

Studies of the metabolism of α -tocopherol stereoisomers in rats using [5-methyl- ^{14}C]SRR- and RRR- α -tocopherol

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Abstract We investigated the distribution and metabolism of SRR- α -tocopherol (SRR- α -Toc), synthetic α -Toc compared with RRR- α -Toc, in rats after a single oral administration of 2 mg (20 μCi) SRR- and RRR- α -[5-methyl- ^{14}C]Toc. In the liver, there was no difference in the recovery of radioactivity until 12 h after administration, and it reached a maximum of 4.4% of the dose after 12 h, but in other tissues, radioactivity derived from RRR- α -Toc was clearly higher than that derived from SRR- α -Toc after 12 h. For 96 h after administration, urinary excretions of SRR- α -Toc were 7.8% of the dose and significantly greater than that of RRR- α -Toc, which was 1.3% of the dose. On the other hand, total fecal excretions of SRR- and RRR- α -Toc were 87.6% and 83.0%, respectively. Therefore, radioactivity in the urine was assumed to have transferred out of the liver. Furthermore, the urine samples were hydrolyzed with 3 N methanolic HCl and analyzed by high performance liquid chromatography (HPLC) and liquid chromatography/mass spectrometry. The results showed that about 73% of the total radioactivity injected into HPLC was found to be 2,5,7,8-tetramethyl-2-(2'-carboxyethyl)-6-hydroxy chroman (α -CEHC), as well as RRR- α -Toc. Thus, there is no difference between SRR- α -Toc and RRR- α -Toc in metabolic pathways, and it is suggested that SRR- α -Toc discriminated in the liver is rapidly metabolized by the liver and excreted as the conjugate of α -CEHC in the urine.—Kaneko, K., C. Kiyose, T. Ueda, H. Ichikawa, and O. Igarashi. Studies of the metabolism of α -tocopherol stereoisomers in rats using [5-methyl- ^{14}C]SRR- and RRR- α -tocopherol. *J. Lipid Res.* 2000. 41: 357–367.

Supplementary key words RRR- α -tocopherol • SRR- α -tocopherol • RRR- α -[5-methyl- ^{14}C]tocopherol • SRR- α -[5-methyl- ^{14}C]tocopherol • α -CEHC • α -tocopherol metabolism in vivo • distribution of radioactivity in rat • detection of LC-MS • urine metabolites

α -Tocopherol (α -Toc) has three chiral centers in its phytyl tail (2, 4' and 8'), which could result in a total of eight different stereoisomers. Naturally occurring α -Toc consists of a single stereoisomer, 2*R*, 4'*R*, 8'*R*- α -Toc (RRR- α -Toc), whereas the synthetic form, all-*rac*- α -Toc, contains equimolar stereoisomers. α -Toc is used as a dietary supplement and a food additive in both its forms. However, in the rat resorption–gestation assay, relative biopotencies of

individual α -Toc acetate (α -Toc Ace) stereoisomers of 100% (RRR), 90% (RRS), 73% (RSS), 57% (RSR), 60% (SSS), 37% (SRS), 31% (SRR), and 21% (SSR) were established (1), and the biopotency of synthetic α -Toc was therefore found to be different from the natural form.

The study of the distribution of α -Toc stereoisomers in vivo suggested that the 2*R*-configuration especially affects the biodiscrimination between the eight α -Toc stereoisomers (2–6). This discrimination is related to the α -Toc transfer protein (α -TTP) that is present in the liver (7–9). α -Toc stereoisomers are equally well absorbed from the intestine (10) and transported to the liver, but only 2*R*-isomers are preferentially secreted in association with nascent very low density lipoprotein. This is dependent on the characterization of α -TTP. After that, 2*S*-isomers remaining in the liver rapidly disappeared (11, 12).

Tocopherol has two possible metabolic pathways. One leads to the degradation of the side chain after opening of the chroman ring and the other to the degradation of the side chain while retaining the chroman ring. In 1956, Simon et al. (13, 14) investigated urinary metabolites of rabbits and humans administered 10 to 15 mg (1.5 to 2.0 μC) of α -Toc. After hydrolysis, these metabolites were identified as 2-(3-hydroxy-3-methyl-5-carboxypentyl)-3,5,6-trimethylbenzoquinone (α -Toc acid) and its lactone, so this was assumed to be the metabolic pathway for α -Toc (13, 14). In this pathway, first the chroman ring is opened, leading to α -tocopherylquinone (α -TQ). α -TQ is then reduced to the α -hydroquinone, conjugated with glucuronic acid, and degraded by β -oxidation, ultimately being excreted to the urine. After that, two additional minor me-

Abbreviations: α -Toc, α -tocopherol; α -Toc-Ace, α -Toc-acetate; α -TTP, α -Toc transfer protein; α -Toc acid, 2-(3-hydroxy-3-methyl-5-carboxypentyl)-3,5,6-trimethylbenzoquinone; α -TQ, α -tocopherylquinone; α -CEHC, 2,5,7,8-tetramethyl-2-(2'-carboxyethyl)-6-hydroxy chroman; SLLU- α , 2,7,8-trimethyl-2-(2'-carboxyethyl)-6-hydroxy chroman; LSC, liquid scintillation counter; HPLC, high performance liquid chromatography; MS, mass spectrometry; UV, ultraviolet detector; RD, radiometric detector; α -CEHC-Me, methyl ester of α -CEHC.

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tabolites of α -Toc, E-acid I [2,3,5-trimethyl-6-(5'-carboxy-3'-methyl-2'-pentenyl)-1,4-benzoquinone], which is formed by dehydration of a part of Toc acid, and E-acid II [2,3,5-trimethyl-6-(3'-carboxybutyl)-1,4-benzoquinone] were identified from the urine of rabbits after an administration of high doses of α -Toc by Watanabe et al. in 1974 (15).

On the other hand, in 1965, Schmandke (16) suggested the presence of 2,5,7,8-tetramethyl-2-(2'-carboxyethyl)-6-hydroxy chroman (α -CEHC), which degrades the side chain without opening the chroman ring in excretions of α -Toc. Later in 1995, α -CEHC was isolated from the urine of humans and administered with large doses of vitamin E supplementation, after hydrolysis, by Schultz et al. (17).

As for the other vitamin E homologues, δ -Toc and γ -Toc, they were reported to be the same as the metabolites with the chroman ring structurally closed to α -CEHC. In 1984, Chiku, Hamamura, and Nakamura (18) analyzed the metabolites excreted in the urine of rats that had δ -

Toc administered intravenously and concluded that the major metabolite of δ -Toc was sulfate conjugate of 2,8-dimethyl-2-(2'-carboxyethyl)-6-chromanol. For γ -Toc, in 1993, Wechter et al. (19) isolated a new endogenous natriuretic factor from the urine of humans who had uremia. As a result of structural analysis of this compound, this compound was found to be a metabolite of γ -Toc, 2,7,8-trimethyl-2-(2'-carboxyethyl)-6-hydroxy chroman (*SLLU*- α) (19).

As noted above, there are various reports for metabolites of Toc. However, it is not clear how and by which organs Toc is metabolized. In our present study, we focused on the later metabolic fate of *SRR*- α -Toc, which is discriminated in the liver, investigated the difference in the distribution, including metabolites between *RRR*- and *SRR*- α -[14 C]-Toc in rats after oral administration, and characterized the urinary metabolite. This study also focuses on the metabolism of *RRR*- α -Toc.

