THE RELATION BETWEEN URINARY EXCRETION OF TRYPTOPHAN AND NIACIN METABOLITES AND PYRIDINE NUCLEOTIDES IN THE LIVER IN RATS FED A TRYPTOPHAN-DEFICIENT DIET

Sada OHGURI

Department of Maternal and Child Health, Institute of Public Health, Shirokanedai, Minato-ku, Tokyo
(Received April 24, 1974)

The determination of nicotinamide adenine nucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) levels in the liver and urinary excretion of tryptophan niacin metabolites were made in rats fed a tryptophan-deficient diet. Under experimental conditions excretion of N1-methyl-2-pyridone-5-carboxiamide (pyridone) decreased by approximately one-third in the tryptophan-deficient group when compared with the control group. A rapid decrease in body weight was shown in the tryptophan-deficient group when compared with the control group. The level of NAD+ and NADP+ and total PN+ in the tryptophan- and niacin-deficient group was decreased significantly when compared with other deficient group (niacin- or nicotinamide-added group) and the control group except for NADP+ . No statistical difference was seen on the addition of nicotinic acid or nicotinamide. But a slightly lower level of urinary nicotinic acid was observed in the group with nicotinamide added when compared with group with nicotinic acid added. Simultaneously, a high level of NAD+ was observed in the group with nicotinamide added. Thus, a difference in availability between nicotinic acid and nicotinamide might be expected.

It is well known that NAD and NADP are synthesized in the body not only from nicotinamide and nicotinic acid, but also from tryptophan. We wished to investigate the change of NAD and NADP level on the basis of whether or not the substrate is present and difference in substrate. We also wanted to study the effect of tryptophan deficiency on the urinary excretion of tryptophan niacin metabolites in rats.

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EXPERIMENT 1

Thirty female Wistar rats (26-30 g weight) were used. They were fed ad libitum for 3 weeks (basal diet), and divided into 2 groups and housed in individual cages. One group received a tryptophan-deficient diet (deficient group) and other group received a basal diet (control group). After the rats had been fed with these diets for 2 weeks, the tryptophan-deficient diet was replaced with a basal diet for the succeeding 2 weeks.

**Diet.** The composition of diet and the administration were similar to those described above.

**Methods.** The urine samples were collected during the last 3 days of each period. Five rats were then killed in the preliminary period, and 7 rats of the control group and 6 rats of tryptophan-deficient group, respectively, were killed in the experimental and rehabilitation period.

**NAD⁺ and NADP⁺ analyses** were carried out on individual samples from each group. The urinary excretion of pyridone and N²-methylnicotinamide (MNA) were estimated by methods described above. The pyridine nucleotides in the liver were also estimated. The animals killed with dried ice and acetone. The complete liver was removed and weighed, then homogenized with 2 N HClO₄ and Tris-buffer (pH 7.2) and ice distilled water. After deproteinization with HClO₄, the sample was treated with alchohol dehydrogenase and isotitric dehydrogenase, and then a methylethylketone procedure was performed. Total pyridine nucleotide was estimated by the method of Jacobson *et al.* using ketone condensation.

**Results and Discussion**

**Gain in weight.** The results obtained agree very well those obtained in the first experiments on the effects of tryptophan deficiency on body weight, that is, rapid decrease in body weight when the tryptophan-deficient group is compared with the control group.

**Excretion of pyridone and MNA.** Urinary excretion level of pyridone and MNA are shown in Table 1. Pyridone excretion decreased by approximately one-third in the deficient group as compared with that of control group. The excretion of the sum of MNA and pyridone was nearly at the same level in both groups in the experimental and rehabilitation period, respectively. These results indicate a decrease in the excretion of pyridone and, simultaneously, an increase of the excretion of MNA. The level of NAD⁺ and NADP⁺ and total PN⁺ in liver and weight are shown in Table 2. The data show a decrease in the level of NAD⁺ and NADP⁺ in the deficient group compared with the control group. Especially, NADP⁺ decreased as much 50 per cent of the level of control group. When tryptophan is deficient, the decrease of NAD⁺ and NADP⁺ level is considered, because of the blocking of the biosynthesis of NAD and NADP from
tryptophan as a substrate.

SHIMOYAMA et al. (1) reported the activity of NAD dehydrogenase and the amount of NAD and other enzymes activity in the rat organ during starvation. They found a slight decrease in the amount of NAD with the progress of starvation.

EXPERIMENT 2

This sequence of experimentation was repeated in order to confirm the experimental results. Furthermore, the experiment was carried out on the effects of different substrates on the formation of NAD and NADP level in rat liver. Experiments were carried out to study the effects on the difference between nicotinic acid and nicotinamide as substrate.

Materials and Methods

Thirty female Wistar rats, 35–49 g in weight, were used in this study. After feeding rats with a basal diet (preliminary period), they were divided into 4 groups. Rats of group 1 received the basal diet, and nicotinamide was injected directly into their stomach. Rats of group 2 received the tryptophan-deficient
diet, and nicotinamide or nicotinic acid were injected into them. Rats of group 3 received the tryptophan deficient diet, and nicotinic acid was injected into their stomach. Rats of group 4 received tryptophan-deficient diet, and nicotinamide was injected into their stomach.

