Biokinetics of Dietary *RRR*-α-Tocopherol in the Male Guinea Pig at Three Dietary Levels of Vitamin C and Two Levels of Vitamin E. Evidence that Vitamin C Does Not "Spare" Vitamin E *in Vivo*[1]

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The net rates of uptake of "new" and loss of "old" *2R,4'R,8'R*-α-tocopherol (*RRR*-α-TOH, which is natural vitamin E) have been measured in the blood and in nine tissues of male guinea pigs over an eight week period by feeding diets containing deuterium-labelled α-tocopheryl acetate (d_6-*RRR*-α-TOAc). There was an initial two week "lead-in" period during which 24 animals [the "high" vitamin E (HE) group] received diets containing 36 mg of unlabelled (d_0) *RRR*-α-TOAc and 250 mg of ascorbic acid per kg diet, while another 24 animals [the "low" vitamin E (LE) group] received diets containing 5 mg d_0-*RRR*-α-TOAc and 250 mg ascorbic acid per kg diet. The HE group was then divided into three equal subgroups, which were fed diets containing 36 mg d_6-*RRR*-α-TOAc and 5000 mg [the "high" vitamin C (HEHC) subgroup], 250 mg [the "normal" vitamin C (HENC) subgroup] and 50 mg [the "low" vitamin C (HELC) subgroup] ascorbic acid per kg diet. One animal from each group was sacrificed each week and the blood and tissues were analyzed for d_0- and d_6-*RRR*-α-TOH by gas chromatography-mass spectrometry. The LE group was similarly divided into three equal subgroups with animals receiving diets containing 5 mg d_6-*RRR*-α-TOAc and 5,000 mg (LEHC), 250 mg (LENC) and 50 mg (LELC) ascorbic acid per kg diet with a similar protocol being followed for sacrifice and analyses. In the HE group the total (d_0- + d_6-) *RRR*-α-TOH concentrations in blood and tissues remained essentially constant over the eight week experiment, whereas in the LE group the total *RRR*-α-TOH concentrations declined noticeably (except in the brain, an organ with a particularly slow turnover of vitamin E). There were no significant differences in the concentrations of "old" d_0-*RRR*-α-TOH nor in the concentrations of "new" d_6-*RRR*-α-TOH found in any tissue at a particular time between the HEHC, HENC and HELC subgroups, nor between the LEHC, LENC and LELC subgroups. We conclude that the long-postulated "sparing" action of vitamin C on vitamin E, which is well documented *in vitro*, is of negligible importance *in vivo* in guinea pigs that are not oxidatively stressed in comparison with the normal metabolic processes which consume vitamin E (e.g., by oxidizing it irreversibly) or eliminate it from the body. This is true both for guinea pigs with an adequate, well-maintained vitamin E status and for guinea pigs which are receiving insufficient vitamin E to maintain their body stores.

The biokinetics of vitamin E uptake and loss in the HE guinea pigs are compared with analogous data for rats reported previously (*Lipids 22*, 163–172, 1987). For most guinea pig tissues the uptake of vitamin E under "steady-state" conditions was faster than for the comparable rat tissues. However, the brain was an exception with the turnover of vitamin E occurring at only one-third of the rate for the rat.

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We have recently employed *2R,4'R,8'R*-α-tocopheryl acetate substituted in a metabolically inactive position with three atoms of deuterium (d_3-*RRR*-α-TOAc) to make the first measurements of the net, long-term uptake of (deuterated) natural vitamin E, d_3-*2R,4'R,8'R*-α-tocopheryl (d_3-*RRR*-α-TOH) in the male rat under normal laboratory dietary conditions, using a diet in which the d_3-*RRR*-α-TOAc (36 mg/kg diet) was the only source of vitamin E (1). We discovered that there were dramatic differences in uptake kinetics between tissues. For example, the equalization time, $t_{1:1}$, which is the time required for the new (deuterium-labelled) α-TOH concentration to become equal to that of the old (unlabelled) α-TOH, was estimated to be ca. 9, 18, 40 and 72 days in the lung, heart, brain and spinal cord, respectively (K.U. Ingold, G.W. Burton, and W. Siebrand, 1990, unpublished results).

Since analogous, tissue-sampling experiments on man are almost inconceivable, we decided to examine the biokinetics of natural vitamin E in a different laboratory animal in order to see if the same general pattern of fast-uptake tissues and slow-uptake tissues obtained. We chose the guinea pig as our experimental animal and carried out the biokinetics under conditions similar to those employed in the earlier study on rats. However, in order to increase the sensitivity of the measurement of small amounts of deuterated tocopherol in the presence of a large amount of the unlabelled compound, we employed *2R,4'R,8'R*-α-tocopheryl acetate labelled in metabolically inactive positions with six atoms of deuterium (d_6-*RRR*-α-TOAc). As will be reported elsewhere (K.U. Ingold, G.W. Burton, and W. Siebrand, 1990, unpublished results), guinea pigs and rats have a somewhat similar pattern of fast-uptake and slow-uptake tissues. That is, corresponding tissues could be classified either as "fast" or "slow." However, the fast-uptake tissues of the guinea pig were somewhat "faster" than those of the rat and, while some of the slow-uptake tissues of the guinea pig were "faster" than for the rat, others were dramatically slower, e.g., for the brain, $t_{1:1}$ was 40 and 107 days in the rat and guinea pig, respectively (K.U. Ingold, G.W. Burton, and W. Siebrand, 1990, unpublished results).

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Abbreviations: AH_2, vitamin C; α-TOH, α-tocopherol; *RRR*-α-TOH, *2R,4'R,8'R*-α-tocopherol; *RRR*-α-TOAc, *RRR*-α-tocopheryl acetate; d_0-*RRR*-α-TOH, unlabelled *RRR*-α-tocopherol; d_3-*RRR*-α-TOH, d_3-*RRR*-α-(5-CD_3)tocopherol; d_3-*RRR*-α-TOAc, *RRR*-α-(5-CD_3)tocopheryl acetate; d_6-*RRR*-α-TOH, *RRR*-α-(5,7-$(CD_3)_2$)tocopherol; d_9-*ambo*-α-TOH, *2RS,4'R,8'R*-α-(5,7,8-$(CD_3)_3$)tocopherol; $t_{1:1}$, equalization time; LE, low vitamin E; HE, high vitamin E; LELC, low E, low (vitamin) C; LENC, low E, normal C; LEHC, low E, high C; HELC, high E, low C; HENC, high E, normal C; HEHC, high E, high C; α-TO•, α-tocopheroxyl radical; RBC, red blood cells; ROO•, peroxyl radical; ROOH, hydroperoxide.

The guinea pig was chosen for our second whole animal biokinetic experiment with vitamin E because, unlike a rat but like man, the guinea pig cannot synthesize ascorbic acid (vitamin C, AH_2). The availability of vitamin C depends, therefore, on the dietary level of this compound, which can be manipulated over a wide range. This is important because both vitamin E (2-5) and vitamin C (6-8) are chain-breaking antioxidants and there is a considerable body of evidence which indicates that there is a synergistic antioxidant interaction between these vitamins in a wide variety of *in vitro* model systems (9-39). There is sound experimental evidence that this synergism is due to the "regeneration" of α-TOH by reduction of its initial oxidation product, the tocopheroxyl radical, α-TO$^{\bullet}$, by the ascorbate anion, AH^-. That is, the lipid-soluble α-TOH traps lipid peroxyl radicals, ROO$^{\bullet}$, forming α-TO$^{\bullet}$ and lipid hydroperoxide, ROOH, and the α-TO$^{\bullet}$ is then reduced by the water-soluble ascorbate rather than being irreversibly oxidized by reaction with a second peroxyl radical. The overall synergistic interaction between these two radical-trapping antioxidants *in vitro* can be represented by reactions 1-3.

$$ROO^{\bullet} + \alpha\text{-TOH} \rightarrow ROOH + \alpha\text{-TO}^{\bullet} \quad [1]$$

$$\alpha\text{-TO}^{\bullet} + AH^- \rightarrow \alpha\text{-TOH} + A^{\bullet -} \quad [2]$$

$$A^{\bullet -} + A^{\bullet -} \xrightarrow{H^+} A + AH^- \quad [3]$$

There is some much less compelling evidence for an interaction between vitamin E and vitamin C *in vivo* for rats (40-47), guinea pigs (48-57), and premature infants (58). Synergistic E/C interactions have generally been reported, a result which lends support to the hypothesis, drawn from *in vitro* experiments, that vitamin C can "regenerate" vitamin E *in vivo*, or at least "spare" vitamin E by some other *in vivo* mechanism. For example, dietary vitamin C has been reported to enhance plasma levels of vitamin E (40,45,47,50,58), to enhance vitamin E levels in other tissues (52,53,56), and to partially reverse effects due to vitamin E deficiency (47). Antagonistic E/C interactions have also been reported (41-43,48,51). Thus, dietary vitamin C has been reported to lower plasma levels of vitamin E (42,51) and to enhance erythrocyte hemolysis (42,43,51). The difficulties involved in determining whether there is any E/C interaction *in vivo* and, if so, whether the interaction is synergistic or antagonistic can be further illustrated by the pioneering measurements of expired pentane as a measure of lipid peroxidation as described by Tappel and co-workers (41,44,54). These studies demonstrated that the level of lipid peroxidation induced in rats by methyl ethyl ketone peroxide is uninfluenced by vitamin C if the rats have an adequate vitamin E status, but is enhanced by vitamin C when the animals are vitamin E deficient (41), while for iron-loaded rats (44) and CCl_4-intoxicated guinea pigs (54), dosing with vitamin C reduced *in vivo* lipid peroxidation. Overall, the literature indicates that a prooxidant effect of vitamin C has generally, though not always (51), been observed in severely vitamin E deficient animals and might be attributed to a prooxidant (chain-initiating) effect on lipid peroxidation by ascorbate, particularly in the presence of iron (59,60), as well as to the reduced molar effectiveness of ascorbate as a chain-breaking antioxidant at high concentrations (7). With both synergistic and antagonistic E/C interactions being reported it is not surprising to find that slight modifications of the experimental conditions or the use of some different measure of antioxidant status can lead to a failure to detect any kind of E/C interaction *in vivo* (40-42,44-48,50-52,55,56).

