Summary  The antiscorbutic effect of dehydro-L-ascorbic acid (DAsA) was investigated in vitamin C-deficient guinea pigs. Male guinea pigs were fed vitamin C-deficient diets for 16 days to deplete body L-ascorbic acid (AsA) pools and then fed the deficient diet supplemented with DHA and/or AsA intraperitoneally for 14 days. During the repletion period, most of the animals injected with 0.5 mg DAsA/day developed scurvy, their body weights decreased and their mortality rate was higher than that of the other groups injected with 0.5 mg AsA/day or 5 mg DAsA/day. Injecting animals with 0.5 mg AsA/day resulted in the disappearance of the typical scorbutic symptoms and regaining of body weight. These data indicate that DAsA has considerably less antiscorbutic activity than AsA in vitamin C-deficient guinea pigs.

Key Words  L-ascorbic acid, dehydro-L-ascorbic acid, vitamin C deficiency, antiscorbutic effect

Dehydro-L-ascorbic acid (DAsA), an oxidized form of L-ascorbic acid (AsA), is known to be reversibly converted to AsA in animal tissues. Various studies have been undertaken to evaluate the antiscorbutic effect of DAsA in a healthy humans (1-3) and guinea pigs fed normal diets (2,4-6). Tsujimura et al. (3) reported DAsA had the same antiscorbutic effects as AsA based on results of urinary AsA excretions of healthy men who were fed normal diets containing AsA and administered DAsA. Tolbert suggested that the vitamin C activity of DAsA is about 70% and absorbed DAsA is probably as effective as ascorbate in meeting nutritional needs (7), although there was no clear evidence for this.

AsA is commonly utilized as an antioxidant in foods and may produce some degradation products during the processing and storage of foods. These products, including DAsA, can be taken into the tissues and may have some influence on the metabolism of other nutrients. Therefore it is necessary to clarify the bioavailability of the degradation products of AsA, especially its oxidized form, DAsA, in animals.
in various AsA nutritional states.

The purpose of this study is to investigate the antiscorbutic effect of DAsA, that is, the ability of DAsA to prevent scurvy in vitamin C-deficient guinea pigs.

**EXPERIMENTAL**

**Reagents.** AsA was obtained from Wako Pure Chemical Industries, Ltd. DAsA was prepared each day before use by oxidation of an aqueous solution of AsA with bromine (5). The freshly prepared oxidized solution was mixed with saline solution to give a final volume of 5 ml (1 mg/ml), containing 95% DHA, 5% DKG and no ASA as determined by the 2,4-dinitrophenylhydrazine method and high-performance liquid chromatography as described below.

**Animals and diets.** Male Hartley strain albino guinea pigs weighing about 250 g were housed in individual cages in a temperature- and humidity-controlled room, and were allowed free access to the experimental diets and water throughout the experimental period.

The animals were fed vitamin C-deficient diets for 16 days (depletion period). The composition of the diets was given in a previous report (8).

On day 16 of feeding, the animals were divided into five groups of 6–11 animals. Each group was fed vitamin C-deficient diets and received intraperitoneal administration of AsA or DAsA solutions in the following doses: Group A-1, 0.5 mg/day AsA; Group D-1, 0.5 mg/day DAsA; Group D-10, 5 mg/day DAsA; Group AD, a mixture of 0.5 mg AsA and 5 mg DAsA/day; Group CN (vitamin C-deficient group), 0.5 ml of saline solution/day. The animals were weighed daily.

The animals were sacrificed on day 14 of the repletion period. The liver, spleen, kidneys and adrenals of each animal were removed, rinsed with saline solution, blotted and weighed.

**Tissue AsA and DAsA analyses.** The preparation of tissue samples for determination of AsA and DAsA was described in a previous report (9). The concentrations of AsA in tissues were determined by high-performance liquid chromatography (HPLC). HPLC conditions were as follows: a Hitachi Liquid Chromatograph type 638-50 was used, and the eluate was monitored with a UV detector at 254 nm. A stainless steel column (250 × 4 mm i.d.) was packed with LiChrosorb-NH2, and phosphate buffer (pH 3.3, 0.01 M NaH2PO4/0.01 M HPO3) was used as the mobile phase at a flow rate of 0.7 ml/min.

