Summary Urinary excretion of nicotinamide and its metabolites in mouse, guinea pig, and hamster with a pharmacological amount of nicotinamide or \( N^1 \)-methylnicotinamide (MNA) was investigated to compare them with those of rat. In mouse, nicotinamide \( N \)-oxide was the most abundant metabolite, accounting for 35% of urinary excretion of nicotinamide and its metabolites, and followed by \( N^1 \)-methyl-2-pyridone-5-carboxamide (2-Pyr), 20%; nicotinamide, 18%; MNA, 16%; and \( N^1 \)-methyl-4-pyridone-3-carboxamide (4-Pyr), 11%. In guinea pig, 2-Pyr was the most abundant metabolite, accounting for 80% of urinary excretion, and was followed by MNA, 11%; nicotinamide, 3%; 4-Pyr, 3%; and nicotinamide \( N \)-oxide, 3%. In hamster, nicotinamide was the most abundant metabolite, accounting for 44%, and followed by 2-Pyr, 21%; nicotinamide \( N \)-oxide, 15%; MNA, 10%; and 4-Pyr, 10%. Urinary excretion of nicotinic acid and nicotinuric acid was not detected in mouse, guinea pig, and hamster. When a pharmacological amount of nicotinamide was intraperitoneally injected into mouse, the excretion of nicotinamide \( N \)-oxide increased to 79.7% of the nicotinamide metabolites, while those of MNA (4.9%), 2-Pyr (11.2%), and 4-Pyr (6.3%) decreased. Nicotinic acid and nicotinuric acid were again not detected. When a pharmacological amount of nicotinamide was intraperitoneally injected into guinea pig and hamster, the deamidated metabolites nicotinic acid and nicotinuric acid occupied significant percentages of the nicotinamide metabolites: 50.4% and 26.3%, respectively, in guinea pig; and 7.7% and 79.5%, respectively, in hamster. The ratio of 2-Pyr to 4-Pyr excretion did not change when nicotinamide or MNA was located into mouse, guinea pig, and hamster. From these results, the catabolism of nicotinamide in rodent is discussed.

Key Words nicotinamide, \( N^1 \)-methylnicotinamide oxidase, rodent, mouse, guinea pig, hamster, catabolism of nicotinamide, \( N^1 \)-methylnicotinamide, \( N^1 \)-methyl-2-pyridone-5-carboxamide, \( N^1 \)-methyl-4-pyridone-3-carboxamide
The catabolic pathway of nicotinamide and nicotinic acid in rat is as shown in Fig. 1. In normal adult rat, N1-methyl-4-pyridone-3-carboxamide (4-Pyr) accounts for 82% of the urinary excretion of nicotinamide and its metabolites, while nicotinamide accounts for 4%, N1-methylnicotinamide (MNA) 7%, N1-methyl-2-pyridone-5-carboxamide (2-Pyr) 6%, and nicotinamide N-oxide 1% as shown in Fig. 2A (1,2). No urinary excretion of nicotinic acid and nicotinuric acid is observed in normal rats (1,2). However, this urinary excretion pattern changed when a pharmacological dose of nicotinamide (2) or MNA (3) was intraperitoneally injected into rats.

Although there are two reports about catabolism of niacin in other rodents, guinea pig (4) and mouse (5), the precise data are not reported. So, we investigated the catabolic pathway of nicotinamide in mouse, guinea pig, and hamster to compare it with that in rat.

MATERIALS AND METHODS

Chemicals. Nicotinamide and nicotinic acid were obtained from Wako Pure Chemical Industries (Osaka). MNA chloride was purchased from Tokyo Kasei Kogyo Co. (Tokyo). 2-Pyr and 4-Pyr were synthesized by the methods of Pullman

Fig. 1. Catabolism of niacin.

