EFFECT OF TRYPTOPHAN DEFICIENCY ON THE URINARY EXCRETION OF TRYPTOPHAN AND NIACIN METABOLITES IN RATS

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The effect of tryptophan deficiency on the urinary excretion of tryptophan and niacin metabolites and riboflavin, and on nitrogen balance has been examined in rats.
The excretion of xanthurenic acid decreases, and the excretion of N\(^1\)-methylnicotinamide (MNA) slightly increases when tryptophan is deficient. But the increase in MNA excretion was not statistically significant, perhaps owing to the scatter of the data.
Decrease in the excretion of N-methyl-2-pyridone-5-carboxamide (pyridone) is as low as 1/2 to 1/20 of the control level when tryptophan is deficient. The excretion of riboflavin increases when tryptophan is deficient.

It has been assumed that protein deficiency results in adverse effects on the formation of body tissues and on growth and development. Moreover, with a high deficiency in protein, expense of body protein and a significant decrease in enzyme activity might occur. The change of free amino acid and related materials in the body may serve as sensitive indices for the assessment of protein nutrition. The metabolism of amino acid is very complicated, and there remain many problems to be resolved. Tryptophan metabolism is particularly unique, and several metabolic pathways for it are known. There are many problems to be resolved regarding metabolism in tryptophan deficiency.

In order to elucidate the effect of tryptophan deficiency on tryptophan and niacin metabolism, we have studied the effect of tryptophan deficiency on the urinary excretion of tryptophan metabolites in rats. Experiments were carried out on the excretion of tryptophan and niacin metabolites when tryptophan deficiency is induced by excluding tryptophan from an amino acid mixture.
**EXPERIMENTAL**

*Materials and Methods.* Thirty female Wistar rats, 30–40 g in weight, were used in the study. After a week on commercial rations, a basal diet was given ad libitum for 7 days, and then the rats were divided into three groups and housed in individual cages. Rats of group I received a tryptophan-deficient diet and rats of group II received a methionine-deficient diet and rats of group III received a control diet.

After having fed the rats with these diets for 10 days, each group received the basal diet for 9 days (rehabilitation period). The composition of the experimental diet is shown in Table 1. The amino acid mixture described by Miyazaki pattern No. 18, was used.

| Table 1. Composition of experimental diet. |
|-------------------------------------------|
| Amino acid mixture<sup>a</sup> | 15% |
| Cornstarch | 47 |
| Sucrose | 30 |
| Salt mixture<sup>b</sup> | 5 |
| Soybean oil and drop of halibut oil | 1 |
| Cellulose powder | 1 |
| Vitamin mixture<sup>c</sup> | 1 |

<sup>a</sup> By Miyazaki pattern No. 18.

<sup>b</sup> By Harper.

<sup>c</sup> Thiamine 20 µg, pyridoxine 20 µg, DL-pantothenic acid 150 µg, riboflavin 20 µg, nicotinic acid 500 µg, choline 5 mg per rat per day.

The amount of food consumed during the period was measured, and the animals weight was measured at weekly intervals. The urine sample were collected under toluene for the last 3 days of the experimental period and, of the rehabilitation period, and usually diluted to 100 ml with distilled water and frozen until ready for analyses.

The urine nicotinic acid was determined by microbiological procedures with L-arabinosus as the test organism, and MNA was determined by the Huff acetone condensation methods, pyridone by the method of Price and xanthurenic acid colorimetrically by the method of Price. Total nitrogen of urine was determined by the micro-Kjeldahl method.

**RESULTS AND DISCUSSION**

The results obtained are shown in Fig. 1. The values presented in Fig. 1 are the averages for the last 3 days of each experimental period for all subjects. The intake amount of diet and N-balance are shown in Table 2. The daily intake amount of diet was reduced both in the tryptophan- and methionine-
deficient groups. With tryptophan or methionine supplement the rats received a diet intake the same or more in amount as in the control rats. The body weight of rats in groups I and II immediately decreases during the deficient period, and increased readily during the rehabilitation period, although there was no difference between rats in group I and II.

Table 2. Average diet intake and nitrogen balance.

|                | Experimental period |                         | Rehabilitation period |
|----------------|---------------------|-------------------------|-----------------------|
|                | Diet intake/day/rat | Nitrogen intake/day    | Output nitrogen/day   |
|                | (g)                 | (g)                     | (g)                   |
|                |                     |                         | Balance nitrogen/day  |
|                |                     |                         | (±)                   |
| Group III      | 12.1                | 0.19                    | 0.11                  | +0.08                 |
| Group I        | 6.5                 | 0.10                    | 0.06                  | +0.04                 |
| Group II       | 4.6                 | 0.07                    | 0.07                  | ±0.00                 |
|                | 10.7                | 0.17                    | 0.11                  | +0.06                 |
|                | 10.8                | 0.17                    | 0.08                  | +0.09                 |
|                | 11.3                | 0.18                    | 0.10                  | +0.08                 |
Nitrogen balance; The retention of nitrogen in control group (group III) during the experimental period was maintained +0.08 g on the average; it was +0.04 g for the tryptophan-deficient group (group I) and ±0.00 g for the methionine-deficient group (group II). In the rehabilitation period, it was +0.09 g for the retention for group I, +0.08 g for group II, and +0.06 for group III.