Guinea pigs on a diet containing d_6-*RRR*-α-TOAc provide a unique opportunity to search for any *in vivo* protective or destructive effect of vitamin C on vitamin E. An experimental protocol carefully designed to measure vitamin E turnover would be very much more sensitive than any of the earlier whole animal studies in detecting the existence, or otherwise, of an *in vivo* interaction between vitamins E and C. It would therefore provide a far more definitive answer to the important question: Does vitamin C "spare" vitamin E *in vivo*?

We chose essentially the same experimental protocol for the present study of the biokinetics of vitamin E uptake and loss in the guinea pig that we had previously employed with rats (1). However, because far more guinea pigs were employed than in the earlier study on rats (48 vs 9 animals) we limited the number of biological tissues and fluids examined to 11 (vs 23 for the rats). Another difference was that the guinea pigs were given the diet containing d_6-*RRR*-α-TOAc after a two week "lead-in" period, during which the animals were fed a diet containing the same concentration of the unlabelled material, d_0-*RRR*-α-TOAc, whereas for the rats the corresponding "lead-in" time was four weeks. The maximum length of time the animals were on deuterated vitamin E was similar (56 days for guinea pigs vs 65 days for rats), but the guinea pigs were sacrificed for tissue analyses at regular seven day intervals whereas the rats had been sacrificed on days 1, 2, 4, 8, 16, 31 and 65 after being switched to the deuterated tocopherol diet.

The rationale behind the protocol (see Methods section and Fig. 1) is that if vitamin C really does "spare" vitamin E *in vivo* then, at high dietary levels of vitamin C, one would expect a slower than normal loss of "old" vitamin E (d_0-*RRR*-α-TOH) from a tissue and a corresponding slower than normal uptake of "new" vitamin E (d_6-*RRR*-α-TOH). Similarly, at low dietary levels of vitamin C one would expect a faster than normal loss of "old" and a faster than normal uptake of "new" vitamin E.

MATERIALS AND METHODS

Materials. *2R,4′R,8′R*-α-(5,7-$(CD_3)_2$)tocopherol (d_6-*RRR*-α-TOH) was prepared by deuteriomethylation of δ-tocopherol (61) and was then converted to the acetate as previously described (1). *2RS,4′R,8′R*-α-(5,7,8-$(CD_3)_3$)tocopherol (d_9-*ambo*-α-TOH) was prepared for use as an internal standard by condensation of hydroquinone with phytol followed by deuteriomethylation.

Methods. Forty-eight male, two-week-old, specific pathogen free guinea pigs (170-222 g) obtained from Charles River Canada, Inc., P.Q. (St. Constant, Quebec, Canada) were divided into two main groups, a high vitamin E group of 24 animals, HE, and a low vitamin E group of 24 animals, LE. The animals were housed four per plastic cage (1720 cm^2 floor area) with ground corn cob bedding and were fed a Reid-Briggs Guinea Pig diet modified as follows: corn oil (which contains α-TOH) was omitted as were vitamins E and C; tocopherol-stripped corn oil (7.3%

by weight) containing d_0-*RRR*-α-TOAc or d_6-*RRR*-α-TOAc (5 or 36 mg/kg diet) was added together with ascorbic acid (50, 250 or 5000 mg/kg diet). During these experiments the guinea pigs consumed ca. 15–20 g of diet per day.

All the animals in the HE group were fed a diet containing 36 mg d_0-*RRR*-α-TOAc/kg diet [the same level as for the rats in the earlier study (1)] and 250 mg ascorbic acid/kg diet for two weeks. After this "lead-in" period, i.e., on day 0 of the actual experiment, the HE group of animals were divided into three equal subgroups, all of which received a diet containing 36 mg d_6-*RRR*-α-TOAc but different levels of vitamin C (Fig. 1). The high vitamin C subgroup, HEHC, were fed the megadose (62) level of 5,000 mg ascorbic acid/kg diet; the normal vitamin C subgroup, HENC, continued with 250 mg ascorbic acid/kg diet; and the low vitamin C subgroup, HELC, received the barely antiscorbutic (49–51,53,55,56,63–68) level of 50 mg ascorbic acid/kg diet.

A similar protocol was followed with the LE group, but for these guinea pigs the levels of d_0- and d_6-*RRR*-α-TOAc wre only 5 mg/kg diet. A two week "lead-in" with d_0-α-TOAc and 250 mg ascorbic acid/kg diet was followed by the d_6-*RRR*-α-TOAc and the same three levels of vitamin C as for the HE subgroups. That is, the three LE subgroups, LEHC, LENC, and LELC, received 5,000 mg, 250 mg, and 50 mg ascorbic acid/kg diet, respectively (Fig. 1).

One guinea pig from each of the six sub-groups was sacrificed weekly on days 7, 14, 21, 28, 35, 42, 50 and 56.

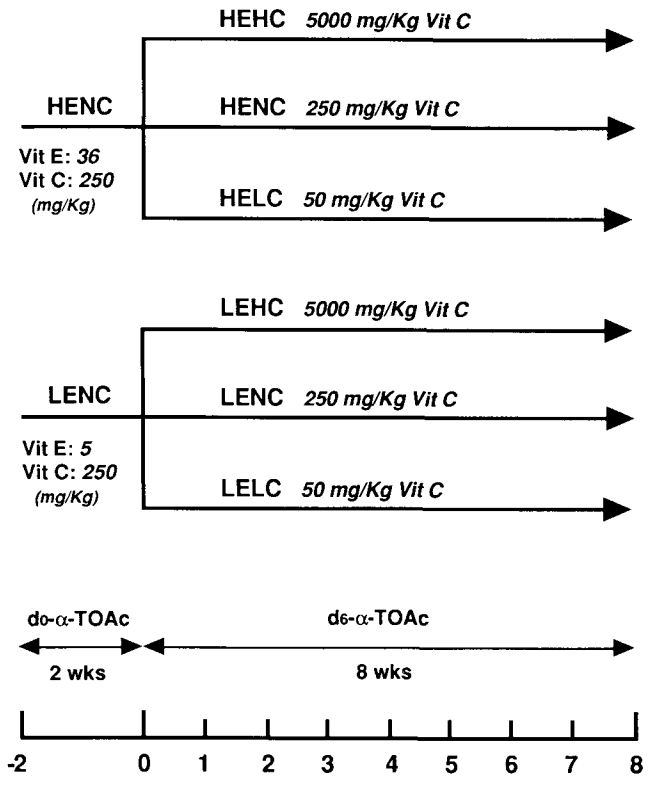

FIG. 1. Dietary regimes for the guinea pigs employed in this study.

Blood samples were obtained by heart puncture with the animals anesthetized with Innovar-Vet™ (Pitman-Moore Ltd., Don Mills, Ontario). The animals were then sacrificed by arterial perfusion with isotonic NaCl. Blood was separated into plasma and red blood cell (RBC) fractions by centrifugation, and the RBC were washed as described previously (1). The plasma, RBC, and nine weighed tissues (adrenal, brain, heart, kidney, liver, lung, muscle [biceps femoris, b.f.], spleen, and testis) were stored at −80°C prior to analysis. The frozen tissue samples were thawed and 7.95 nmol d_9-*ambo*-α-TOH in 50 μl heptane was immediately added to them; this was followed by tissue homogenization and extraction of α-TOH into heptane, as described previously. The entire brain, the two adrenals, one kidney, and one testis were utilized while weighed portions (0.5–1.0 g) of the heart, spleen, b.f. muscle, liver, and lung were employed. The plasma (500 μl) and RBC (in phosphate buffered saline, 1.3 ml, hematocrit 45%) were mixed with 3.98 nmol and 7.95 nmol d_9-*ambo*-α-TOH in heptane, respectively, as soon as they thawed and the α-TOH was extracted immediately.

The heptane extracts were assayed using a Varian model 5000 high performance liquid chromatography (HPLC) (Varian Associates, Palo Alto, CA) equipped with a 250 × 4 mm Lichrosorb Si 60 (5μ particle size) using 90% hexane/10% butyl methyl ether as the eluent, and the α-TOH fraction in each sample was collected automatically. The relative proportions of d_0-*RRR*-α-TOH, d_6-*RRR*-α-TOH, and d_9-*ambo*-α-TOH in each sample were determined as described previously (1) by gas chromatography-mass spectrometry (GC-MS) analysis following conversion of these tocopherols to their trimethylsilyl ethers. Since the absolute amount of d_9-*ambo*-α-TOH added to each sample was known, the absolute concentrations of "old" d_0-*RRR*-α-TOH and "new" d_6-*RRR*-α-TOH could be readily calculated.

RESULTS

Our complete results are presented in Table 1 which lists the absolute concentrations of d_0-*RRR*-α-TOH and d_6-*RRR*-α-TOH as a function of time in the plasma, RBC, and nine tissues for the six dietary regimes employed in these experiments.

In accordance with our experience with rats (1), the total concentration of vitamin E (i.e., d_0- + d_6-*RRR*-α-TOH) in the tissues of the HE group of guinea pigs remained essentially constant during the eight week experimental period, as is best seen in Figure 2. However, for the LE group of guinea pigs the total concentration of vitamin E declined significantly during the eight weeks in all tissues examined except the brain (where turnover is extremely slow).

