA.

References