Kinetics of Rat Peripheral Nerve, Forebrain and Cerebellum α-Tocopherol Depletion: Comparison with Different Organs[1]

JEAN-MARIE BOURRE[2] AND MICHEL CLEMENT

INSERM U 26, Hôpital Fernand Widal, 200 rue du Faubourg Saint-Denis, 75475 Paris Cedex 10, France

ABSTRACT Forty-two 60-d-old rats were fed a vitamin E–deficient diet for up to 8 wk and the kinetics of α-tocopherol depletion were measured in nervous tissue and various organs. In most tissues examined, including the sciatic nerve but not the cerebellum, the disappearance was biphasic, suggesting the existence of two pools of vitamin E. In forebrain the disappearance was poorly biphasic. After an 8-wk period of vitamin E deprivation, forebrain, cerebellum, sciatic nerve endoneurium, liver, heart, muscle, testes and serum vitamin E concentrations were 51, 64, 65, 10, 20, 20, 40 and 19%, respectively, of initial values. J. Nutr. 121: 1204–1207, 1991.

INDEXING KEY WORDS:

- *vitamin E* • *α-tocopherol*
- *peripheral nerve* • *brain* • *rats*

Vitamin E (α-tocopherol) seems to be the only significant lipid-soluble chain-breaking (peroxyl radical–trapping) antioxidant in vivo (1). Therefore, its principal function is to scavenge free radicals in the lipid phase, protecting the polyunsaturated fatty acids against peroxidation. In addition, specific effects of α-tocopherol that do not involve its antioxidant function and that act upon the architecture of membranes by controlling the profile of their lipids have been suggested (2, 3). Because nervous tissue contains very high amounts of lipids (all of them found in membranes and not used for energy), it is assumed that vitamin E plays an important role in nervous tissue (4, 5). It is known that vitamin E is very important for the maintenance of normal neurological structure and function in humans (5–7) as well as in experimental animals (8). The long delay in defining the role of vitamin E in human nutrition probably resulted from the difficulty in exhausting the stores of the vitamin.

Studies of vitamin E accumulation in the body, though numerous, have generally been restricted to one tissue or have considered only one or two points in time. Unfortunately, studies concerning the kinetics of tissue α-tocopherol depletion and repletion are less numerous, even in animals. Bieri (9) did not analyze nervous tissue, but found that rat liver, heart and plasma lost one half or more of their α-tocopherol within 1–2 wk when rats were fed a diet deficient in α-tocopherol. This occurred in both young and mature animals, but was more marked in growing rats because the increasing tissue mass further diluted the concentration. Machlin and Gabriel (10) found that the accumulation rate of tocopherol in adult rats fed high doses of α-tocopherol was highest in the liver and adipose tissue and lowest in muscle and the brain.

Using the same animals after they had accumulated vitamin E, Machlin and Gabriel (10) found that the rate of depletion of tocopherol from all tissues (except adipose tissue) was more rapid than the rate of accumulation. Gross-Sampson et al. (11) found that after an extended period of deficiency, neurological tissue retained a greater percentage of vitamin E than did other tissues. We found (12) that the brain is protected against vitamin E loss compared with other tissues, because in animals fed a deficient diet for 4 mo and born to mothers receiving a deficient diet 4 d before parturition, vitamin E content by weight was ~67% lower than that measured on the day of weaning, in spite of a sevenfold increase in body weight. Thus, this present work (using HPLC techniques) was performed to determine the kinetics of α-tocopherol depletion in several tissues at the same time, of adult rats fed a vitamin E–deficient diet.

[1]Supported by l'Institut National de la Santé et de la Recherche Médicale (INSERM), l'organisation Nationale Interprofessionnel des Oléagineux (ONIDOL) and le centre Technique Interprofessionnel des Oléagineux Métropolitains (CETIOM).

[2]To whom correspondence should be addressed.

0022-3166/91 $3.00 Received 18 June 1990. Accepted 17 January 1991.