DAsA was reduced with dithioerythritol (DTE) to AsA and determined as total AsA by HPLC. Sample solutions were adjusted to neutral (pH 7.0) with a saturated barium hydroxide solution, then mixed with a 1% DTE aqueous solution and centrifuged at 1,000 × g for 5 min. The supernatant of the solutions was analyzed by HPLC. The recovery of DAsA with this reducing method was approximately 100%.
RESULTS AND DISCUSSION

Mortality and body weight changes

Table 1 shows the mortality of the animals during the repletion period. Clayton et al. (2) reported that guinea pigs (initial body weight, 300–350 g) fed vitamin C-deficient diets survived for an average of 28.6 ± 1.0 days. In group CN, 3 animals which survived for 30 days revealed serious scurbutic symptoms such as loss of hair, inflexibility of the limbs, collapse and hemorrhages. The mortality rates of groups D-1 and D-10 injected with DAsA were higher than groups A-1 and AD which were supplemented with AsA. In group D-1, the following number of animals died of typical acute scurvy after initiating repletion: day 5, 2; day 7, 1; day 9, 1; day 11, 1; day 13, 3.

Figure 1 shows the changes in the relative body weights of animals remaining alive after 14 days of repletion. The body weights of guinea pigs fed vitamin C-deficient diets decreased slightly after day 12 of depletion, and some of the animals showed some of the scurbutic symptoms described above. The average body weights at the beginning of the repletion period were about 294 ± 7 g, expressed as 100% in Fig. 1. The relative body weights of animals in groups A-1, D-10 and AD increased gradually after day 5 of repletion and these animals showed no apparent symptoms of scurvy at the end of the repletion period. On the other hand, the animals in group D-1 lost weight similar to those in group CN, and 2 animals in group D-1 developed inflexibility of their limbs, evidence of scurvy.

Table 2 gives the ratio of tissue weight to body weight. The ratio of adrenals and kidneys in group D-1 were significantly higher than those in groups D-10, A-1 and AD (p < 0.01), but similar to those of group CN.

In the study of the bioavailability of DAsA carried out by Clayton et al. (2), long-term (4-month) injection of guinea pigs with DAsA produced toxic symptoms, although it had an antiscorbutic effect and raised the tissue level of AsA. As the amount of DAsA injected in the present study was considerably lower than in any of the previous work cited, autopsy revealed no apparent pathological changes.

Table 1. Mortality rate of guinea pigs during repletion period.

| Experimental group | Initial number of animals | Number of deaths |
|--------------------|---------------------------|------------------|
| A-1                | 9                         | 1                |
| D-1                | 11                        | 8                |
| D-10               | 11                        | 4                |
| AD                 | 9                         | 2                |
| CN                 | 6                         | 3                |

The animals fed vitamin C-deficient diets were given the following doses intraperitoneally for 14 days. A-1, 0.5 mg AsA/day; D-1, 0.5 mg DAsA/day; D-10, 5 mg DAsA/day; AD, 0.5 mg AsA + 5 mg DAsA/day; CN, 0.5 ml of saline.

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Fig. 1. Body weight changes of guinea pigs. The animals fed vitamin C-deficient diets were given the following doses intraperitoneally for 14 days. A-1, 0.5 mg AsA/day; D-1, 0.5 mg DAsA/day; D-10, 5 mg DAsA/day; AD, 0.5 mg AsA + 5 mg DAsA/day; CN, 0.5 ml of saline.

Table 2. Tissue weight to body weight ratios.

| Group | Adrenals  | Spleen    | Kidneys   | Liver     |
|-------|-----------|-----------|-----------|-----------|
| A-1 (8) | 0.074 ± 0.005<sup>a</sup> | 0.20 ± 0.01<sup>a</sup> | 1.03 ± 0.04<sup>a</sup> | 5.48 ± 0.39 |
| D-1 (3) | 0.108 ± 0.013<sup>b</sup> | 0.48 ± 0.26 | 1.25 ± 0.14<sup>b</sup> | 5.44 ± 0.76 |
| D-10 (7) | 0.054 ± 0.005<sup>a</sup> | 0.14 ± 0.01<sup>b</sup> | 0.90 ± 0.05<sup>a</sup> | 5.36 ± 0.44<sup>a</sup> |
| AD (7) | 0.060 ± 0.005<sup>a</sup> | 0.16 ± 0.02 | 0.89 ± 0.05<sup>a</sup> | 4.86 ± 0.35<sup>a</sup> |
| CN (3) | 0.124 ± 0.014<sup>b</sup> | 0.61 ± 0.33 | 1.39 ± 0.05<sup>b</sup> | 7.28 ± 1.09<sup>b</sup> |

Values are means ± SEM. Numbers of animals are in parentheses. <sup>a</sup>Significantly different from <sup>b</sup>(p < 0.01).

The sub-optimal level of AsA ingestion required to produce adequate growth, but not tooth development, in guinea pigs has been reported to be 0.5 mg/100 g body weight (10). In this study, 0.5 mg of AsA/animal/day given intraperitoneally was enough to induce recovery from the vitamin C-deficient state.

