Synergistic Action of Vitamin E and Vitamin C *in Vivo* Using a New Mutant of Wistar-Strain Rats, ODS, Unable to Synthesize Vitamin C

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It is well known that vitamins E and C exhibit synergistic Summary action in in vitro systems, but with regard to in vivo systems, much of the available data are confusing. To elucidate this problem we used a new mutant of Wistar-strain rats that cannot synthesize vitamin C, namely, ODS rats. Two experiments were planned: (1) during development of vitamin E deficiency, whether vitamin C could spare the consumption of vitamin E; and (2) under conditions of a regular level of vitamin E intake, whether different dose levels of vitamin C can affect vitamin E concentration in tissues. The results obtained show that with vitamin C intake, higher levels of vitamin E were deposited in tissues in both experiments. With the development of vitamin E deficiency, rats in the group with a higher dose of vitamin C deposited higher concentrations of α -tocopherol. With simultaneous administration of vitamin E and vitamin C to the same mutant rats, the rats in the group with a higher dose of vitamin C deposited higher levels of vitamin E in all tissues tested. Thus, we concluded that vitamin C can spare the consumption of vitamin E in vivo as well as in vitro.

Key Words vitamin E, vitamin C, α -tocopherol, ODS rats, *in vivo* synergistic action of vitamins E and C, vitamin E deficiency, tissue concentration of α -tocopherol

Many studies have showed evidence that ascorbate can assist in regenerating α -tocopherol from the α -tocopherol radical in *in vitro* model systems, such as liposomes (1-3), and in uniform solutions (4-6). The overall synergistic interaction between vitamins E and C *in vitro* can be represented by the following reactions.

$$ROO \cdot + \alpha - TOH \longrightarrow ROOH + \alpha - TO \cdot$$

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α -TO·+AH⁻ $\longrightarrow \alpha$ -TOH+A⁻·

$$A^- \cdot + A^- \cdot \longrightarrow A + AH^-$$

 $(\alpha$ -TOH = α -tocopherol, AH⁻ = ascorbate, ROO · = peroxy radical, A⁻ · = ascorbyl radical, α -TO · = α -tocopheroxyl radical)

In *in vitro* model systems or in foods, ascorbate and 2-O-fatty acid esters of ascorbic acid are used as a synergist of tocopherols. But, with regard to *in vivo* conditions, clear evidence has not yet been established. A few papers suggest some interrelationship of vitamins E and C *in vivo*, but they involve only indirect evidence in rats (7-10), guinea pigs (11-15), and humans (16, 17).

Very recently Burton *et al.* published a paper in which vitamin C did not spare vitamin E *in vivo* using guinea pigs (18). The animals were given doses of ascorbate at three dietary levels (50, 250, 5,000 mg/kg diet) and two levels of 6 deuterated RRR- α -tocopherol (d_6 -TOH acetate, 36 and 5 mg/kg) for 8 weeks. Overall results suggest no relationship between vitamins E and C *in vivo*, because no differences in d_0 -* and d_6 - α -tocopherol concentrations could be found in all of the tissues tested among groups given differing doses of ascorbate. Researchers thus concluded that no regeneration of α -tocopherol from α -tocopherol radicals had occurred by synergistic action of ascorbate *in vivo*.

In addition, we published results regarding the synergistic action of vitamins E and C *in vivo* using guinea pigs (19). In this experiment we found a tendency for some synergistic action between the two vitamins. But, since the difference in α -tocopherol concentration in all tested tissue among animals of the same dose level of ascorbate was quite high, we cannot find any significant difference among groups of different dose levels for both vitamins.

Recently in Japan a new mutant of rats has been developed: these rats cannot synthesize ascorbate and require dietary supplementation of ascorbate, as do humans (20-22). The new mutant, called osteogenic disorder Shionogi (ODS rat), shows very little individual difference between animals and is now commercially available from Japan Clear Co., Ltd., Tokyo. As it is useful for vitamins C and E deficiency studies, we used the new ODS rat for the purpose of elucidating the synergistic action of vitamins E and C *in vivo*.

EXPERIMENTAL

Animals and diets. i) Animals: ODS male rats (50–70 g, 5 weeks old) were purchased from Japan Clear Co., Ltd. They were housed individually in cages and kept at 24° C.

ii) Diets: In Exp. 1, a low-level vitamin C- and vitamin E-deficient diet

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^{*} d_0 - α -tocopherol means nondeuterated RRR- α -tocopherol.