DISCUSSION

Does vitamin C "spare" vitamin E in vivo? The rationale for having both the HE and the LE groups of guinea pigs is that at high dietary levels of vitamin E any "sparing" action by vitamin C might be masked from experimental observation because adequate "new" vitamin E would always be available to replace any used, "old" vitamin E. However, with sufficiently low dietary levels of vitamin E the rate of depletion of "old" vitamin E in a tissue

TABLE 1

Time-Dependence of the Absolute Concentrations of d_0- and d_6-*RRR*-α-TOH in Blood and Tissue[a]

Tissue	Day	HEHC		HENC		HELC		LEHC		LENC		LELC	
		d_0	d_6	d_0	d_6	d_0	d_6	d_0	d_6	d_0	d_6	d_0	d_6
Adrenal	7	437	458	401	358	689	383	217	52	217	56	341	54
	14	238	467	117	410	145	518	74	46	152	86	106	44
	21	196	800	108	525	163	726	66	52	58	41	44	36
	28	104	786	61	521	49	561	58	64	129	128	40	34
	35	114	790	64	520	93	617	39	35	22	17	31	59
	42	57	779	41	544	85	755	16	23	28	35	14	14
	49	79	840	—	—	33	437	14	23	20	31	10	16
	56	20	364	33	384	44	672	7.8	22	15	24	27	45
Brain	7	38	2.0	31	1.5	35	1.5	34	0.3	29	0.4	34	0.3
	14	18	1.4	21	1.5	21	2.2	30	0.8	35	1.0	18	0.8
	21	31	4.1	30	5.8	31	6.0	35	1.2	49	1.3	46	1.4
	28	18	4.6	17	4.0	15	3.3	42	1.5	40	1.8	46	1.9
	35	66	18	26	6.0	33	7.4	41	2.3	43	1.3	41	2.6
	42	20	8.1	13	4.2	16	5.8	36	2.3	37	2.4	32	1.4
	49	39	17	—	—	26	10	31	2.5	35	2.4	36	2.1
	56	15	4.9	14	4.8	16	5.8	26	1.4	26	2.2	32	3.8
Heart	7	19	13	21	12	24	10	17	2.5	17	2.5	17	1.9
	14	18	18	11	19	14	23	5.2	2.5	11	5.9	14	7.1
	21	13	27	10	30	10	27	11	6.4	13	7.2	7.4	5.6
	28	7.0	35	6.7	27	3.6	19	7.6	6.3	11	8.3	8.1	6.0
	35	7.2	40	5.7	31	7.7	35	6.9	6.9	3.7	3.2	5.0	10
	42	3.6	34	3.3	28	4.7	33	2.7	3.9	4.7	5.7	4.0	4.0
	49	4.4	44	—	—	3.9	43	3.4	5.3	3.4	4.9	3.1	5.0
	56	1.7	27	2.7	26	2.4	32	0.9	2.5	2.3	3.8	5.0	8.5
Kidney	7	13	11	12	9	18	9	12	1.6	13	1.7	12	1.5
	14	8.0	8.8	8.8	12	10	16	4.5	1.9	9.1	3.6	5.6	2.4
	21	5.7	14	5.7	17	8.6	23	5.1	3.2	7.3	3.8	4.3	2.8
	28	6.8	22	3.7	16	3.6	17	5.0	3.8	8.3	5.9	5.8	4.1
	35	6.6	30	3.9	21	3.8	18	5.0	3.9	2.1	1.7	2.3	4.1
	42	3.1	22	2.1	16	3.7	20	2.3	2.7	3.6	3.8	2.3	2.1
	49	3.5	29	—	—	2.0	20	1.9	2.7	2.1	2.7	2.6	3.7
	56	0.9	12	1.7	14	1.5	16	0.8	1.9	1.5	2.2	2.9	4.6
Liver	7	16	41	17	41	34	55	22	7.0	14	5.5	22	5.6
	14	13	33	11	46	13	67	8.4	5.1	17	13	16	9.0
	21	6.7	42	7.4	41	8.8	53	7.9	7.4	11	9.5	5.3	5.2
	28	8.0	43	4.5	31	6.4	59	7.3	7.5	8.2	7.8	14	12
	35	8.7	67	6.6	54	6.1	48	5.9	6.3	2.1	1.8	4.3	9.4
	42	3.7	55	3.7	43	5.8	51	2.4	3.8	5.4	7.3	4.4	5.0
	49	4.9	55	—	—	3.8	45	3.5	6.7	5.2	8.8	3.9	7.1
	56	1.5	22	2.7	23	2.5	34	0.9	2.6	3.4	5.8	4.6	7.4
Lung	7	25	21	17	12	20	10	20	2.8	28	4.8	34	4.1
	14	11	15	9.5	19	17	39	18	7.3	13	7.2	20	5.0
	21	10	28	14	43	14	49	12	7.3	28	14	11	8.0
	28	11	50	8.6	44	4.5	25	9.3	9.4	15	13	18	13
	35	6.8	40	7.6	47	11	55	12	11	5.7	5.3	6.7	13
	42	4.1	42	6.0	55	9.3	64	5.3	7.1	6.9	8.7	4.7	5.5
	49	5.7	59	—	—	2.9	35	3.4	5.3	4.8	7.3	5.9	10
	56	2.9	44	4.2	44	4.1	53	1.1	3.5	4.0	7.0	8.3	14
Biceps femoris	7	12	3.9	8.9	2.3	12	2.6	9.9	0.5	10	0.6	10	0.4
	14	10	4.5	8.2	4.3	10	5.2	7.2	1.1	7.1	1.2	7.8	1.2
	21	7.3	5.8	7.9	6.3	6.5	4.7	6.8	1.5	6.8	1.6	5.3	1.2
	28	—	—	6.3	7.2	4.2	7.4	5.6	1.5	6.4	2.1	6.5	1.7
	35	8.2	14	5.6	11	5.2	8.0	5.9	2.0	2.5	1.0	2.2	2.0
	42	9.1	17	3.4	9.2	6.2	12	2.8	1.4	4.3	2.1	1.9	1.0
	49	4.2	13	—	—	2.8	9.5	1.7	1.2	2.4	1.4	1.8	1.3
	56	2.3	9.4	2.6	8.7	2.6	12	0.7	0.8	1.5	1.0	2.8	2.4
Plasma	7	2.2	5.1	1.7	4.0	4.8	8.5	2.9	0.7	4.2	1.4	4.3	1.0
	14	1.5	4.1	1.4	3.8	1.4	6.2	1.2	1.1	1.9	2.0	—	—
	21	1.0	7.1	1.5	7.0	1.5	6.9	2.2	1.8	4.3	2.3	1.5	1.3
	28	1.2	7.6	0.5	3.7	0.4	3.9	1.3	3.7	1.5	1.4	1.4	1.1
	35	1.4	10	0.7	4.9	0.9	6.7	1.9	1.7	1.2	0.7	1.5	2.1
	42	0.6	8.6	0.5	5.6	0.7	5.4	1.5	1.8	2.5	1.9	1.0	0.9
	49	0.9	9.7	—	—	0.4	4.9	1.7	1.2	0.8	1.5	0.6	1.1
	56	0.4	3.4	0.6	4.9	0.3	4.6	0.4	1.2	0.5	0.9	0.6	1.2

(Continued next page)

TABLE 1 *(Continued)*

Tissue	Day	HEHC		HENC		HELC		LEHC		LENC		LELC	
		d_0	d_6	d_0	d_6	d_0	d_6	d_0	d_6	d_0	d_6	d_0	d_6
Red cells	7	2.5	5.5	2.1	4.1	3.3	4.8	3.1	1.2	3.9	1.2	4.1	0.9
	14	1.4	3.3	0.3	1.2	1.0	4.3	1.2	1.1	1.3	1.2	—	—
	21	1.1	5.0	2.7	6.0	1.1	6.2	2.5	1.5	1.2	0.6	1.9	1.1
	28	1.2	7.5	0.7	4.8	0.5	4.0	1.8	1.4	1.7	1.5	1.8	1.6
	35	1.1	7.6	0.8	4.3	0.8	6.1	2.3	1.2	1.0	0.5	1.4	1.9
	42	0.5	5.8	0.4	3.9	0.6	4.6	1.3	1.2	1.6	1.3	1.2	0.8
	49	0.6	6.5	—	—	0.5	5.3	0.7	1.2	0.8	1.1	0.8	1.4
	56	0.0	4.8	1.1	4.7	0.0	6.7	0.2	0.7	0.6	1.0	0.8	1.3
Spleen	7	17	38	14	28	22	32	15	6.9	17	6.4	17	4.5
	14	—	—	8.8	40	7.7	40	6.3	4.7	11	8.7	12	7.4
	21	12	45	7.3	44	7.2	46	10	8.5	11	8.2	6.3	6.1
	28	6.9	56	4.3	39	3.3	39	6.3	6.9	11	11	11	10
	35	6.7	56	5.0	40	4.7	38	6.6	7.0	4.4	3.6	3.4	7.6
	42	3.1	47	2.5	34	4.9	43	2.1	3.6	4.6	7.0	3.4	4.2
	49	4.8	56	—	—	3.1	41	3.3	6.2	3.9	7.2	3.5	6.7
	56	1.7	26	2.4	24	2.2	32	0.9	2.8	2.8	5.0	5.5	9.2
Testis	7	14	7.7	15	6.0	17	5.0	14	1.2	12	1.4	16	1.3
	14	14	11	5	5.5	9.5	9.2	13	5.1	15	4.9	13	2.6
	21	4.9	9.2	10	16	31	42	7.1	3.3	5.9	2.9	6.4	2.8
	28	11	29	6.2	16	5.6	15	4.7	2.7	6.6	3.7	6.8	3.0
	35	6.4	18	3.6	11	4.8	13	4.2	2.5	2.5	1.5	2.5	4.0
	42	4.0	22	3.7	18	3.1	18	3.3	2.8	3.5	2.9	2.6	1.9
	49	3.6	24	—	—	2.1	15	2.3	2.7	2.5	2.8	2.2	2.4
	56	3.2	16	2.4	13	2.3	16	0.6	1.2	1.4	1.6	2.5	3.5

[a]Concentrations are in nmol/ml of plasma or packed red cells or nmol/g of tissue. HE and LE, 36 and 5 mg α-TOAc/kg diet; HC, NC and LC, 5,000, 250 and 50 mg ascorbic acid/kg diet, respectively.

should exceed its rate of replacement by "new" vitamin E. Under such dietary conditions, any *in vivo* "sparing" action of vitamin C on vitamin E should become obvious as a fairly dramatic difference in the rates of loss of "old" vitamin E (and, indeed, of total vitamin E) between the LEHC, LENC, and LELC subgroups of animals, with this rate being least for the LEHC subgroup and greatest for the LELC subgroup. Thus, the LEHC, LENC, and LELC subgroups should provide an even more sensitive probe for any *in vivo* vitamin C/vitamin E interaction than would the three HE subgroups.