After the rats were fed these diets for 2 weeks, urine samples were collected and 4 rats were killed, and in next 2 weeks, after urine was collected, 3 rats of each deficient group and 4 rats of the control group were killed.

The composition and administration of diets were similar to the experiment described above. The urinary excretion of tryptophan and niacin metabolites were estimated. In this experiment, urinary excretion of nicotinic acid was estimated by a microbiological procedure with L-arabinosus. NAD\(^+\) and NADP\(^+\) and Total PN\(^+\) in liver were estimated by the previously described method.

**Results and Discussion**

Body weight, liver weight, level of NAD\(^+\) and NADP\(^+\), total PN\(^+\) and the ratio of NAD\(^+\) to NADP\(^+\) are shown in Table 3. These results are in agreement with our previous experiment showing lower levels of NAD\(^+\) and NADP\(^+\) and total PN\(^+\) in tryptophan deficiency that are lower than those of the control group. Rats of tryptophan and niacin-deficient group especially, showed significantly lower levels of NAD\(^+\) and total PN\(^+\) when compared with those of other deficient groups and the control group. NADP\(^+\) was also low, but not significantly different. However, there was no difference between the administration of nicotinamide or nicotinic acid when the tryptophan was

|                  | Body weight g | Liver weight g | Per rat per liver g m\(\mu\)mol | NAD/NADP |
|------------------|---------------|----------------|----------------------------------|----------|
| Trp def. diet    |               |                |                                  |          |
| - NiA            | 82            | 3.80           | 208                              | 2.5      |
|                  | 75            | 3.83           | 124                              |          |
|                  | 64            | 3.00           | 223                              |          |
|                  |               |                | 185                              |          |
| Trp def. diet    | 65            | 2.93           | 193                              | 3.32     |
| + NiA            | 72            | 3.66           | 238                              | 3.88     |
|                  | 53            | 1.90           | 306                              | 4.25     |
|                  |               |                | 246                              |          |
| Trp def. diet    | 67            | 3.74           | 213                              | 2.3      |
| + NiAA           | 76            | 4.12           | 304                              | 3.9      |
|                  | 59            | 3.12           | 323                              |          |
|                  |               |                | 280                              |          |
| Hasal diet       | 85            | 3.41           | 471                              | 2.3      |
| + NiA            | 113           | 4.61           | 379                              |          |
|                  | 120           | 5.85           | 375                              |          |
|                  | 123           | 5.15           | 563                              |          |
|                  |               |                | 447                              |          |

Table 3. Body weight, liver weight, NAD\(^+\), NADP\(^+\) and total PN\(^+\) per rat per liver g m\(\mu\)mol.
Table 4. Daily urinary excretion of N1-methylnicotinamide, nicotinic acid and pyridone in rats (µg).

| Group          | No. of rat | Nicotinic acid | N1-Methylnicotinamide | Pyridone |
|---------------|------------|----------------|------------------------|----------|
|               |            | per rat/day    | per g food consumed    | per rat/day | per g food consumed |
| Trp def. diet -NiA | 5          | 10.7           | 1.8                    | 15.8      | 2.4                  | 0.4  | 0.06 |
| Trp def. diet +NiA | 5          | 19.2           | 2.0                    | 57.8      | 6.0                  | 0.8  | 0.08 |
| Trp def. diet +NiAA | 5          | 15.0           | 2.6                    | 143.0     | 24.9                 | 0.5  | 0.09 |
| Basal diet +NiA | 4          | 19.3           | 2.7                    | 22.3      | 4.2                  | 1.0  | 0.14 |

deficient. The urinary excretion of MNA, nicotinic acid and pyridone is shown in Table 4.

**Nicotinic acid excretion.** As might have been expected, nicotinic acid excretion level was lower in the tryptophan and nicotinic acid-deficient group when compared with other 3 groups. There is no statistical difference in nicotinic acid excretion between the group with nicotinic acid added and the group with nicotinamide added. But slightly lower level of urinary nicotinic acid was observed in the group with nicotinamide added when compared with the group with nicotinic acid added. Simultaneously, slight high level of NAD⁺ was observed in the group with nicotinamide added. Thus it might be expected that there is difference in the availability of nicotinic acid and nicotinamide.

**MNA excretion.** A very low level of urinary MNA excretion was found when the tryptophan- and nicotinic acid-deficient group was compared with 3 other groups. A high level of excretion was found in the tryptophan-deficient group and the group with nicotinic acid added or nicotinamide added when compared with the control group (p<0.01). Especially, significant high level of MNA was found in the tryptophan deficient group with nicotinamide added (p<0.05).

**Pyridone excretion.** A low level of excretion was found in the tryptophan-deficient groups, whether nicotinic acid or nicotinamide was present or not when compared with the control group. The difference of level of pyridone between control and tryptophan and nicotinic acid-deficient group was statistically significant (p<0.05).

**REFERENCES**

1) SHIMOYAMA, M., OTA, M., YAMAGUCHI, K., and UEDA, I., *J. Jap. Soc. Food and Nutr.* (in Japanese), 19, 2 (1966).