Looking at mortality and body weight changes, the animals injected with 0.5 mg DAsA/day (group D-1), showed less recovery from AsA-depleted states than those injected with the same amount of AsA/day (group A-1), suggesting that DAsA does not have the same antiscorbutic effect as AsA.

Contents of AsA and DAsA in tissues

Figure 2 reveals the values of AsA and total AsA (DAsA + AsA) contained in
ANTISCORBIC EFFECT OF DAsA

Fig. 2. Contents of total AsA and AsA in the tissues of guinea pigs. Significantly different from group A-1 at $p<0.05$ (*), $p<0.01$ (**), $p<0.001$ (***) each whole tissue. DAsA contents were estimated by subtracting AsA values from total AsA values. The contents of AsA and total AsA in 4 tissues of the groups injected with AsA and/or DAsA were significantly higher than those of group CN, which received no treatment ($p<0.05$–0.001). Although the contents of AsA in adrenals of group D-1 were significantly lower than those of group A-1 ($p<0.05$), AsA and total AsA contents in the other tissues were not significantly different between these two groups. However, 3 animals in group D-1 died on day 13 of repletion, were dissected within 12 h and contents of AsA and total AsA in their tissues were measured, but these data are not shown in Fig. 2. The contents of AsA and total AsA in these livers (17±14, 20±1 μg) were much lower than those of group A-1 ($p<0.001$), at the same level as those of group CN. It is assumed that the extreme shortage of AsA and total AsA in the tissues, especially the liver, caused high mortality in group D-1, indicating that the injected DAsA was not biologically available in those animals.

Clayton et al. (2) reported that injecting vitamin C-deficient guinea pigs with 40 mg of DAsA intraperitoneally for 3 consecutive days produced the same levels of AsA in tissues as those injected with 20 mg AsA, and the antiscorbutic effect of

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DAsA was about one-half that of AsA. The contents of AsA in adrenals and liver of group D-10 were only about twice those of group A-1 ($p<0.05$), although the amount of DAsA injected in the former was ten times that in the latter. Since the amount of AsA used in this study was the minimum needed for body weight gain, and quite small compared with that in Clayton’s experiment, the amount of injected DAsA required to produce the same AsA contents in the tissues is probably over five times that of AsA. This suggests that intraperitoneally injected DAsA was less well utilized as AsA in vitamin C-deficient guinea pigs compared with the results reported by Clayton.

Tsujimura et al. (11) reported that in the case of healthy guinea pigs administered a single dose of 16–22 mg DAsA/day, the tissue contents of AsA increased and reached a maximum 60 min after DAsA injection. These data reveal the reduction of DAsA to AsA in the body; however, they provide no accurate information on the antiscorbutic effect of DAsA.

The contents of AsA and total AsA in the tissues in group AD were almost equal to the total amount in those in group A-1 and group D-10. Simultaneous injection of AsA and DAsA had no remarkable effect on the contents of AsA and total AsA.

The ratio of AsA to total AsA

Table 3 shows the ratio of DAsA to AsA, which is thought to reveal the physiological state of cell function, for example as an index of cell division (12). The ratios of AsA/total AsA in adrenals and liver of groups D-1 and D-10 were significantly higher than those of group A-1 ($p<0.01$, except adrenals and liver of D-10: $p<0.05$), that is, scarcely any DAsA was detected in the tissues of these groups, suggesting that DAsA was readily converted to AsA or other forms.

The low levels of these ratios in the spleens of all the experimental groups except group D-1 may be a characteristic of guinea pigs during recovery from vitamin C-deficient states.

The ratios of DAsA were very low in the surviving guinea pigs administered DAsA, suggesting that DAsA was actively reduced to AsA even in an AsA-deficient...
state. Banerjee (12) reported that glutathione, which may be an important hydrogen donor of the DAsA-reducing system, decreased in AsA-deficient guinea pigs. This was also observed in this study (data not shown). If glutathione were the main reducing agent of DAsA, vitamin C-deficient guinea pigs would produce less AsA from DAsA and exhibit fewer antiscorbutic effects than in healthy animals. The bioavailability of DAsA in the animals may be controlled by other reducing systems, for example, by coupling with the glutathione regenerating system (13). From these data, the vitamin C status of animals is thought to be one of the most important factors determining utilization of DAsA as AsA, and should be considered in estimating daily vitamin C requirements.

In order to clarify the reduction mechanism of DAsA to AsA, the distribution of DAsA in the tissues must be investigated. The details of the behavior of DAsA in the tissues of animals intravenously injected with DAsA will be reported in a subsequent paper.

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