We have recently analyzed the biokinetics of vitamin E in rats, guinea pigs and man under "steady-state" conditions (K. U. Ingold, G. W. Burton, and W. Siebrand, 1990, unpublished results), i.e., under conditions where the total concentration of "new" (deuterium-labelled) and "old" (unlabelled) vitamin E in a tissue remained essentially constant during the experiment. In Figure 2, we show the total concentration of *RRR*-α-TOH (i.e., d_0 + d_6) for plasma and eight tissues from the HEHC, HENC, and HELC subgroups of guinea pigs at each time point. Not surprisingly, the data show considerable scatter because these comparisons of the total tocopherol concentration in a tissue at any particular time are based on samples obtained from three different animals. Furthermore, "identical" guinea pigs unfortunately show more individual variations in vitamin E levels than do "identical" rats in similar experiments. Nevertheless, despite the scatter two things are immediately obvious: First, in each of the subgroups the total concentration of vitamin E in a given tissue remained approximately constant for the eight week experiment. Second, the total concentration of vitamin E (given as its eight week average in Table 2) does not differ significantly in any tissue between the HEHC, HENC, and HELC subgroups. Hence, it appears that any "sparing" or "regenerating" action by vitamin C on the rate at which vitamin E is consumed (by all metabolic routes) is negligible in comparison with the normal turn-over of vitamin E in guinea pigs under the conditions of the HE experiment.

This conclusion was somewhat unexpected in view of the extensive and conclusive evidence that vitamin C "spares" and/or "regenerates" vitamin E in a wide variety of *in vitro* model systems (9–37) and the extensive, though less conclusive, evidence for the same phenomenon *in vivo* (40,45–47,50,52,53,56,58). We have therefore confirmed this conclusion by reanalyzing the data in Table 1 to demonstrate that the rate of loss of "old" vitamin E from a particular tissue is not significantly different between the HEHC, HENC, and HELC subgroups of guinea pigs. Figure 3 shows plots for plasma and eight tissues of the ratio of "old" vitamin E/total vitamin E, i.e., d_0-*RRR*-α-TOH/[d_0- + d_6-*RRR*-α-TOH]. We use "old" vitamin E/total vitamin E ratios in order to minimize the effect of differences between individual animals and temporal fluctuations within an animal. Figure 3 provides convincing proof that the rate of loss of "old" vitamin E from a tissue is quite uninfluenced by the level of vitamin C in the diet of the HE group of guinea pigs. Indeed, plots of log[d_0-*RRR*-α-TOH] vs time, which were found to be approximately linear (vide infra), showed no statistically significant differences between the HEHC, HENC, and HELC slopes for each tissue.

Confirmation that increased levels of vitamin C in the

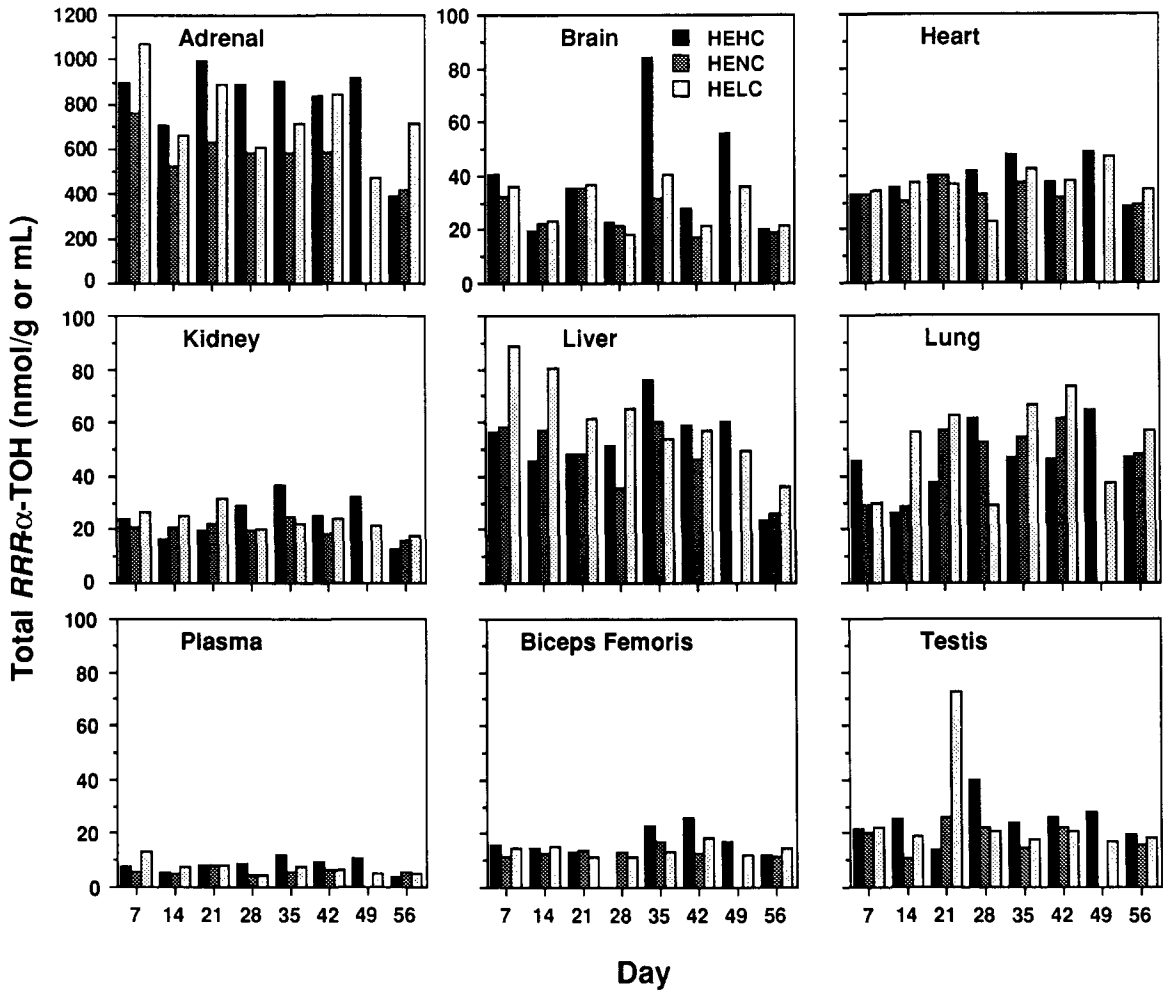

FIG. 2. Total (i.e., d_0- + d_6-) *RRR*-α-TOH concentrations for plasma and eight tissues from the HEHC, HENC, and HELC subgroups of guinea pigs have been plotted at weekly intervals. Note the different vertical scale for adrenal.

TABLE 2

Effect of Vitamin C on Total α-Tocopherol Concentrations in Blood and Tissues of Guinea Pigs[a]

	HEHC	HENC	HELC
Adrenal	816 ± 194	584 ± 103	746 ± 185
Brain	38 ± 22	26 ± 7	29 ± 9
Heart	39 ± 7	33 ± 4	37 ± 7
Kidney	25 ± 8	20 ± 3	24 ± 4
Liver	53 ± 15	47 ± 13	62 ± 17
Lung	47 ± 12	47 ± 13	51 ± 17
Biceps femoris	17 ± 5	13 ± 2	14 ± 2
Plasma	8 ± 3	6 ± 1	7 ± 3
Red cells	7 ± 2	5 ± 2	6 ± 1
Spleen	47 ± 22	42 ± 8	46 ± 7
Testis	25 ± 7	19 ± 5	26 ± 19

[a]Guinea pigs were maintained on high vitamin E (HE) diets (36 mg acetate/kg diet) containing vitamin C at high (HEHC, 5000 mg/kg), normal (HENC, 250 mg/kg) or low (HELC, 50 mg/kg) levels. Concentrations (nmol/g or ml), are the mean and standard deviation of values obtained from eight animals killed at the rate of one per week over an eight week period. The only statistically significant difference found was between adrenal HEHC and HENC ($p < 0.05$).

diet did, indeed, lead to increased levels in the animals was obtained by measuring, using an HPLC method (69), the ascorbate levels in extracts of homogenized samples of a selection of frozen tissues from animals in the HE group. The concentrations of ascorbate in the livers of each of the LC, NC, and HC animals were found to be 42, 237, and 750 nmol/g, respectively, at two weeks, and 100, 179 and 788 nmol/g, respectively, at five weeks. Values obtained for lung in the LC and HC groups at two weeks were 47 and 199 nmol/g, respectively, and the corresponding values for testis were 156 and 749 nmol/g, respectively.

In the LE group of guinea pigs the total concentration of vitamin E decreased substantially over eight weeks in all tissues except the brain (Fig. 4). [The absence of a noticeable decline in the brain is due to the very slow loss of "old (and gain of "new") vitamin E by this organ (Table 1). The literature suggests that had we examined adipose tissue it also would have shown a very slow turnover of vitamin E (70).] The LE group certainly does not have a "steady-state" vitamin E status but is instead progressing towards deficiency or a very much lower steady-

