

Effect of Graded Doses of Erythorbic Acid on Ascorbic Acid Content of Tissues of Guinea Pigs

Emiko SUZUKI, Tadao KURATA, Norlita SANCEDA,
and Nobuhiko ARAKAWA¹

*Department of Food and Nutrition, Ochanomizu University,
2-1-1, Otsuka, Bunkyo-ku, Tokyo 112, Japan*

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Summary The effect of graded doses of erythorbic acid (ErA) on the content of ascorbic acid (AsA) in the tissues of guinea pigs administered AsA was studied. The guinea pigs were administered 5 mg AsA and 1, 5, 20, and 100 mg ErA; or 1 mg AsA and 1 and 20 mg ErA; or 20 mg AsA and 20 mg ErA for 16 days. The animals were then sacrificed, and the liver, adrenal glands, spleen, and kidneys were removed to determine the contents of AsA and ErA by using HPLC. The content of AsA in the tissues of the animals administered less than 5 mg ErA together with 5 mg AsA was not significantly different from that of the animals administered 5 mg AsA. The administration of 100 mg ErA together with 5 mg AsA caused a decrease in the amount of AsA in the tissues. The content of AsA in the tissues of the animals administered ErA together with 1 mg AsA was not significantly different from that of the animals administered 1 mg AsA. In the case of animals administered an equal amount of both AsA and ErA, the AsA tissue content was consistently much higher than that of ErA. These results indicated that the administration of relatively small amounts of ErA did not appear to reduce the availability of AsA.

Key Words ascorbic acid, erythorbic acid, guinea pig

Erythorbic acid (D-isoascorbic acid), which is the epimer of L-ascorbic acid (AsA), is used widely as an antioxidant in foods. Hornig *et al.* (1) reported that erythorbic acid (ErA), when administered to guinea pigs, reduced their body weight gain and also decreased the uptake of AsA into various tissues, and they concluded that ErA decreased the bioavailability of AsA.

In our previous study (2), we found that AsA levels in the tissues of guinea pigs administered AsA and ErA were lower than those in the animals administered only AsA, which concurred with the results reported by Hornig (1). However, the

¹ 鈴木恵美子, 倉田忠男, ノーリタ・サンセダ, 荒川信彦

decrease in body weight gain and acceleration in AsA catabolism as reported by Hornig (1) were not observed.

In our previous report (2), the administration of 100 mg ErA in addition to daily supplementation of 5 mg AsA was adequate to maintain normal body weight gain and to prevent the occurrence of scurvy in guinea pigs. As yet, no report has been made on the effect of the administration of various amounts of ErA on guinea pigs administered adequate (5 mg) or marginal (1 mg) levels of AsA.

In this study, the effect of graded doses of ErA on the AsA content in tissues of both normal and chronically AsA-deficient guinea pigs was examined.

MATERIALS AND METHODS

Animals. Male Hartley guinea pigs, initially weighing about 220 g, were fed *ad libitum* the AsA-deficient diet described by Arakawa *et al.* (2). The animals were housed individually in wire cages and were weighed daily. All animals were randomly divided into nine groups according to the following experimental plan.

Experimental plan. The animals were divided into nine groups. Group A (control animals) was orally supplemented with 5 mg AsA/day, group B with 5 mg AsA and 1 mg ErA/day, group C with 5 mg AsA and 5 mg ErA/day, group D with 5 mg AsA and 20 mg ErA/day, group E with 5 mg AsA and 100 mg ErA/day, group F with 1 mg AsA/day, group G with 1 mg AsA and 1 mg ErA/day, group H with 1 mg AsA and 20 mg ErA/day, and group I with 20 mg AsA and 20 mg ErA/day. AsA and ErA were dissolved in water immediately before use. The experimental feeding period was 16 days. At the end of this period, the animals were sacrificed after a 24-h fast and the liver, adrenal glands, kidneys, and spleen were removed to determine the AsA and ErA contents.

Determination of AsA and ErA. After extracting AsA and ErA from the tissues with metaphosphoric acid solution, the contents of AsA and ErA were simultaneously determined using HPLC as described in our previous paper (2).

Statistical tests. The significant difference between the means of two groups was statistically analyzed by Student's *t*-test or the Cochran-Cox test, depending on whether the variances were equal or different.

