Biokinetics of and Discrimination Between Dietary *RRR*- and *SRR*-α-Tocopherols in the Male Rat[1]

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The net rates of uptake of the natural (2*R*,4′*R*,8′*R*) diastereoisomer of α-tocopherol (α-T) and the biodiscrimination relative to its 2*S*-epimer (2*S*,4′*R*,8′*R*) have been measured, in two experiments, for the blood and 21 tissues of male Sprague-Dawley rats fed over a period of several months diets containing deuterium-substituted forms of the α-T acetates. Gas chromatography–mass spectrometry was used to measure the amount of deuterated tocopherols taken up relative to the amount of nondeuterated tocopherol remaining. The measurements were performed at different times after the rats, placed for one month on a basal diet containing nondeuterated, natural α-T acetate, were switched to a diet containing the same total quantity of deuterated forms of either natural α-T acetate or a mixture of the acetates of the 2*R*- and 2*S*-epimers (i.e., *ambo*-α-T acetate). In experiment 1 the source of vitamin E in the replacement diet was trideuterio-2*R*,4′*R*,8′*R*-α-T acetate. The data obtained provide the first direct measure of the rate at which natural vitamin E is replaced and augmented in the tissues of growing animals under normal laboratory dietary conditions. There are dramatic differences in the tissue kinetics; for example, the apparent half-life of vitamin E, i.e., the time at which the total amount of ingested trideuterio-α-T taken up is the same as the amount of nondeuterated α-T remaining, varies from ca. 1 wk for the lung to ca. 11 wk for the spinal cord. In experiment 2 the vitamin E in the replacement diet was an equimolar mixture of trideuterio-2*S*,4′*R*,8′*R*- and hexadeuterio-2*R*,4′*R*,8′*R*-α-T acetates. The results show that there is a preferential uptake of the natural diastereoisomer of α-T by all tissues (except the liver during the first month). Examination of fecal material reveals that the biodiscrimination begins in the gut; the incomplete hydrolysis of the acetates shows clearly that this reaction proceeds to a greater extent with the natural diastereoisomer. The greatest discrimination of all the tissues examined was found to occur in the brain. After five months, the level of the deuterated natural diastereoisomer was more than five times that of the deuterated 2*S*-epimer. These results have potential implications for human nutrition.
Lipids 22, 163–172 (1987).

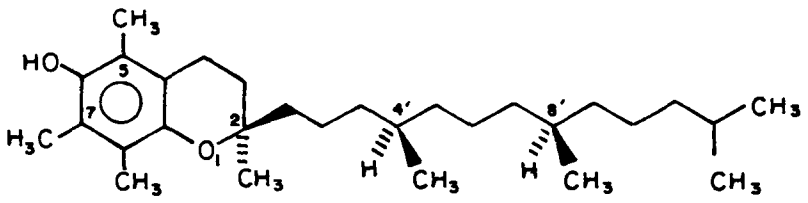
Vitamin E appears to owe its bioactivity mainly or entirely to its ability to inhibit lipid peroxidation in vivo (1). This it does by "trapping" the chain-carrying lipid peroxyl radicals, thus "breaking" the free-radical chain process (for concise reviews of the mechanism of lipid peroxidation and its prevention by antioxidants, see refs. 2 and 3). The major component of natural vitamin E, 2*R*,4′*R*,8′*R*-α-T (*RRR*-α-T; Scheme 1) has long been known to show in various animal bioassays a greater activity than synthetic 2*RS*,4′*RS*,8′*RS*-α-T (*all-rac*-α-T), which is an equimolar mixture of all of the eight possible stereoisomers (1).

More recently, it has been shown that the acetates of each of the eight diastereoisomers of α-T all have different activities in the rat fetal gestation-resorption assay, with the natural diastereoisomer, i.e., *RRR*-α-T, being the most active form (4). The differences in biopotencies of the α-T diastereoisomers appear to originate largely from the difference in the chirality at carbon atom 2 (5–10). Thus, the four diastereoisomers with the 2*R* configuration are generally more active than their corresponding 2*S* epimers (in which the CH_3 group and $C_{16}H_{33}$ phytyl group at position 2 are interchanged). Also, single dose experiments in which radioactively labeled 2*R*,4′*RS*,8′*RS*-α-T and 2*S*,4′*RS*,8′*RS*-α-T (or *RRR*-α-T and 2*S*,4′*R*,8′*R*-α-T [*SRR*-α-T]) have been administered to rats (11,12) and chicks (13,14) show that the uptake of the 2*R*-diastereoisomers into tissues is greater than for the 2*S* compounds, strongly suggesting that this is the reason for their greater bioactivity.

We have conducted the first experiments that measure the long-term uptake under normal laboratory dietary conditions of deuterium-substituted *RRR*-α-T and *SRR*-α-T into rat tissues, either singly (*RRR*-α-T only) or competitively (i.e., *RRR*-α-T vs *SRR*-α-T), using diets in which the acetates of these compounds were the only source of vitamin E.

MATERIALS AND METHODS

Materials. [5-CD_3]-2*R*,4′*R*,8′*R*-α-T (d_3-*RRR*-α-T) and [5,7-$(CD_3)_2$]-2*R*,4′*R*,8′*R*-α-T (d_6-*RRR*-α-T) were prepared by the $SnCl_2$-catalyzed deuteriomethylation with perdeutero-



RRR-α-T

SCHEME 1

[1]NRCC No. 26850.
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paraformaldehyde of natural (2*R*,4′*R*,8′*R*) γ- and δ-tocopherols, respectively (15). [5-CD_3]-2*S*,4′*R*,8′*R*-α-T (d_3-*SRR*-α-T) was obtained after repeated recrystallization of an approximate 80:20 mixture of *SRR*- and *RRR*-[5-CD_3]-α-T-*p*-phenylazobenzoates produced by the $ZnCl_2$-catalyzed cyclization of [5-CD_3]-α-T quinol (16). The quinol was obtained by the $NaBH_4$ reduction of [5-CD_3]-α-T quinone, which in turn was obtained by the $FeCl_3$-catalyzed oxidation (17) of deuteriomethylated 2*R*,4′*R*,8′*R*-γ-T (15). Natural γ-T and δ-T were obtained from soybean oil concentrate. The chiral purity of the deuterated *RRR*- and *SRR*-tocopherols was confirmed by measurement of the optical rotation of the potassium ferricyanide oxidation product mixture (16). Tocopherol acetates were prepared by reaction with sodium acetate in acetic anhydride.