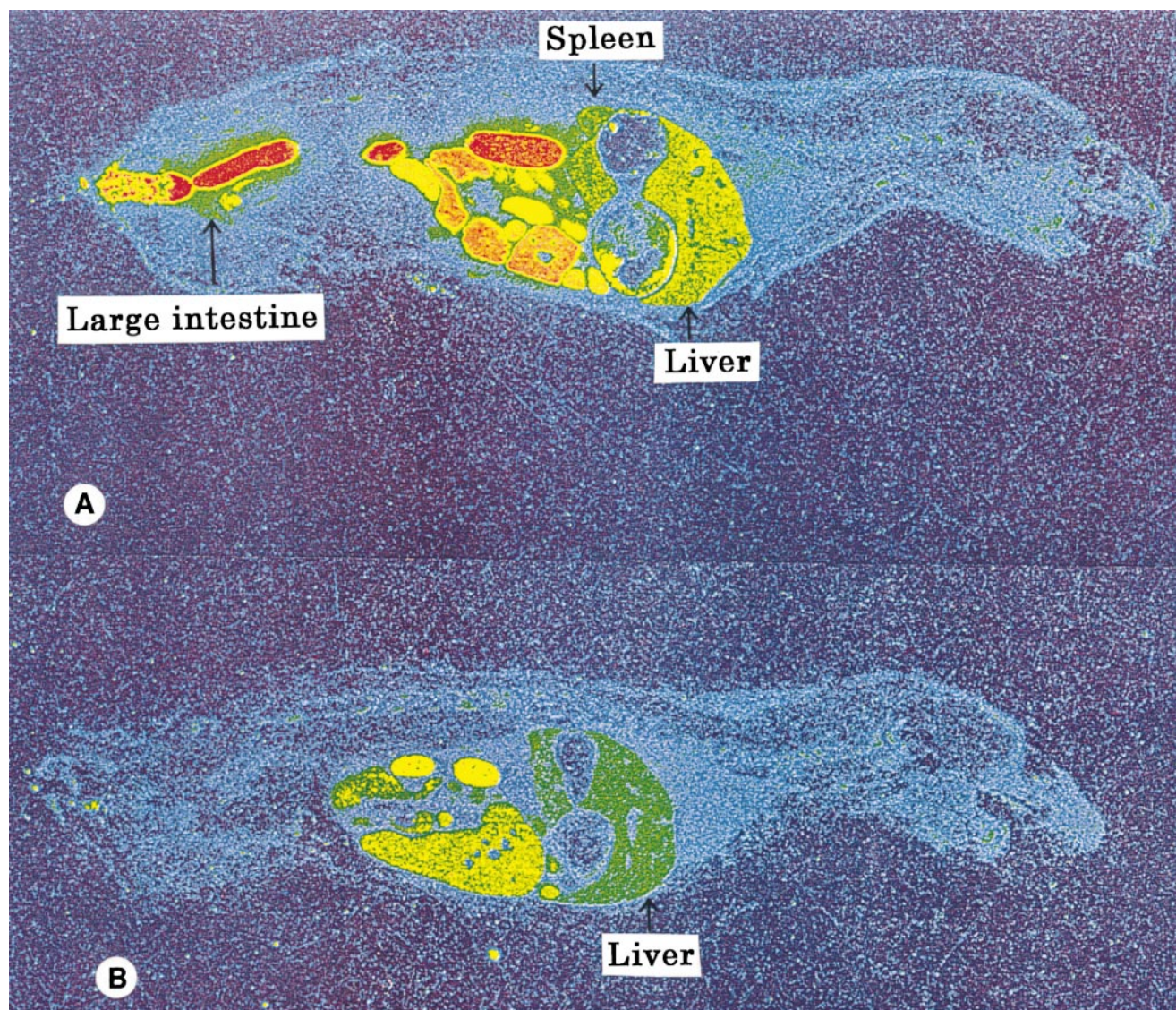


Fig. 1. Autoradiogram showing distribution pattern of *SRR*- α -[14 C]tocopherol in rat. Rats were administered orally a single dose of 2 mg (20 μ Ci) *SRR*- α -[14 C]tocopherol; A: 12 h after administration; B: 24 h after administration.

EXPERIMENTAL PROCEDURES

Materials

SRR and *RRR*- α -[5-methyl- ^{14}C]-Toc (specific activity: 4.74 MBq/mg and 4.81 MBq/mg, radiochemical purity: 95.4% and 97.4% as determined by HPLC, respectively) was obtained by methylation of *SRR* and *RRR*- γ -Toc with [^{14}C]paraformaldehyde and subsequent reduction at Tokai research laboratories, Daiichi Pure Chemicals Co., Ltd. (Ibaraki, Japan). The detailed method was reported by Nakamura, Hamamura, and Ueda (20). *2-ambo*- α -Toc Ace and α -CEHC were kindly donated by Eisai Co., Ltd. (Tokyo, Japan).

Preparation of *SRR*- α -Toc

Free *SRR*- α -Toc was prepared from *SRR*- α -Toc Ace, which was separated from *2-ambo*- α -Toc Ace as described (21). To *SRR*- α -Toc Ace in ethanol was added 6% ethanolic pyrogallol solution and 60% KOH. The mixture was saponified with occa-

sional shaking at 70°C for 30 min under nitrogen. After being cooled in ice water, the reaction mixture was diluted with 1% NaCl solution and extracted two times with hexane. Then the hexane layer was evaporated. The residue dissolved in methanol was passed through a column of octadecylsilyl silica (Sep Pak C₁₈; Waters Associates, Milford, MA), then again extracted with hexane. The purity of *SRR*- α -tocopherol was 99% as determined by HPLC.

Animals

Ten-week-old (5-week-old for autoradiography) male Fisher 344/DuCrj strain rats were purchased from Charles River Co. (Yokohama, Japan). They were fed on a commercial diet (CE-2; Nippon Clea Co., Tokyo, Japan) containing 30.31 mg *dl*- α -Toc per kg. For the isolation of the metabolites, 3-week-old male Sprague-Dawley strain rats were purchased from Nippon Clea Co. These animals were initially fed on CE-2 for 1 week, then given a vitamin E-deficient diet (AIN-76; Funabashi Farms,