TABLE 1

Composition of the purified diet deficient in vitamin E

Ingredient	Concentration
	g/100 g
Casein (delipided, vitamin-free)	20
Glucose	56
Starch	9.6
Hydrogenated lipids and glycerol	5
Linoleic acid, 18:2 (*n*-6)	1.2
Linolenic acid, 18:3 (*n*-3)	0.2
Mineral mixture[1]	7
Vitamin mixture[2]	1

[1]The mineral mixture had the following composition (mg/100 g mixture): P, 775; Ca, 1000; K, 600; Na, 400; Mg, 100; Mn, 8; Fe, 30; Cu, 1.25; Zn, 4.5; Co, 0.009; I, 0.049.

[2]The vitamin mixture had the following composition (mg/100 g mixture): thiamine, 2.0; riboflavine, 1.5; pyridoxine, 1.0; cyanocobalamin, 0.005; ascorbic acid, 80.0; menadione, 4.0; biotin, 0.03; folic acid, 0.5; *p*-aminobenzoic acid, 5.0; Ca pantothenate, 7.0; niacin, 10.0; choline chloride, 136.0; vitamin E (all-*rac*-α-tocopherol) <1.5 IU; vitamin A acetate 1980 IU; vitamin D (cholecalciferol) 250 IU.

MATERIALS AND METHODS

Male Sprague-Dawley rats bred in our laboratory were fed a standard diet from Iffa-Credo (L'Arbresle, France). Lipid, fatty acid and vitamin E content of the diet were described previously (12). Animals had free access to water and were maintained under standardized conditions of light (0700–1900 h), temperature (22 ± 1°C) and humidity (70%). When 60 d old, 42 animals were fed a purified diet deficient in vitamin E (U.A.R., Villemoisson, France) as previously described (12) (Table 1). At different times after being fed the deficient diet, the rats were fasted overnight and then killed. To eliminate blood contamination, animals were anesthetized with diethyl ether and were infused with ice-cold isotonic saline containing 10 mg/L heparin and 0.5% pyrogallol through the left ventricle of the heart. Six animals were used at each time point.

Vitamin E was measured on lyophilized tissue samples. In a preliminary experiment, we verified that lyophilization does not alter the vitamin E content of the tissue; using the experimental conditions described by Vatassery et al. (13), tocopherylquinone was not detected after tissue desiccation. The retention time of tocopherylquinone was determined using a standard synthesized according to Nair et al. (14). Vitamin E was determined by HPLC on lyophilized tissue stored at –30°C, according to Katsui's method (15) with slight modifications (16). All tissues were homogenized in an all-glass homogenizer with 1 mL of ice-cold water. The samples (0.8 mL of homogenate or 0.1 mL of serum + 0.7 mL of ice-cold water) were placed in a centrifuge tube with 1 mL of 6% pyrogallol in ethanol and 0.05 mL of 5–7-dimethyltocol (40 mg/L in ethanol) as an internal standard. Hexane (5 mL) was then added to the tube and the tube vigorously shaken. The mixture was centrifuged at 3000 × *g* for 10 min; the hexane layer was transferred to another test tube and evaporated, and the residue redissolved in methanol:water (95:5) and filtered.

For HPLC determination, a 12.5-cm lichosphere RP 18 column containing 4-μm particles was used (Merck Clevenot, Nogent Marne, France). The eluant [methanol:water (95:5)] was pumped at a rate of 1 mL/min with a 2150 LKB pump (LKB Instruments, Les Ullis, France). Vitamin E was detected by its fluorescence (excitation of 295 nm, emission 320 nm) using a Schoffel FS 970 fluorometer (Cunow, Clichy, France) to eliminate the UV-absorbing compounds that migrated close to vitamin E. The concentration of vitamin E was calculated with an integrator (Delsi, Argenteuil, France). Results are expressed as mg/L for serum and μg/g fresh weight for tissues except for sciatic nerve, which was expressed in μg/g dry weight (because endoneurium was separated from the whole sciatic nerve in saline under a dissecting microscope to avoid contamination by perineurium and fat cells).