J. Nutr. Sci. Vitaminol.
and Colowick (6) and Shibata et al. (7), respectively. Nicotinamide N-oxide was purchased from Aldrich Chemical Co. (Milwaukee, Wis., USA). Nicotinuric acid was purchased from Sigma Chemical Co. (St. Louis, Mo., USA). All other chemicals used were of the highest purity obtainable from commercial sources.

**Animals and diet.** Male mice of the CD-1 (ICR) strain (8 weeks old; 30.6 ± 0.2 g, means ± SEM; n = 5) were obtained from Charles River (Atsugi, Japan). The mice were individually housed in metabolic cages and fed a commercial nonpurified diet CE-2 [from Clea Japan (Tokyo) and water ad libitum throughout the experimental period. On day 42, five mice (38.1 ± 0.6 g body wt) were injected intraperitoneally at 09:00 a.m. with a pharmacological amount of nicotinamide (500 mg/kg body wt) dissolved in sterilized saline (1 ml). Urine samples (24 h) from the previous day (day 41) and the next 4 days (day 42–45) after injection were collected into flasks containing 1 ml of 1 M HCl and stored at −25°C until analysis for nicotinamide and its metabolites. On day 56, five mice (38.3 ± 0.4 g body wt) were injected intraperitoneally at 09:00 a.m. with a pharmacological amount of MNA chloride (500 mg/kg body wt) dissolved in sterilized saline (1 ml). Urine samples (24 h) from the previous day (day 55) and the next 4 days (day 56–59) after injection were collected as described above.

Male guinea pigs of the Hartley strain (3 weeks old; 198.3 ± 8.7 g) were purchased from Charles River. These were treated in the same way as the mice. The body weight at the times of nicotinamide injection and MNA injection was 425.2 ± 30.6 g and 508.4 ± 29.6 g, respectively.

Male hamsters (8 weeks old; 97.2 ± 1.7 g) were purchased from a local animal supplier [Awazu Animal Laboratory (Kadoma, Japan)]. These were also treated in the same way as the mice. The body weight at the times of nicotinamide injection and MNA injection was 124.7 ± 6.9 g and 126.0 ± 7.2 g, respectively.

**Analyses.** The methods used for measuring liver activity of nicotinamide methyltransferase [EC 2.1.1.1] (8), 2-Pyr-forming MNA oxidase [EC 1.2.3.1: MNA + H$_2$O + A (unknown acceptor) → 2-Pyr + AH$_2$ + H$^+$] (9) and 4-Pyr-forming MNA oxidase [MNA + H$_2$O + A (unknown acceptor) → 4-Pyr + AH$_2$ + H$^+$] (9) have been described.

The determinations of nicotinamide (7), 2-Pyr (7), 4-Pyr (7), nicotinamide N-oxide (10), MNA (11), nicotinic acid (12), and nicotinuric acid (12) have been described.

**RESULTS**

**Mouse**

Urinary excretion of nicotinamide, MNA, 2-Pyr, 4-Pyr, nicotinamide N-oxide, nicotinic acid, and nicotinuric acid before and after injection of a pharmacological amount of nicotinamide is shown in Table 1. Under physiological conditions (the day before injection of nicotinamide), urinary excretion of major niacin metabolites comprised about 35% nicotinamide N-oxide, 20% 2-Pyr, 18% nicotinamide, 16%
Table 1. Urinary excretion of nicotinamide and its metabolites before and after injecting mouse, guinea pigs, and hamsters with a pharmacological dose of nicotinamide (500 mg/kg body wt)*.