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(ascorbate 50 mg/kg, α -tocopherol 1.0 mg/kg, LowC-DE diet) and a vitamin Csupplemented and vitamin E-deficient diet (ascorbate 600 mg/kg, α -tocopherol 1.0 mg/kg HighC-DE diet) were purchased from Japan Clear Co., Ltd. Both diets contained other nutrients at the same levels as follows: cornstarch, 36%; vitamin-free casein, 25%; α -wheat starch, 10%; granulated sugar, 5%; mineral mixture (final composition is the same as the AIN-76) diluted with cellulose powder, 6%; vitamins mixture (final composition is the same as the AIN-76 except for C and E) diluted with sucrose, 2%; stripped corn oil (vitamin E free), 8%; and cellulose powder, 8%.

In Exp. 2, a low-level vitamin C- and vitamin E-supplemented diet (ascorbate 50 mg/kg, α -tocopherol acetate 50 IU/kg, LowC-VE diet) and a vitamin C- and vitamin E-supplemented diet (ascorbate 600 mg/kg, α -tocopherol acetate 50 IU/kg, HighC-VE diet) were also purchased from Japan Clear Co., Ltd. Both diets contain other nutrients as in Exp. 1 except that stripped corn oil was substituted for cuttlefish oil; the stripped corn oil contains 25% EPA (20:5, *n*-3) and 25% DHA (22:6, *n*-3) and was kindly offered by Japan Oil Co., Tokyo.

Vitamin C content in all diets in Exp. 1 and 2, as shown below, was adjusted by changing the mixing ratio of HighC-DE and LowC-DE diets in Exp. 1 and HighC-VE and LowC-VE diets in Exp. 2, respectively. The oil in all diets was added to the mixture of other nutrients every day and blended.

Commercial chow (CE-2, Japan Clear Co. Ltd., α -tocopherol content 30 mg/kg diet) was used during prefeeding.

Determination of tocopherols and ascorbate in plasma, erythrocytes, and tissues. Tocopherols in these biological specimens were analyzed by HPLC following the method of Ueda and Igarashi (23-25). Ascorbate concentration in plasma and tissues was analyzed by the 2,4-dinitrophenylhydrazine method (26).

Experiment 1. To examine the effect of ascorbate upon vitamin E status during the progress of vitamin E deficiency in ODS rats, rats were fed on three levels of vitamin C- and vitamin E-deficient diets.

After prefeeding for 11 days on commercial chow (CE-2, Japan Clear Co., α tocopherol content 30 mg/kg) in order to match the vitamin E level in all animals, 21 animals were divided into 3 groups (1 group=7 animals), namely the low vitamin C- and E-deficient (V.C 150 mg/kg diet, C150-DE group), middle vitamin C- and E-deficient (V.C 300 mg/kg, C300-DE group), and high vitamin C- and E-deficient (V.C 600 mg/kg, C600-DE group) groups. Before feeding and after 2 and 4 weeks on diets, heparinized blood was drawn from the tail vein of each animal to determine tocopherols in plasma and erythrocytes. After 6 weeks all animals were sacrificed following overnight fasting, and the plasma, erythrocytes, heart, liver, kidney, spleen, and lung were used to analyze tocopherols and ascorbate.

Experiment 2. To know the effect of ascorbate upon vitamin E status under the normal feeding of vitamin E, ODS rats were fed at three different levels of vitamin C, but at the same normal level of vitamin E, in their diets.

After prefeeding on commercial CE-2 diet for 7 days, 21 ODS rats were

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divided into three groups, namely the low vitamin C (V.C 150 mg/kg diet, C150-VE group), middle vitamin C (V.C 300 mg/kg, C300-VE group), and high vitamin C (V.C 600 mg/kg, C600-VE group) groups. Before feeding and after 3 and 5 weeks on diets, heparinized blood was drawn from tail vein of each animal. After 6 weeks all animals were sacrificed following overnight fasting, and the concentration of tocopherols and ascorbate in the same tissues as described in Exp. 1 were measured.