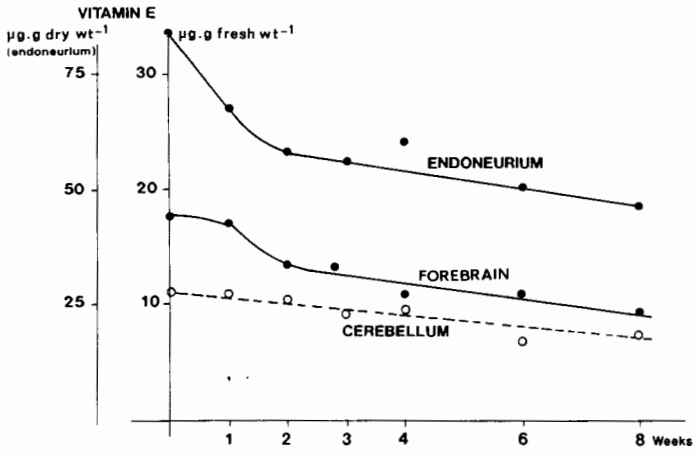

FIGURE 1 Kinetics of vitamin E depletion in forebrain, cerebellum and sciatic nerve endoneurium. Rats previously fed a standard diet were fed a purified vitamin E–deficient diet when 60 d old. Animals were killed at different times after receiving the deficient diet. Six animals were used at each time point. Standard deviations are not shown because each was <10% of the respective mean.

RESULTS AND DISCUSSION

Figure 1 shows that kinetics of the vitamin E depletion were dissimilar in the different parts of the nervous system. The tocopherol depletion rate was clearly biphasic for sciatic endoneurium, not biphasic for cerebellum, and poorly biphasic for forebrain. The

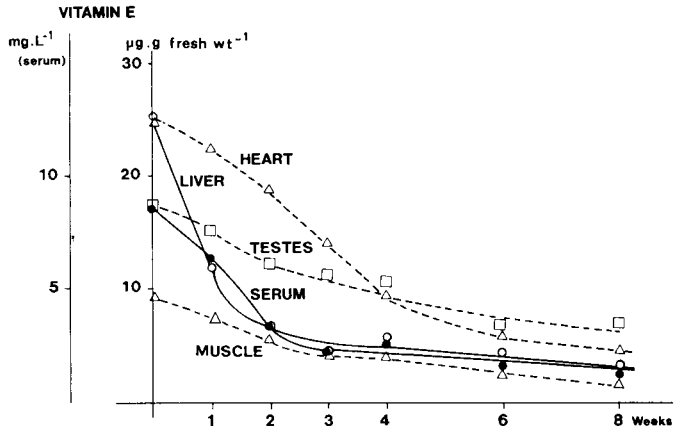

FIGURE 2 Kinetics of vitamin E depletion in various tissues. Rats previously fed a standard diet were fed a purified vitamin E–deficient diet when 60 d old. Animals were killed at different times after receiving the deficient diet. Six animals were used at each time point. Standard deviations are not shown because each was <10% of the respective mean.

results obtained in this study using adult animals after 8 wk of deficiency are at variance with disappearance rates found in young weanling animals by other authors: 40% in brain in adult rats vs. 70% in brain of young rats; 45% vs. 90% in nerve of young rats; 30% in adult rat cerebellum vs. ~50% in young rat cerebellum (11). Figure 2 shows the kinetics of vitamin E disappearance in various tissues. Liver showed a marked drop in the first week to about one-half the initial values. Serum also decreased markedly in 2 wk to one-half the initial concentrations. At the end of 8 wk of vitamin E deficiency, the concentrations of α-tocopherol in the liver and heart were similar but the depletion rate in the liver was greater than in the heart. Testis and muscle showed a very gradual decrease. Our values are in agreement with those of Bieri (9) and confirm that the depletion is more marked in the growing rat (9, 11). However, the quantities of vitamin E we found at all time points were somewhat higher in comparison with those of Bieri (9), possibly due to a higher content in the diet or to a different measurement technique. Interestingly, for all tissues examined, except the forebrain and the cerebellum, there were two phases of depletion: an initial rapid loss, followed by a second phase of slower and prolonged depletion. The first phase may correspond to a rapidly mobilized pool of labile vitamin E, and the second phase to a less labile pool, which we speculate might be bound to membranes and subcellular structures. Loss of this latter pool may be more critical and correspond to neurological defects. However, it is not known why nervous tissue seems to maintain a greater proportion of vitamin E in this pool than do other tissues.

