Studies on the Excretion of Ascorbic Acid 2-Sulfate and Total Vitamin C into Human Urine after Oral Administration of Ascorbic Acid 2-Sulfate

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Summary The excretion of AsS and total vitamin C into urine after oral administration of AsS to humans was investigated. When 10 mmol of AsS was administered to the subjects, the excretion of AsS into urine continued for 60 hr in males and 48 hr in females. The average amount excreted per hour was less than 5 mg. These results differed from those for AsA and DAsA orally administered to humans. The determination of vitamin C after oral administration of AsS to the subjects consisting of ten males and six females showed no vitamin C effect in humans, similarly to the case with the guinea pig and the rhesus monkey.

Key Words ascorbic acid 2-sulfate, ascorbic acid, antiscorbutic activity, humans, vitamin C-deficient test diet.

AsS is a natural form of AsA which has also been reported to occur in the urine of man (1–3). The vitamin C effect of AsS has been studied by the use of various vitamin C-requiring animals (4–7). AsS orally administered to rainbow trout, coho salmon, and ayu is partially hydrolyzed at the sulfate group by the AsS-sulfohydrolase [EC 3.1.6.1] to form AsA (6, 7). The antiscorbutic effect of AsS in rainbow trout is due to the resulting AsA, while the AsS structure itself seems to have no vitamin C activity (6, 8). On the other hand, the guinea pig and the rhesus monkey, which have no ability to hydrolyze AsS, develop scurvy resulting from the decrease in vitamin C content of the bodies, after being fed AsS as vitamin C supplement (4, 5). Vitamin C effect of AsS, however, has not yet been studied in man. The present experiment was undertaken to determine whether vitamin C content in urine increases when AsS is orally administered to man, or, whether AsA

Abbreviations: AsS, L-ascorbic acid 2-sulfate; AsA, L-ascorbic acid; DAsA, dehydro L-ascorbic acid; T.C., total vitamin C (AsA+DAsA); DNPH, 2,4-dinitrophenylhydrazine; TLC, thin layer chromatography; HPLC, high-performance liquid chromatography.
EXPERIMENTAL

Subjects. The subjects in this experiment were students and faculty members of Kagawa Nutrition College: twelve men (A ~ L) and eleven women (M ~ W), all of whom were healthy adults. The age, height, and body weight of the subjects were as follows: males, from 18 to 37 years of age, from 156 to 177 cm in height, and from 49 to 67 kg in body weight; females, from 19 to 23 years of age, from 155 to 165 cm in height, and from 42 to 56 kg in body weight.

Diets. Vitamin C-deficient test diets given to the subjects during the experiment were prepared according to our previous paper (9). The menu was made out for three days and used repeatedly throughout the experiment. The foods used were selected from the food composition table (10) but foods possibly containing D-arabosecorbic acid were omitted. The intakes of side dishes for each subject were equal for the same sex in the following experiments, while the calorie intakes for the same sex were controlled using rice or bread.

AsS crystals. For the purposes of oral administration, AsS·2Na·2H₂O (Mwt 336) was synthesized using the procedure of the authors (11, 12). The crystals were free from any vitamin C-related compounds and had a purity of 99%.

Determination methods for vitamin C and AsS. Total vitamin C in the urine was determined according to the DNPH method (13). Vitamin C contents of some dry foods in the test diets were measured by the TLC method (14). AsS was determined by the Bromine-DNPH method, and the purity of the AsS crystals was checked by the HPLC method (15, 16).

Procedures. The excretion of AsS and vitamin C content in the urine after oral administration of AsS were investigated in experiments I and II. In experiment I, the excretion of AsS was determined from the urine collected over 24 hr and the urine collected for 96 hr, at 2 or 3 hr intervals and 12 hr intervals, respectively. In experiment II, vitamin C content was measured with time for the urine collected for 120 hr in order to investigate whether AsS orally administered is converted to AsA.

I-(1): The experiment was carried out for a period of three days. The seven subjects, five females and two males, were fed on vitamin C-deficient test diets during the experimental period. Then, 10 mmol of AsS was orally administered to each subject at 9 o’clock a.m. on the third day of the experiment. The urine was collected at two-hour intervals from 9 a.m. to 9 p.m. and at three-hour intervals from 9 p.m. to 9 a.m. on the next day, and the amounts of AsS excreted were measured.

I-(2): This experiment was undertaken for six days on four subjects, three females and a male, because the I-(1) experiment revealed that the excretion of AsS continued over a period of 24 hr following oral administration. Vitamin C-deficient test diets and the AsS tolerance test were similar to those of I-(1). The amounts of AsS excreted were measured in the urine collected for 12 hr from 9 a.m. to 9 p.m.
METABOLISM OF AsS IN HUMANS

Scheme 1. The Explanation of experiment II.