Methods. Two experiments were conducted. In both experiments, nine 3-wk-old male Sprague-Dawley rats bred at the NRCC specific pathogen-free facility and housed in individual wire cages were placed for four weeks on a standard AIN-76 diet (major components: 50% sucrose, 20% casein, 15% cornstarch and 5% tocopherol-stripped corn oil) (18) that contained extra menadione [10 times the level in the original diet (19,20)] and 36 mg of 2*R*,4′*R*,8′*R*-α-T acetate (d_0-*RRR*-α-T-Ac) per kg of diet. After four weeks, the rats were switched to diets in which the (nondeuterated) α-T was replaced by a nominally equivalent amount of either 36 mg of d_3-*RRR*-α-T acetate (d_3-*RRR*-α-T-Ac; experiment 1) or a combination of 18 mg of d_6-*RRR*-α-T acetate (d_6-*RRR*-α-T-Ac) and 18 mg of d_3-*SRR*-α-T acetate (d_3-*SRR*-α-T-Ac) per kg of diet (experiment 2; this combination of diastereoisomers is known as *ambo*-α-T). (All three diets were prepared at NRCC and the tocopherols were incorporated—along with the recommended amount [18] of butylated hydroxytoluene—into each diet after dissolving them in tocopherol-stripped corn oil.)

Blood samples and tissues were obtained from animals after 1, 2, 4, 8, 16, 31-32 and 64-65 days (and 154 days in experiment 2) on the diets containing the deuterated tocopherols.

The blood samples (1 ml), obtained by heart puncture with animals under halothane anesthesia, were withdrawn from one to seven animals (fasted for at least 4 hr) on each of the specified days. The blood was placed in microcentrifuge tubes coated with disodium ethylenediaminetetraacetate and spun at 2000 rpm for 3/4 min in an Eppendorf microcentrifuge. The plasma was removed and stored frozen at −50 C. The red blood cells (RBC) were washed by resuspending them in ice-cold phosphate-buffered saline (5 mM, pH 8.0), spinning for 30 sec, removing the supernatant and repeating the whole procedure twice. The washed RBC were resuspended in the same buffer containing fresh sodium ascorbate (1 mg per ml of buffer) and stored frozen at −50 C. (The ascorbate was added to protect the α-T from oxidation by hemoglobin-derived iron during storage, thawing and extraction.)

Rapid extractions of α-T into heptane were performed on the plasma immediately after thawing by the usual ethanol/heptane procedure (21,22). Typically, 200 μl of ethanol was added to and mixed with 200 μl of plasma followed by 250 μl of heptane. The heptane extracts were stored at −50 C as 2 × 100 μl samples for the purpose of performing replicate analyses.

RBC were extracted using a modified version of the sodium dodecyl sulfate (SDS) method (22). Although volumes and hematocrits varied with each sample, the average volume of RBC suspension in the phosphate/saline/ascorbate buffer used in each extraction was 700 μl, and the average hematocrit was 25% (i.e., 175 μl of packed RBC). The following is an example of a typical extraction: the RBC suspension (700 μl), thawed in ice water, was transferred to a 30-ml glass centrifuge tube. The vial that contained the frozen cells was rinsed with 300 μl of the phosphate/saline/ascorbate buffer, and the rinsings were added to the RBC suspension in the centrifuge tube. SDS (0.1 M; 1.8 ml; 10 × volume of packed RBC) was added to the RBC and mixed briefly on a vortex stirrer, followed by absolute ethanol (2.8 ml, equal to the total aqueous volume), which was also briefly vortex-stirred. *n*-Heptane (1.0 ml) was added, and the mixture was vortex-stirred for 1 min and centrifuged for 1-2 min. A large fraction (0.8 ml) of the heptane extract was carefully removed and concentrated down under a stream of nitrogen to a volume of less than 200 μl.

Tissues were obtained from rats that had been killed either by decapitation (experiment 1) or by perfusion of saline into the anesthetized animals (experiment 2). The effect of perfusion of the tissues upon the measured ratios of deuterated and nondeuterated tocopherols was determined by using both methods on the two rats that were killed on day 4 in each experiment. All tissues were stored at −50 C. Vitamin E was extracted from aqueous homogenates of the tissues using the SDS method (22). The concentration of the SDS solution used was 0.1 M unless noted otherwise.

All extracts (including those from plasma and RBC) were purified, and the total α-T was quantitated by high performance liquid chromatography (HPLC) (Varian model 5000) using a Lichrosorb Si 60 column (5 μ; Merck, Darmstadt, Federal Republic of Germany) eluted with hexane/*t*-butylmethyl ether (3%)/2-propanol (0.05%) at 2 ml min^{-1}. 2,2,5,7,8-Pentamethyl-6-hydroxychroman was used as the internal standard (22). Often, the collected fraction containing the α-T was purified further by a second "pass" through the HPLC after being concentrated under a stream of nitrogen. Some modifications of the extraction and purification procedures were necessary depending on the type and amount of the particular tissue.

Liver, lung, kidney, heart, brain, testes, small intestine (duodenum), biceps femoris (1-2 g of each) and spleen (ca. 0.75 g) were homogenized in 6 ml of water (2 × 15 sec using a Brinkmann/Kinematic Polytron PT 10/35 equipped with an anaerobic generator). (In light of the known differences in uptake of vitamin E by different regions of the rat brain [23], the whole brain was homogenized.) A carefully measured volume (2.0 ml) of the homogenate was placed in a 30-ml glass centrifuge tube. SDS (2.0 ml), ethanol (4.0 ml) and *n*-heptane (2.5 ml) were each added to the homogenate in succession and vortex-mixed briefly (except *n*-heptane, which was mixed for 1 min). The mixture was centrifuged (1-2 min), and 2 × 1.0 ml of the heptane extract was carefully removed and placed in separate vials. One sample was concentrated to ca. 200 μl under a stream of nitrogen and injected onto the HPLC column after the addition of the internal standard. The other sample was stored at −50 C for a replicate analysis later, if desired.

Epididymal, inguinal and retroperitoneal white adipose tissue (1-2 g of each) were homogenized in a mixture of water (5.5 ml) and SDS (0.5 ml). SDS (1.5 ml), ethanol (6.0 ml) and *n*-heptane (2.5 ml) were each added in succession to the homogenate (4.5 ml) with vortex-mixing in the manner already described. The heptane extract was divided into two 1.0 ml samples. Preliminary HPLC analysis indicated a lot of interference in the region of the α-T peak. Therefore, the sample was subjected to an alkaline hydrolysis to reduce the possibility of interference by ion fragments from other species in the subsequent gas chromatography-mass spectrometry (GC-MS) analysis of the isotopically substituted tocopherols. The sample (1.0 ml) was placed in a 15-ml glass tube equipped with a teflon-lined screw cap, blown down to dryness under a stream of nitrogen and redissolved in ethanol (2 ml) containing sodium ascorbate (1%). Anhydrous sodium methoxide (0.5 M, 4 ml) was then added and the mixture heated at 80 C for 20 min. After the mixture cooled, glacial acetic acid (200 μl) was added followed by water (6 ml) and *n*-heptane (2.5 ml). The mixture was then vortex-stirred for 1 min. A portion (2.0 ml) of the heptane extract was removed, concentrated down to 200 μl under a stream of nitrogen and injected onto the HPLC column. The α-T fraction was collected and blown down, and the residue was subjected to a second alkaline hydrolysis. Again, the heptane extract was passed through the HPLC column and the α-T fraction collected and concentrated.