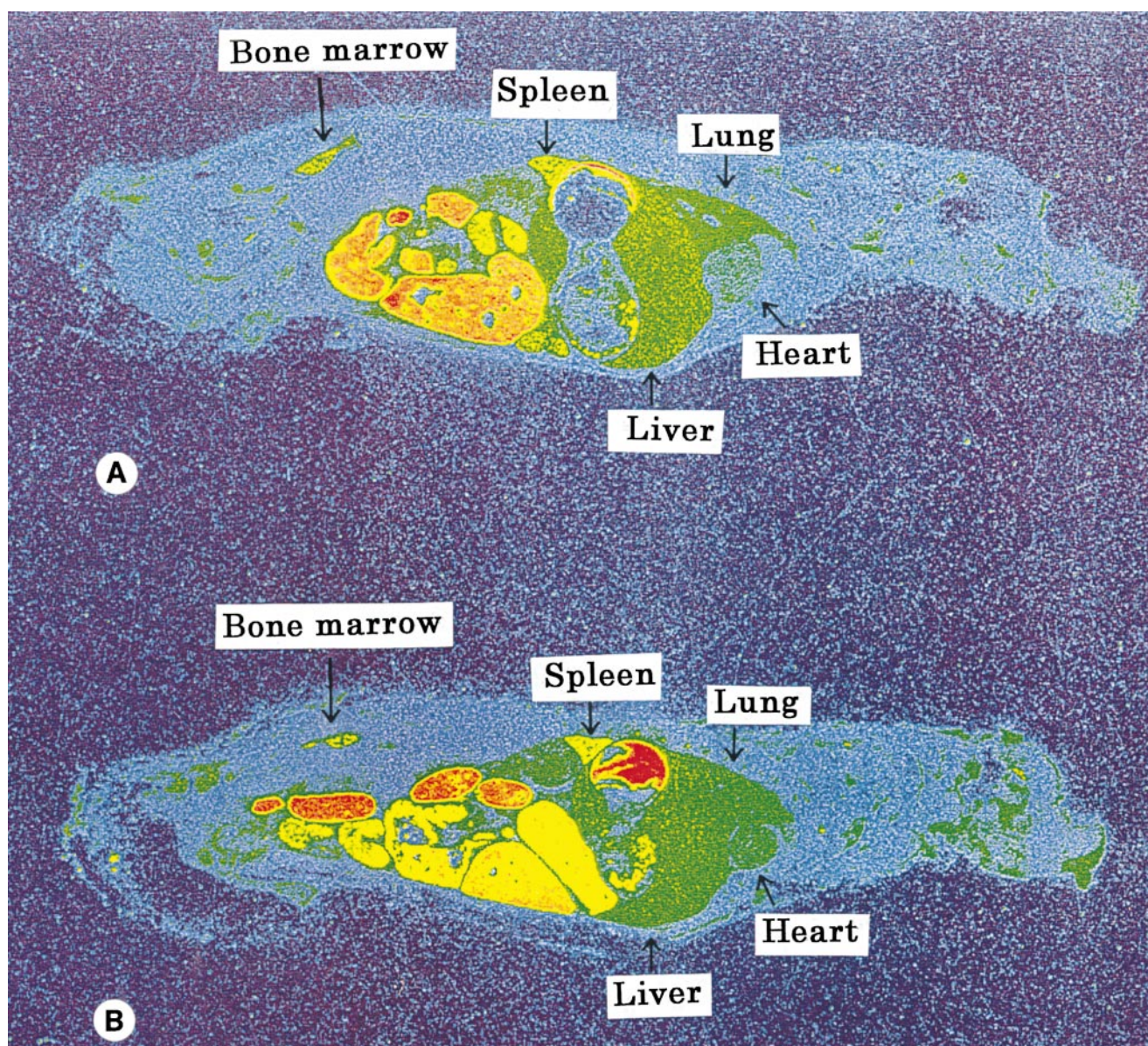


Fig. 2. Autoradiogram showing distribution pattern of *RRR*- α -[^{14}C]tocopherol in rat. Rats were administered orally a single dose of 2 mg (20 μCi) *RRR*- α -[^{14}C]tocopherol; A: 12 h after administration; B: 24 h after administration.

Chiba, Japan), to which was added 10% stripped corn oil (Funabashi Farms), for 6 weeks. All animals were housed individually in cages at 22–24°C and 50–60% humidity, with a 12-h light/dark cycle. The food and water were supplied ad libitum.

Dosing and sample collections

The rats (weighing about 200 g) were separated into *SRR*- α -Toc groups and *RRR*- α -Toc groups (each of four rats). After 18 h without food, *SRR*- or *RRR*- α -[5-methyl-¹⁴C]Toc dissolved with stripped corn oil (2 mg/20 μ Ci/1 ml) was administered by stomach tube to each rat. Tissues were obtained from animals dissected at 4, 12, 24, 48, 72, and 96 h after the dosage. Two other groups of four rats each, dissected 96 h after administration, were placed into metabolic cages individually for blood, urine, and feces collections at 4, 12, 24, 48, 72, and 96 h postdose. The total volumes of the urine voided were recorded after each collection. The blood was centrifuged at 2,500 rpm for 10 min to obtain plasma. All samples were stored at –80°C until analysis.

Determination of total radioactivity

Total radioactivity was measured in an LS5801 liquid scintillation counter (LSC) (Beckman Instruments, Inc., Tokyo, Japan) with 5 ml of Hionic-Fluor scintillator (Hewlett-Packard Japan, Ltd., Tokyo, Japan). For pretreatment, 50 μ l of the plasma or 50 mg of the tissues cut into small pieces or feces ground in a mortar were incubated at 50°C to 60°C overnight after the addition of 0.5 ml of Soluene-350 (Hewlett-Packard Japan, Ltd.) as a solubilizer in a scintillation vial. The radioactivity in urine was determined by direct liquid scintillation counting of a 0.1-ml aliquot of samples. Each sample vial was counted for 10 min, and the counting efficiency was corrected by external standardization.

Autoradiography

Four rats were separated into two groups. After 12 h without food, *SRR*- or *RRR*- α -[5-methyl-¹⁴C]Toc dissolved with stripped corn oil (2 mg/20 μ Ci/1ml) was administered by stomach tube to each rat. Whole-body autoradiography was carried out at 12 and 24 h after the dosage, following the procedure described by Matsuoka, Joshima, and Kashima (22, 23). Autoradioluminogram was prepared by Nemoto & Co., Ltd. (Tokyo, Japan).

Preparation of urinary samples for HPLC analysis

Urinary samples, excreted over a period of 12–48 h after administration, were pooled and applied to a Sep Pak C₁₈ to remove salts. After concentration, it was extracted with methanol and filtrated by 45 μ m microfilter for removing salts again. As the radioactivity of methanol extract was measured, more than 95% of radioactivity in the urine was recovered. The extract was dried and dissolved in a mobile phase.

An aliquot was analyzed by reversed-phase HPLC. The HPLC was performed on a Waters Symmetry C₁₈ column (4.6 mm \times 250 mm, 5 μ m; Japan Millipore Ltd., Tokyo, Japan) at 30°C by using a CCEP pump (TOSOH Co., Tokyo, Japan) and an E5CS column oven (OMRON Co., Tokyo, Japan). A mobile phase was 50% (v/v) acetonitrile–water (pH was adjusted to 3.6 with acetic acid) at a flow rate of 1.0 ml/min. The effluent was monitored with an ultraviolet detector (UV) (UV-8010; TOSOH Co., Tokyo, Japan) set at 295 nm and radiometric detector (RD) (raytest, RAMONA; M&S Instruments Trading Inc., Tokyo, Japan) connected in series.

Isolation of urinary metabolite

Vitamin E-deficient rats (weighing about 400 g) were administered a single dose of *SRR*- α -Toc dissolved with stripped corn oil (10 mg/1 ml) after 17 h without food. After administration, the rats were placed into metabolic cages individually. Urine was collected

into the flask on dry ice every 12 h for 48 h. Urine samples were immediately lyophilized and stored at –30°C until analysis. The hydrolysis was performed at 60°C for 1 h under nitrogen after the addition of 3 N methanolic HCl to the lyophilized urine powder. The reaction mixture was diluted with a 3-fold volume of water, then extracted with hexane. The hexane phase was removed with a stream of nitrogen. The residue dissolved in the mobile phase was injected into HPLC as described above. The collected metabolite fraction was evaporated and stored at –30°C until LC/MS analysis.