|                | Day before injection | Day 1     | Day 2     |
|----------------|----------------------|-----------|-----------|
| **Mouse**      |                      |           |           |
| Nam            | 0.18 ± 0.03          | 28.9 ± 7.0| 0.43 ± 0.08|
| MNA            | 0.16 ± 0.04          | 1.29 ± 0.38| 0.45 ± 0.11|
| 2-Pyr          | 0.20 ± 0.06          | 2.70 ± 0.80| 0.77 ± 0.23|
| 4-Pyr          | 0.10 ± 0.04          | 1.40 ± 0.62| 0.84 ± 0.54|
| Nam N-oxide    | 0.35 ± 0.04          | 17.7 ± 4.09| 0.67 ± 0.06|
| NiA            | N.D.                 | N.D.      | N.D.      |
| NuA            | N.D.                 | N.D.      | N.D.      |
| **Guinea pig** |                      |           |           |
| Nam            | 0.10 ± 0.03          | 30.4 ± 9.12| 0.40 ± 0.01|
| MNA            | 0.35 ± 0.01          | 6.23 ± 0.74| 1.24 ± 0.33|
| 2-Pyr          | 2.60 ± 0.09          | 33.5 ± 1.62| 4.94 ± 0.14|
| 4-Pyr          | 0.10 ± 0.01          | 1.34 ± 0.05| 0.20 ± 0.01|
| Nam N-oxide    | 0.10 ± 0.03          | 65.4 ± 23.7| 0.13 ± 0.02|
| NiA            | N.D.                 | 120.3 ± 33.7| N.D.     |
| NuA            | N.D.                 | 230.5 ± 34.6| N.D.     |
| **Hamster**    |                      |           |           |
| Nam            | 0.58 ± 0.04          | 61.4 ± 12.7| 1.71 ± 0.55|
| MNA            | 0.13 ± 0.02          | 1.40 ± 0.12| 0.39 ± 0.20|
| 2-Pyr          | 0.27 ± 0.06          | 2.77 ± 0.86| 1.85 ± 0.51|
| 4-Pyr          | 0.13 ± 0.03          | 1.46 ± 0.47| 1.04 ± 0.31|
| Nam N-oxide    | 0.20 ± 0.04          | 7.25 ± 0.29| 0.87 ± 0.29|
| NiA            | N.D.                 | 7.20 ± 0.93| N.D.     |
| NuA            | N.D.                 | 73.1 ± 18.4| N.D.     |

*Values are expressed as μmol/day and means ± SEM for 4–5 rodents. Nam, nicotinamide; MNA, N¹-methylnicotinamide; 2-Pyr, N¹-methyl-2-pyridone-5-carboxamide; 4-Pyr, N¹-methyl-4-pyridone-3-carboxamide; Nam N-oxide, nicotinamide N-oxide; NiA, nicotinic acid; NuA, nicotinuric acid; N.D., not detected.

MNA, and 11% 4-Pyr as shown in Fig. 2B. Urinary excretion of nicotinic acid and nicotinuric acid was not detected.

On day 1 after nicotinamide injection, 18.8% of the dose was excreted as unchanged nicotinamide, 11.4% as nicotinamide N-oxide, 1.6% as 2-Pyr, 0.9% as 4-Pyr, and 0.7% as MNA. The increases in MNA, 2-Pyr, and 4-Pyr excretion were each around 10-fold and that of nicotinamide N-oxide was 70-fold. Even on day 1 after nicotinamide injection, urinary excretion of nicotinic acid and nicotinuric acid was not detected. By day 3, urinary excretion of nicotinamide and its metabolites had returned to normal levels (data not shown).

Urinary excretion of nicotinamide and its metabolites before and after the
Fig. 2. Urinary excretory pattern of nicotinamide and its metabolites in rat, mouse, guinea pig, and hamster under physiological conditions. Urinary excretion of nicotinic acid and nicotinuric acid was not detected.

Injection of a pharmacological amount of MNA is shown in Table 2. On day 1 after the injection, the urinary excretion of 2-Pyr and 4-Pyr increased by 44- and 57-fold, respectively; 28% of the dose was excreted as unchanged MNA, 8.8% as 2-Pyr, and 6.0% as 4-Pyr; slight increase in nicotinamide and nicotinamide N-oxide was observed. By day 4, urinary excretion of MNA and its metabolites 2-Pyr and 4-Pyr had returned to normal levels (data not shown).