- The urine of female subjects was collected at 24 hr intervals from 9 a.m. to 9 a.m. on the next day. The urine of male subjects was collected at 12 hr intervals from 9 a.m. to 9 p.m. and from 9 p.m. to 9 a.m. on the next day.
- Each subject was orally administered with 1.0g of AsA.
- Female subjects: AsS 0.5mmol/kg, body weight (R, S: n=2)
  - AsS 0.25mmol/kg, body weight (T, U: n=2)
  - NaCl 0.9% soln. (V, W: n=2)
- Male subjects: AsS 0.5 mmol/kg, body weight (C, D, E, F, G: n=5)
  - NaCl 0.9% soln. (H, I, J, K, L: n=5)
- Male subjects: AsS 0.5mmol/kg, body weight (H, I: n=2)

and from 9 p.m. to 9 a.m. on the next day for 96 hr following the administration of AsS. One-tenth of the urine specimen for each subject was collected in a 100ml polyethylene bottle for analysis.

II: This experiment was carried out as shown in Scheme 1 on the basis of experiment I. To each subject 1g (5.7mmol) of AsA was orally administered for a week before the AsS administration in order to cause the repletion of the body pool of vitamin C, followed by feeding the test diets for three days prior to the AsS tolerance test. The subjects, consisting of six females and ten males, were respectively divided into three groups as shown in the Scheme. The explanation that the tolerance tests of AsS in different amounts would be applied to all subjects was given for preventing the influence of the stress of each group on the excretion of vitamin C. The urine of the female subjects receiving the test diets was collected at 24 hr intervals from 9 a.m. to 9 a.m. on the next day, while that of male subjects was collected at 12 hr intervals from 9 a.m. to 9 p.m. and from 9 p.m. to 9 a.m. on the next day for five days. Two polyethylene bottles for the urine specimen were prepared for each subject, one of which was used for the determination of AsS and the other, containing 1g of oxalic acid, for vitamin C determination.

RESULTS AND DISCUSSION

Nutritional intakes from vitamin C-deficient test diets

The average values of nutritional intake from the test diets for female and male
Table 1. The average values of daily nutritional intake from the vitamin C-deficient test diets.
The diets were available to the subjects three times a day. The values for females are the data for the diets of females for 63 man-days (experiment I-(1): five females for 3 days, I-(2): three for 6 days, and II: six for 5 days); the values for males are the data on the diets for 62 man-days (I-(1): two males for 3 days, I-(2): one for 6 days, and II: ten for 5 days).

|          | Energy (Cal) | Protein (g) | Fat (g) | Fiber (g) | Calcium (mg) | Iron (mg) | Vitamin A value (IU) | Thiamin (mg) | Riboflavin (mg) | Vitamin C (mg) |
|----------|-------------|-------------|---------|-----------|--------------|-----------|---------------------|---------------|-----------------|----------------|
| Female   | 1,697       | 65.7        | 53.6    | 1.7       | 525          | 7.7       | 651                 | 0.64          | 0.76            | 0.6            |
| Male     | 2,596       | 102.0       | 84.1    | 1.8       | 892          | 10.0      | 2,036               | 0.85          | 1.79            | 1.4            |
subjects in experiments I and II are listed in Table 1. Although there are some differences in the nutritional intake between males and females resulting from the different intake of the principal food and side dishes, such differences are negligible, because vitamin C content of the test diets is low. Other nutritive elements are sufficient for the diet used for such short-period experiments.

Table 2. The excretion of AsS in the urine after the oral administration of 10 mmol AsS.

(1) Case of urine collected in 24 hr.

| Intervals (hr) | Female (M, N, O, P, Q: n = 5) | Male (A, B: n = 2) |
|----------------|--------------------------------|-------------------|
|                | Urine (ml)^c                  | AsS (mg)^a,b      | Urine (ml)^c | AsS (mg)^a,c |
| 0–2            | 76                             | 3.7±0.8           | 113          | 9.8          |
| 2–4            | 113                            | 6.2±1.4           | 151          | 8.1          |
| 4–6            | 82                             | 4.9±2.0           | 75           | 5.0          |
| 6–8            | 97                             | 5.0±2.1           | 75           | 4.7          |
| 8–10           | 88                             | 5.3±1.6           | 72           | 3.5          |
| 10–12          | 109                            | 5.4±1.3           | 70           | 3.9          |
| 12–15          | 135                            | 7.4±1.2           | 81           | 6.1          |
| 15–18          | 118                            | 6.6±1.0           | 120          | 7.8          |
| 18–21          | 128                            | 3.9±1.3           | 135          | 6.9          |
| 21–24          | 104                            | 5.2±1.1           | 225          | 8.2          |
| Total          | 1,050                          | 53.6              | 1,117        | 64.0         |

(2) Case of urine collected in 96 hr.

| Intervals (hr) | Female (M, N, O: n = 3) | Male (A: n = 1) |
|----------------|-------------------------|-----------------|
|                | Urine (ml)^c            | AsS (mg)^a,c    | Urine (ml)^c | AsS (mg)^a |
| 0–12           | 633                     | 35              | 675          | 75          |
| 12–24          | 480                     | 26              | 585          | 36          |
| 24–36          | 687                     | 17              | 1,175        | 32          |
| 36–48          | 580                     | 3               | 405          | 11          |
| 48–60          | 985                     | 0               | 760          | 5           |
| 60–72          | 480                     | 0               | 365          | 0           |
| 72–84          | 657                     | 0               | 900          | 0           |
| 84–96          | 575                     | 0               | 345          | 0           |
| Total          | 5,077                   | 81              | 5,210        | 159         |

^a As the acid form (C₆H₆O₈S, Mwt 256). ^b Mean ± SD. ^c Mean.