Liquid chromatography/mass spectrometry

LC/MS analysis was performed on a TSQ 7000 LC/APCI MS system (Thermo Quest K.K., Tokyo, Japan). Conditions were as follows: auxiliary gas flow, 10 units, sheath gas pressure, 70 psi, capillary temperature, 150°C, vaporizer temperature, 400°C; corona current, 5 μ amps; scan time, 1 sec. For LC, a CAPCEL PAK UG120 column (5 μ m, 1.5 \times 250 mm; Shiseido Co., Ltd., Tokyo, Japan) was used. The mobile phase was 50% (v/v) acetonitrile–water at a flow rate of 0.1 ml/min.

Statistical analysis

All values are expressed as means \pm SD. The significance of the difference between radioactivity derived from *SRR*- α -Toc and *RRR*- α -Toc was evaluated by paired-comparison *t* tests. The differences were considered significant at *P* < 0.05.

RESULTS

Isotope distribution within the body by macro autoradiography

The distribution of *SRR*- α -[¹⁴C]Toc in rat tissues by autoradiography is shown in Fig. 1. Twelve hours after administration, a high level of radioactivity was shown in the liver, the spleen, and the large intestine. After 24 h, there was no change in the isotope distribution, but radioactivity decreased as a whole so that most of the administered

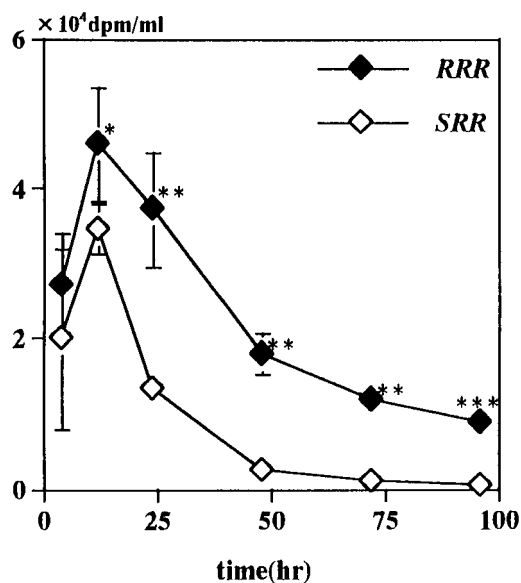


Fig. 3. Change in the radioactivity level of the plasma. Rats were administered orally a single dose of 2 mg (20 μ Ci) *SRR*- or *RRR*- α -[¹⁴C]tocopherol. Results are shown as mean values \pm SD of four rats. Values with asterisks denote a significant difference between *SRR* and *RRR*; *** *P* < 0.001, ** *P* < 0.01, * *P* < 0.05.

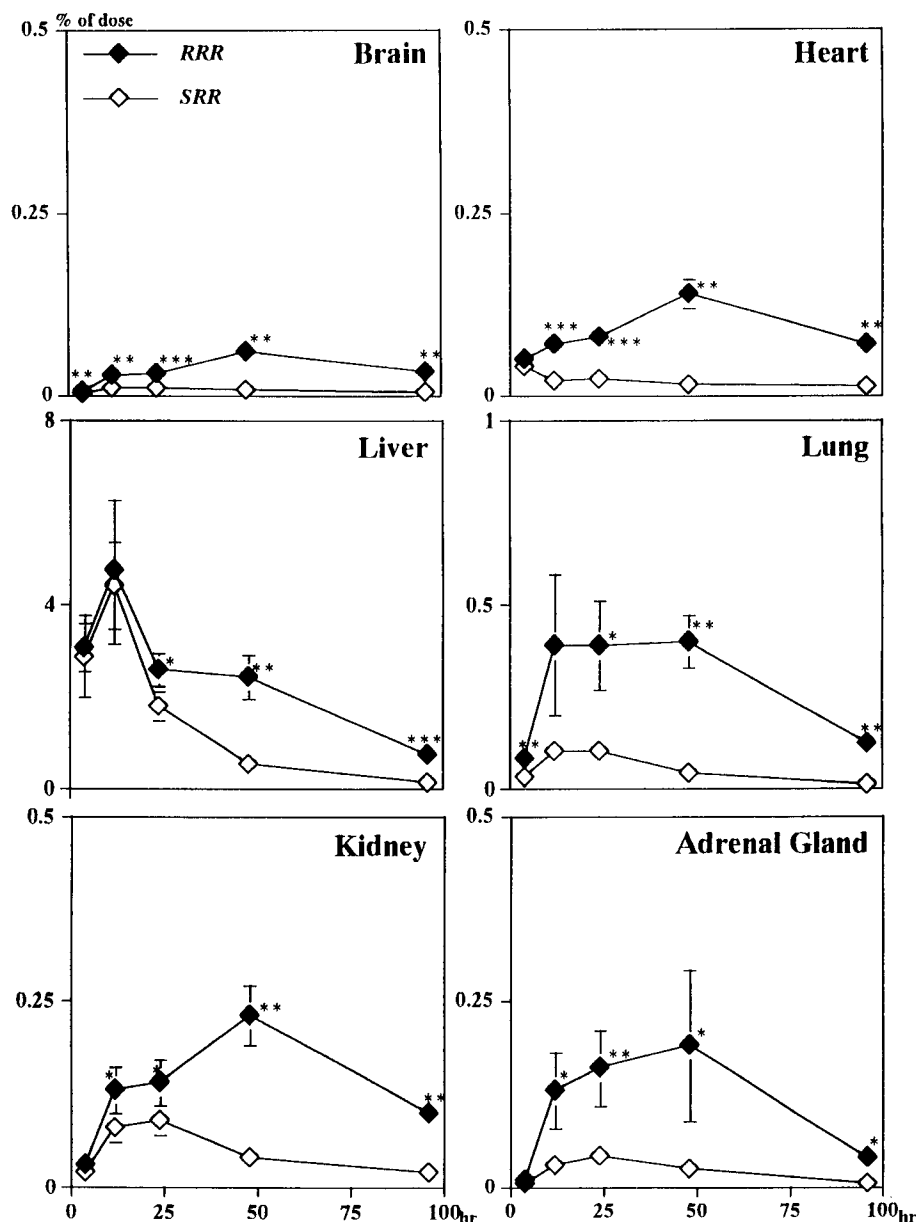


Fig. 4. Change in the recovery of radioactivity in the tissues. Rats were administered orally a single dose of 2 mg (20 μ Ci) *SRR*- or *RRR*- α - 14 C]tocopherol. Results are shown as mean values \pm SD of three or four rats. See Fig. 3.

Toc was already excreted out of the body. In contrast, the distribution of *RRR*- α - 14 C]Toc in rat tissues (Fig. 2), 12 h after administration, showed high levels of radioactivity in various tissues such as the heart, the lung, the liver, the spleen, the bone marrow, and the large intestine. Radioactivity in the spleen and the bone marrow was probably from radioactivity in the blood. After 24 h, there was no change of isotope distribution or radioactivity level, and the isotope was distributed throughout the body by blood circulation.