Guinea pig

Urinary excretion of nitotinamide, MNA, 2-Pyr, 4-Pyr, nicotinamide N-oxide, nicotinic acid, and nicotinuric acid before and after injection of a pharmacological amount of nicotinamide is shown in Table 1. Under physiological conditions urinary excretion of major niacin metabolites comprised about 80% 2-Pyr, 11% MNA, 3% nicotinamide, 3% 4-Pyr, and nicotinamide N-oxide 3% as shown in Fig. 2C. Urinary excretion of nicotinic acid and nicotinuric acid was not detected.

On day 1 after nicotinamide injection, 1.7% of the dose was excreted as unchanged nitocinamide, 13.4% as nicotinic acid, 7.9% as nicotinuric acid, 3.8% as nicotinamide N-oxide, 2% as 2-Pyr, 0.3% as MNA, and 0.1% as 4-Pyr. The increases in MNA, 2-Pyr, and 4-Pyr excretion were each around 20-fold and that of nicotinamide N-oxide was 654-fold. Urinary excretion of nicotinic acid and nicotinuric acid accounted for about 70% of the urinary excretion of nicotinamide and its metabolites on day 1 after nicotinamide injection; but, on day 2, urinary
Table 2. Urinary excretion of nicotinamide and its metabolites before and after injecting mouse, guinea pig, and hamster with a pharmacological dose of MNA (500 mg/kg body wt).*

|                 | Day before injection | Day 1       | Day 2       |
|-----------------|----------------------|-------------|-------------|
| **Mouse**       |                      |             |             |
| Nam             | 0.20±0.03            | 0.52±0.11   | 0.32±0.03   |
| MNA             | 0.19±0.13            | 31.5±6.17   | 1.05±0.42   |
| 2-Pyr           | 0.23±0.04            | 10.0±1.64   | 1.15±0.42   |
| 4-Pyr           | 0.12±0.04            | 6.79±1.10   | 1.53±0.54   |
| Nam N-oxide     | 0.41±0.02            | 0.80±0.09   | 0.48±0.03   |
| **Guinea pig**  |                      |             |             |
| Nam             | 0.07±0.03            | 0.13±0.02   | 0.19±0.04   |
| MNA             | 0.28±0.03            | 410±19.9    | 0.35±0.07   |
| 2-Pyr           | 2.96±0.15            | 156±35.2    | 3.70±0.06   |
| 4-Pyr           | 0.14±0.02            | 6.73±2.04   | 0.15±0.01   |
| Nam N-oxide     | 0.10±0.04            | 0.09±0.03   | 0.08±0.03   |
| **Hamster**     |                      |             |             |
| Nam             | 0.55±0.14            | 1.11±0.33   | 0.75±0.08   |
| MNA             | 0.12±0.06            | 149±5.70    | 0.32±0.07   |
| 2-Pyr           | 0.18±0.06            | 67.1±6.60   | 0.64±0.08   |
| 4-Pyr           | 0.09±0.03            | 31.4±6.81   | 0.35±0.03   |
| Nam N-oxide     | 0.27±0.07            | 0.47±0.15   | 0.33±0.05   |

*Values are expressed as μmol/day and means±SEM for 4-5 rodents. Abbreviations, see Table 1.

Urinary excretion of nicotinic acid and nicotinuric acid was not observed. By day 4, urinary excretion of nicotinamide and its metabolites had returned to normal levels (data not shown).

Urinary excretion of nicotinamide and its metabolites before and after the injection of a pharmacological amount of MNA is shown in Table 2. On day 1 after injection, the urinary excretion of 2-Pyr and 4-Pyr increased by 57- and 52-fold, and 28% of the dose was excreted as unchanged MNA, 10.4% as 2-Pyr, and 0.5% as 4-Pyr. By day 2, urinary excretion of MNA and its metabolites 2-Pyr and 4-Pyr had returned to normal levels.

