RELATIONSHIP BETWEEN TRYPTOPHAN INTAKE AND URINARY EXCRETION OF 3-HYDROXYKYNURENINE, 3-HYDROXYANTHRANILIC ACID, XANTHURENIC ACID AND KYNURENIC ACID

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Summary Some metabolites of tryptophan such as 3-hydroxyanthranilic acid (3-OHAA), 3-hydroxykynurenine (3-OHKY) and xanthurenic acid (XA) have been suspected of being carcinogenic, and the relationship between bladder cancer and the urinary excretion of these metabolites has been discussed. However, the relationship between the intake of tryptophan and the excretion of these metabolites has not yet been reported. In this work, the urinary excretion of 3-OHAA, 3-OHKY, XA and kynurenic acid (KA) was determined when an experimental diet containing 770 or 850mg/day of tryptophan was given to six normal female human subjects and when 900mg/day of tryptophan was added to the experimental diet. Under the experimental diet, 3-OHAA, 3-OHKY, XA and KA in urine (μmole/day) were 3.57±1.35, 1.95±1.11, 10.11±3.25 and 15.80±2.35, respectively. Under the additional intake of tryptophan, the values increased to 7.07±5.31, 3.90±2.44, 18.85±7.20 and 31.51±7.52, respectively. The averages of excretion were proportional to the tryptophan intake. Mean excretion ratios (% of 3-OHAA, 3-OHKY, XA and KA (μmoles) to tryptophan intake (μmoles) were 0.086±0.056, 0.053±0.032, 0.245±0.085 and 0.390±0.084, respectively. However, individual differences and daily fluctuations were observed and it was especially remarkable in 3-OHAA in the period of additional tryptophan intake.

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Some metabolites of tryptophan have been suspected of being carcinogenic, and the relationship between bladder cancer and the urinary excretion of these compounds has been discussed. Induction of bladder cancer in mice by implantation of cholesterol pellets containing the metabolites of tryptophan, e.g. 3-hydroxykynurenine (3-OHKY), 3-hydroxyanthranilic acid (3-OHAA) or xanthurenic acid (XA), has been reported by Allen et al. (1) and Bryan et al. (2). Since Boyland and Williams (3) suggested that such metabolites in urine may be related to the induction of bladder cancer, several investigators have studied the urinary excretion of these compounds in patients with bladder cancer and have reported on abnormal excretion of tryptophan metabolites (4-6). However, the analytical methods applied to these compounds were not really satisfactory in sensitivity or specificity. In our previous papers, reliable and sensitive methods of fluorometry for 3-OHKY and 3-OHAA were devised and the urinary excretion of these compounds in normal persons and in patients with bladder cancer was compared (7, 8). The results showed that 3-OHAA in the urine was higher in patients, while 3-OHKY levels were not significantly different. The relationship between the intake of tryptophan and the excretion of these carcinogenic metabolites has not yet been reported on, although it should be most fundamentally important. In this paper, the urinary excretion of 3-OHAA, 3-OHKY, XA and kynurenic acid (KA) was determined when experimental diets containing 770 or 850 mg/day of L-tryptophan were given to normal human subjects and when the experimental diet was supplemented with 900 mg/day of L-tryptophan.

MATERIALS AND METHODS

Subjects. Six healthy women, ranging in age from 20 to 22, were used.

Experimental diets. Serious consideration was given to the preparation of the experimental diets so as to maintain the daily intake of tryptophan at a constant level. The following foods were used: white rice, wheat flour, processed cheese, pork sausage, dried whole eggs, milk powder, miso powder, soy sauce, mashed potato flakes, vegetables, black tea, sucrose, corn starch, corn oil and margarine. The amount of food given to each subject were weighed daily and cooking was done separately for each meal of the individual subject. Two kinds of diets, A and B, were used. Diet A and B differed in the amount of rice and the subjects selected diet A or diet B in advance. Subjects 1, 2, 3 and 5 took diet A and subjects 4 and 6 took diet B. Besides the experimental diets the subjects were permitted to take sucrose, corn starch and margarine ad libitum for supply of energy. Total nitrogen was determined by the Kjeldahl method. The content of amino acids was calculated from the amino acid composition of foods in Japan (9). Diet A and B contained 770 and 850 mg/day of tryptophan, respectively. The other nutrients were calculated from the standard
tables of food composition in Japan (10). All of the nutrients contained therein apparently met the subjects’ requirements.