RESULTS AND DISCUSSION

Growth curve and symptoms of scurvy (Exp. 1 and Exp. 2)

Growth curves of three groups of ODS rats administered different levels of vitamin C in Exp. 1 are shown in Fig. 1a. Only rats of the C150-DE group started to decrease their body weight after 2 weeks, as shown in the first drawing of blood, and showed symptoms of scurvy, such as bleeding around the nose and convulsion in the legs. Rats of the other groups did not show any symptom of scurvy and their growth was normal.

In Exp. 2 the same symptoms of scurvy were observed only in the rats of C150-VE group. The other two groups showed normal growth as illustrated in Fig. 1b.



Fig. 1. Growth curve of ODS rats fed with vitamin E-deficient or supplemented diets at three levels of ascorbate content. a) Exp. 1, vitamin E-deficient diet groups; b) Exp. 2, vitamin E-supplemented diet groups.

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Fig. 2. Effect of dietary ascorbate contents upon the changes of α -tocopherol levels in plasma and erythrocytes of rats fed with a vitamin E-deficient diet for 6 weeks (Exp. 1). Data show mean of each group.

Table 1. α -Tocopherol concentration in plasma, erythrocytes, and tissues after 6 weeks of vitamin E deficiency (Exp. 1).

	Group		
	C150-DE	C300-DE	C600-DE
Dietary AsA (mg/kg diet)	150	300	600
Plasma $(\mu g/ml)$	2.69±0.41	0.97 ± 0.05	0.73 ± 0.11^{a}
RBC (μ g/ml packed cells)	4.15±0.90	1.39±0.29	1.12±0.15 ^b
Heart $(\mu g/m)$ puesed county	20.89 ± 1.07	14.13±0.60	15.62 ± 0.35^{a}
Liver $(\mu g/g)$	15.25 ± 0.28	7.10±0.31	8.14 ± 0.27^{a}
Kidney $(\mu g/g)$	11.06 ± 0.88	5.62 ± 0.38	5.85±0.22
Spleen $(\mu g/g)$	25.35 ± 1.79	10.46±0.48	12.18 ± 0.34^{a}
Lung $(\mu g/g)$	21.32 ± 0.81	12.67 ± 0.52	13.93 ± 0.41^{a}

Values are means \pm SD. The values sharing a superscript letter in group C600 are significantly different from those in group C300; ^ap < 0.005, ^bp < 0.05.

Progress of vitamin E deficiency and effect of dietary levels of ascorbate (Exp. 1)

The progress of vitamin E deficiency plasma and erythrocytes α -tocopherol concentrations decreased in every group as shown in Fig. 2. But the C150-DE group showed the highest level of α -tocopherol among the three groups as seen in the α -tocopherol concentrations in tissues shown in Table 1. The reason for this is

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	Group		
	C150-DE	C300-DE	C600-DE
Dietary AsA (mg/kg diet)	150	300	600
Heart $(\mu g/g)$	11.07 ± 0.82	21.90±2.34	29.48±3.13ª
Liver $(\mu g/g)$	34.61±2.74	125.14±6.92	195.20±10.11ª
Kidney (μ g/g)	21.23 ± 1.20	55.08±3.98	75.60 ± 4.48^{a}
Lung $(\mu g/g)$	54.37±1.04	164.47±8.91	231.46 ± 10.56^{a}
Serum (μ g/ml)	$2.88\!\pm\!0.52$	7.84±0.82	$10.05\!\pm\!0.93^{\mathtt{a}}$

Table 2. Ascorbate concentration in serum and tissues after 6 weeks of vitamin E deficiency (Exp. 1).

Values are means \pm SD. The values sharing a superscript letter in group C600 are significantly different from group those in C300; ^ap < 0.005.