RESULTS

Figure 1 shows the average body weights of the animals in the nine experimental groups. The growth rate of the guinea pigs administered 5 mg AsA (group A) was quite normal and similar to that of animals administered 5 mg AsA (groups B, C, D, and E). The animals administered only 1 mg AsA (group F) showed a slight decrease in weight gain as compared to that of group A. However, in groups G and H, the weight gain was larger than that of group F and was similar to that of group A. The animals administered 20 mg AsA and 20 mg ErA (group I) showed weight gain similar to the animals administered 5 mg AsA (group A).

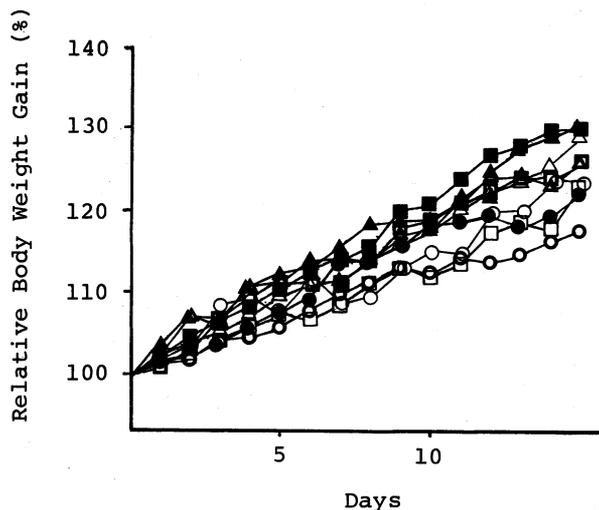


Fig. 1. Body weight changes in guinea pigs.

- , group A, 5 mg AsA-supplemented group.
- ▲, group B, 5 mg AsA and 1 mg ErA-supplemented group.
- , group C, 5 mg AsA and 5 mg ErA-supplemented group.
- △, group D, 5 mg AsA and 20 mg ErA-supplemented group.
- , group E, 5 mg AsA and 100 mg ErA-supplemented group.
- , group F, 1 mg AsA-supplemented group.
- △, group G, 1 mg AsA and 1 mg ErA-supplemented group.
- , group H, 1 mg AsA and 20 mg ErA-supplemented group.
- , group I, 20 mg AsA and 20 mg ErA-supplemented group.

Table 1 shows the AsA and ErA contents in the tissues of guinea pigs given various levels of ErA with an adequate amount of AsA. The AsA content of the four tissues of group A was not significantly different from that of groups B and C. In the liver the AsA content seemed to decrease with increasing ErA dosage. The AsA content of groups D and E was about 50% that of group A. ErA was retained in groups D and E but not in groups B and C. In group D the amount of ErA administered was 4 times larger than that of AsA, but the amount of ErA retained was much less than that of AsA. Furthermore, ErA administered at a level 20 times that of AsA gave almost the same result (group E). In the case of the adrenals, the AsA content of group A was not significantly different from groups B, C, and D, but was significantly higher than that of group E. The content of ErA increased with increasing ErA dosage, though in group E this amount was not significantly different from the AsA content. The content of AsA in the spleen and kidneys was almost the same in groups A, B, and C, but was higher than that of groups D and E. The AsA content in the spleen tended to decrease with increasing ErA dosage. ErA was retained in a relatively small amount in the spleen of groups C, D, and E. In the kidneys, the AsA content of groups D and E was about 50% that of group A. ErA

Table 1. AsA and ErA contents in tissues of guinea pigs supplemented with various levels of ErA at an adequate AsA level. (mg/100 g tissues)

Group	Liver		Adrenal glands		Spleen		Kidneys	
	AsA	ErA	AsA	ErA	AsA	ErA	AsA	ErA
A	2.29 ± 0.42 ^{*,ab}	—	23.6 ± 4.1 ^a	—	10.6 ± 1.1 ^{ad}	—	1.69 ± 0.32 ^c	—
B	2.12 ± 0.63	ND	33.9 ± 3.8 ^{bcd}	0.2 ± 0.1 ^{ac}	10.5 ± 1.8 ^c	ND	1.96 ± 0.52 ^a	ND
C	1.68 ± 0.37	ND	21.8 ± 3.9 ^{be}	0.5 ± 0.3 ^{be}	9.9 ± 1.4 ^g	0.2 ± 0.1 ^a	1.73 ± 0.33 ^b	0.01 ± 0.01 ^a
D	0.86 ± 0.21 ^a	0.04 ± 0.03 ^a	18.4 ± 2.1 ^{ef}	3.4 ± 0.7 ^c	7.6 ± 0.8 ^{ab}	0.2 ± 0.1 ^b	0.63 ± 0.19 ^{abc}	ND
E	0.94 ± 0.30 ^b	0.22 ± 0.07 ^a	10.1 ± 2.2 ^{def}	6.1 ± 1.9 ^{abc}	4.1 ± 0.9 ^{bcdg}	1.5 ± 0.4 ^{ab}	0.95 ± 0.29	0.32 ± 0.10 ^a