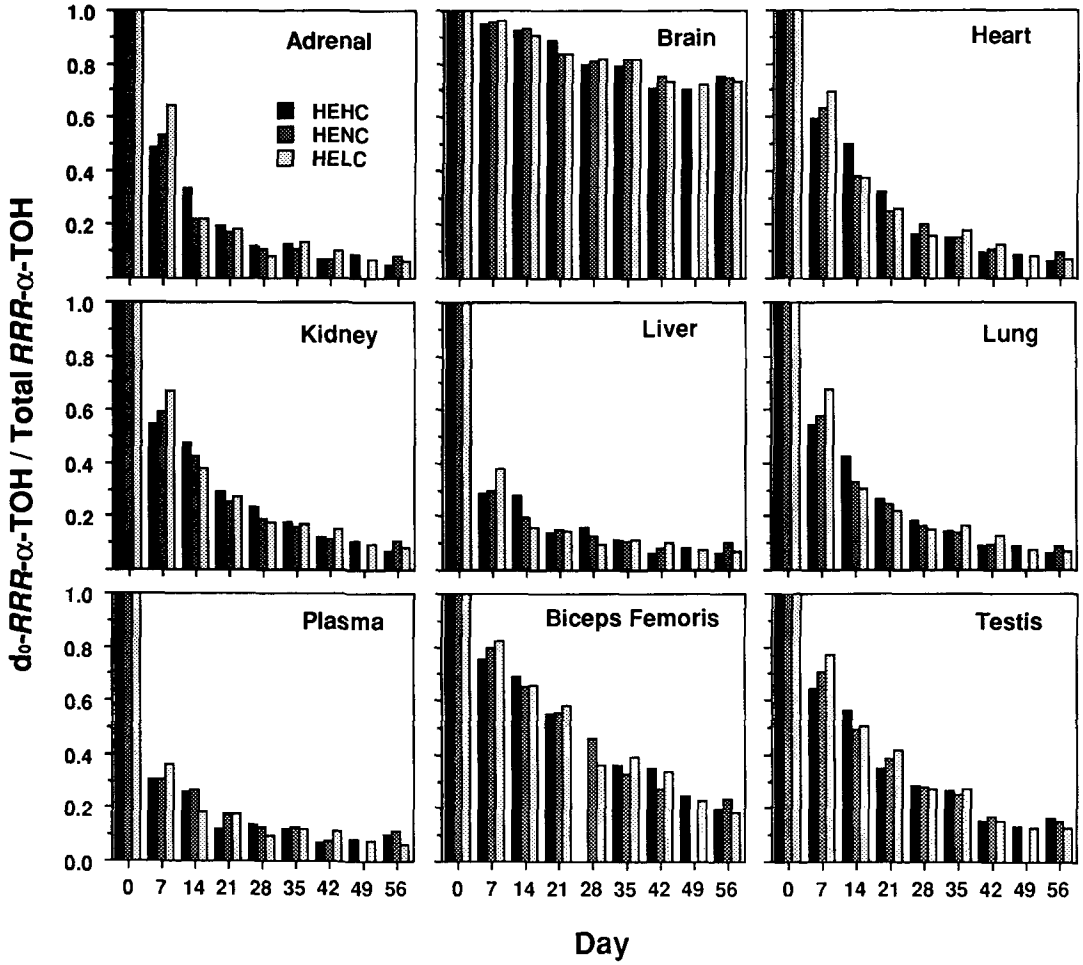

FIG. 3. "Old" d_0-*RRR*-α-TOH/total (d_0- + d_6-) *RRR*-α-TOH ratios for plasma and eight tissues from the HEHC, HENC, and HELC subgroups of guinea pigs have been plotted at weekly intervals.

state level of vitamin E, during the eight week experiment. Nevertheless, the data shown in Figure 4 demonstrate that there is no statistically significant difference in the total concentration of vitamin E in a particular tissue between the LEHC, LENC, and LELC subgroups of guinea pigs over the eight week course of this experiment. We conclude that any "sparing" or "regenerating" of vitamin E by vitamin C is negligible in comparison with those metabolic processes which consume vitamin E or eliminate it from the body, even in a guinea pig which is receiving insufficient vitamin E in its diet to maintain its body stores.

This conclusion was also unexpected. Therefore, in Figure 5 we present plots of the ratio of "old" vitamin E/total vitamin E for plasma and eight tissues. Again, differences between the slopes of plots of log[d_0-*RRR*-α-TOH] vs time for the LEHC, LENC, and LELC subgroups for each tissue were not statistically different. Obviously, the rate of loss of "old" vitamin E from a tissue was quite uninfluenced by the level of vitamin C in the diet of the LE group just as was the case for the HE group of guinea pigs.

In summary, we can find no evidence for an interaction between vitamin C and vitamin E *in vivo* despite a careful, sensitive, and sophisticated search using an appropriate animal model, i.e., an animal which does not synthesize ascorbic acid. We conclude that any synergistic (i.e., "sparing") or antagonistic interaction between these two vitamins *in vivo* in animals not subject to enhanced oxidative stress is negligible in comparison to other metabolic processes. Strictly speaking, this conclusion applies only to guinea pigs. However, we can see no reason why it should not also apply to other animals, including man.

To conclude this section we note that our present results demonstrate that even the most carefully modeled *in vitro* system may fail to reproduce the *in vivo* reality. In this case, the most careful models have involved α-tocopherol dissolved in dilinoleoylphosphatidylcholine (26) or soybean phosphatidylcholine (27) multilamellar liposomes dispersed in water containing ascorbic acid which was subjected to attack at 37°C by thermally-generated, water- or lipid-soluble peroxyl radicals (71). On addition of vitamin C, the water-soluble peroxyl radicals were efficiently trapped which prevented them from attacking the phospholipid bilayer and hence "spared" the vitamin E until all the vitamin C had been consumed (26,27). This result has been confirmed not only

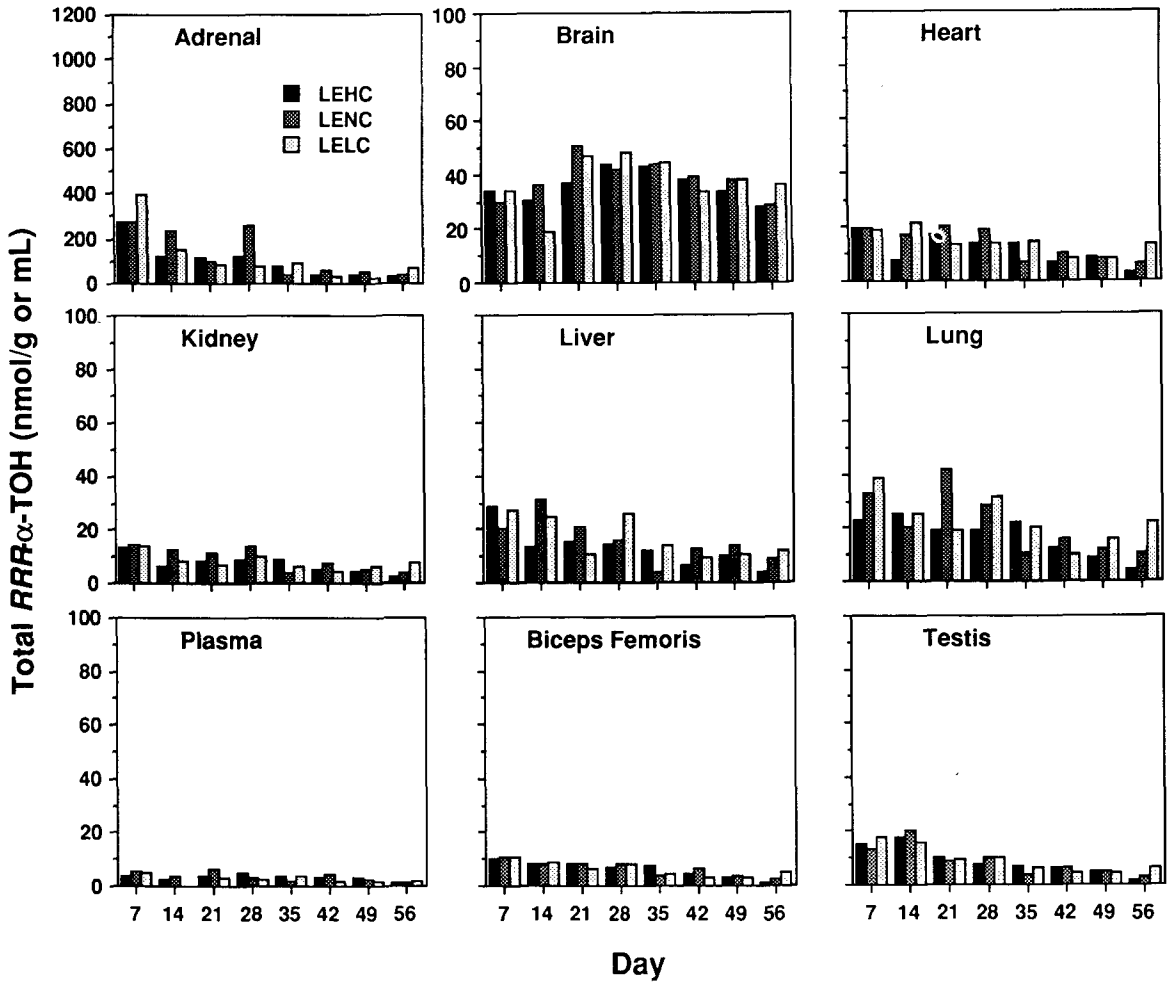

FIG. 4. Total (i.e., d_0- + d_6-) *RRR*-α-TOH concentrations for plasma and eight tissues from the LEHC, LENC and LELC subgroups of guinea pigs have been plotted at weekly intervals. Note that the vertical scales in this figure (including that for adrenal) have been made the same as those in Figure 2 for comparative purposes.

in analogous liposomal and micellar model systems (24,25,28,33) but also in plasma when the plasma was subjected to attack by water-soluble peroxyl radicals (69,72, 73). However, vitamin C is not unique in such systems since other water-soluble, radical-trapping antioxidants (e.g., plasma proteins, glutathione, cysteine, urate, bilirubin, etc.)—both in plasma (69,72,73) and in liposomal model systems (74–77)—also "spare" vitamin E from attack by water-soluble peroxyl radicals. More interesting are the liposomal systems in which lipid-soluble peroxyl radicals are generated from a lipid-soluble initiator (71). In such systems ascorbate by itself was ineffective at protecting the phospholipid from peroxidation (26,27,78). The same is true for most other potentially available water-soluble physiological antioxidants [except for conjugated bilirubin and biliverdin (78)] both by themselves and [except for cysteine (77)] in the presence of vitamin E (74,76,78). Ascorbate is virtually unique in that it regained its antioxidant capabilities in the presence of vitamin E (26,27). That is, when oxidation is initiated in the lipid phase, vitamin C is the only water-soluble antioxidant which becomes active when vitamin E is present. Presumably this "sparing" by vitamin C of vitamin E occurs via the "regenerating" reaction [2], as was suggested over 40 years ago by Golumbic (79).