very apparent, because C150-DE rats retained the same amounts of α -tocopherol as did the rats of the other two groups as the beginning of the progress of the vitamin E deficiency, but they could not grow during the progress of the vitamin E deficiency due to the scurvy. Therefore, during the progress of scurvy, rats of C150-DE group still retained the same amounts of α -tocopherol in their bodies as did the other two groups. But, since the body weight of C150-DE was quite small (about 1/2) compared to the other two groups, the highest concentration of α tocopherol in plasma, RBC (erythrocytes), and tissues found in the C150-DE group might only be reflected low body weight during the experiment periods. In comparing the C300-DE and C600-DE groups, two important results are found in Fig. 2 and Table 1. The body weights and tissues weights of both groups were virtually the same and not significantly different. In plasma and RBC after 2, 4, and 6 weeks on the vitamin E-dificient diet, the concentration of α -tocopherol in the C 300-DE group was significantly higher than that of the C600-DE group. Whereas with increasing dose levels of ascorbate in the diet, higher concentrations of α tocopherol in tissues were shown. In all tissues, such as liver, heart, spleen, and lung, excluding the kidney, the α -tocopherol concentration of C600-DE rats was significantly higher than in the C300-DE group. Table 2 shows the ascorbate concentrations in tissues of both groups. Ascorbate concentration in the three groups C150-DE, C300-DE, and C600-DE paralleled the dose of vitamin C in diets. Thus, this relationship between the ascorbate and α -tocopherol concentration shows that ascorbate spares consumption of vitamin E during progress of vitamin E deficiency. Next, in blood, low intake of vitamin C might mobilize vitamin E from the tissues and create higher levels in C300-DE than in C600-DE. The changes in serum lipid level remain to be examined, since changes in serum vitamin C are known to reflect the serum lipid change.



Fig. 3. Effect of dietary ascorbate concentrations upon the α -tocopherol levels in plasma and erythrocytes of rats during normal vitamin E feeding for 6 weeks (Exp. 2). Data show mean of each group.

α -Tocopherol concentration in tissues at normal feeding of vitamin E and the effect of dietary levels of ascorbate (Exp. 2)

In the C300-VE and C600-VE groups, growth curves (Fig. 1b) and tissues weights were virtually the same and not significantly different. The serum concentration of α -tocopherol at 0 week was 11.72 \pm 1.30 (n=21). α -Tocopherol in plasma and RBC of the C150-VE group showed the lowest concentration after 3 weeks of feeding, and then the animals of this group showed symptoms of scurvy and lost body weight. After 5 and 6 weeks the RBC α -tocopherol level of the C150-VE group increased the most among the three groups, and the plasma α -tocopherol level stayed at the same level as that after 3 weeks of feedings (Fig. 3). Comparing this group with the other two groups, the latter two maintained the same level of α tocopherol in RBC and slightly decreased the level of plasma α -tocopherol. Moreover, the highest level of α -tocopherol in tissues among the three groups was found in the C150-VE group, shown in Table 3. The reason for this is similar to that in Exp. 1 and very apparent, because C150-VE rats might retain a slightly lower level of α -tocopherol in the tissues before scurvy set in, as assumed from the finding of plasma and RBC concentrations of α -tocopherol, than that retained by But after 3 weeks the rats lost body weight and the the other two groups. incorporated vitamin E in the bodies of rats of the C150-VE group was still retained, as well as an increased concentration of α -tocopherol in tissues following

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	Group		
	C150-VE	C300-VE	C600-VE
Dietary AsA (mg/kg diet)	150	300	600
Plasma (µg/ml)	4.80±0.72	$2.32 {\pm} 0.35$	3.37 ± 0.47^{a}
RBC (μ g/ml packed cells)	12.18 ± 0.62	6.97±0.75	9.46±0.59ª
Heart $(\mu g/g)$	68.34±2.72	51.51±1.85	56.66±2.41ª
Liver $(\mu g/g)$	39.99±3.20	26.33 ± 1.50	31.54 ± 2.40^{a}
Kidney ($\mu g/g$)	32.16±2.10	26.55 ± 1.27	31.08 ± 1.76^{a}
Spleen $(\mu g/g)$	45.25±3.05	39.65±0.95	41.91 ± 1.15^{a}
Lung $(\mu g/g)$	70.53±5.40	54.05±2.94	62.78 ± 3.46^{a}

Table 3. α -Tocopherol concentration in plasma, erythrocytes, and tissues after 6 weeks of normal feeding of vitamin E (Exp. 2).

Values are means \pm SD. The values sharing a superscript letter in group C600 are significantly different from those in group C300; ^ap < 0.005.

Table 4. Ascorbate concentration in serum and tissues after 6 weeks of normal feeding of vitamin E (Exp. 2).