Interscapular brown adipose tissue (0.4 g) was treated essentially in the same manner as white adipose tissue. Homogenization was performed in water (4 ml), and the entire homogenate was extracted with SDS (2 ml), ethanol (6 ml) and *n*-heptane (2.5 ml). The extract (1 ml) was hydrolyzed and purified twice by HPLC using the method already described.

Diaphragm, soleus, tensor fascia latae and tibialis anterior muscle tissues (0.2-0.6 g) were cut into small pieces with scissors, homogenized in a mixture of water (3 ml) and SDS (0.5 ml, 20 sec) and extracted in the usual manner by adding a further amount of SDS (0.5 ml) followed by ethanol (4 ml) and *n*-heptane (2.5 ml). A portion (1 ml) of the recovered heptane extract (2.0 ml) was purified by HPLC in the usual way.

Aorta (40 mg) was homogenized with a mixture of water (0.5 ml) and SDS (0.2 ml). The probe/generator of the homogenizer was rinsed with water (ca. 1.3 ml) to bring the final aqueous volume to 2 ml. The entire homogenate was treated in the usual way with ethanol (2 ml) and *n*-heptane (2 ml), and the heptane extract (1.8 ml) was purified by HPLC in the normal manner.

Skin (0.4-0.7 g) was cut into small pieces and converted to a powder by cooling with liquid nitrogen and grinding the mixture in a stainless steel mortar and pestle. The powder was homogenized first with water (5 ml, 10 sec) and then with added SDS (0.5 ml, 10 sec). The entire homogenate was extracted in the usual way by adding more SDS (0.5 ml) followed by ethanol (6 ml) and *n*-heptane (2.5 ml). A fraction (1.0 ml) of the recovered extract (2.0 ml) was purified by HPLC.

Fecal pellets (ca. 0.5 g), recovered from the lower part of the large intestine, were homogenized with water (4 ml). A sample of the homogenate (2 ml) was extracted with SDS (2 ml), ethanol (4 ml) and *n*-heptane (2.5 ml). A portion (1.0 ml) of the recovered extract (2.0 ml) was injected onto the HPLC column. The α-T fraction was collected as well as an earlier eluting fraction containing α-T acetate. The latter fraction was blown down to dryness under a stream of nitrogen and the α-T acetate hydrolyzed by heating the residue at 70 C for 30 min with sodium methoxide (0.5 M) in methanol (2 ml) plus methanol (1 ml) containing sodium ascorbate (1%). Glacial acetic acid (100 μl) was added to the cooled mixture followed by SDS (2 ml) and *n*-heptane (2 ml), which were vortex-mixed. The heptane extract recovered after centrifugation was injected onto the HPLC column and the α-T fraction was recovered.

The content and composition of α-T acetate in each diet were checked after saponification of the food to extract the α-T. The diet (0.5 g) was made into a slurry with water (0.5 ml) and warmed at 70 C for 2 min, with a 2% solution of sodium ascorbate in ethanol (2 ml). Saturated aqueous potassium hydroxide (1 ml) and ethanol (1.5 ml) were added, and the mixture was heated at 70 C for 40 min. Water (2 ml) and hexane (6 ml) were added to the cooled mixture and vortex-stirred. A sample (1 ml) of the hexane layer that separated after centrifugation of the mixture for 2 min was removed and concentrated down to 200 μl under a stream of nitrogen (after the addition of an internal standard). The α-T in the concentrated sample was then simultaneously quantitated and purified for GC-MS by the usual HPLC method.

The relative proportions of d_0-, d_3- and d_6-α-Ts were determined by GC-MS of their trimethylsilyl ethers (24). Although the free tocopherols could be successfully analyzed by GC-MS, the trimethylsilyl ether derivatives were used because there was no "tailing" of the peaks. This feature was considered desirable for obtaining more accurate peak area integrations.

The tocopherol trimethylsilyl ethers were prepared by evaporating the purified extracts of α-T in heptane down to dryness under a stream of nitrogen, adding pyridine (100 μl) and N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) (50 μl; Pierce, Rockford, Illinois) and heating at 65 C for 15 min. After cooling, the mixture was blown down to a small volume (ca. 20 μl). A sample (2 μl) was injected onto a Hewlett Packard Ultra 1 fused silica capillary column (10 m × 0.2 mm ID, OV-101 type, cross-linked, bonded phase) maintained at 275 C in a Hewlett Packard 5790A Series GC, which was connected to a Hewlett Packard 5970A Series Mass Selective Detector set up to monitor continuously the 502 (d_0), 505 (d_3) and 508 (d_6) molecular ions. The peak area data for the 505 and 508 ions were corrected for the contribution (2.37%) from the M+3 ions originating from d_0-α-T and d_3-α-T, respectively.

RESULTS

Table 1 indicates the high degree of purity of the deuterium-substituted α-T. It is clear that by using three mass units separation to distinguish the different α-Ts, the M+3 contributions to the next highest molecular ion are very small and in good agreement with the contribution (2.37%) expected from natural abundance isotopes.

GC-MS analysis of α-T extracted from the saponified diets containing deuterated tocopherols confirmed the absence of the nondeuterated form in each diet and verified that the d_6-*RRR*-α-T-Ac/d_3-*SRR*-α-T-Ac ratio was 1.0 in the diet used in experiment 2.

TABLE 1

Relative Ion Intensities, Measured as Peak Areas by Single Ion Monitoring, of the 502, 505 and 508 Ions Obtained from the Gas Chromatography–Mass Spectrometry of the Trimethylsilyl Ethers of d_0-*α*-T, d_3-*RRR*-*α*-T, d_3-*SRR*-*α*-T and d_6-*RRR*-*α*-T[a]

Compound	502	505	508
d_0-*RRR*-*α*-T	100.00	2.25	<0.01
d_3-*RRR*-*α*-T	1.30	100.00	2.17
d_3-*SRR*-*α*-T	0.26	100.00	2.22
d_6-*RRR*-*α*-T	0.13	1.70	100.00

[a]The ion intensities for each compound are expressed as a percentage of the parent molecular ion, which is arbitrarily set at 100%.

TABLE 2

Body Weights (g) of Rats at Time of Death for Tissue Analysis

Day[a]	Experiment 1	Experiment 2
1	260	264
2	293	250
4	281	277
4	282	279
8	308	292
16	365	336
31	417	—
32	—	347
64	—	480
65	485	—
65	459	—
154	—	499

[a]Number of days after 7-wk-old rats were switched to diet containing deuterated *α*-tocopherol acetate.

Table 2 shows for each experiment the weights of the rats at the time they were killed for tissue analysis.

We will use the symbol ∅ to describe the ratio of total deuterated *α*-T [$\Sigma(d_x$-*α*-T); x = 3,6] to nondeuterated *α*-T, i.e., $\varnothing = \Sigma(d_x$-*α*-T)/(d_0-*α*-T), which is d_3-*RRR*-*α*-T/d_0-*RRR*-*α*-T in experiment 1 and (d_6-*RRR*-*α*-T + d_3-*SRR*-*α*-T)/d_0-*RRR*-*α*-T in experiment 2. The complete set of data are presented in Table 3. (Note that the data presented for plasma and RBC are average values.) A comparison of the results from animals that were perfused with saline with those that were not (day 4 in both experiments) indicates that the results were not affected by the presence of blood in the tissue.