**Experimental period.** (1) Period I (8 days): The subjects ate experimental diets. (2) Period II (7 days): The subjects ate experimental diets and 900 mg of L-tryptophan. The subjects were given 300 mg of tryptophan in enteric coated capsules three times a day at each meal. (3) Period III (2 days): The subjects ate experimental diets.

**Determination of tryptophan metabolites.** From the 6th day of period I to the 2nd day of period III, 24 hr urine from each subject was collected. However, subjects 2 and 3 failed to collect the sample, respectively on the 2nd day of period III and from the 1st to 4th day of period II because of menstruation, respectively. 3-OHKY in the urine of subject 6 on the 2nd day of period III could not be determined because of difficulty in extracting this sample. 3-OHAA and 3-OHKY in the urine were determined by the methods reported by Watanabe et al. (7, 8) and XA and KA by the method reported by Satoh and Price (11).

**RESULTS**

The results of determination of tryptophan metabolites are shown in Tables 1, 2, 3 and 4. There were individual differences and daily fluctuations, especially regarding 3-OHAA and 3-OHKY levels. The mean value of each of the four metabolites was significantly higher in period II than in period I (3-OHAA and 3-

| Period | Day & mean | Subjects | Pooled mean Mean ± SD |
|--------|------------|---------|----------------------|
|        |            | 1  2  3  4  5  6 |                          |
|       |            |         |                      |
| I     | 6th        | 2.25 5.81 4.02 5.96 4.68 3.29 |                      |
|       | 7th        | 3.11 2.23 2.52 2.99 4.49 3.87 |                      |
|       | 8th        | 2.74 1.63 1.68 2.99 5.34 4.69 |                      |
|       | Mean       | 2.70 3.22 2.74 3.98 4.84 3.95 | 3.57 ± 1.35          |
| II    | 1st        | 3.86 2.61 — 7.88 19.78 4.11 |                      |
|       | 2nd        | 5.88 5.74 — 4.71 14.69 1.50 |                      |
|       | 3rd        | 0.59 4.81 — 6.33 17.71 7.09 |                      |
|       | 4th        | 22.13 6.44 — 5.54 9.15 4.54 |                      |
|       | 5th        | 8.50 0.91 4.81 0.31 5.81 3.64 |                      |
|       | 6th        | 0.28 8.60 9.47 0.88 12.80 2.05 |                      |
|       | 7th        | 6.01 4.85 11.06 8.06 11.94 13.50 |                     |
|       | Mean       | 6.75 4.85 8.44 4.82 13.13 5.20 | 7.07 ± 5.31          |
| III   | 1st        | 0.26 1.68 4.60 6.88 6.11 0.72 |                      |
|       | 2nd        | 2.95 — 1.09 3.36 1.91 4.50 |                      |
|       | Mean       | 1.61 1.68 2.85 5.12 4.01 2.61 | 3.10 ± 2.21          |
Table 2. Urinary excretion of 3-OHKY (μmole/day).

| Period | Day & mean | Subjects | Pooled mean Mean ± SD |
|--------|------------|---------|----------------------|
|        |            | 1 2 3 4 5 6 |                      |
| I      | 6th        | 1.03 1.43 0.81 1.62 2.89 1.40 |                      |
|        | 7th        | 4.83 2.01 1.59 4.30 1.65 1.50 |                      |
|        | 8th        | 1.05 3.71 1.22 1.26 1.17 2.06 |                      |
|        | Mean       | 2.15 2.38 1.21 2.39 1.90 1.65 | 1.95 ± 1.11          |
| II     | 1st        | 5.62 8.62 — 7.84 1.40 3.15 |                      |
|        | 2nd        | 5.07 4.52 — 10.11 3.13 2.33 |                      |
|        | 3rd        | 6.40 2.67 — 2.03 3.55 8.94 |                      |
|        | 4th        | 2.20 1.29 — 6.59 4.95 3.61 |                      |
|        | 5th        | 2.43 0.62 2.27 4.82 2.49 4.83 |                      |
|        | 6th        | 3.47 0.70 1.07 1.67 2.93 1.70 |                      |
|        | 7th        | 2.27 1.97 5.72 7.54 2.43 5.07 |                      |
|        | Mean       | 3.92 2.91 3.02 5.80 2.98 4.23 | 3.90 ± 2.44          |
| III    | 1st        | 3.76 0.61 3.03 2.75 4.72 1.47 |                      |
|        | 2nd        | 5.14 — 2.33 4.94 2.51 — |                      |
|        | Mean       | 4.45 0.61 2.68 3.85 3.62 1.47 | 3.13 ± 1.51          |

