METABOLIC RELATIONSHIP BETWEEN TRYPTOPHAN, VITAMIN B₆ AND NICOTINIC ACID IN RATS

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Experiments were carried out in regard to the effect of tryptophan and vitamin B₆ deficiency on tryptophan and niacin metabolites. Tryptophan-deficient rats promptly lost weight, and the administration of tryptophan to the diet resulted in a rapid gain in weight. Nitrogen loss was found in tryptophan-deficient rats, and nitrogen retention occurred when tryptophan was supplemented to the diet. There was no apparent effect of vitamin B₆ supplement on the excretion of nitrogen. The increase of urinary xanthurenic acid by vitamin B₆-deficient rats was seen when tryptophan was supplemented. Vitamin B₆ had no effect on the excretion of niacin and N¹-methylnicotinamide (MNA) and N¹-methyl-2-pyridone-5-carboxiamide (pyridone).

It is well known that vitamin B₆ acts a coenzyme in the catalysis of a large number of biochemical reactions, particularly in those related to protein and amino acid metabolism. The ingestion of a large amount of tryptophan, either as a free amino acid or as casein, accentuates a deficiency of vitamin B₆ in rats and mouse (Miller and Bauman (1)). The amount of xanthurenic acid excreted by the vitamin B₆-deficient animal is markedly increased when the animal is fed a high level of tryptophan (Miller and Bauman (2)).

These findings suggested that vitamin B₆-deficiency may affect the metabolic conversion of tryptophan to nicotinic acid and MNA. Experiments were carried out on the effect of tryptophan and vitamin B₆ deficiency on tryptophan and nicotinic acid metabolites.

EXPERIMENT 1

Materials and Methods

Thirty-four female Wistar rats, 30-40 g in weight, were used in these studies. After 7 days on commercial rations, they were given a basal diet ad libitum for
7 days, divided into 3 groups and housed in individual cages. Rats in group 1 received a tryptophan and vitamin B₆-deficient diet, and nicotinic acid was injected directly into their stomach. Rats in group 2 received a tryptophan-deficient diet, and nicotinic acid was injected with vitamin B₆ directly into their stomach. Rats in group 3 received a tryptophan-deficient diet, and nicotinic acid and a large amount of vitamin B₆ were injected directly into their stomach (3 times as much as B₆ given in rats of group 2).

After 2 weeks on these diets, the deficient diets were replaced with a basal diet during the 4 weeks in all three groups.

The urine samples were collected during last 3 days of each period. The amount of food consumed was measured during each period. Animals were weighed at weekly intervals. Diets composition and administration were similar to those described previously. Urinary excretion of nicotinic acid, MNA, pyridone and xanthurenic acid were estimated by previously described methods.

Results and Discussion

The results for the nicotinic acid, MNA and pyridone, xanthurenic acid excretion per day and also per g of food consumed are shown in Table 1.

Gain in weight. The tryptophan-deficient rats promptly lost weight and when tryptophan was added to diet, body weight quickly increased. The gain in body weight of rats in the group with vitamin B₆-added was greater than rats in vitamin B₆-deficient groups, especially, greater in rats fed a large amount of vitamin B₆.

Table 1. Effect of vitamin B₆ on the urinary excretion of tryptophan and niacin metabolites in rats (µg). The figures in the parenthese are average excretion per day per cent of tryptophan consumed.

| Group | Diet regimen | No. | NiA per day /rat | MNA per day /rat | Pyridone per day /rat | XA per day /rat |
|-------|---------------|-----|-----------------|-----------------|----------------------|--------------|
|       |               |     | per g food consumed | per g food consumed | per g food consumed | per g food consumed |
| -B₆   | Trp def.      | 2w  | 491.6           | 88.9            | 166.6               | 29.4          | 1.44          | 0.25          | 31.4          | 5.5          |
|       | Basal         | 10  | 676.8           | 228.3           | 29.7                | 0.98          | 2.69          | 0.32          | 397.6         | 46.1         |
|       | Basal         | 4w  | 702.8           | 264.9           | 26.0                | 3.32          | 0.33          | 959.6         | 93.7         |
| +B₆   | Trp def.      | 2w  | 412.9           | 67.1            | 171.0               | 29.2          | 1.37          | 0.23          | 27.0          | 4.4          |
|       | Basal         | 10  | 522.9           | 262.9           | 23.6                | 0.91          | 3.06          | 0.28          | 110.9         | 10.0         |
|       | Basal         | 4w  | 492.3           | 293.7           | 26.3                | 0.96          | 4.17          | 0.36          | 187.0         | 16.3         |
| +B₆   | Trp def.      | 2w  | 513.2           | 81.5            | 221.1               | 36.5          | 1.25          | 0.20          | 44.7          | 7.4          |
|       | Basal (large) | 10  | 592.5           | 325.2           | 20.1                | 0.79          | 1.93          | 0.18          | 78.4          | 7.1          |
|       | Basal (large) | 4w  | 637.7           | 332.6           | 25.5                | 1.0           | 4.27          | 0.33          | 215.6         | 16.8         |