In both experiments, plots of ∅ vs time were linear for almost all tissues. This result was not anticipated and seems remarkable considering the fact that each point in the plots was obtained from a different animal. Some examples of these plots are shown in Figure 1.

In view of the demonstrated linearity of ∅ with time, the data were fitted by the method of least squares to the equation $\varnothing = kt$, where t is time and k is a proportionality constant, the value of which depends on the tissue, diet, etc. The results are shown in Table 4.

The two k values obtained for each tissue in experiment 2 (i.e., with or without inclusion of the final point for day 154) are in satisfactory agreement except in the case of lung. If this tissue is excluded from consideration, with only one or two exceptions the k values derived from experiment 2 are somewhat lower than the k values derived from experiment 1. This means that the rate of uptake of *α*-T by the rats is smaller when the vitamin is provided as the mixture of two stereoisomers in experiment 2 than when it is provided as the natural stereoisomer in experiment 1.

The reciprocal of each k value listed for experiment 1 in Table 4 yields the apparent half-life, $t_{1/2}$, in days for natural *RRR*-*α*-T in each tissue; $t_{1/2}$ is the time it takes for the net amount of ingested deuterated *RRR*-*α*-T taken up by a tissue to equal the amount of the nondeuterated *RRR*-*α*-T remaining. This time is shortest for the lung ($t_{1/2} \approx 7$ days, experiment 1), liver and small intestine and is longest for the brain, testes ($t_{1/2} \approx 30$ days, experiment 1) and spinal cord ($t_{1/2} \approx 76$ days, experiment 1).

Figure 2 shows the time dependence of the discrimination in the net uptake of d_6-*RRR*-*α*-T vs d_3-*SRR*-*α*-T in plasma, RBC, liver and brain of rats fed the diet containing equal amounts of the acetates of these diastereoisomers (experiment 2). After a single day there is a 1.4-fold enrichment of d_6-*RRR*-*α*-T in the plasma, the enrichment increasing to ca. 2.5 over the 5-mo duration of this experiment. The RBC consistently show a higher enrichment in d_6-*RRR*-*α*-T than the plasma throughout the experiment; the enrichment ratio, i.e., $(RRR/SRR)_{rbc}/(RRR/SRR)_{plasma}$ has a mean value of 1.35 ± 0.13 (standard deviation for 30 values).

Three remarkable results are also shown in Figure 2. First, the liver, in contrast to all the other tissues, shows an initial excess, by a factor of two, of d_3-*SRR*-*α*-T. This lasts for ca. 3 wk until eventually the liver begins to show a slight excess of d_6-*RRR*-*α*-T. The initial excess of the *SRR*-epimer is similar to the result reported from an early experiment by Weber et al. (12), in which fasted rats received single doses of either 2*S*,4′*RS*,8′*RS*-*α*-T or 2*R*,4′*RS*,8′*RS*-*α*-T radioactively labeled acetates. In that experiment, the average level of the 2*S*-diastereoisomers found in the liver half an hour after the doses were orally administered was approximately twice that of their 2*R*-epimers (12). However, after two hours the situation was reversed and the 2*R*-diastereoisomers exceeded their 2*S* counterparts by a small margin. Although in our own experiment the rats were deprived of food for at least four hours before their livers were removed, it is very likely that some food was still present in the gut at the time they were killed. There will, therefore, have been a continuing infusion of vitamin E into the animals. This may explain why our liver data appear to be similar to the trends noted in the very early stages of the single dose experiment (12).

Second, the brain shows an extraordinary, progressive discrimination in favor of natural *RRR*-*α*-T, especially after one month, with the *RRR/SRR* ratio exceeding a value of five after five months and showing no sign of leveling off. This result has been confirmed in subsequent related experiments. In one experiment, two rats were placed on the diet used in experiment 2 (i.e., 1:1 d_6-*RRR*-*α*-T/d_3-*SRR*-*α*-T acetates) immediately after weaning and were found to have in their brains d_6-*RRR*-*α*-T/d_3-*SRR*-*α*-T ratios of 5.7 and 4.8 after 120 and 128 days, respectively. In a second experiment in which two weanling rats

TABLE 3

Time-Dependence of ∅ (Ratio of [Total] Deuterated α-T to Nondeuterated α-T) for Blood and Tissues in Experiments 1 and 2[a]