Distribution of radioactivity in the plasma and tissues

A graph of total radioactivity time duration in the plasma after administration of *SRR*- or *RRR*- α - 14 C]Toc is

shown in Fig. 3. Total radioactivity in the plasma derived from both forms of α -Toc reached its highest levels 12 h after the dose, then decreased. After 12 h, however, radioactivity in the plasma derived from *RRR*- α -Toc was significantly higher than that derived from *SRR*- α -Toc. It was shown that *RRR*- α -Toc was preferentially released from the liver to the plasma, compared to *SRR*- α -Toc which remained in the liver. *SRR*- α -Toc disappeared rapidly from the plasma. In comparing the respective ratios of decrease of radioactivity, it was found that radioactivity derived from *SRR*- α -Toc was down 92.4%, and from *RRR*- α -Toc it was down only 61.2%, from 12 to 48 h postdose. This result was because of the recirculation of *RRR*- α -Toc.

Time-dependent recovery of radioactivity in the tissues

is shown in Fig. 4. In the liver, there was no difference in the recovery of radioactivity derived from both forms of α -Toc for 12 h after administration. The maximum total radioactivity after 12 h occupied 4.4% of the original dose. But after 24 h, the radioactivity derived from *RRR*- α -Toc was higher compared with that derived from *SRR*- α -Toc. In other tissues, there were traces throughout, but after 12 h, radioactivity derived from *RRR*- α -Toc was clearly higher than that derived from *SRR*- α -Toc. Furthermore, radioactivity derived from *SRR*- α -Toc reached its maximum level 24 h after the dosage, and that derived from *RRR*- α -Toc reached maximum at 48 h. Therefore, it was shown that *SRR*- α -Toc disappeared rapidly from the liver. The radioactivity of the carcass 96 h after administration of [14 C]*SRR*- α -Toc and *RRR*- α -Toc was $0.99 \pm 0.10\%$ and $4.94 \pm 0.95\%$ of the dose, respectively, and *RRR*-isomer was higher than *SRR*-isomer. It was proved that *SRR*- α -Toc has poor retention in the body compared with *RRR*- α -Toc. Table 1 shows the quantitative difference of radioactivity in rat tissues between the *RRR* group and the *SRR* group. The radioactivities are expressed as $\text{dpm} \times 10^3$ per gram wet tissue. Radioactivity in all tissues derived from *RRR*- α -Toc was significantly higher than that derived from *SRR*- α -Toc. Radioactivity in adrenal glands was the highest in all tissues because the whole weight of adrenal glands was the smallest in all tissues.

Urinary and fecal excretion of radioactivity

The cumulative urinary and fecal elimination patterns after administration of *SRR* or *RRR*- α -[14 C]Toc are shown in Fig. 5. The urinary excretion of radioactivity derived from *SRR*- α -Toc was significantly greater than that derived from *RRR*- α -Toc, indicating total excretion rates of 7.8% and 1.3%, respectively, for 96 h postdose. On the other hand, fecal excretion of radioactivity in the *SRR*- α -Toc group at only 12 h after administration was clearly greater than in the *RRR*- α -Toc group, but thereafter no differences were found. For 96 h after administration, total fecal excretion of *SRR* and *RRR*- α -Toc was 87.6% and 83%, respectively.

Characterization of urinary radioactive substances

Because a significant portion of radioactivity was recovered in the urine within 48 h, the samples from collections from 12 to 48 h after the dose (maximum excretion) were pooled and used for further analysis. A representative HPLC chromatogram of the urine extract is shown in Fig. 6. In RD, only one peak was detected at the retention time of around 2 min, but not detected in UV 295 nm because of other peaks. The effluent corresponding to this peak was collected, and an aliquot was measured in LSC. Radioactivity corresponding to this peak derived from *SRR*- α -Toc was 75% of the total radioactivity injected into HPLC. Therefore it was suggested that the peak detected in RD was due to a major urinary metabolite. As for *RRR*- α -Toc, because it has much less radioactivity, a 10-fold concentration of the urine sample was used. The same chromatograms of *SRR*- α -Toc were obtained. Radioactivity of radioactive substances was 67% of that injected initially.

TABLE 1. Radioactivities in rat tissues after oral administration of *RRR*- α -[5-methyl- 14 C]tocopherol or *SRR*- α -[5-methyl- 14 C]tocopherol

Tissue	4 H		12 H		24 H		48 H		96 H	
	RRR	SRR	RRR	SRR	RRR	SRR	RRR	SRR	RRR	SRR
Brain	1.83 \pm 0.25 ^b	0.83 \pm 0.20	8.53 \pm 2.26 ^b	2.65 \pm 0.45	9.55 \pm 1.57 ^a	2.90 \pm 0.32	19.35 \pm 4.51 ^b	1.99 \pm 0.54	10.46 \pm 1.96 ^b	1.41 \pm 0.17
Heart	32.34 \pm 6.78 ^b	29.39 \pm 11.40	44.11 \pm 10.98 ^a	14.36 \pm 3.11	52.58 \pm 10.00 ^a	16.14 \pm 4.16	90.65 \pm 20.36 ^b	10.37 \pm 2.05	47.39 \pm 7.43 ^b	8.47 \pm 0.93
Lung	49.76 \pm 6.97 ^b	20.76 \pm 6.25	237.53 \pm 114.25	67.21 \pm 12.01	238.79 \pm 76.71 ^c	61.43 \pm 11.38	248.48 \pm 47.04 ^b	25.24 \pm 6.11	75.58 \pm 11.96 ^b	9.06 \pm 1.43
Liver	214.80 \pm 38.38	211.65 \pm 63.76	259.97 \pm 92.80	250.60 \pm 59.13	119.22 \pm 14.40 ^c	109.32 \pm 23.87	113.00 \pm 29.34 ^b	26.53 \pm 7.93	36.54 \pm 5.10 ^a	7.15 \pm 0.47
Kidney	7.76 \pm 1.01	6.56 \pm 2.03	38.09 \pm 9.02 ^c	25.01 \pm 6.30	39.70 \pm 7.62 ^c	25.16 \pm 7.11	63.55 \pm 11.44 ^b	10.41 \pm 1.47	29.81 \pm 4.69 ^b	5.24 \pm 0.38
Adrenal glands	283.55 \pm 67.55	80.01 \pm 29.49	2157.31 \pm 865.01 ^c	521.42 \pm 104.65	3042.47 \pm 878.30 ^c	768.84 \pm 155.67	3889.82 \pm 1198.74 ^b	453.45 \pm 151.61	674.62 \pm 196.70 ^b	67.41 \pm 6.79

Rats were administered a single oral dose of 2 mg (20 μ Ci) *SRR*- or *RRR*- α -[14 C]tocopherol. Results are expressed as $\text{dpm} \times 10^3/\text{g}$ wet tissue and as means \pm SD of four rats. ^a $P < 0.001$; ^b $P < 0.01$; ^c $P < 0.05$, significant difference between *SRR* and *RRR* at a time point (by t-test).