Excretion of nicotinic acid. Low excretion of nicotinic acid was observed in 3 groups during tryptophan-administration, regardless of presence or absence of vitamin B₆. However, when tryptophan is deficient, the greatest excretion of nicotinic acid in rats of vitamin B₆-deficient group as compared with vitamin B₆ added group was observed. HENDERSON et al. (3) found that the excretion of nicotinic acid by vitamin B₆-deficient rats was less than in control following tryptophan loading. SPECTOR (4) reported that vitamin B₆ had no significant influence on the transformation of amino acid to nicotinic acid or MNA in rats.

Excretion of MNA. Excretion of MNA in tryptophan-deficient rats was much more than that of rats administered tryptophan, regardless of presence or absence of vitamin B₆. When tryptophan was administered, the percentage of excretion of MNA to the intake of tryptophan was the same whether vitamin B₆ was administered or not; in vitamin B₆-deficient group the percentage was 0.98, vitamin B₆ administered group 0.94, the group fed vitamin B₆ in large amount 0.90.

Excretion of pyridone. The excretion of pyridone was larger in the group fed with tryptophan-added diet than in that with a tryptophan-deficient diet, regardless of presence or absence of vitamin B₆. The percentage of excretion of pyridone to the intake amount of tryptophan was also the same (0.01%) regardless of presence or absence of vitamin B₆.

Excretion of xanthurenic acid. There was significant change in excretion when tryptophan was added. Excretion of xanthurenic acid was significantly greater during the period when tryptophan was added than in the period when tryptophan was deficient, especially, in vitamin B₆-deficient group as compared with vitamin B₆ administered groups.

EXPERIMENT 2

The experiment sequence of experimentation was repeated in order to ascertain the effect of vitamin B₆ deficiency on the excretion of niacin and tryptophan metabolites.

Materials and Methods

Fifty Wistar rats, 21 days old, were fed commercial animal ration for one week following one week of basal diet, and were then divided into three groups. One group received a tryptophan-deficient diet which was deficient in vitamin B₆ (represented as the B₆-deficient group), and other group received a tryptophan-deficient diet which includes vitamin B₆ (represented as the B₆ administration group). The third group received a basal diet supplemented with vitamin B₆ (represented as the control group).

After 2 weeks on these diets, the tryptophan-deficient diet was replaced with a basal diet for 2 weeks.
Urine samples were collected during last 3 days of each period. The urinary excretion of nicotinic acid, MNA, pyridone were estimated by the described methods.

Results and Discussion

Food intake. The daily food intake of the rats fed tryptophan-deficient diets decreased to one-half that of control rats, regardless of presence or absence of vitamin $B_6$. When the diet was supplemented with tryptophan, food intake increased, but not in rats of the control group.

Nitrogen balance. Nitrogen loss was observed when tryptophan was deficient, and nitrogen retention occurred when tryptophan was supplemented. Supplementing vitamin $B_6$ has no apparent effect on the amount of nitrogen excreted.

Gain in weight. The tryptophan-deficient rats promptly lost weight, and when returned to a basal diet, their body weight immediately increased. Weight gain in rats of the group with vitamin $B_6$-added was better than that of vitamin $B_6$-deficient group, but the difference not statistically significant.

The amount of urinary nicotinic acid, MNA, and pyridone per day and also per g of food consumed, and the nitrogen-balance are shown in Table 2. The excretion of nicotinic acid increased by about twofold when rats were fed with tryptophan-deficient diet and a comparison was made with a basal diet, regardless of presence or absence of vitamin $B_6$ ($p<0.01$).

| Group | Diet regimen | No. | N-balance | NiA per rat/day | MNA per rat/day | Pyridone per rat/day |
|-------|--------------|-----|-----------|-----------------|-----------------|---------------------|
| $-B_6$ | Trp def.     | 8   | −         | 953.9           | 180.6           | 12.6                |
|       | Basal        | 8   | +         | 868.8           | 91.2            | 27.9                |
| $+B_6$ | Trp def.     | 8   | −         | 887.1           | 178.3           | 17.2                |
|       | Basal        | 8   | +         | 759.9           | 74.9            | 21.9                |
| $+B_6$ | Basal        | 9   | +         | 837.3           | 66.4            | 28.2                |
|       | Basal        | 9   | +         | 876.3           | 63.1            | 28.0                |

The excretion of MNA was considerably greater in the tryptophan-deficient diet group regardless of presence or absence of vitamin $B_6$ ($p<0.05$).

When tryptophan was deficient, the excretion of pyridone was clearly at a low level by vitamin $B_6$ deficient as compared to that in a basal diet ($p<0.05$). There was no difference in excretion of pyridone between $B_6$-added and $B_6$-deficient group.

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