A, 5 mg AsA-supplemented group; B, 5 mg AsA and 1 mg ErA-supplemented group; C, 5 mg AsA and 5 mg ErA-supplemented group; D, 5 mg AsA and 20 mg ErA-supplemented group; E, 5 mg AsA and 100 mg ErA-supplemented group. * Values are means ± SE, *n* = 5–9 (except group A, *n* = 12 or 13). Means in the same column with a common superscript letter are significantly different. ^{a,b,c,d,e,f} *p* < 0.05, ^g *p* < 0.01, ^d *p* < 0.001. ND, not detected.

Table 2. AsA and ErA contents in tissues of guinea pigs supplemented with various levels of ErA at a marginal AsA level. (mg/100 g tissues)

Group	Liver		Adrenal glands		Spleen		Kidneys	
	AsA	ErA	AsA	ErA	AsA	ErA	AsA	ErA
F	0.20±0.09*	—	8.3±1.7	—	2.4±0.6	—	0.24±0.12	—
G	0.27±0.11	ND	9.2±2.0	0.5±0.2 ^a	1.7±0.8	ND	0.33±0.11	ND
H	0.56±0.26	0.07±0.03	7.3±1.2	4.5±0.7 ^a	2.6±0.8	0.4±0.2	0.41±0.21	0.15±0.06

F, 1 mg AsA-supplemented group; G, 1 mg AsA and 1 mg ErA-supplemented group; H, 1 mg AsA and 20 mg ErA-supplemented group. * Values are means ± SE, $n=9-11$ (except group F, $n=17$). Means in the same column with a common superscript letter are significantly different. ^a $p < 0.001$. ND, not detected.

Table 3. AsA and ErA contents in tissues of guinea pigs supplemented with the same amounts of ErA and AsA. (mg/100 g tissues)

Group	Liver		Adrenal glands		Spleen		Kidneys	
	AsA	ErA	AsA	ErA	AsA	ErA	AsA	ErA
G	0.27±0.11 ^{a,cd}	ND	9.2±2.0 ^{ab}	0.5±0.2 ^a	1.7±0.8 ^{ae}	ND	0.33±0.11 ^{ac}	ND
C	1.68±0.37 ^c	ND	21.8±3.9 ^a	0.5±0.3	9.9±1.4 ^e	0.2±0.1	1.73±0.33 ^c	0.01±0.01
I	2.56±0.26 ^d	ND	40.4±8.0 ^b	0.9±0.1 ^a	14.3±2.9 ^a	ND	2.20±0.49 ^a	ND

G, 1 mg AsA and 1 mg ErA-supplemented group; C, 5 mg AsA and 5 mg ErA-supplemented group; I, 20 mg AsA and 20 mg ErA-supplemented group. * Values are means ± SE, $n=8-11$ (except group I, $n=4$ or 5). Means in the same column with a common superscript letter are significantly different. ^{a,b} $p < 0.05$, ^{c,d} $p < 0.01$, ^e $p < 0.001$. ND, not detected.

was retained in group E, but only a trace amount was found in group C.

Table 2 shows the AsA and ErA contents in the tissues of the guinea pigs administered ErA at a marginal AsA level. The content of AsA in the tissues of the animals administered a marginal level of AsA (1 mg) was apparently lower than that of those with an adequate level of AsA (5 mg) (Table 1). The AsA contents of the four tissues in the animals administered 1 mg AsA (groups F, G, and H) were not significantly different from each other.

ErA was found in the adrenal glands but not in the liver, spleen, or kidneys of the animals administered both 1 mg AsA and 1 mg ErA. However, ErA was found in the four tissues when 1 mg AsA plus 20 mg ErA was administered, although the amount of ErA was lower than that of AsA.