Table 3. Urinary excretion of XA (μmole/day).

| Period | Day & mean | Subjects | Pooled mean Mean ± SD |
|--------|------------|---------|----------------------|
|        |            | 1 2 3 4 5 6 |                      |
| I      | 6th        | 6.84 12.22 8.06 10.23 13.30 7.03 |                      |
|        | 7th        | 7.22 15.32 7.22 8.22 13.26 15.18 |                      |
|        | 8th        | 6.45 14.22 6.96 8.00 10.09 10.08 |                      |
|        | Mean       | 6.84 14.59 7.41 8.82 12.22 10.76 | 10.11 ± 3.25         |
| II     | 1st        | 12.04 13.40 — 19.12 12.71 23.97 |                      |
|        | 2nd        | 14.02 25.30 — 20.29 16.92 19.72 |                      |
|        | 3rd        | 10.01 26.50 — 15.59 16.66 45.81 |                      |
|        | 4th        | 13.02 23.24 — 15.12 12.77 27.83 |                      |
|        | 5th        | 13.31 28.12 17.86 9.12 13.50 27.87 |                      |
|        | 6th        | 11.75 24.93 15.88 16.30 16.92 16.72 |                      |
|        | 7th        | 11.75 30.36 17.10 16.83 21.23 22.54 |                      |
|        | Mean       | 12.27 24.55 16.95 15.62 15.82 26.35 | 12.85 ± 7.20         |
| III    | 1st        | 8.67 15.27 12.12 8.94 12.62 14.61 |                      |
|        | 2nd        | 9.08 — 9.30 10.80 11.00 13.55 |                      |
|        | Mean       | 8.88 15.27 10.71 9.87 11.81 14.08 | 11.45 ± 2.36         |

OHKY: p < 0.05, XA and KA: p < 0.01. Then in period III, 3-OHAA, XA and KA decreased significantly (p < 0.05, p < 0.01 and p < 0.001, respectively), but the decrease in 3-OHKY was not significant. The difference between period I and III
The mean excretion ratio of each of the four metabolites was slightly lower in the period II than in period I, but the difference was not significant. Namely, on average excretion was found to be proportional to tryptophan intake in spite of individual differences and daily fluctuations in each period.

**DISCUSSION**

Boyland *et al.* (12) reported that β-glucuronidase activity was at high level in the urine of patients with bladder cancer and suggested that glucuronides of excreted 3-OHAA and 3-OHKY show carcinogenic action after enzymic hydrolysis of the conjugation. However, the results of a previous paper (13) showed that the conjugated forms of these substances in human urine were minor and it is supposed that the excretion of free forms of these substances which have carcinogenic action may be affected by the intake of tryptophan or by the metabolism of tryptophan in the body.

In this work, the excretion of 3-OHKY and 3-OHAA in period I was within the same range as in previous experiments (7, 8) on normal Japanese subjects whose tryptophan intakes were not estimated. The excretion levels of 3-OHAA and 3-OHKY were significantly increased in period II by the addition of 900 mg of L-
Table 5. Mean excretion ratios (%).a