	Group		
	C150-VE	C300-VE	C600-VE
Dietary AsA (mg/kg diet)	150	300	600
Heart $(\mu g/g)$	15.69 ± 3.05	18.55 ± 3.34	30.04 ± 2.40^{a}
Liver $(\mu g/g)$	57.60±5.81	89.80±6.21	166.60 ± 12.00^{a}
Kidney (μ g/g)	24.39±1.02	43.43±2.36	66.70±3.66 ^a
Lung $(\mu g/g)$	47.33±3.72	140.80±5.85	195.33±8.97*
Serum (µg/ml)	4.63±0.82	6.28±1.14	8.73 ± 0.80^{a}

Values are means \pm SD. The values sharing a superscript letter in group C600 are significantly different from those in group C300; ^ap < 0.005.

the decrease of organ weights. Thus, all of the tissues of the C150-VE group maintained the highest concentration among the three groups. Except for the C150-VE group, the α -tocopherol concentration in RBC and plasma of the C300-VE and C600-VE groups are parallel during 6 weeks of feeding. The concentrations of α -tocopherol in both plasma and RBC were significantly higher in the C600-VE group than in the C300-VE group at any time. Table 3 shows the results regarding the α -tocopherol concentration in tissues tested (heart, liver, kidney, spleen, and lung) in the three groups. Ascorbate concentration in tissues of the three groups are shown in Table 4. The ascorbate concentration in tissues depended upon the dose level of vitamin C. These results explain more clearly than those in Exp. 1 the interrelationship between vitamins E and C *in vivo*. Since in this experiment vitamin E was supplemented to the animals, the differences in α -tocopherol concentrations between the C300-VE groups was much higher than those in Exp. 1.

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Whereas, in plasma and RBC, as in tissues, the higher level of α -tocopherol was found in the C600-VE rather than the C300-VE group. In Exp. 2, since vitamin E was supplemented, plasma and RBC α -tocopherol concentrations might reflect tissue levels of vitamin E. And at normal nutritional levels of vitamin E, the higher intake of vitamin C spares consumption of vitamin E *in vivo*.

These results are just the reverse of Burton *et al.* (18) because in these experiments we used ODS rats. This paper shows the first biological evidence regarding an interrelationship between ascorbate and α -tocopherol *in vivo* using intact animals. This result owes to the good uniformity of the ODS rats regarding the nutritional status of vitamin E. Mutant ODS rats show very low individual differences for both vitamin E and C concentrations in tissues, plasma, and RBC. Several years ago we used the guinea pig for the same purpose (19). But because that species shows great individual differences, we could not find any significant difference among groups. Thus the new ODS mutant rat is useful for research on the interrelationship between vitamins C and E.

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REFERENCES

- 1) Scarpa, M. (1984): Formation of α -tocopherol radical and recycling of α -tocopherol by ascorbate during peroxidation of phosphatidylcholine liposomes. *Biochim. Biophys.* Acta, 801, 215–219.
- Niki, E., Kawakami, A., Yamamoto, Y., and Kamiya, Y. (1985): Synergistic inhibition of oxidation of soybean phosphatidylcholine liposomes in aqueous dispersions by vitamin E and vitamin C. Bull. Chem. Soc. Jpn., 58, 1971–1975.
- 3) McCay, P. B. (1985): Vitamin E: interactions with free radicals and ascorbate. Ann. Rev. Nutr., 5, 323-340.
- Niki, E., Saito, T., Kawakami, A., and Kamiya, Y. (1984): Inhibition of oxidation of methyl linoleate in solution by vitamin E and vitamin C. J. Biol. Chem., 259, 4177– 4182.
- 5) Mukai, K. (1987): Stop-flow investigation of the reaction between vitamin E radical and vitamin C in solution. *Biochim. Biophys. Acta*, **991**, 276–279.
- 6) Niki, E. (1987): Interaction of ascorbate and α -tocopherol. Ann. NY. Acad. Sci., 498, 186–199.
- 7) Chen, L. H. (1981): An increase in vitamin E requirement induced by high supplementation of vitamin C in rats. Am. J. Clin. Nutr., 34, 1036-1041.
- 8) Dillard, C. J., Downey, J. E., and Tappel A. L. (1984): Effect of antioxidants on lipid peroxidation in iron-loaded rats. *Lipids*, **19**, 127–133.
- 9) Chen, L. H., and Thacker, R. R. (1986): Effects of dietary vitamin E and high level of ascorbic acid on iron distribution in rat tissus. *Int. J. Vit. Nutr. Res.*, 56, 253–258.
- 10) Chen, L. H., and Thacker, R. R. (1987): Effect of ascorbic acid and vitamin E in biochemical changes associated with vitamin E deficiency in rats. Int. J. Vit. Nutr.