Tissue	Expt.	Day											
		1	2	4	4	8	16	31	32	64	65	65	154
Lung	1	0.08	0.17	0.53	0.40	0.80	1.80	3.30			10.20	6.90	
	2	0.08	0.14	0.40	0.31	0.86	1.60		2.00	2.80			25.00
Liver	1	0.16	0.35	0.79	0.65	1.00	1.90	3.40			6.30	7.20	
	2	0.32	0.47	0.96	0.90	1.70	2.30		3.10	5.60			17.00
Small intestine	1	0.06	0.37	1.30	0.51	0.78	1.90	3.70			6.30	6.40	
	2	0.08	0.19	0.43	0.42	0.94	1.80	2.90	4.50				11.00
Plasma[b]	1	0.50	0.50	0.77		1.00	2.00	3.40			6.10		
	2	0.15	0.33	0.65		1.00	1.70		2.50	3.80			12.00
Kidney	1	0.05	0.09	0.27	0.25	0.49	1.30	2.70			5.60	5.80	
	2	0.04	0.08	0.20	0.22	0.73	1.20		2.10	3.20			9.30
Red blood cells[b]	1	0.15	0.35	0.73		1.30	1.90	3.20			5.20		
	2	0.14	0.26	0.65		1.10	1.70		2.40	3.70			9.60
Heart	1	0.02	0.13	0.13	0.11	0.28	0.88	2.30			4.70	4.80	
	2	0.01	0.03	0.10	0.13	0.28	0.81		1.10	3.10			12.00
Thymus	1	0.03	0.07	0.30	0.36	0.55	1.29	1.62			4.50	4.67	
	2	0.03	0.06	0.31	0.31	0.75	1.39		2.18	3.93			8.46
Muscle (biceps femoris)	1	0.02	0.04	0.11	0.09	0.20	0.67	1.70			4.70	3.80	
	2	0.02	0.03	0.08	0.09	0.22	0.56		1.20	3.50			9.70
Muscle (tibialis anterior)	1	0.01	0.03	0.09	0.10	0.21	0.62	1.33			4.89	3.61	
	2	0.01	0.02	0.06	0.06	0.19	0.54		1.42	2.66			12.35
Muscle (soleus)	1	0.02	0.04	0.10	0.12	0.26	0.68	1.47			3.92	4.19	
	2	0.02	0.04	0.09	0.10	0.25	0.64		1.21	2.99			10.12
Spleen	1	0.13	0.48	0.74	0.66	0.98	2.00	3.20			6.40	1.80	
	2	0.11	0.21	0.53	0.53	0.99	1.20		2.20	4.10			11.00
Muscle (tensor fascia latae)	1	0.02	0.03	0.10	0.10	0.24	0.74	1.58			2.98	4.50	
	2	0.02	0.04	0.09	0.10	0.24	0.60		1.21	3.44			9.84
White adipose tissue (retroperitoneal)	1	0.03	0.03	0.04	0.08	0.21	0.46	1.30			3.40	3.20	
	2	0.02	0.02	0.10	0.12	0.26	0.52		ND[c]	3.50			5.50
Aorta	1	0.02	0.07	0.10	0.10	0.28	0.64	1.31			2.83	2.95	
	2	0.03	0.05	0.11	0.10	0.22	0.56		0.95	2.25			5.40
Brown adipose tissue (interscapular)	1	0.01	0.02	0.07	0.08	0.14	0.43	1.17			2.56	3.06	
	2	0.01	0.01	0.06	0.07	0.17	0.46		0.98	2.46			8.71
Diaphragm	1	0.02	0.03	0.09	0.12	0.22	0.59	1.41			2.00	3.51	
	2	0.02	0.02	0.09	0.09	0.21	0.57		1.17	2.72			8.49
Skin	1	0.02	0.04	0.12	0.10	0.25	0.79	1.37			2.72	2.75	
	2	0.02	0.04	0.11	0.11	0.27	0.65		1.29	2.30			6.98
White adipose tissue (inguinal)	1	0.01	0.02	0.07	0.08	0.17	0.45	0.98			2.26	2.26	
	2	0.01	0.02	0.07	0.08	0.19	0.53		0.94	2.12			5.95
Brain	1	<0.01	0.02	0.06	0.03	0.06	0.21	0.64			1.50	2.80	
	2	<0.01	0.01	0.04	0.03	0.10	0.25		0.51	1.20			4.10
Testes	1	0.01	0.04	0.14	0.12	0.21	0.31	0.79			1.90	2.00	
	2	0.02	0.03	0.08	0.08	0.20	0.56		0.61	1.70			7.10
White adipose tissue (epididymal)	1	<0.01	0.01	0.03	0.07	0.16	0.32	0.84			1.70	1.94	
	2	<0.01	0.02	0.01	0.01	0.17	0.36		0.70	1.56			4.02
Spinal cord	1	0.01	0.02	0.04	0.04	0.07	0.15	0.39			0.90	0.78	
	2	0.01	0.01	0.04	0.05	0.10	0.15		0.28	0.54			1.56

[a]Ratios of the deuterated to nondeuterated forms, d_3-*RRR*-α-T/d_0-*RRR*-α-T (experiment 1) and (d_6-*RRR*-α-T + d_3-*SRR*-α-T)/d_0-*RRR*-α-T (experiment 2), respectively, are given for various times after switching the rats to the diets containing the deuterated α-tocopherols. In experiment 1, only the animal corresponding to the data for the first set of day 4 results was perfused with saline. In experiment 2, all animals were perfused except the animal corresponding to the second set of day 4 results.

[b]Mean values for blood drawn from several rats (except day 154): number of rats (day no.)—4 (1), 7 (2), 6 (4), 4 (8), 3 (16), 2 (31), 2 (32), 2 (64), 2 (65). Single values for day 111 (experiment 2 only) were 5.94 and 10.35 for plasma and red blood cells, respectively.

[c]ND, not determined.

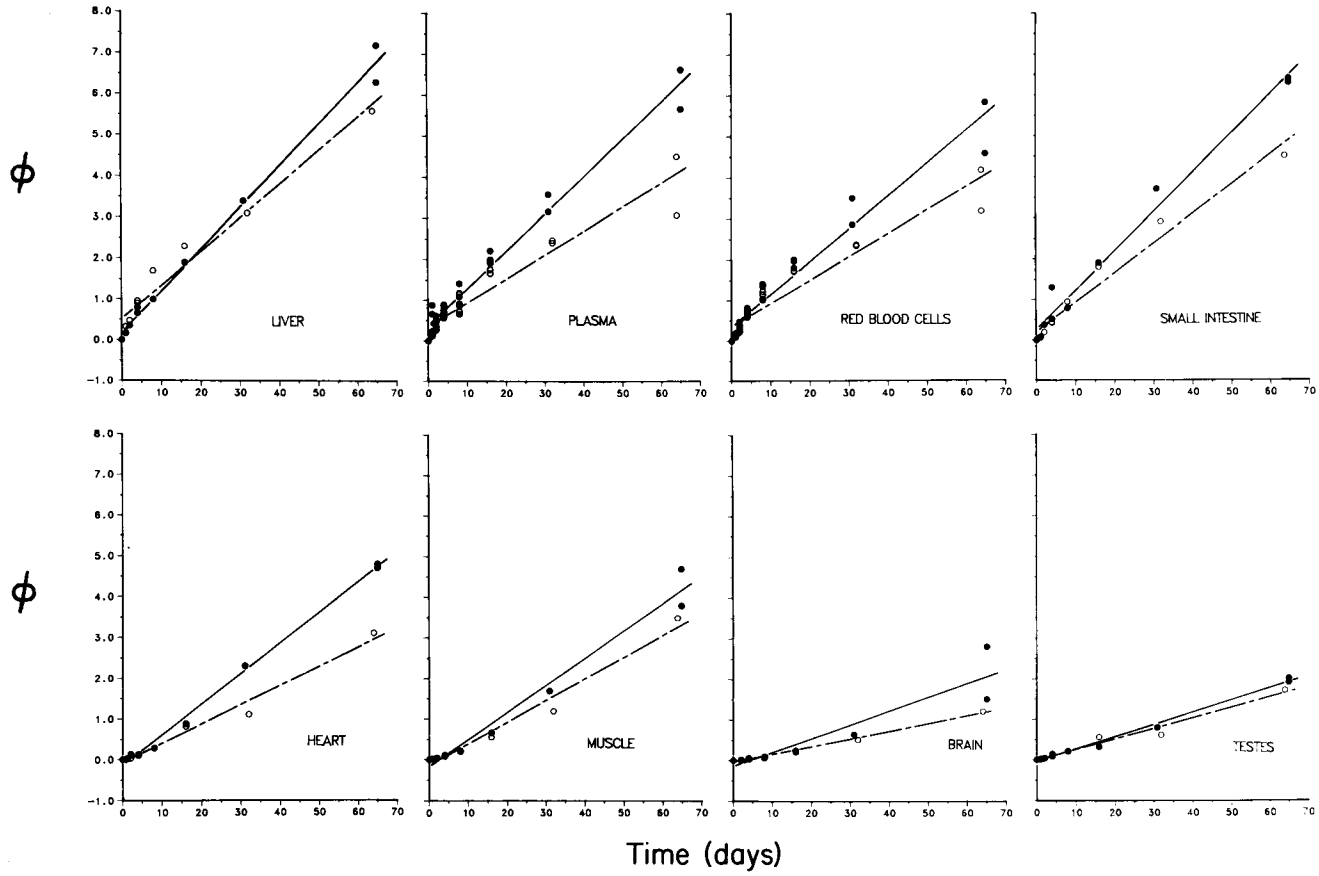

FIG. 1. Plots of ∅ [ratio of the total amount of deuterated to nondeuterated α-tocopherols, Σ(d_x-α-T)/(d_0-α-T)] vs time for liver, plasma, red blood cells, small intestine, heart, muscle (soleus), brain and testes. Each graph includes data points from experiment 1 (d_3-*RRR*-α-T/d_0-*RRR*-α-T; solid line) and experiment 2 [(d_6-*RRR*-α-T + d_3-*SRR*-α-T)/d_0-*RRR*-α-T; broken line)]. Lines were obtained by a least squares fit of the data collected over a 64–65 day period for each experiment. The data ponts are from single rats (9 and 8 points for each tissue in experiments 1 and 2, respectively, and 30 data points for plasma and red blood cells in both experiments).