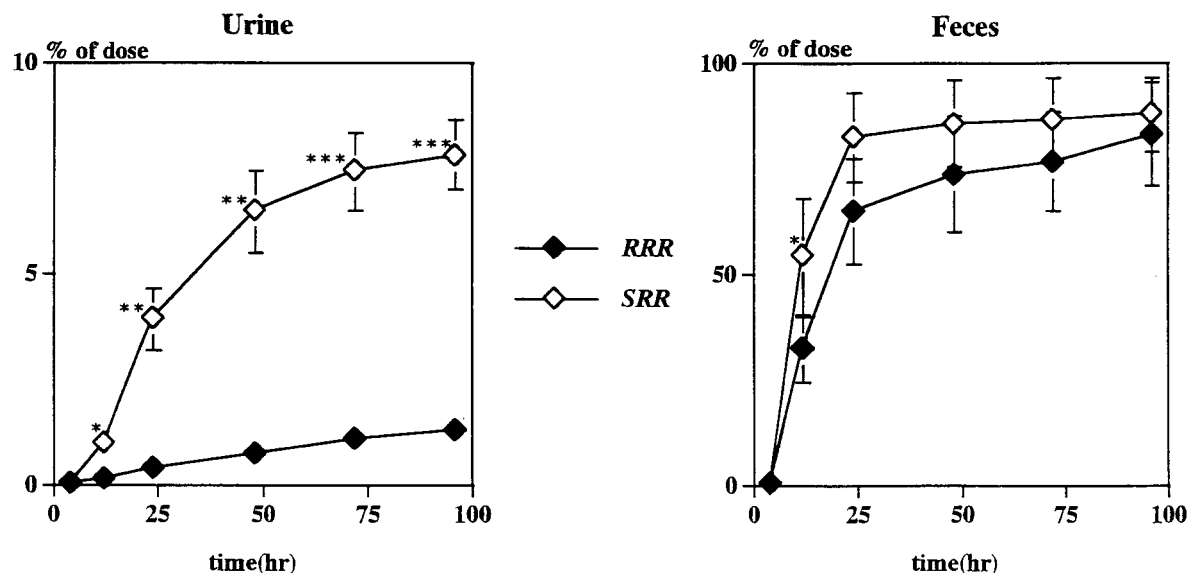


Fig 5. Cumulative excretion of radioactivity in urine and feces. Rats were administered orally a single dose of 2 mg (20 μ Ci) *SRR*- or *RRR*- α - 14 C]tocopherol. Results are shown as mean values \pm SD of three or four rats. See Fig. 3.

Next, the dry extract was treated with 3 N methanolic HCl at room temperature overnight under nitrogen to hydrolyze conjugates and promote esterification. After evaporation, the mixture was dissolved in the mobile phase

and injected into HPLC. The retention time of the radioactive substance shifted to 16.7 min after methylation. Moreover, in UV 295 nm, the peak at the same retention time was detectable (Fig. 7). Radioactivity corresponding

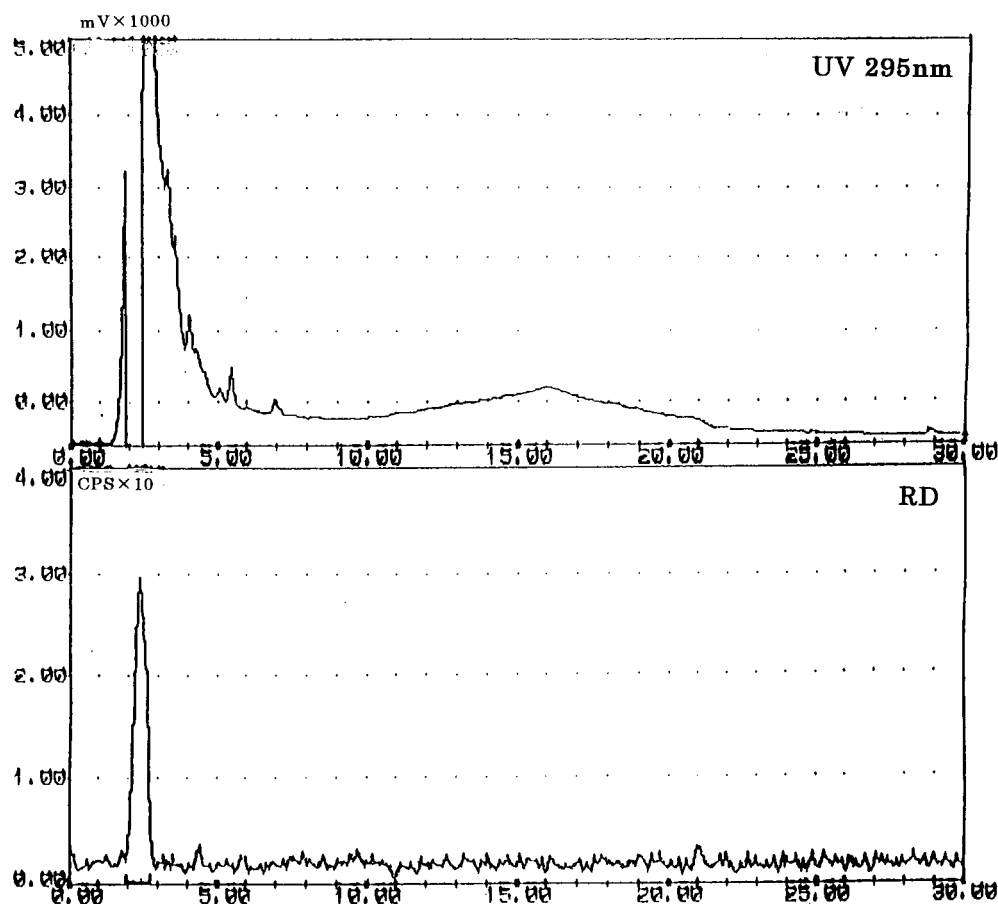


Fig. 6. HPLC chromatogram of urinary radioactive substances after single oral administration of *SRR*- α - 14 C]tocopherol. Urine excreted from 12 to 48 h after the dose was used.