The question as to why water- and lipid-soluble peroxyl radicals in the presence of phospholipid bilayers and water-soluble peroxyl radicals in plasma do not reflect the situation in guinea pigs with steady or declining vitamin E status is intriguing. The simplest answer (Occam's answer) would be that under normal conditions the flux of peroxyl radicals which enters the lipids of a healthy animal is not nearly as high as has frequently been supposed. This answer receives support from Tappel's finding that expired pentane levels from animals are extremely low, even for animals that are receiving inadequate or no vitamin E, relative to the levels reached for animals that are oxidatively stressed in various ways (41,44,54,80) (A. L. Tappel, 1989, private communication). This would mean that for oxidatively unstressed animals, only a very small fraction of the available vitamin E is actually destroyed by trapping the peroxyl radicals. A much more interesting possibility is that the tocopheroxyl radical is formed extensively even in a healthy animal, but is reduced *in vivo* not by ascorbate but by some other, possibly enzymic, process. There is, in fact, considerable

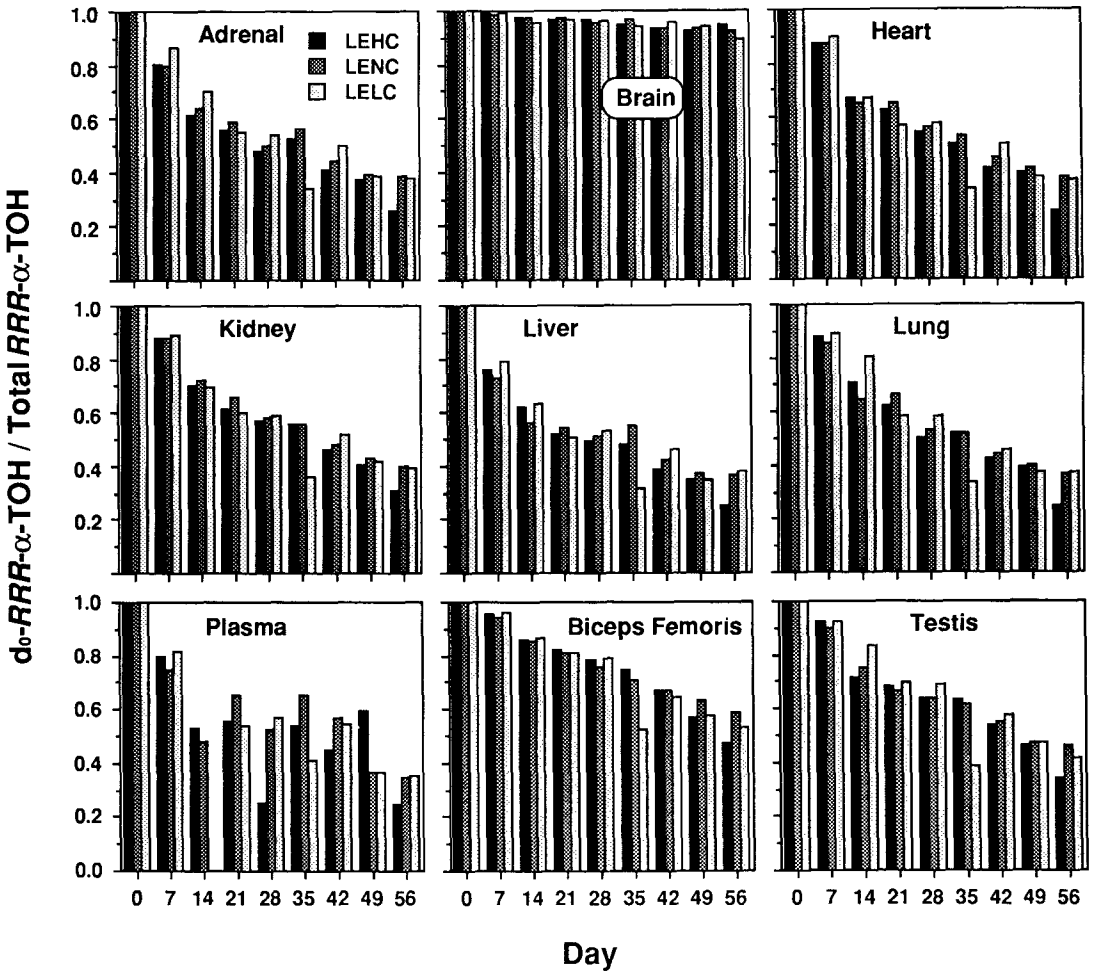

FIG. 5. "Old" d_0-*RRR*-α-TOH/total (d_0- + d_6-) *RRR*-α-TOH ratios for plasma and eight tissues from the LEHC, LENC, and LELC subgroups of guinea pigs have been plotted at weekly intervals.

evidence that rat liver microsomes and other organelles and tissues contain a membrane-bound, heat-labile, glutathione-dependent, free-radical reductase which probably acts by converting the tocopheroxyl radical to tocopherol (38,81-90) and which therefore participates in the *in vivo* protective system against lipid peroxidation. Free radical reductase activity in microsomes may also be NADPH dependent (91). A quantitative and unequivocal determination of the peroxyl radical flux in the lipids of healthy animals would add enormously to our understanding of free radical biology.

Biokinetics of vitamin E in guinea pigs. Comparison with rats. There are no significant differences in the biokinetics of vitamin E between the HEHC, HENC, and HELC subgroups of animals and between the LEHC, LENC, and LELC subgroups. This allows us to combine the biokinetic data for all the HE and all the LE guinea pigs, which simplifies biokinetic comparisons between these groups. Moreover, the HE biokinetic data can be compared with the analogous data for the same tissues obtained from the HE rats (1).

Under "steady-state" conditions of vitamin E the tissues of an animal can be divided broadly into two kinetic groups (K. U. Ingold, G. W. Burton, and W. Siebrand, 1990, unpublished results). The first group, which includes brain, heart, muscle, and testes, shows slow, first order (i.e., exponential) loss of "old" and gain of "new" vitamin E. The second group, which includes plasma, liver, lung, adrenal gland, and kidneys, shows non-exponential behavior with an initial, rapid change in vitamin E concentration compared with later stages. Formally, the behavior of the fast tissues can be represented as the sum of two (or more) first-order processes (K. U. Ingold, G. W. Burton, and W. Siebrand, 1990, unpublished results).

Since this detailed kinetic analysis of the HE ("steady-state") group of guinea pigs will be reported elsewhere (K. U. Ingold, G. W. Burton, and W. Siebrand, 1990, unpublished results), though without the supporting raw data which is given in Table 1, we will not repeat it here. For present purposes it is sufficient to give the time required under "steady-state" conditions for the concentration of deuterium-labelled, "new" vitamin E in a tissue to become equal to the concentration of unlabelled, "old" vitamin E. These equalization times, $t_{1:1}$, give a simple measure of the speed with which vitamin E turns over in different tissues under "steady-state" conditions. Equalization times can be estimated by inspection of the

TABLE 3

Comparison of Tissue Equalization Times ($t_{1:1}$; days) in Young Guinea Pigs and Rats[a]

Tissue	Guinea pig	Rat
Plasma	3.7	6.2
Liver	3.0	6.9
Lung	9.9	8.8
Kidney	9.8	13
Heart	14	18
Testis	17	40
Biceps femoris	24	23
Brain	107	40

[a]Values for all tissues, except guinea pig brain, were obtained by interpolation of concentration data plotted in single- or multiexponential form vs time. Because of a very slow rate of turnover, the value for guinea pig brain was estimated by extrapolation.

raw data but can be determined more reliably from exponential (slow tissues) or multiexponential (fast tissues) plots (K. U. Ingold, G. W. Burton, and W. Siebrand, 1990, unpublished results). Values of $t_{1:1}$ for the HE guinea pigs' tissues, obtained in this way after combining the HEHC, HENC, and HELC data, are given in Table 3. For comparison this table also includes the $t_{1:1}$ values for the same classes of tissue obtained from HE rats, i.e., from rats fed a diet containing 36 mg d_3-*RRR*-α-TOAc/kg diet and having essentially constant concentrations of vitamin E in their tissues. Some of the rat $t_{1:1}$ values differ from those previously reported (1) because of our current application of a more sophisticated kinetic treatment (K. U. Ingold, G. W. Burton, and W. Siebrand, 1990, unpublished results).

Inspection of Table 3 reveals that all but one of the fast (multiexponential kinetics) tissues from guinea pigs exchange vitamin E more rapidly than the corresponding rat tissues, whereas the slow (monoexponential kinetics) tissues from the guinea pig may exchange vitamin E more, or less, rapidly than the corresponding rat tissues. The most striking difference in the tissue biokinetics of vitamin E between guinea pigs and rats lies in the brain [and probably in adipose tissue (cf., 1,70)]; under "steady-state" conditions the transport of vitamin E into or out of the guinea pigs' brain occurs at only ca. 40% of the rate for the rat. It would be extremely interesting to have analogous data for humans.

There appears to be only one earlier "comparative" study of vitamin E uptake by rats and guinea pigs and only blood, adrenals, heart, and liver were examined (92). On diets containing 30 mg *all-racemic*-α-TOAc per kg of diet the rats after 46 weeks had from 1.4 to 2.0 times as much α-TOH (per ml or per g tissue) as did the guinea pigs in the same tissues after 32 weeks (92). The relevance of this observation to our own measurements is not obvious.

Equalization times for the LE group of guinea pigs have not been calculated because the vitamin E status of these animals is not at a "steady-state," but declines in all tissues except the brain (Table 1 and Fig. 4). This fact is especially evident in plots of the logarithm of total tissue vitamin E vs time (Fig. 6).