TABLE 4

Values of *k* and Apparent Half-Lives, $t_{1/2}$, of α-T in Tissues and Blood[a]

Tissue	Experiment 1	Experiment 2		
	k (Day 65)[b]	*k* (Day 64)[b,c]	*k* (Day 154)[b]	$t_{1/2}$[d]
Lung	0.132 ± 0.011	0.045 ± 0.006	0.151 ± 0.017	7.6
Liver	0.102 ± 0.003	0.082 ± 0.006	0.105 ± 0.004	9.8
Small intestine	0.096 ± 0.004	0.072 ± 0.005	0.070 ± 0.002	10.4
Plasma	0.091 ± 0.003	0.059 ± 0.004	0.067 ± 0.003	10.9
Kidney	0.089 ± 0.001	0.052 ± 0.004	0.059 ± 0.002	11.2
Red blood cells	0.080 ± 0.004	0.057 ± 0.004	0.068 ± 0.003	12.5
Heart	0.075 ± 0.002	0.047 ± 0.003	0.076 ± 0.005	13.3
Thymus	0.070 ± 0.003	0.062 ± 0.003	0.055 ± 0.002	14.4
Muscle (biceps femoris)	0.067 ± 0.004	0.053 ± 0.003	0.063 ± 0.002	14.9
Muscle (tibialis anterior)	0.066 ± 0.005	0.043 ± 0.001	0.078 ± 0.006	15.1
Muscle (soleus)	0.063 ± 0.002	0.046 ± 0.002	0.065 ± 0.003	15.8
Spleen	0.060 ± 0.016	0.062 ± 0.003	0.070 ± 0.002	16.6
Muscle (tensor fascia latae)	0.059 ± 0.005	0.053 ± 0.003	0.064 ± 0.002	17.0
White adipose tissue (retroperitoneal)	0.052 ± 0.002	0.056 ± 0.002	0.038 ± 0.003	19.3
Aorta	0.045 ± 0.001	0.035 ± 0.001	0.035 ± 0.000	22.2
Brown adipose tissue (interscapular)	0.044 ± 0.002	0.038 ± 0.002	0.056 ± 0.003	22.6
Diaphragm	0.043 ± 0.005	0.043 ± 0.001	0.054 ± 0.002	23.0
Skin	0.043 ± 0.001	0.037 ± 0.001	0.045 ± 0.001	23.4
White adipose tissue (inguinal)	0.035 ± 0.001	0.033 ± 0.001	0.038 ± 0.001	28.2
Brain	0.034 ± 0.005	0.019 ± 0.001	0.026 ± 0.001	29.4
Testes	0.030 ± 0.001	0.026 ± 0.002	0.044 ± 0.003	33.3
White adipose tissue (epididymal)	0.029 ± 0.001	0.025 ± 0.001	0.026 ± 0.000	34.8
Spinal cord	0.013 ± 0.001	0.008 ± 0.000	0.010 ± 0.000	76.3

[a]Obtained by least squares analysis of data fit to the equation ∅ = *k*t, where ∅ is the ratio of (total) deuterated to nondeuterated α-tocopherols and t is time in days. Units are day^{-1} (± standard deviation) for *k* and days for $t_{1/2}$.

[b]Duration of experiment.

[c]Results obtained by excluding the one additional data point for day 154.

[d]Calculated from the reciprocal of the *k* values from experiment 1.

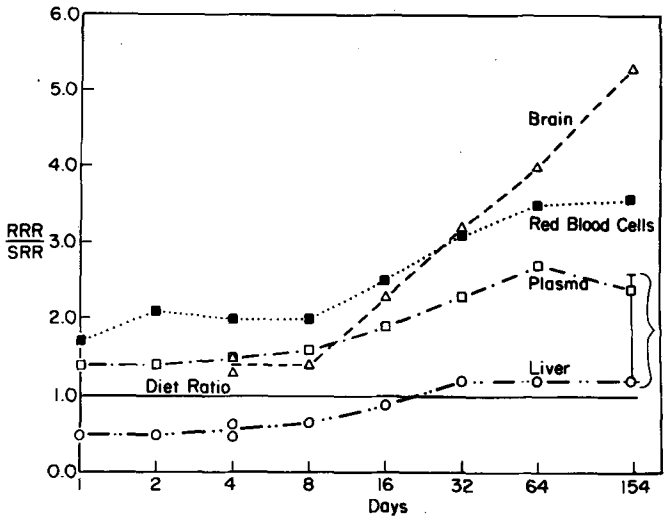

FIG. 2. Time-dependence of the discrimination in the net uptake of deuterated *RRR*- and *SRR*-α-tocopherols (d_6-*RRR*-α-T/d_3-*SRR*-α-T) into plasma, red blood cells, liver and brain of male rats fed a diet containing equal amounts of the corresponding tocopherol acetates (experiment 2). Points for plasma and red blood cells represent mean values from two or more animals (except for day 154). The points for liver and brain are from single animals. The bar at 154 days represents the full range in the *RRR*/*SRR* ratio found at 154 days for all of the other tissues examined (see Table 4).

were placed on a diet with a d_6-*RRR*-α-T/d_3-*SRR*-α-T acetate ratio of 0.5, the d_6-*RRR*-α-T/d_3-*SRR*-α-T ratios in the rats' brains were 2.2 and 2.5 after 64 days; that is, relative to the dietary ratio, the enrichments of d_6-*RRR*-α-T to d_3-*SRR*-α-T were 4.4 and 5.0, respectively.

Third, all the other tissues examined (except brain, RBC, liver and spinal cord; see Table 5 for complete data) show discriminations that after one month lie between that of plasma on one hand and liver on the other (see bar at day 154 in Fig. 1).

Analysis of fecal material from the large intestine (Table 6) reveals that the hydrolysis of the dietary tocopherol acetates in the gut was incomplete, particularly in experiment 2. It is also very clear that *RRR*-α-T-Ac underwent hydrolysis to a greater extent than *SRR*-α-T-Ac. This latter observation is probably the reason why the amount of fecal d_6-*RRR*-α-T was generally larger than the amount of d_3-*SRR*-α-T, and the ratio of α-T-Ac to α-T was generally higher in experiment 2 than in experiment 1.