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Res., 57, 385-390.

- 11) Chen, L. H., and Chang, H. M. (1978): Effect of dietary vitamin E and vitamin C on respiration and swelling of guinea pig liver mitochondria. J. Nutr., 108, 1616–1620.
- 12) Kunert, K. J., and Tappel, A. L. (1983): The effect of vitamin C on *in vivo* lipid peroxidation in guinea pigs as measured by pentane and ethane production. *Lipids*, 18, 271–274.
- 13) Hruba, F., Novakova, V., and Ginter, E. (1982): The effect of chronic marginal vitamin C deficiency on the α -tocopherol content of the organs and plasma of guinea pigs. *Experimentia*, **38**, 1454–1455.
- 14) Bendich, A., D'Apolito, P., Gabriel, E. and Machlin, L. K. (1984): Interaction of dietary vitamin C and vitamin E on guinea pig immune responses to mitogens. J. Nutr., 114, 1588–1593.
- 15) Miyazawa, T., Ando, T., and Kaneda, T. (1986): Effect of dietary vitamin C and vitamin E on tissue lipid peroxidation of guinea pigs fed with oxidized oil. Agric. Biol. Chem., 50, 71–78.
- 16) Arad, I. D., Dgani, Y., and Eyal, F. G. (1985): Vitamin E and vitamin C plasma levels in premature infants following supplementation of vitamin C. Int. J. Vit. Nutr. Res., 55, 395–397.
- 17) Keith, R. E., Chrisley, B. M., and Driskell, J. A. (1980): Dietary vitamin C supplementation and plasma vitamin E levels in humans. Am. J. Clin. Nutr., 33, 2394–2400.
- 18) Burton, G. W., Wronska, U., Stone, L., Foster, D. O., and Ingold, K. U. (1990): Biokinetics of dietary RRR-α-tocopherol in the male guinea pig at three levels of vitamin C and two levels of vitamin E. Evidence that vitamin C does not "spare" vitamin E in vivo. Lipids, 25, 199–210.
- Igarashi, O., Mouri, K., and Chen, L.-M. (1987): Nutritional factors affecting vitamin E levels in tissues, *in* Clinical and Nutritional Aspects of Vitamin E, ed. by Hayaishi, O., and Mino, M., Elsevier, Holland, pp. 63–72.
- 20) Mizushima, Y., Harauchi, T., Yoshizaki, T., and Makino, S. (1984): A rat mutant unable to synthesize vitamin C. *Experimentia*, 40, 359–361.
- 21) Horio, F., Ozaki, K., Yoshida, A., Makino, S., and Hayashi, Y. (1985): Ascorbic acid requirement in a rat mutant unable to synthesize ascorbic acid. J. Nutr., 115, 1630– 1640.
- 22) Horio, F., Ozaki, K., Oda, H., Makino, S., Hayashi, Y., and Yoshida, A. (1987): Effect of dietary ascorbic acid, cholesterol and PCB on cholesterol concentrations in serum and liver in a rat mutant unable to synthesize ascorbic acid. J. Nutr., 117, 1036– 1044.
- 23) Ueda, T., and Igarashi, O. (1987): Effect of coexisting fat on the extraction of tocopherols from tissues after saponification as a pretreatment for HPLC determination. J. Micronutr. Anal., 3, 15-25.
- 24) Ueda, T., and Igarashi, O. (1987): New solvent system for extraction of tocopherols from biological specimens for HPLC determination and the evaluation of 2,2,5,7,8-pentamethyl-6-chromanol as an internal standard. J. Micronutr. Anal., 3, 185–198.
- 25) Ueda, T., and Igarashi, O. (1990): Determination of vitamin E in biological specimens and foods by HPLC. Pretreatment of samples and extraction of tocopherols. J. Micronutr. Anal., 7, 79–96.

26) Roe, J. M., and Oestering, M. (1943): J. Biol. Chem., 147, 399; Arakawa, N., and Okamura, M. (1989): in Vitamins Analysis in Japanese, ed. by Japanese Society of Vitaminology, Kagakudoujin, Kyoto, pp. 135–143.