In fact, turnover of vitamin E in the brain is very slow, as this tissue still retains 5% of the normal concentration after 52 wk deficiency (11). Although vitamin E is taken up by the brain of rats (17, 18) and mice (19), the mechanism and regulation of its transfer through the blood-brain barrier needs clarification. The half-life of vitamin E is higher in the nervous tissue compared with other tissues (20). This strongly suggests that neurological tissue preferentially conserves vitamin E and that its turnover may be reduced. It is difficult to determine in which structure of the sciatic nerve the labile pool is found. We have previously speculated (using peripheral nerve dysmyelinating trembler neurological mutants) that vitamin E could be a component of some nonmembrane material, such as the extracellular matrix or the basal lamina (21). The present study shows that a dramatic reduction of vitamin E in the serum corresponds to a dramatic decrease of this compound in various organs (with a lesser decrease in muscle, however, and no correspondence for nervous tissue). Thus, there is probably either a weak transfer of vitamin E between nervous tissue and intestitial fluid, or vitamin E is degraded more slowly in nervous tissue. In addition, vitamin E could be actively protected, and regenerated after oxidation, in agreement with our previous results (12) showing that the level of vitamin E in the brain is maintained for weeks at nearly the level found at birth in animals fed a deficient diet and born to mothers receiving a deficient diet 4 d before parturition.

Changes in vitamin E concentration were not due to differences in the polyunsaturated fatty acid levels in the commercial and the purified vitamin E–deficient diet, as these two diets contained similar quantities of linoleic and α-linolenic acid. It is known that increasing the amount of polyunsaturated fatty acids in the diet changes the requirement for and utilization of vitamin E (22–25); it also changes the ratio between vitamin E and polyunsaturated fatty acids (26, 27). The high ratio we found in sciatic nerve (16) in comparison with nonneurological tissues (26) further suggests that vitamin E may have an important structural role in addition to its antioxidant properties in peripheral nervous tissue. In fact, we found in the dysmyelinating neurological mutant that alteration of α-tocopherol content in the peripheral nervous tissue was not correlated with polyunsaturated fatty acid content (21). Moreover, in the developing and aging peripheral nervous system, vitamin E content is highly correlated only with total and specific (*n*-6) polyunsaturated fatty acids (16). There is no correlation between alteration of vitamin E content and myelination or polyunsaturated fatty acid content in the neurological dysmyelinating trembler mutant (21).

It would be interesting to measure other systems involved in free radical protection, as it has been shown that antioxidant enzymes in young animals do not compensate for the reduced concentrations of lipid soluble vitamin E (11, 12).