The data in Table 6 also show that the original, non-deuterated tocopherol persists in the gut longer than would be expected after switching the animals to a diet containing 100% deuterated tocopherol. From the GC-MS data provided in Table 1, the apparent limiting ratio of d_3-*RRR*-α-T to d_0-*RRR*-α-T in experiment 1 can be calculated to be 100/1.3 = 76.9. Similarly, the apparent limiting ratio in experiment 2 (i.e., for a 1:1 mixture of

TABLE 5

The Dependence with Time of the Discrimination in the Uptake of Deuterated *RRR*- and *SRR*-α-Tocopherols in Experiment 2[a]

Tissue	Day								
	1	2	4	4	8	16	32	64	154
Lung	1.40	1.30	1.30	1.40	1.20	1.50	1.90	1.90	2.60
Liver	0.49	0.49	0.47	0.62	0.67	0.89	1.20	1.20	1.20
Small intestine	1.30	1.30	1.20	1.20	1.10	1.40	1.50	1.70	1.80
Plasma[b]	1.40	1.40	1.50		1.60	1.90	2.30	2.70	2.40
Kidney	1.60	1.50	1.30	1.40	1.30	1.50	1.80	1.20	1.80
Red blood cells[b]	1.70	2.10	2.00		2.00	2.50	3.10	3.50	3.60
Heart	0.96	0.99	1.00	1.40	0.88	1.20	0.99	1.40	1.90
Thymus	1.60	1.44	1.32	1.40	1.25	1.61	1.81	1.84	1.58
Muscle (biceps femoris)	1.40	1.40	1.30	1.40	1.30	1.40	1.70	1.90	1.70
Muscle (tibialis anterior)	—[c]	—[c]	1.33	1.39	1.37	1.74	2.22	1.99	2.12
Muscle (soleus)	—[c]	1.49	1.38	1.47	1.31	1.64	1.92	2.12	2.22
Spleen	1.40	1.40	1.10	1.20	0.97	1.50	1.80	1.30	2.10
Muscle (tensor fascia latae)	—[c]	1.51	1.26	1.42	1.23	1.53	1.69	2.02	1.65
White adipose tissue (retroperitoneal)	1.40	1.30	1.40	1.40	1.20	1.50	—[d]	2.10	1.90
Aorta	1.54	1.25	1.16	1.34	1.20	1.41	1.73	1.70	1.68
Brown adipose tissue (interscapular)	—[c]	—[c]	1.66	1.61	1.42	1.55	1.57	1.40	1.19
Diaphragm	—[c]	1.04	1.40	1.40	1.36	1.66	1.92	2.07	2.22
Skin	1.04	1.00	1.14	1.22	1.09	1.33	1.26	1.44	1.39
White adipose tissue (inguinal)	—[c]	1.65	1.56	1.52	1.28	1.52	1.60	1.61	1.55
Brain	—[c]	—[c]	1.50	1.30	1.40	2.30	3.20	4.00	5.30
Testes	1.60	1.40	1.40	1.40	1.30	1.70	1.80	1.90	2.00
White adipose tissue (epididymal)	—[c]	—[c]	—[c]	—[c]	1.33	1.99	1.89	2.25	2.14
Spinal cord	—[c]	—[c]	1.34	1.31	1.24	1.70	2.40	2.89	3.69

[a]Values of the ratio d_6-*RRR*-α-T/d_3-*SRR*-α-T are given for various times after switching the rats to the diet containing equal amounts of the two deuterated α-tocopherols.

[b]Mean values for blood drawn from several rats (except day 154): number of rats (day no.)—4 (1), 7 (2), 6 (4), 4 (8), 3 (16), 2 (32), 2 (64). Single values for day 111 were 2.66 and 4.17 for plasma and red blood cells, respectively.

[c]Insufficient deuterated tocopherols available to determine ratio accurately.

[d]Not determined.

TABLE 6

Absolute Concentrations of α-T and α-T Acetate (per Wet Wt) and Ratios d_3-*RRR*-α-T/d_0-*RRR*-α-T (Experiment 1), d_6-*RRR*-α-T/d_3-*SRR*-α-T and (d_6-*RRR*-α-T + d_3-*SRR*-α-T)/d_0-*RRR*-α-T (Experiment 2) in Fecal Material Recovered from Large Intestine

Experiment 1				Experiment 2					
		α-Tocopherol			α-Tocopherol acetate		α-Tocopherol		
Day	α-T-Ac (nmol/g)	nmol/g	d_3-*RRR*/ d_0-*RRR*	Day	nmol/g	d_6-*RRR*/ d_3-*SRR*	nmol/g	d_6-*RRR*/ d_3-*SRR*	(d_6-*RRR* + d_3-*SRR*)/ d_0-*RRR*
1	10	87	3.66	1	36	0.22	99	1.34	4.23
2	8	71	10.55	2	19	0.43	99	0.97	6.53
4	18	95	20.98	4	7	0.74	76	0.95	8.76
4	19	75	17.04	4	42	0.45	109	1.06	12.64
8	17	83	20.06	8	33	0.26	95	1.08	25.41
16	16	95	24.91	16	90	0.21	108	1.95	31.90
31	41	67	36.08	32	33	0.29	99	1.20	12.25
65	34	84	69.91	64	137	0.33	66	2.59	58.74
65	8	78	33.20	154	36	0.42	51	1.13	44.48

d_3-*SRR*-α-T and d_6-*RRR*-α-T) can be calculated to be (100 + 100)/(0.26 + 0.13) = 513. It is evident that these limits are not attained in either experiment.

DISCUSSION

The advantage of using deuterium-labeled α-T in conjunction with GC-MS for studies of relatively long-term uptake and discrimination of vitamin E is evident from the present results. Our two experiments have yielded remarkably consistent results for the net rates of uptake of deuterated α-T by various tissues. The plots of Ø vs time for the tissues and blood show an unexpected and surprising degree of linearity. Even though there is a steady influx of vitamin E from the diet, this fact alone is not sufficient to explain the linearity. Nevertheless, the empirically observed linearity of Ø has allowed us to obtain the first comprehensive picture of the biokinetics in the tissues and blood of α-T acquired under normal dietary conditions. Particularly noteworthy is the large range in the rates of net uptake of deuterated α-T into different tissues. This is dramatically illustrated by the α-T half-lives ($t_{1/2}$), which range from 8 days in the lung to 76 days in the spinal cord (see Table 4). Of course, the animals underwent significant growth during the experiment (see Table 2) and therefore the total quantity of tocopherol was increasing in many organs and tissues because the size of the organs and tissues was increasing. Growing tissue would be expected to take up more tocopherol than it loses, and the new cells would be expected to contain mainly the deuterated (i.e., new) α-T. Growth would therefore have the effect of increasing the Ø values compared to the values that might be obtained in mature animals of stable weight. In growing tissues, the $t_{1/2}$ values will therefore tend to underestimate somewhat the actual time for a 50% turnover of α-T.