**Hamster**

Urinary excretion of nicotinamide, MNA, 2-Pyr, 4-Pyr, nicotinamide N-oxide, nicotinic acid, and nicotinuric acid before and after injection of a pharmacological amount of nicotinamide is shown in Table 2. Under physiological conditions, urinary excretion of major niacin metabolites comprised about 44% nicotinamide, 21% 2-Pyr, 15% nicotinamide N-oxide, 10% MNA, and 10% 4-Pyr as shown in Fig. 2D. Urinary excretion of nicotinic acid and nicotinuric acid was not detected.
On day 1 after nicotinamide injection, 12% of the dose was excreted as unchanged nicotinamide, 14.4% as nicotinuric acid, 1.4% as nicotinic acid, 1.4% as nicotinamide N-oxide, 0.4% as 2-Pyr, 0.3% as 4-Pyr, and 0.2% as MNA. The increases in MNA, 2-Pyr, and 4-Pyr excretion were each around 10-fold and that of nicotinamide N-oxide was 36-fold. Urinary excretion of nicotinic acid and nicotinuric acid was observed only on day 1 after injection. By day 3, urinary excretion of nicotinamide and its metabolites had returned to normal levels (data not shown).

Urinary excretion of nicotinamide and its metabolites before and after the injection of a pharmacological amount of MNA is shown in Table 2. On day 1 after injection, the urinary excretion of 2-Pyr and 4-pyr increased by 373- and 350-fold, respectively; 41% of the dose was excreted as unchanged MNA, 17% as 2-Pyr, and 9% as 4-Pyr; and slight increases in nicotinamide and nicotinamide N-oxide were observed. By day 4, urinary excretion of MNA and its metabolites, including 2-Pyr and 4-Pyr, had returned to normal levels (data not shown).

The ratio of 2-Pyr to 4-Pyr excretion in mouse, guinea pig, and hamster

Table 3 shows the ratio of 2-Pyr to 4-Pyr excretion in mouse, guinea pig, and hamster before and after injection of nicotinamide or MNA. Under physiological conditions, this ratio was around 2, 25, and 2, respectively, in mouse, guinea pig, and hamster. After intraperitoneal injection of a pharmacological amount of nicotinamide or MNA, the ratio changed little in all animals.

Nicotinamide methyltransferase, 2-Pyr-forming MNA oxidase, and 4-Pyr-forming MNA oxidase activity and the ratio of 2-Pyr-forming MNA oxidase to 4-Pyr-forming MNA oxidase

Nicotinamide methyltransferase activity was considerably lower than that of MNA oxidase in mouse, guinea pig, and hamster as shown in Table 4. 2-Pyr-forming MNA oxidase activity was higher than 4-Pyr-forming MNA oxidase activity in all animals. The ratio of 2-Pyr-forming MNA oxidase to 4-Pyr-forming MNA oxidase activity was about the same as the ratio of 2-Pyr to 4-Pyr excretion in all animals, as shown in Tables 3 and 4.

DISCUSSION

We have reported that in omnivora, 2-Pyr accounted for 60–90% of the total urinary excretion of nicotinamide and its metabolites; in carnivora, MNA accounted for more than 90% of the same; in herbivora, nicotinamide accounted for 80–100%; and in all of these mammals, the urinary excretion of nicotinic acid, nicotinuric acid, and nicotinamide N-oxide was below the limit of detection (13).

In rat, the urinary excretion of 4-Pyr accounted for about 80% of the total urinary excretion of nicotinamide and its metabolites (1, 2). To determine whether it is only the rat that excretes such high levels of 4-Pyr, the urinary excretion of
Table 3. Ratio of 2-Pyr to 4-Pyr excretion before and after injection of a pharmacological dose of nicotinamide\textsuperscript{a} or MNA.\textsuperscript{b}