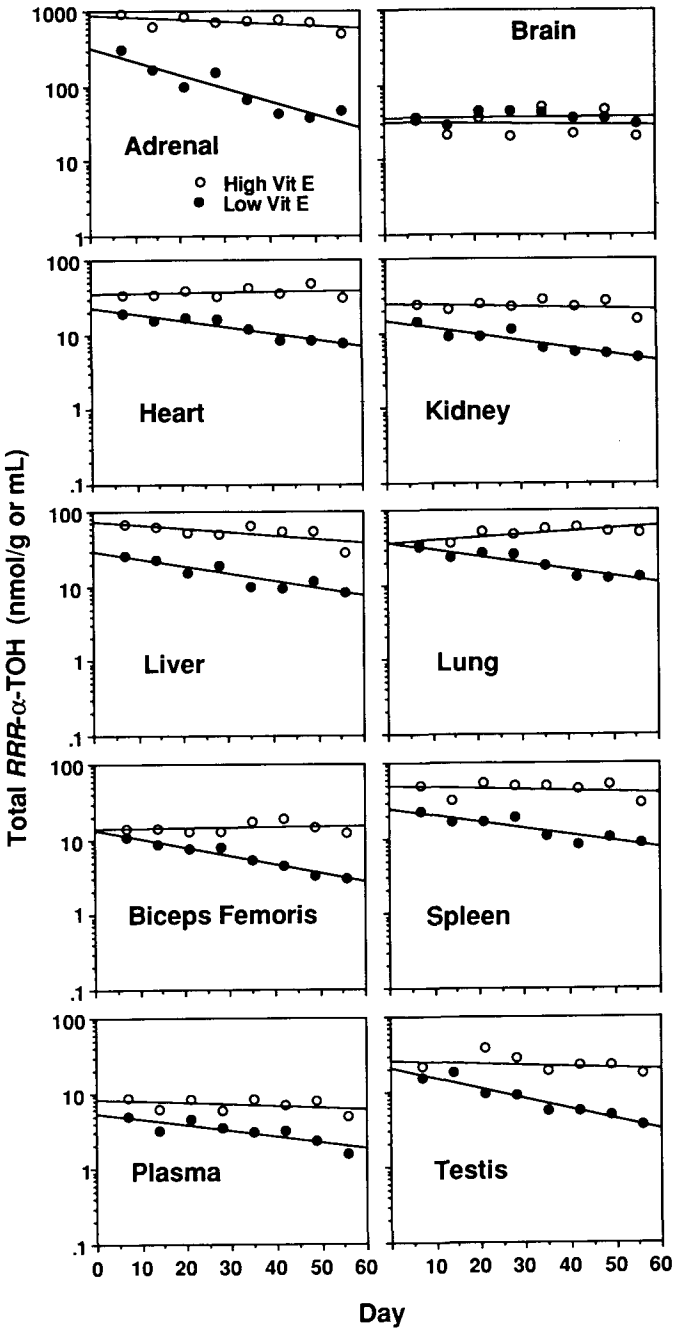

FIG. 6. Semi-logarithmic plots of total (d_0- + d_6-) *RRR*-α-TOH concentrations vs time for plasma and nine tissues from the HE (○) and LE (●) groups of guinea pigs. The individual points which are displayed are the averaged values found for the HC, NC and LC subroups of animals. However each line is the least squares fit to all of the HC, NC and LC data points. Note the different vertical scale for adrenal and brain.

Interestingly, the rates of loss of "old" vitamin E from the tissues of the LE animals were not different from the corresponding rates of the HE animals. This is shown in Figure 7 in which we have plotted log[d_0-*RRR*-α-TOH] vs time for both fast and slow tissues. Although, under "steady-state" conditions, the biokinetics for the fast tissues can be somewhat better described in terms of a

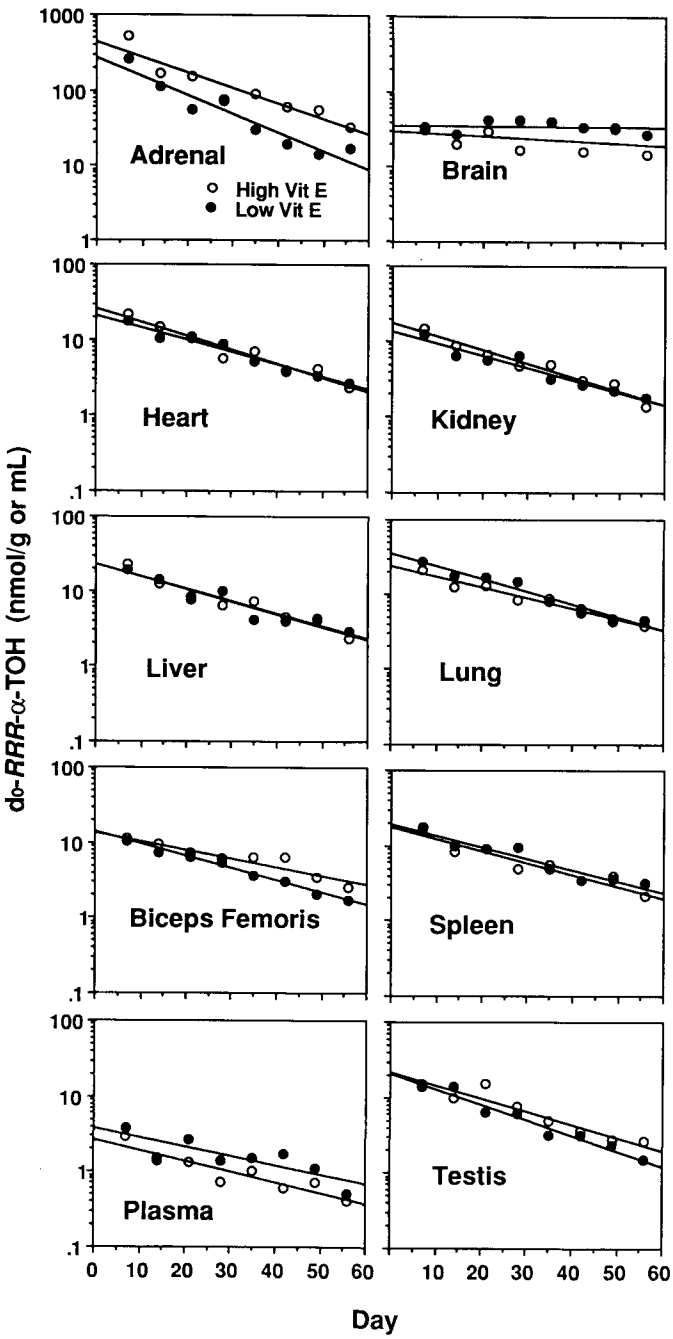

FIG. 7. Semi-logarithmic plots of "old" d_0-*RRR*-α-TOH concentrations vs time for plasma and nine tissues from the HE (○) and LE (●) groups of guinea pigs. The individual points which are displayed are the averaged values found for the HC, NC and LC subgroups of animals. However each line is the least squares fit to all of the HC, NC and LC data points. Note the different vertical scale for adrenal and brain.

multiexponential (K. U. Ingold, G. W. Burton, and W. Siebrand, 1990, unpublished results), it is reasonable to use a single exponential because the data in Figure 7 do not include the initial rapid decline that occurs in the first few days.

It is not unreasonable that "old" vitamin E should be lost from a specific tissue of the LE and HE groups of animals at the same rate, since different rates would imply the existence of some physiological "feed-back" mechanism which could "sense" the vitamin E status of the animal. We hypothesize that such a physiological "feed-back" process may be present in certain "critical" tissues which have very slow rates of uptake and loss of vitamin E. The brain would appear to be a prime candidate for possession of a control mechanism which would ensure that the rate at which it lost vitamin E would depend on the animal's overall vitamin E status, i.e., the rate of loss would be lower when the animal was put on a diet containing an inadequate (or no) vitamin E compared with an animal on a normal diet. Unfortunately, our eight-week-long study with the guinea pigs was of too short a duration for any such effect (or lack of such effect) to demonstrate itself. We therefore plan to carry out further experiments to determine whether certain critical tissues do or do not possess a "feed-back" mechanism.

ACKNOWLEDGMENTS

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REFERENCES

1. Ingold, K.U., Burton, G.W., Foster, D.O., Hughes, L., Lindsay, D.A., and Webb, A. (1987) *Lipids 22*, 163-172.
2. Burton, G.W., and Ingold, K.U. (1981) *J. Am. Chem. Soc. 103*, 6472-6477.
3. Burton, G.W., Joyce, A., and Ingold, K.U. (1983) *Arch. Biochem. Biophys. 221*, 281-290.
4. Burton, G.W., and Ingold, K.U. (1986) *Accounts Chem. Res. 19*, 194-201.
5. Ingold, K.U., Webb, A.C., Witter, D., Burton, G.W., Metcalf, T.A., and Muller, D.P.R. (1987) *Arch. Biochem. Biophys. 259*, 224-225.
6. Pryor, W.A., Kaufman, M.J., and Church, D.F. (1985) *J. Org. Chem. 50*, 281-283.
7. Wayner, D.D.M., Burton, G.W., and Ingold, K.U. (1986) *Biochim. Biophys. Acta 884*, 119-123.
8. Bendich, A., Machlin, L.J., Scandurra, O., Burton, G.W., and Wayner, D.D.M. (1986) *Adv. Free Radical Biol. Med. 2*, 419-444.
9. Golumbic, C., and Mattill, H.A. (1941) *J. Am. Chem. Soc. 63*, 1279-1280.
10. Watts, B.M., and Wong, R. (1951) *Arch. Biochem. 30*, 110-120.
11. Privett, O.S., and Quackenbush, F.W. (1954) *J. Am. Chem. Soc. 31*, 321-323.
12. Harrison, W.H., Gander, J.E., Blakley, E.R., and Boyer, P.D. (1956) *Biochim. Biophys. Acta 21*, 150-158.
13. Lew, Y.T., and Tappel, A.L. (1956) *Food Technol. 10*, 285-289.
14. Tappel, A.L., Brown, W.D., Zalkin, H., and Maier, V.P. (1961) *J. Am. Oil Chem. Soc. 38*, 5-9.
15. Cort, W.M. (1974) *J. Am. Oil Chem. Soc. 51*, 321-325.
16. Packer, J.E., Slater, T.F., and Willson, R.L. (1979) *Nature 278*, 737-738.
17. Niki, E., Tsuchiya, J., Tanimura, R., and Kamiya, Y. (1982) *Chem. Letters*, 789-792.
18. Leung, H.-W., Vang, M.J., and Mavis, R.D. (1981) *Biochim. Biophys. Acta 664*, 266-272.
19. Bascetta, E., Gunstone, F.D., and Walton, J.C. (1983) *Chem. Phys. Lipids 33*, 207-210.
20. Niki, E., Saito, T., and Kamiya, Y. (1983) *Chem. Letters*, 631-632.
21. Rousseau, C., Richard, C., and Martin, R. (1983) *J. Chim. Phys. Phys.-Chim. Biol. 80*, 827-829.