It is interesting to note that the Ø values were higher in lung, liver and small intestine than in plasma. This is easy to understand for the small intestine, since it is here that the new (deuterated) α-T is being absorbed from food containing only deuterated α-T. We suggest that the Ø values for the other two organs are higher than in plasma because uptake of α-T occurs most rapidly soon after feeding, when both the total α-T concentration and the Ø ratio in the plasma are temporarily elevated (25). This enhanced rate of absorption at elevated α-T levels in plasma may be combined with a fairly rapid cell turnover in these tissues (i.e., the death of old cells with "old" d_0-*RRR*-α-T and the birth of new cells with "new" d_x-α-T).

For organs whose weights remained essentially the same or increased only marginally during the experiment (e.g., brain, testes and heart), $t_{1/2}$ must correspond quite closely to the time it took for 50% of the natural α-T to be lost from a tissue by all chemical and physical processes. It remains to be seen to what extent the rate of turnover is determined by chemical consumption (by, for example, oxidation of α-T by lipid peroxyl radicals, which is not repaired by ascorbate or other water-soluble reducing agents [2; 26 and references cited therein]) and by physical loss (e.g., cell death, exchange into plasma or lymph).

A second major aspect of this work is that it provides direct information regarding the pattern of tissue discrimination between two of the eight diastereoisomers of α-T. This gives us new insights into the reasons for the different bioactivities of individual stereoisomers, including, for example, the difference between natural and *all-rac*-α-T.

The results obtained from fecal material suggest that discrimination in favor of the natural compound begins with the hydrolysis of the α-T acetates in the gut, i.e., before there has even been any absorption of the free phenol into the lymph. The simplest explanation for this would be that *RRR*-α-T-Ac is more rapidly hydrolyzed than *SRR*-α-T-Ac, presumably because of the chirality of the active enzymes in the pancreatic juice (e.g., carboxyl ester hydrolase) or the bile salts that are necessary for hydrolysis to occur (25,27) or to both. However, this explanation is not consistent with the result obtained by Weber et al. (12), which showed that the 2S-diastereoisomers are absorbed more rapidly within the first half-hour after dosing. A possible explanation that reconciles the results from both experiments is that the hydrolysis of

the 2*S*-diastereoisomers is faster but is more susceptible to inhibition by free tocopherol. The effects of inhibition and chiral discrimination upon the in vitro enzymic hydrolysis of α-T acetates are currently being investigated.

Our discovery that a small percentage of the α-T present in the fecal material is undeuterated after two months (experiments 1 and 2) and even after five months (experiment 2; see Table 5) suggests that α-T is "returned" to the gut from at least some tissues in the body of the rat. We presume that this return occurs by transport in the plasma and lymph with eventual excretion in the bile (experiments underway).

RBC exhibit a moderate chiral selectivity, relative to plasma, in favor of *RRR*-α-T [$(RRR)/(SRR)_{rbc}/(RRR)/(SRR)_{plasma} = 1.35$, see Fig. 2]. We presume that chiral discrimination by the (chiral) phospholipids, cholesterol, proteins, etc., will be accentuated by the more structured lipid environment of the RBC membranes compared with the more "relaxed" environment of the plasma lipoproteins. In this regard, attempts to measure chiral effects in model phospholipid vesicles by various nuclear magnetic resonance methods have not been successful (28).

The unique behavior of the liver, in which there is an initial discrimination in favor of the unnatural *SRR*-α-T for ca. 3 wk, suggests that it possesses a mechanism by which *SRR*-α-T is selectively extracted from the lymph (25) and/or a mechanism that selectively "exports" *RRR*-α-T into lipoproteins that enter the blood plasma. Lipid "export" from the liver is known to involve the very low density lipoproteins (VLDL) and is likely to be accompanied by vitamin E "export." Indeed, an α-T transport protein, which is present in rat liver cytosol but not in other tissues (29–32), has been identified and shown to be fairly specific for the natural stereoisomer (31). We therefore suggest that this protein is active in transporting *RRR*-α-T into the lipoproteins synthesized in the liver, i.e., *RRR*-α-T is selectively "exported." There is, presumably, also some mechanism for the removal of *SRR*-α-T from the liver. However, it appears to take some weeks to become fully activated.

The very strong long-term discrimination by rat brain in favor of (natural) *RRR*-α-T and the very slow uptake of α-T into spinal cord have potential consequences for human nutrition and the treatment of neurological diseases associated with vitamin E deficiency (33,34). The high degree of discrimination implies that the rat brain will take up less of the *ambo*-α-T than *RRR*-α-T from diets that contain the same amount of both. The biokinetic data support this. The difference between the k values for experiments 1 and 2 is most marked for the brain (see Table 4). This result strongly suggests that a diet (dose) of natural α-T will be significantly more "available" to the brain than an equivalent diet (dose) of synthetic *all-rac*-α-T.

It seems likely that the high discrimination shown by the brain and to a lesser extent by the spinal cord is a result of the "blood-brain barrier." Possibly, α-T enters these tissues by passive diffusion across several membranes. This would be slow, and by analogy to the RBC, would tend to favor the natural stereoisomer.

The other tissues show no enhancement of the *RRR*/*SRR* ratio relative to plasma. This is probably because α-T uptake by tissue occurs in conjunction with the uptake of other lipidic material, there being, therefore, little chance of chiral discrimination at the point of entry of the tocopherol into the cell. Thus, α-T is known to be present in all lipoprotein fractions, and two mechanisms have been identified by which α-T is transferred from human lipoproteins to tissue. First, the specific, high affinity, low density lipoprotein (LDL) receptor mechanism (35) has been shown to be involved in the delivery of vitamin E from human LDL to human fibroblast cells (36,37). Second, α-T present in human chylomicrons has been shown to transfer to human erythrocytes and fibroblasts during the hydrolysis of triglycerides by lipoprotein lipase, and for this transfer to occur the lipase must itself bind to the cell membrane (38). The second of these two mechanisms is probably the more important in the rat (39); the *RRR*/*SRR* ratio in most tissue should at least parallel the *RRR*/*SRR* ratio in the plasma. The mechanism by which α-T is transferred to or from tissue by the high density lipoproteins (HDL) has not apparently been identified, although α-T in human HDL has been shown to transfer readily to human erythrocytes (40).

Finally, our results raise serious questions regarding the validity of and reliance upon current animal bioassays for evaluating the biopotencies of different tocopherols. As far as the stereoisomers of α-T are concerned, it would seem that both the duration of the test period and the tissue(s) that give the observed symptoms of deficiency and cure could affect the derived biopotency.

ACKNOWLEDGMENTS

The Association for International Cancer Research partially supported this work. E. Lusztyk, M. Slaby, B. E. Nadeau and S.W.Y. Ma gave valuable help, T. F. Slater and N. Cohen lent advice and encouragement and the Central Soya Corp., Fort Wayne, Indiana, donated soybean concentrate.

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[Received May 26, 1986]