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Table 3. The excretion of AsS in the urine.
Case of female

| Time after administration (hr) | Groups | AsS (0.5 mmol/kg, body weight)$^a$ | AsS (0.25 mmol/kg, body weight)$^a$ | NaCl$^a$ |
|-------------------------------|--------|-----------------------------------|-----------------------------------|---------|
|                               |        | Urine (ml)$^d$ | AsS (mg)$^{c,d}$ | Urine (ml)$^d$ | AsS (mg)$^{c,d}$ | Urine (ml)$^a$ | AsS (mg)$^{c,d}$ |
| −72−−48                      | R, S ($n=2$) | 416 | 0 | 464 | 0 | 641 | 0 |
| −48−−24                      |        | 483 | 0 | 545 | 0 | 720 | 0 |
| −24− 0                       |        | 440 | 0 | 441 | 0 | 748 | 0 |
| 0−24                         |        | 668 | 72 | 693 | 55 | 739 | 0 |
| 24−48                        |        | 550 | 74 | 573 | 40 | 672 | 0 |
| Time after administration (hr) | Urine (ml)d | AsS (mg)c,e | Urine (ml)d | AsS (mg)c,d | Urine (ml)d | AsS (mg)c,d |
|-------------------------------|-------------|-------------|-------------|-------------|-------------|-------------|
| 0–12                          | 443         | 84±14       | 500         | 0           | 627         | 0           |
| 12–24                         | 396         | 45±15       | 485         | 0           | 340         | 0           |
| 24–36                         | 498         | 31±10       | 520         | 82          | 433         | 0           |
| 36–48                         | 444         | 10±5        | 365         | 38          | 413         | 0           |

\(^a\) On day 8, 9 a.m., of experiment II (at 0 hr in this table). \(^b\) On day 9, 9 a.m., of experiment II (at 24 hr in this table). \(^c\) As the acid form (C₆H₈O₉S, Mwt 256). \(^d\) Mean. \(^e\) Mean ± SD.
Changes of the AsS contents with time after oral administration

The AsS contents of the urine were determined with time after the oral administration of different amounts of AsS to male and female subjects. The results are summarized in Tables 2 and 3. As shown in Table 2, the excretion of AsS in the urine is less than 5 mg per hour on average (weight as the acid form (Mwt 256); the amount of AsS is hereinafter represented by the acid form), but continues after 21 to 24 hr. The examination of the urine collected over 96 hr showed that the excretion of AsS continued for 60 hr in males and 48 hr in females after the administration. The data of Table 3, in which a higher dose of AsS was used, indicates that the excretion of AsS tends to continue after these time periods, particularly in females. The rate of the amount of AsS excreted to the size of the dose was 2.5% (64 mg) in males and 2.1% (54 mg) in females, when the dose was 10 mmol (2.56 g) (Table 2-(1)). When 0.5 mmol per kg body weight was given (the average in males was 7.5 g, and in females 5.9 g), the rate was 1.7% in males and 1.2% in females (Table 3).

These results indicate that the amounts of AsS excreted increase with the increase in the size of the dose, but are not proportional to the latter. Thus, it has been demonstrated that the excretion of AsS in the urine is low and does not rise during any particular period, in contrast to the case with the oral administration of...
AsA and DAsA. This seems to indicate that most of the AsS administered orally in this experiment was excreted in the feces (5, 17), because AsS was hardly absorbed in humans.

Changes of the vitamin C contents of the urine with time after the oral administration of AsS

The amount of vitamin C excreted in the urine was measured with time for the urine collected over 48 hr following the administration of 0.25 and 0.5 mmol respectively of AsS to females and 0.5 mmol to males. Figure 1 shows the results, together with the values of vitamin C contents of the urine from three days before the AsS tolerance test for comparison. The amount of vitamin C excreted in the urine of each subject decreased during the test with vitamin C-deficient diet and at last became less than 7 mg for all the subjects on the day before the AsS tolerance test. Thus, the appropriate conditions were set up for detecting AsA produced from AsS, even in the case of the sulfonate anion of AsS being hydrolyzed by only 0.1 to 0.2%. Vitamin C excreted into the urine exceeded the value of the day before the tolerance test in all groups including the NaCl control group. This result may be due to the stress of the AsS load. The excretion of vitamin C into the urine during the test period was almost equal for male and female subjects. The formation of AsA from orally administered AsS was not found in either sex. The result wherein vitamin C content of the urine decreased even after the oral administration of AsS is consistent with that for the guinea pig and the rhesus monkey. Consequently, AsS is considered to have no vitamin C activity in man under the experimental conditions.

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