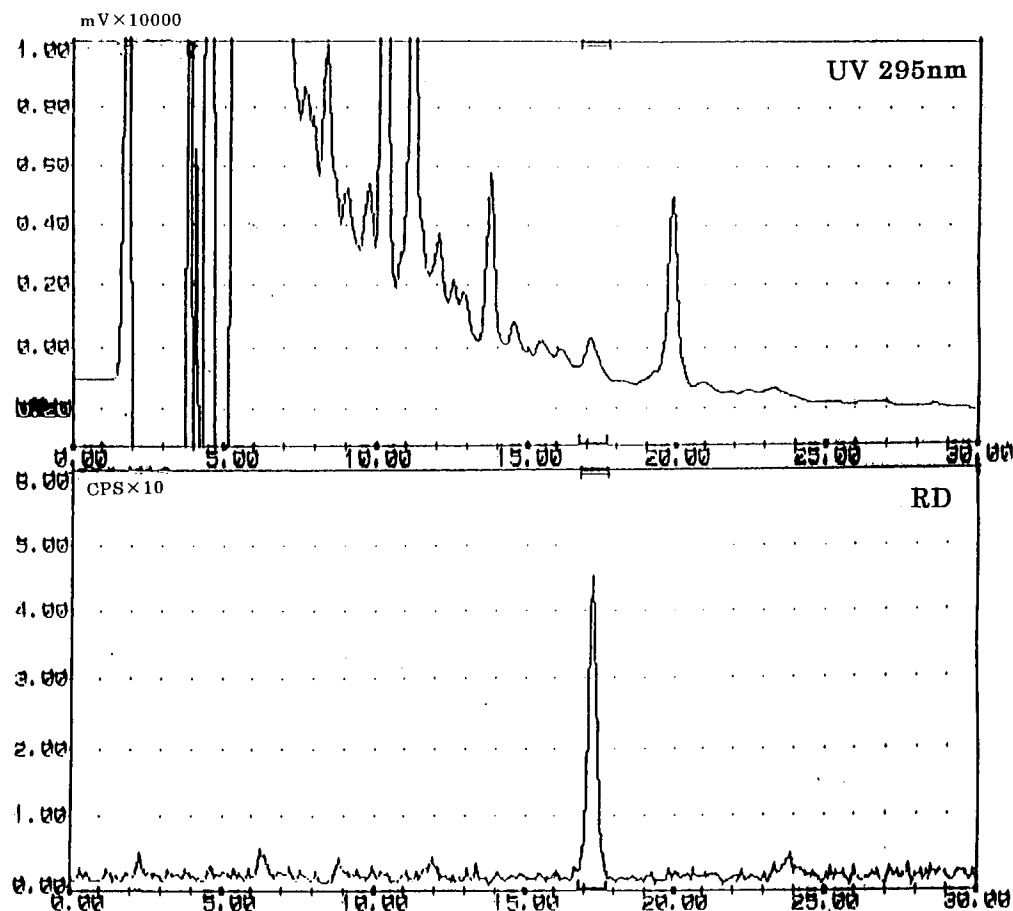


Fig. 7. HPLC chromatogram of hydrolyzed radioactive substances in urine after single oral administration of *SRR*- α - ^{14}C tocopherol. Urine excreted from 12 to 48 h after the dose was used. A urine sample was hydrolyzed with 3 N methanolic HCl. For experimental details, see the text.

to this peak derived from *SRR*- α -Toc was 73% of that injected initially. *RRR*- α -Toc gave the same results (Fig. 8). Consequently, it was proved that no difference exists between *SRR*- and *RRR*- α -Toc in the metabolic pathway.

Meanwhile, an unlabeled α -CEHC standard was methylated similarly and injected into HPLC. In UV 295 nm, α -CEHC standard eluted at the retention time of 6.8 min. After methylation, the retention time shifted to 16.7 min (data not shown). As methylated urinary radioactive substances have the same retention time as that of the methyl ester of α -CEHC (α -CEHC-Me), it was considered that the major urinary metabolite of *SRR*- α -Toc is a conjugate of α -CEHC.

Identification of urinary metabolite

The mass spectra of methylated urinary metabolites and methyl esters of α -CEHC are shown in Fig. 9. The basic structure of the major urinary metabolite of *SRR*- α -Toc was identified as α -CEHC because the mass spectra of both were identical.

DISCUSSION

This study demonstrated the distribution and metabolism of *SRR*- α -Toc compared with *RRR*- α -Toc in rats after a

single oral administration of *SRR*- and *RRR*- α - ^{14}C Toc at a dose of 2 mg. In both cases, the total recovery of radioactivity 96 h after the dosage was more than 90%.

It was only in the liver that the isotope distribution in tissues was detected in high levels. From 24 to 48 h post-dose, radioactivity in the liver derived from *RRR*- α -Toc was at the plateau, but that derived from *SRR*- α -Toc decreased. In the other tissues, there were traces of radioactivity throughout, but with respect to stereoisomers, radioactivity derived from *RRR*- α -Toc was higher compared with that derived from *SRR*- α -Toc after 12 h (Fig. 5). This is due to preferential discrimination and transport of *RRR*- α -Toc by α -TTP.

For 96 h after administration, the cumulative urinary excretion of radioactivity derived from *SRR*- α -Toc was clearly larger than that from *RRR*- α -Toc, but the cumulative fecal excretion of radioactivity was similar in both *RRR*- and *SRR*- α -Toc. Therefore it is inferred that *SRR*- α -Toc absorbed within the body is excreted mainly by way of the urine. Weber and Wiss (24) reported comparative studies with ^3H -labeled *d*- and *l*- α -Toc in rats after a single oral administration. As radioactivity excreted in the urine derived from *l*- α -Toc was higher compared with *d*- α -Toc 24 h after the dose, it was observed that the elimination rate of *l*- α -Toc was rapid. In this case, *d*- and *l*- α -Toc Ace con-

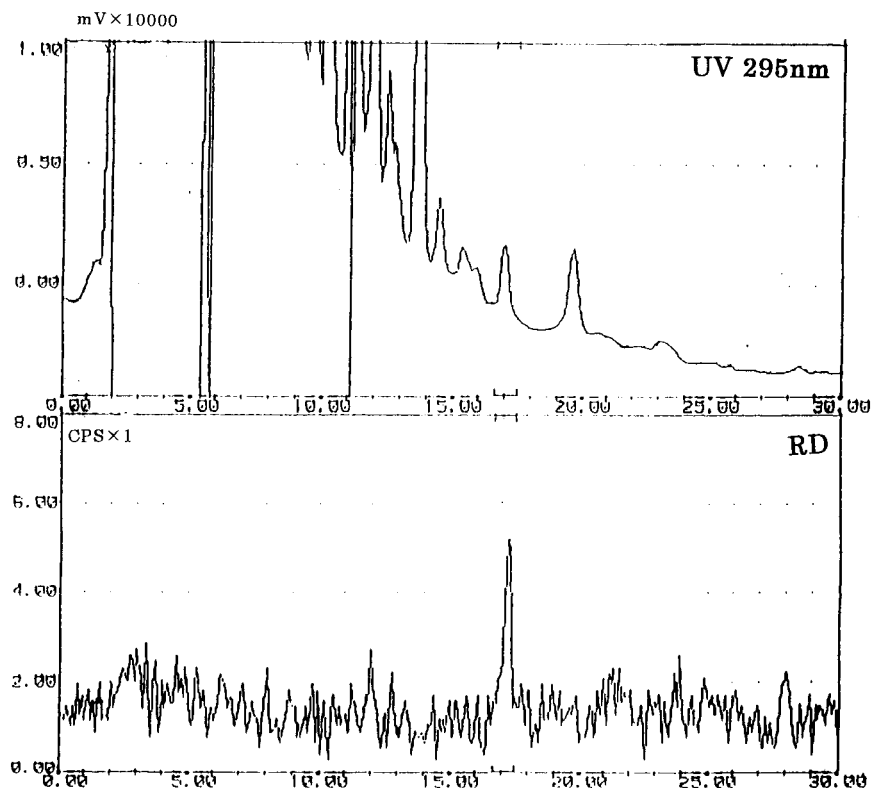


Fig. 8. HPLC chromatogram of hydrolyzed radioactive substances in urine after single oral administration of *RRR*- α - ^{14}C tocopherol. Urine excreted from 12 to 48 h after the dose was used. A urine sample was hydrolyzed with 3 N methanolic HCl. For experimental details, see the text.

tain an equimolar of four *2R*-isomers and one of four *2S*-isomers, respectively. In our study, however, at only 12 h postdose, radioactivity excreted in the feces was significantly higher in the *SRR*- α -Toc group than in the *RRR*- α -Toc group. It has been shown that the discrimination of α -Toc stereoisomers does not occur during absorption in the small intestine (10); therefore it was thought that part of Toc is eliminated via the bile. After 96 h, the cumulative fecal radioactivity of the *RRR*- α -Toc group was not significantly different from that of the *SRR*- α -Toc group. Also, the percentages of radioactivity detected in the rat feces of both *RRR* group and *SRR* group were high. We considered that most of the radioactivity in feces was unabsorbed α -Toc.