| Period | Subjects mean & SD | 3-OHAA | 3-OHKY | XA | KA |
|--------|-------------------|--------|--------|----|----|
| I      | 1                 | 0.072  | 0.057  | 0.081 | 0.342 |
|        | 2                 | 0.085  | 0.063  | 0.387 | 0.389 |
|        | 3                 | 0.073  | 0.032  | 0.196 | 0.496 |
|        | 4                 | 0.096  | 0.057  | 0.212 | 0.406 |
|        | 5                 | 0.128  | 0.051  | 0.323 | 0.462 |
|        | 6                 | 0.095  | 0.040  | 0.258 | 0.338 |
|        | Mean              | 0.092  | 0.050  | 0.260 | 0.406 |
|        | SD                | 0.034  | 0.029  | 0.086 | 0.065 |
| II     | 1                 | 0.083  | 0.048  | 0.150 | 0.266 |
|        | 2                 | 0.059  | 0.036  | 0.300 | 0.382 |
|        | 3                 | 0.103  | 0.037  | 0.207 | 0.391 |
|        | 4                 | 0.056  | 0.068  | 0.182 | 0.408 |
|        | 5                 | 0.161  | 0.036  | 0.193 | 0.423 |
|        | 6                 | 0.061  | 0.049  | 0.308 | 0.382 |
|        | Mean              | 0.085  | 0.047  | 0.225 | 0.374 |
|        | SD                | 0.065  | 0.029  | 0.085 | 0.088 |
| III    | Mean              | 0.079  | 0.066  | 0.293 | 0.422 |
|        | SD                | 0.055  | 0.052  | 0.060 | 0.089 |
| I, II  | Mean              | 0.086  | 0.053  | 0.245 | 0.390 |
| & III  | SD                | 0.056  | 0.032  | 0.085 | 0.084 |

a Metabolite (μmole) / Tryptophan intake (μmole) × 100

Tryptophan in enteric coated capsules. The averages of excretion, however, were proportional to tryptophan intake, although marked individual and daily differences were observed. The results suggested that the free tryptophan added in period II was metabolized the same as tryptophan taken as protein. However, calculating from the data in a previous paper (13), the increases in 3-OHAA and 3-OHKY in human urine due to administration of 2,000 mg of L-tryptophan were 13.65 ± 8.21 and 11.08 ± 5.96 μmoles/day, respectively. Accordingly, the excretion ratios of 3-OHAA and 3-OHKY to 2,000 mg (9.79 μmoles) of tryptophan were 0.14 ± 0.08 and 0.11 ± 0.06%, respectively, and these values were significantly larger than those obtained in the present study. It is assumed that this difference may be due to the load of the larger amount of free tryptophan without enteric coating, which may produce an imbalance in the amino acid pattern.

The cause of the marked individual differences and daily fluctuations, especially in 3-OHAA in period II is not known. After 2,000 mg of L-tryptophan was administered to humans when their stomachs were empty, the peaks of urinary excretion of 3-OHAA were at about the same time (3–4 hr after administration),
while the quantities were markedly different (14). These data suggest that excretion of 3-OHAA is remarkably variable when tryptophan is administered. The significance of such individual differences in the etiology of bladder cancer is an interesting problem that should be further studied.

When the excretion ratios in periods I and II were compared, the excretion of XA and KA was proportional to the intake of tryptophan with smaller individual differences than with 3-OHAA and 3-OHKY. It was shown that the increase in the intake of tryptophan produced an almost proportional increase in the excretion of XA and KA in previous experiments (15) using an amino acid mixture as the protein source.

There were no significant differences among periods I, II and III in the mean excretion ratio of each of the four metabolites except between periods II and III for XA, but there was a tendency for the mean excretion ratio of 3-OHKY, XA and KA to be smaller in period II and larger in period III than in period I. Such a tendency may suggest a time lag in the metabolic reaction to the addition and removal of tryptophan.

The metabolic process of tryptophan via the kynurenine pathway is as follows (16, 17).

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  l-tryptophan
     ↓
     N-formyl-l-kynurenine
      ↓
     l-kynurenine → KA
         /  \             \  →
        XA   3-OHKY  anthranilic acid
      \   \        ↓
       3-OHAA degradation via glutarate pathway or NAD pathway
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The data for 3-OHKY, XA and KA in this work were approximately parallel, while those for 3-OHAA showed large variations in period II and comparatively low levels in period III. It is possible that this complexity of data for 3-OHAA might be related to the fact that 3-OHAA can be formed via two pathways (17) as shown in the chart above.

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