|                | Day before injection | Day 1       | Day 2       |
|----------------|----------------------|-------------|-------------|
| **Mouse**      |                      |             |             |
| (Nicotinamide load) | 2-Pyr/4-Pyr          | 2.29 ± 0.22 | 1.86 ± 0.16 | 1.77 ± 0.34 |
|                | (MNA load)           | 2.14 ± 0.21 | 1.68 ± 0.06 | 2.21 ± 0.11 |
| **Guinea pig** |                      |             |             |
| (Nicotinamide load) | 2-Pyr/4-Pyr          | 26.6 ± 0.70 | 24.9 ± 0.45 | 25.1 ± 1.64 |
|                | (MNA load)           | 22.5 ± 1.65 | 26.0 ± 2.10 | 24.2 ± 0.53 |
| **Hamster**    |                      |             |             |
| (Nicotinamide load) | 2-Pyr/4-Pyr          | 2.12 ± 0.19 | 1.92 ± 0.05 | 1.98 ± 0.08 |
|                | (MNA load)           | 2.24 ± 0.12 | 1.98 ± 0.01 | 1.82 ± 0.01 |
| **Rat**        |                      |             |             |
| (Nicotinamide load) | 2-Pyr/4-Pyr          | 0.1 ± 0.01  | 0.6 ± 0.05  | 0.5 ± 0.05  |
|                | (MNA load)\textsuperscript{d} | 0.08 ± 0.02 | 1.50 ± 0.16 | 0.17 ± 0.04 |

Values are means ± SEM for 4–5 rodents. \textsuperscript{a} Rodent was injected intraperitoneal with a pharmacological amount of nicotinamide (500 mg/kg body wt). \textsuperscript{b} Rodent was injected intraperitoneal with a pharmacological amount of MNA-Cl (500 mg/kg body weight). \textsuperscript{c} Cited from Shibata, K. (1989): *J. Nutr.*, 119, 892–895. \textsuperscript{d} Cited from Shibata, K. and Matsuo, H. (1989): *Agric. Biol. Chem.*, 53, 1161–1162.

Table 4. Liver nicotinamide methyltransferase, 2-Pyr-forming MNA oxidase, and 4-Pyr-forming MNA oxidase activities in mouse, guinea pig, and hamster.

|                | Nicotinamide methyltransferase | 2-Pyr-forming MNA oxidase | 4-Pyr-forming MNA oxidase |
|----------------|-------------------------------|---------------------------|--------------------------|
| **Mouse**      | 113 ± 23.7                    | 4,122 ± 395               | 2,862 ± 270              |
| **Guinea pig** | 113 ± 26                      | 7,811 ± 643               | 332 ± 42                 |
| **Hamster**    | 57 ± 7.1                      | 7,035 ± 40.2              | 3,838 ± 30.8             |

Values are expressed as nmol/h/g liver and means ± SEM for 4–5 rodents.

*J. Nutr. Sci. Vitaminol.*
nicotinamide and its metabolites were examined in other rodents, namely, mouse, guinea pig, and hamster. In mouse, nicotinamide N-oxide excretion was the highest of nicotinamide and its metabolites as reported by Chaykin et al. (5), in guinea pig, 2-Pyr as reported by Perlzweig et al. (4), and in hamster, nicotinamide (Fig. 2). In mouse and hamster, nicotinamide was generally metabolized equally into MNA, 2-Pyr, 4-Pyr, and nicotinamide N-oxide. Nicotinamide N-oxide might be a characteristic niacin metabolite excreted in the urine in rodent.

Urinary excretory patterns of nicotinamide metabolites in rat (1,2), mouse, guinea pig, and hamster were changed by a pharmacological dose of nicotinamide as shown in Fig. 3. Urinary excretion of nicotinic acid and nicotinuric acid was observed in rat (1, 2), guinea pig, and hamster but not in mouse. Chaykin et al (5)
reported that there are two types of mice: one can convert nicotinamide into nicotinic acid, and one cannot. None of the mice used here could convert nicotinamide into nicotinic acid. In rat, deamidated metabolites nicotinic acid and nicotinuric acid accounted for 36.1% of the total nicotinamide metabolites (1, 2), in guinea pig 76.7%, and in hamster 87.2%. Therefore, the nicotinamidase reaction occurs only when a higher concentration of nicotinamide is present in the body. Perlzweig et al. (4) also report that nicotinuric acid excretion was detected only when a large amount of nicotinamide was fed to guinea pig. The $K_m$ value of nicotinamide for nicotinamidase is 0.1 m in rat (14), so in guinea pig and hamster it might be also be around 0.1 m.