22. Barclay, L.R.C., Locke, S.J., and MacNeil, J.M. (1983) *Can. J. Chem. 61,* 1288-1290.
23. Niki, E., Saito, T., Kawakami, A., and Kamiya, Y. (1984) *J. Biol. Chem. 259,* 4177-4182.
24. Yamamoto, Y., Haga, S., Niki, E., and Kamiya, Y. (1984) *Bull. Chem. Soc. Jpn. 57,* 1260-1264.
25. Scarpa, M., Rigo, A., Maiorino, M., Ursini, F., and Gregolin, C. (1984) *Biochim. Biophys. Acta 801,* 215-219.
26. Doba, T., Burton, G.W., and Ingold, K.U. (1985) *Biochim. Biophys. Acta 835,* 298-303.
27. Niki, E., Kawakami, A., Yamamoto, Y., and Kamiya, Y. (1985) *Bull. Chem. Soc. Jpn. 58,* 1971-1975.
28. Barclay, L.R.C., Locke, S.J., and MacNeil, J.M. (1985) *Can. J. Chem. 63,* 366-374.
29. McCay, P.B. (1985) *Annu. Rev. Nutr. 5,* 323-340.
30. Lambelet, P., Saucy, F., and Löliger, J. (1985) *Experientia 41,* 1384-1388.
31. Rousseau, C., Richard, C., and Martin, R. (1985) *J. Chim. Phys. Phys.-Chim. Biol. 82,* 527-529.
32. Takahashi, M., Niki, E., Kawakami, A., Kumasaka, A., Yamamoto, Y., Kamiya, Y., and Tanaka, K. (1986) *Bull. Chem. Soc. Jpn. 59,* 3179-3183.
33. Liebler, D.C., Kling, D.S., and Reed, D.J. (1986) *J. Biol. Chem. 261,* 12114-12119.
34. Mukai, K., Fukuda, K., Ishizu, K., and Kitamura, Y. (1987) *Biochem. Biophys. Res. Comm. 146,* 134-139.
35. Niki, E. (1987) *Ann. N.Y. Acad. Sci. 498,* 186-199.
36. Niki, E. (1987) *Chem. Phys. Lipids 44,* 227-253.
37. Niki, E. (1987) *Br. J. Cancer 55* (Suppl. VIII), 153-157.
38. Wefers, H., and Sies, H. (1988) *Eur. J. Biochem. 174,* 353-357.
39. Davies, M.J., Forni, L.G., and Willson, R.L. (1988) *Biochem. J. 255,* 513-522.
40. Chen, L.H., Lee, M.S., Hsing, W.F., and Chen, S. (1980) *Internat. J. Vit. Nutr. Res. 50,* 156-162.
41. Litov, R.E., Matthews, L.C., and Tappel, A.L. (1981) *Toxicol. Appl. Pharmacol. 59,* 96-106.
42. Chen, L.H. (1981) *Am. J. Clin. Nutr. 34,* 1036-1041.
43. Bai, N.J., Kumar, P.S., George, T., and Krishnamurthy, S. (1982) *Internat. J. Vit. Nutr. Res. 52,* 386-392.
44. Dillard, C.J., Downey, J.E., and Tappel, A.L. (1984) *Lipids 19,* 127-133.
45. Chen, L.H., and Thacker, R.R. (1985) *FASEB Fed. Proc. 44,* 934.
46. Chen, L.H., and Thacker, R.R. (1986) *Biotechnol. Appl. Biochem. 8,* 40-45.
47. Chen, L.H., and Thacker, R.R. (1987) *Internat. J. Vit. Nutr. Res. 57,* 385-390.
48. Nandi, B.K., Majumder, A.K., Subramanian, N., and Chatterjee, I.B. (1973) *J. Nutr. 103,* 1688-1695.
49. Chen, L.H., and Barnes, K.J. (1976) *Nutr. Reports Internat. 14,* 89-96.
50. Chen, L.H. and Chang, M.L. (1978) *J. Nutr. 108,* 1616-1620.
51. Chen, L.H. and Chang, H.M. (1979) *Internat. J. Vit. Nutr. Res. 49,* 87-91.
52. Kanazawa, K., Takeuchi, S., Hasegawa, R., Okada, M., Makiyama, I., Hirose, N., Toh, T., Cho, S.H., and Kobayashi, M. (1981) *Nihon Univ. J. Med. 23,* 257-265.
53. Hrubá, F., Nováková, V., and Ginter, E. (1982) *Experientia 38,* 1454-1455.
54. Kunert, K.J., and Tappel, A.L. (1983) *Lipids 18,* 271-274.
55. Ginter, E., Kosinova, A., Hudecova, A., and Mlynarcikova, U. (1984) *J. Nutr. 114,* 485-492.
56. Bendich, A., D'Apolito, P., Gabriel, E., and Machlin, L.J. (1984) *J. Nutr. 114,* 1588-1593.
57. Miyazawa, T., Ando, T., and Kaneda, T. (1986) *Agric. Biol. Chem. 50,* 71-78.
58. Arad, I.D., Dgani, Y., and Eyal, F.G. (1985) *Internat. J. Vit. Nutr. Res. 55,* 395-397.
59. Rees, S., and Slater, T.F. (1987) *Acta Biochim. Biophys. Hung. 22,* 241-249.
60. Yamamoto, K., Takahashi, M., and Niki, E. (1987) *Chem. Letters,* 1149-1152.
61. Ingold, K.U., Hughes, L., Slaby, M., and Burton, G.W. (1987) *J. Labelled Compds. Pharmac. 24,* 817-831.
62. Nandi, B.K., Majumder, A.K., Subramanian, N., and Chatterjee, I.B. (1973) *J. Nutr. 103,* 1688-1695.
63. Ginter, E., Ondreicka, R., Bobek, P., and Simko, V. (1969) *J. Nutr. 99,* 261-266.
64. Ginter, E. (1975) *Ann. N.Y. Acad. Sci. 258,* 410-421.
65. Ginter, E., Bobek, P., and Jurcovicova, M. (1982) *Advances Chem. Series 200,* 381-393.
66. Spanheimer, R.G., Bird, T.A., and Peterkovsky, B. (1986) *Arch. Biochem. Biophys. 246,* 33-51.
67. Hatch, G.E., Slade, R., Selgrade, M.K., and Stead, A.G. (1986) *Toxicol. Appl. Pharm. 82,* 351-359.
68. Berger, J., Shepard, D., Morrow, F., and Taylor, A. (1989) *J. Nutr. 119,* 734-740.
69. Wayner, D.D.M., Burton, G.W., Ingold, K.U., and Locke, S. (1987) *Biochim. Biophys. Acta 924,* 408-419.
70. Machlin, L.J., Keating, J., Nelson, J., Brin, M., Filipski, R., and Miller, D.N. (1979) *J. Nutr. 109,* 105-109.
71. Barclay, L.R.C., Locke, S.J., MacNeil, J.M., VanKessel, J., Burton, G.W., and Ingold, K.U. (1984) *J. Am. Chem. Soc. 106,* 2479-2481.
72. Wayner, D.D.M., Burton, G.W., Ingold, K.U., and Locke, S. (1985) *FEBS Lett. 187,* 33-37.
73. Frei, B., Stocker, R., and Ames, B.N. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85,* 9748-9752.
74. Niki, E., Yamamoto, Y., and Kamiya, Y. (1985) *Chem. Letters,* 1267-1270.
75. Niki, E., Saito, M., Yoshikawa, Y., Yamamoto, Y., and Kamiya, Y. (1986) *Bull. Chem. Soc. Jpn. 59,* 471-477.
76. Barclay, L.R.C. (1988) *J. Biol. Chem. 263,* 16138-16142.
77. Motoyama, T., Miki, M., Mino, M., Takahashi, M., and Niki, E. (1989) *Arch. Biochem. Biophys. 270,* 655-661.
78. Stocker, R., and Peterhans, E. (1989) *Biochim. Biophys. Acta 1002,* 238-244.
79. Golumbic, C. (1946) in *Biological Antioxidants* (Mackenzie, C.G., ed.), Josiah Macy, Jr. Foundation, New York, pp. 42-48.
80. Dillard, C.J., and Tappel, A.L. (1988) in *Cellular Antioxidant Defense Mechanisms* (Chow, C.K., ed.), CRC Press, Boca Raton, Florida, Vol. 1, pp. 103-115.
81. Reddy, C.C., Scholz, R.W., Thomas, C.E., and Massaro, E.J. (1982) *Life Sci. 31,* 571-576.
82. Haenen, G.R.M.M., and Bast, A. (1983) *FEBS Lett. 159,* 24-28.
83. Burk, R.F. (1983) *Biochim. Biophys. Acta 757,* 21-28.
84. Hill, K.E., and Burk, R.F. (1984) *Biochem. Pharmacol. 33,* 1065-1068.
85. Franco, D.P., and Jenkinson, S.G. (1986) *J. Appl. Physiol. 61,* 785-790.
86. McCay, P.B., Lai, E.K., Powell, S.R., and Brueggemann, G. (1986) *Fed. Proc. 45,* 451.
87. Haenen, G.R.M.M., Tai Tin Tsoi, J.N.L., Vermeulen, N.P.E., Timmerman, H., and Bast, A. (1987) *Arch. Biochem. Biophys. 259,* 449-456.
88. McCay, P.B., Lai, E.K., Brueggemann, G., and Powell, S.R. (1987) *NATO ASI Ser., Ser. A 131,* 145-156.
89. Bast, A., and Haenen, G.R.M.M. (1988) *Biochim. Biophys. Acta 963,* 558-561.
90. Tirmenstein, M.A., and Reed, D.J. (1989) *J. Lipid Res. 30,* 959-965.
91. Packer, L., Maguire, J.J., Mehlhorn, R.J., Serbinova, E., and Kagan, V.E. (1989) *Biochem. Biophys. Res. Comm. 159,* 229-235.
92. Elmadfa, I., and Walter, A. (1981) *Internat. J. Vit. Nutr. Res. 51,* 284-292.

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