Excretion of riboflavin; The daily excretion of urinary riboflavin increased in rats of group I and II during the experimental period, but decreased after the administration of tryptophan or methionine in the rehabilitation period (average excretion of rats of group I, 5.6 µg, group II, 6.2 µg in experimental period, and in rehabilitation period 3.6 µg and 3.8 µg, respectively). In the control group the excretion remained at a fairly constant level throughout the study (on the average, 3.4 and 2.3 µg).

The amounts of riboflavin excreted in the deficient groups were higher than those of the control. The results obtained agree with those obtained by POLLACK and BOOKMAN (1) or SMITH et al. (2).

According to POLLACK and BOOKMAN (1), labile protein, which includes flavoproteins, increases or decreases rapidly as the body shifts from a negative to a positive nitrogen balance or from a positive to a negative balance, respectively. The amount of riboflavin stored in conjunction with these protein would likewise change rapidly.

When nitrogen balance became negative by exclusion of amino acid, the excretion of riboflavin increased as in the case of protein. SMITH et al. (2) reported that when riboflavin intake was held constant and the nitrogen balance became negative by decreasing the intake of protein, urinary riboflavin increased.

Excretion of xanthurenic acid; The excretion of xanthurenic acid in rats of the deficient groups was apparently lower than that of the control group. Rats of tryptophan-deficient group excreted 102 µg of xanthurenic acid on the average, the methionine-deficient group, 133 µg, and the control group, 270 µg per day. Excretion of xanthurenic acid in urine of rats of deficient groups increased following the administration of tryptophan or methionine (rehabilitation period): in rats of the tryptophan-deficient group, it increased to 191 µg and in the methionine-deficient group to 255 µg, but there is no difference statistically between the two groups. The control group increased to 280 µg per day (see Table 3). SAMUEL et al. (3) found that in pyridoxine-deficient rats, xanthurenic acid originated from dietary tryptophan.

The feeding of a tryptophan-deficient diet should result in the disappearance of xanthurenic acid from urine of pyridoxine-deficient rats and the addition of tryptophan should cause reappearance of xanthurenic acid in the urine of such rats. Tryptophan-deficient rats excreted 0.03 per cent xanthurenic acid in the urine, and 0.7 per cent was excreted by rats receiving tryptophan in the diet (rehabilitation period).

MILLER and BAUMAN (4) observed that after the intraperitoneal injection
of L-tryptophan, pyridoxine-deficient rats excreted 12 to 16 per cent xanthurenic acid in the urine, while only 1 per cent was excreted by rats receiving pyridoxine in the diet.

Table 3. Tryptophan intake and xanthurenic acid excretion.

| Group   | Tryptophan intake (mg) | Excretion per day (µg) | per cent of dose (%) | Tryptophan intake (mg) | Excretion per day (µg) | per cent of dose (%) |
|---------|------------------------|------------------------|----------------------|------------------------|------------------------|----------------------|
| Group I | 31                     | 270                    | 0.9                  | 27.6                   | 280                    | 1.0                  |
| Group II| 0                      | 102                    | 0.9                  | 27.7                   | 191                    | 0.7                  |
| Group III | 11.8                  | 133                    | 1.1                  | 16.0                   | 255                    | 0.6                  |

Excretion of MNA; Rats of control group excreted 206 µg of MNA in the experimental period; the tryptophan-deficient group, 127 µg and the methionine-deficient group, 227 µg. In the rehabilitation period, rats of control group excreted 200 µg of MNA; the tryptophan-deficient group, 108 µg; and the methionine-deficient group, 173 µg.

It has been observed that the urinary excretion of MNA increased when tryptophan was excluded from an amino acid mixture, and the increase of MNA excretion was also observed in methionine-deficient group. But these differences were not statistically significant.

According to Nakagawa et al. (5) urinary excretion of MNA also increased during lysine-deficient period. Huff and Perlzweig (6, 7) observed that in humans and rat, only 30 to 40 per cent of niacin administered could be recovered as RQA in the urine, even after prolonged feeding of niacin. In our experiment, MNA was recovered in the urine. The recovery in control group was 64 per cent; in tryptophan-deficient group in experimental period, the recovery was 57 per cent and in the rehabilitation period, 33 per cent; methionine-deficient group in the experimental period 133 per cent, and in the rehabilitation period, 50 per cent, respectively.

Excretion of pyridone; Rats of control group excreted 10.6 µg of pyridone in experimental period; the tryptophan-deficient group, 5.6 µg and the methionine-deficient group 0.5 µg; approximately pyridone as low as 1/2 to 1/20 was excreted in both deficient groups as compared with that of control group. The urinary levels of pyridone increased at control level in the rehabilitation period (control group 7.0 µg, tryptophan-deficient group 12.1 µg, methionine-deficient group 6.2 µg).

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