The percentage of 2-Pyr excretion decreased greatly when a pharmacological dose of nicotinamide was administered in mouse (24% vs. 11.2%), guinea pig (82.5% vs. 7.5%), and hamster (37.5% vs. 2.2%), but not in rat (6.3% vs. 6.5%) (1, 2) as shown in Fig. 3. The percentage of 4-Pyr excretion also greatly decreased on administration of a pharmacological dose of nicotinamide in rat (85.4% vs. 13%) (1, 2), mouse (13% vs. 6.3%), guinea pig (3.1% vs. 0.4%), and hamster (17.9% vs. 1.6%) as shown in Fig. 3. That is, the decrease in the percentage of 2-Pyr excretion by nicotinamide loading was about the same as that of 4-Pyr excretion in mouse, guinea pig, and hamster. The same results were observed when MNA was loaded, as shown in Table 3. These findings mean that 2-Pyr and 4-Pyr are synthesized from MNA by a single enzyme in mouse, guinea pig, and hamster, but separate enzymes in rat (15). The percentage of pyridones excreted decreased on nicotinamide loading in all the other rodents examined here as well as in rat (1, 2). 2-Pyr-forming MNA oxidase and 4-Pyr-forming MNA oxidase activities might be inhibited by nicotinamide loading. In fact, these two enzyme activities were inhibited by nicotinamide loading in rat: 2-Pyr-forming MNA oxidase was 45% of the control and 4-Pyr-forming MNA oxidase was 7% of the control, when rat was killed 5 h after nicotinamide injection (2).

The percentage of MNA excreted also decreased on nicotinamide loading in mouse (20% vs. 4.9%), guinea pig (11.3% vs. 1.1%), and hamster (17.8% vs. 1.1%); but in rat, it increased (7.3% vs. 28%) (1, 2) as shown in Fig. 3. Nicotinamide methyltransferase activity in rat is slightly activated by nicotinamide loading (2); but, in mouse, guinea pig, and hamster, it might be inhibited.

The percentage of nicotinamide $N$-oxide excreted increased in rat (1% vs. 16.2%) (2), mouse (43% vs. 79.7%), and guinea pig (3.1% vs. 14.3%); but, it decreased in hamster (26.8% vs. 7.7%) as shown in Fig. 3.

When a large amount of MNA was injected intraperitoneally into rat, the ratio of 2-Pyr to 4-Pyr excreted increased greatly (3). This is a result of higher inhibition of 4-Pyr-forming MNA oxidase than 2-Pyr-forming MNA oxidase by MNA loading (3). This result also supports the hypothesis that 2-Pyr and 4-Pyr are synthesized by separate enzymes in rat. However, the ratio of 2-Pyr to 4-Pyr excretion in mouse, guinea pig, and hamster was not changed by MNA loading (Table 3). This result strengthens the hypothesis that 2-Pyr and 4-Pyr are synthesized from

\[ J. \text{ Nutr. Sci. Vitaminol.} \]
NIACIN CATABOLISM IN RODENTS

MNA by a single enzyme.