As for *SRR*- α -Toc, total dpm decreased in the liver by about 3% of the dose, but it increased in urine by about 2.3% of the dose from 12 to 48 h after the dose. Thus it was assumed that the radioactivity was transferred out of the liver and excreted into the urine. However, total dpm of the kidney was very low at more than 12 h after administration; radioactivity derived from *RRR*- α -Toc was higher compared with that derived from *SRR*- α -Toc. Therefore we supposed that α -Toc remaining in liver was rapidly metabolized, and was secreted into the bile or throughout the kidney to the urine without uptake by renal cells.

There is no difference between *SRR* and *RRR*- α -Toc in metabolic pathways. Because chemical treatment of urinary excreted of *SRR*- α -Toc resulted in the formation of α -CEHC, a metabolite of *SRR*- α -Toc, it was presumed that *SRR*- α -Toc is rapidly metabolized in the liver and ultimately ex-

creted in the urine, mainly as α -CEHC. Traber, Elsner, and Brigelius-Flohe (25) compared conversion to α -CEHC from synthetic vitamin E with that from natural form; six humans consumed 150 mg each of deuterated d_3 -*RRR*- and d_6 -all-*rac*- α -Toc Ace. Plasma was enriched with *RRR*- α -Toc, and urine was enriched with α -CEHC derived from all-*rac*- α -Toc. Thus they found that synthetic α -Toc compared with natural vitamin E is preferentially metabolized to α -CEHC and excreted (25). These findings agreed with the results from our present study. In regard to conjugation, however, further structural analysis may be needed to characterize the metabolite of *SRR*- α -Toc completely.

In our previous study, we focused on the distribution of α -TQ stereoisomers based on Simon's metabolites (13, 14). The total concentration of α -TQ in tissues was less than 1% of total Toc. Furthermore, no significant differences were found in the concentration levels between *RRR*- and *SRR*- α -TQ in all tissues. According to these results, it was revealed that the distribution of α -TQ has no relation to that of α -Toc, and the excretion of *SRR*- α -Toc from the liver was limited as an unchanged form or TQ (26). Chow et al. (27) investigated the metabolism of ^{14}C - $\text{d}\alpha$ -TQ and its hydroquinone. The radioactivity in the liver 46 h after intraperitoneal administration of either compound to rats was present almost exclusively in ^{14}C - α -TQ. No conversion to α -Toc or other metabolites was observed. The main metabolite excreted in the urine was a conjugate of α -Toc-acid, and the main metabolite excreted in the feces was a conjugate of α -TQ. Also, it ac-

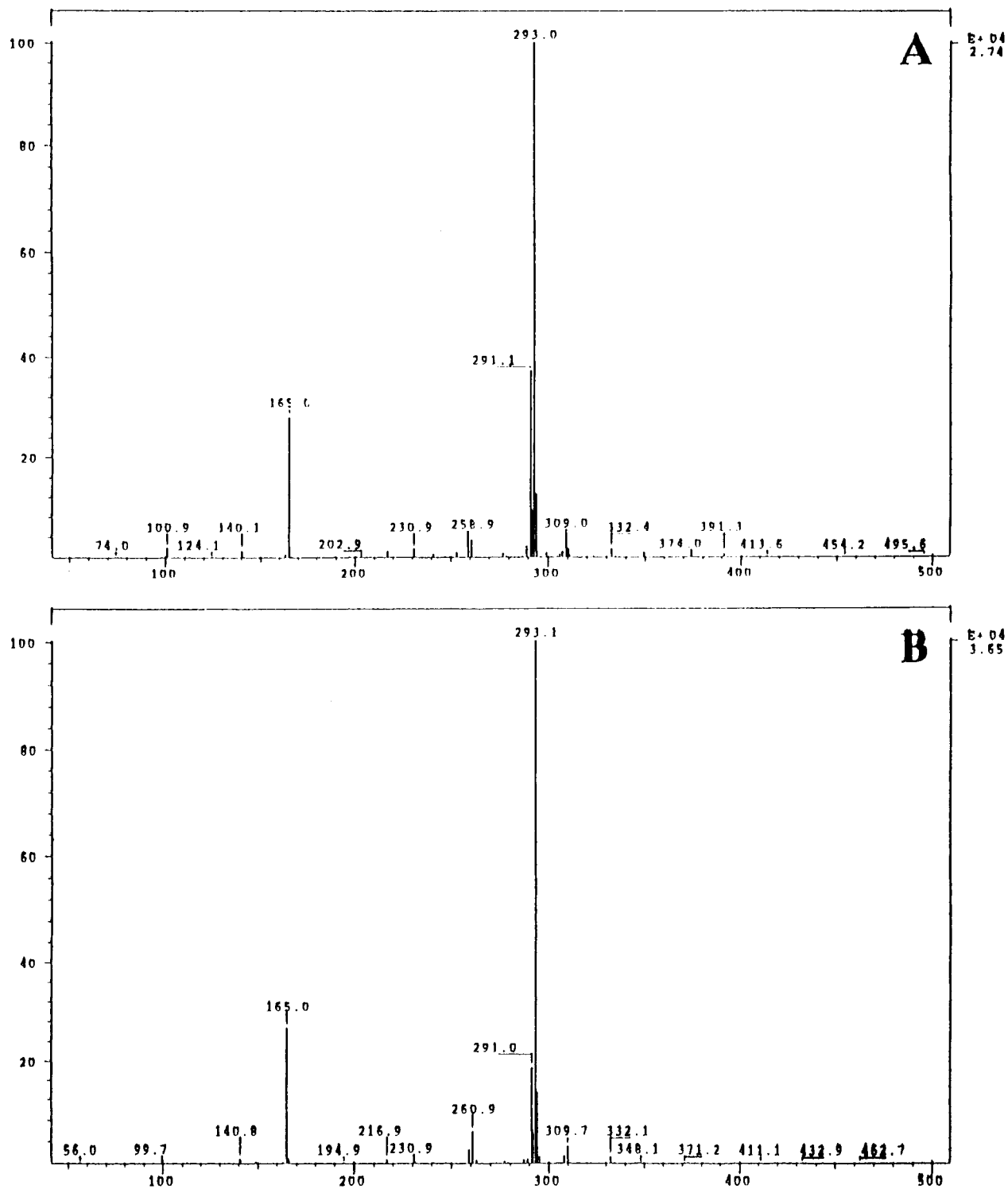



Fig. 9. Mass spectra of methylated urinary metabolite and α -CEHC methyl ester. Urine samples excreted from 12 to 24 h after a single oral administration of *SRR*- α -tocopherol(A) and α -CEHC standard (B) were treated with 3 N methanolic HCl. For experimental details, see the text. α -CEHC, 2,5,7,8-tetramethyl-2-(2'-carboxyethyl)-6-hydroxy chroman.

counts for the observation that only traces of radioactivity (<1% of the dose) were found in rat urine after [14 C] α -Toc administration (28). Because there is only a limited conversion of α -Toc to α -TQ in vivo, it is evident that α -Toc-acid under normal conditions is a minor metabolite of vitamin E.

In summary, α -TQ is one of the oxidative products of α -Toc resulting from the redox system. The major metabolic pathways of *SRR*- α -Toc remaining in the liver or of excess *RRR*- α -Toc in the liver do not involve α -TQ, but degrade the side chain while retaining the chroman ring, with ultimate excretion in the urine being an α -CEHC form. We

are now examining the excretion of CEHC in rat urine and bile to define more clearly the metabolic pathway of vitamin E. 

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