Table 3 shows the AsA and ErA contents in the tissues of guinea pigs administered the same level of AsA and ErA. The AsA contents of the four tissues, the liver, adrenal glands, spleen, and kidneys, increased with the increasing amount of AsA administered. However, ErA was not found in the liver of the three groups, although the adrenal glands contained a very small amount of ErA. The ErA content in the adrenal glands of group G was the same as that of group C, but was significantly lower than that of group I. ErA was found only in the spleen and kidneys of group C.

DISCUSSION

The AsA content in the tissues of guinea pigs administered a marginal level of AsA was lower than those administered an adequate level of AsA, but no significant difference in their growth and appearance was observed between these two groups. Some AsA-dependent metabolic mechanisms, such as collagen biosynthesis (3-5), hydroxylation of proline (6, 7), and tyrosine metabolism (8, 9), could be affected by the AsA contents of the tissues. However, the effects of such disturbances in those metabolic mechanisms, if they occur, may be latent.

The administration of 100 mg ErA together with 5 mg AsA caused a decrease in the levels of AsA in the tissues, although the administration of less than 5 mg of ErA together with 5 mg AsA showed no effect on the AsA contents. In the case of animals administered 5 mg AsA, the intake of a small amount of ErA had no effect on the AsA levels in the tissues, and a very small amount of ErA was retained in them. The amount of ErA administered, which was 4 times greater than that of AsA, showed a tendency to decrease the AsA content in the tissues. Thus, the contents of AsA in various tissues were considerably reduced when a large amount of ErA was administered orally. Moreover, the amounts of ErA incorporated in these tissues were relatively small compared to the decrease in their AsA content. The tissues seemed to retain AsA more selectively than ErA. The patterns of tissue retention of AsA in animals administered various levels of ErA seemed to differ from tissue to tissue, which may be attributed to the different characteristics of these tissues. This suggests that ErA does not competitively inhibit the storage of AsA in the tissues.

The administration of ErA did not affect the AsA levels in the tissues of animals administered 1 mg AsA. The contents of AsA in the tissues of animals administered both 1 mg AsA and 20 mg ErA were not significantly different from those in animals administered only 1 mg AsA (Table 2). On the contrary, in animals administered both 5 mg AsA and 100 mg ErA, the AsA content was about 50% that of the animals administered only 5 mg AsA (Table 1). The administration of ErA did not seem to reduce the content of AsA in the tissues of animals administered a marginal level of AsA. Since 1 mg AsA is the minimum amount to maintain health and prevent the occurrence of scurvy, the tissues seemed to retain as much AsA as possible. In addition to our results, Hughes (10) reported that when guinea pigs with high AsA or ErA tissue contents were maintained on a scorbutic diet, the rate of loss of ErA was more rapid than that of AsA, as described in our previous paper (2). This might suggest that the tissues selectively retained AsA over ErA.

In the case of animals administered with equal amounts of both AsA and ErA, the tissue retention pattern of AsA was much different from that of ErA (Table 3). The AsA contents of the tissues increased with increasing dosage of AsA, but this tendency was not observed in the ErA tissue contents. This also suggests that AsA may be selectively retained in the tissues. When equal amounts of AsA and ErA were administered simultaneously, ErA was scarcely retained in the tissues and had no effect on the AsA tissue content.

In animals administered 20 mg ErA (groups D, H, and I), the ErA contents of the tissues seemed to decrease with the increasing AsA dosage. The ErA contents in animals administered 1 mg AsA and 20 mg ErA were highest among the three groups, but were lower than those of AsA, which was the lowest among these groups. On the other hand, the AsA contents increased with increasing amount of AsA administered. Therefore, ErA may be retained easily in the tissues when the level of AsA is low.

The administration of low doses of ErA to animals with an adequate AsA level did not affect the contents of AsA, and the administration of ErA seemed to be rather beneficial in terms of weight gain in animals administered a marginal level of AsA and did not seem to reduce their tissue AsA content.

These results in the guinea pigs suggested that the intake of ErA from daily food may not have any effect on AsA availability in humans who ingest an adequate amount of AsA. Since the daily intake of ErA by Japanese is small, and much less than that of AsA, there seems to be little possibility that the availability of AsA can be diminished by ErA.

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