Nicotinamide methyltransferase, 2-Pyr-forming MNA oxidase, and 4-Pyr-forming MNA oxidase activities in rat liver under physiological conditions are around 500, 650, and 2,500 nmol/h/g liver (2). Compared with rat (2), nicotinamide methyltransferase activities in mouse, guinea pig, and hamster were lower. 2-Pyr-forming MNA oxidase activities were much higher in mouse, guinea pig, and hamster than in rat. 4-Pyr-forming MNA oxidase activities in mouse and hamster were similar to those in rat, but the activity in guinea pig was much lower than in rat. The ratio of 2-Pyr-forming MNA oxidase to 4-Pyr-forming MNA oxidase activity was the same as the ratio of 2-Pyr to 4-Pyr excretion in mouse, guinea pig, and hamster. But in rat, the enzyme ratio was not the same as urinary excretion ratio. This is due to the fact that rat liver 4-Pyr-forming MNA oxidase is inactivated during catalysis in vitro. Therefore, in all rodents examined here, the ratio of 2-Pyr to 4-Pyr excretion reflects the activity ratio of liver 2-Pyr-forming MNA oxidase to 4-Pyr-forming MNA oxidase in vivo.

REFERENCES

1) Shibata, K. (1989): Fate of excess nicotinamide and nicotinic acid differs in rats. J. Nutr., 119, 892–895.
2) Shibata, K., and Matsuo, H. (1989): Inhibition of N1-methylnicotinamide oxidase activity by a large nicotinamide injection into rats. Agric. Biol. Chem., 53, 2031–2036.
3) Shibata, K., and Matsuo, H. (1989): Inhibition of N1-methylnicotinamide oxidase activity by a large amount of N1-methylnicotinamide injected into rats. Agric. Biol. Chem., 53, 2393–2397.
4) Perlzweig, W. A., Rosen, F., and Pearson, P. B. (1950): Comparative studies in niacin metabolism. The fate of niacin in man, rat, dog, pig, rabbit, guinea pig, goat, sheep and calf. J. Nutr., 40, 453–469.
5) Chaykin, S., Dagani, M., Johnson, L., and Samli, M. (1965): The fate of nicotinamide in the mouse. J. Biol. Chem., 240, 932–938.
6) Pullman, M. E., and Colowick, S. P. (1954): Preparation of 2- and 6-pyridones of N1-methylnicotinamide. J. Biol. Chem., 206, 121–127.
7) Shibata, K., Kawada, T., and Iwai, K. (1988): Simultaneous micro-determination of nicotinamide and its major metabolites, N1-methyl-2-pyridone-5-carboxamide and N1-methyl-4-pyridone-3-carboxamide, by high-performance liquid chromatography. J. Chromatogr., 424, 23–28.
8) Shibata, K. (1986): Nutritional factors affecting the activity of nicotinamide methyltransferase and urinary excretion of N1-methylnicotinamide in rats. Agric. Biol. Chem., 50, 1489–1493.
9) Shibata, K., Taguchi, H., and Iwai, K. (1988): Effects of dietary protein levels on the enzyme activities involved in tryptophan-niacin metabolism in rats. Agric. Biol. Chem., 52, 3165–3167.
10) Shibata, K. (1989): High-performance liquid chromatographic measurement of nicotinamide N-oxide in urine after extracting chloroform. Agric. Biol. Chem., 53, 1329–1331.
11) Shibata, K. (1987): Ultramicro-determination of N1-methylnicotinamide in urine by Vol. 36, No. 2, 1990
high-performance liquid chromatography. *Vitamins (J. Vitamin Soc. Jpn.),* 61, 599–604.

12) Shibata, K. (1988): Simultaneous measurement of nicotinic acid and its major metabolite, nicotinuric acid in blood and urine by a reversed-phase high-performance liquid chromatography. *Agric. Biol. Chem.*, 52, 2973–2976.

13) Shibata, K., Taguchi, H., and Sakakibara, Y. (1989): Comparison of urinary excretion of niacin and its metabolites in various mammals. *Vitamins (J. Vitamin Soc. Jpn.),* 63, 369–372.

14) Petrack, B., Greengard, P., Craston, A., and Sheppy, F. (1965): Nicotinamide deamidase from mammalian liver. *J. Biol. Chem.*, 240, 1725–1930.

15) Stanulovic, M., and Chaykin, S. (1971): Metabolic origins of the pyridones of N1-methylnicotinamide in man and rats. *Arch. Biochem. Biophys.*, 145, 35–42.