LITERATURE CITED

1. Burton, G. W., Joyce, A. & Ingold, K. U. (1983) Is vitamin E the only lipid-soluble, chain-breaking antioxidant in human blood plasma and erythrocyte membranes? Arch. Biochem. Biophys. 221: 281–290.
2. Diplock, A. T. (1983) The role of vitamin E in biological membranes. In: Biology of Vitamin E, Ciba Foundation, Symposium 101, pp. 45–55, Pitman, London, U.K.
3. Buttriss, J. L. & Diplock, A. T. (1988) The relationship between alpha-tocopherol and phospholipid fatty acids in rat liver subcellular membrane fractions. Biochim. Biophys. Acta 962: 81–90.
4. Sokol, R. J. (1988) Vitamin E deficiency and neurologic disease. Annu. Rev. Nutr. 8: 351–373.
5. Traber, M. G., Sokol, R. J., Ringel, S. P., Neville, H. E., Thellman, C. A. & Kayden, H. J. (1987) Lack of tocopherol in peripheral nerves of vitamin-deficient patients with peripheral neuropathy. N. Engl. J. Med. 317: 262–265.
6. Muller, D.P.R., Lloyd, J. K. & Wolff, O. H. (1983) Vitamin E and neurological function. Lancet 29: 225.
7. Satya-Murti, S., Howard, L., Krohel, G. & Wolf, B. (1986) The spectrum of neurologic disorder from vitamin E deficiency. Neurology 36: 917–921.
8. Towfighi, J. (1981) Effects of chronic vitamin E deficiency on the nervous system of the rat. Acta Neurpathol. 54: 261–267.
9. Bieri, J. G. (1972) Kinetics of tissue α-tocopherol depletion and repletion. Ann. N.Y. Acad. Sci. 203: 181–191.
10. Machlin, L. J. & Gabriel, E. (1982) Kinetics of tissue α-tocopherol uptake and depletion following administration of high levels of vitamin E. Ann. N.Y. Acad. Sci. 393: 48–59.
11. Gross-Sampson, M. A., MacEvilly, C. J. & Muller, D.P.R. (1988) Longitudinal studies of the neurobiology of vitamin E and other antioxidant systems, and neurological function in the vitamin E deficient rat. J. Neurol. Sci. 87: 25–35.
12. Chaudiére, J., Clément, M., Gérard, D. & Bourre, J. M. (1988) Brain alteration induced by vitamin E deficiency and intoxication with methylethyl ketone peroxide. Neurotoxicology (Little Rock) 9: 173–180.
13. Vatassery, G. T. & Smith, W. E. (1987) Determination of α tocopherol quinone (vitamin E quinone) in human serum, platelets and red cell membrane samples. Anal. Biochem. 167: 411–417.
14. Nair, P. P. & Machiz, J. (1967) Gas-liquid chromatography of isomeric methyltocols and their derivatives. Biochem. Biophys. Acta 144: 446–451.
15. Katsui, G. (1981) Assay methods of vitamin E: 4. High performance liquid chromatographic methods. Vitamins (Kyoto) 55: 267–273.
16. Clément, M. & Bourre, J. M. (1990) Alteration of alpha-tocopherol content in the developing and aging peripheral nervous system: persistence of high correlations with total and specific *n*-6 polyunsaturated fatty acids. J. Neurochem. 54: 2110–2117.
17. Vatassery, G. T., Angerhofer, C. K. & Knox, C. A. (1984) Effect of age on vitamin E concentrations in various regions of the brain and a few selected peripheral tissues of the rat and on the uptake of radioactive vitamin E by various regions of rat brain. J. Neurochem. 43: 409–412.
18. Vatassery, G. T., Angerhofer, C. K., Knox, C. A. & Deshmukh, D. S. (1984) Concentrations of vitamin E in various neuroanatomical regions and subcellular fractions, and the uptake of vitamin E by specific areas, of rat brain. Biochim. Biophys. Acta 792: 118–122.
19. Vatassery, G. T., Angerhofer, C. K. & Peterson, F. J. (1984) Vitamin E concentrations in the brains and some selected peripheral tissues of selenium-deficient mice. J. Neurochem. 42: 554–558.
20. Ingold, K. U. (1987) Biokinetics of and discrimination between dietary *RRR*- and *SRR*-α-tocopherol in the male rat. Lipids 22: 163–172.
21. Bourre, J. M., Clément, M. & Chaudière, J. (1987) Alteration of the α-tocopherol content in the brain and peripheral nervous tissue of dysmyelinating mutants. Neurochem. Pathol. 7: 91–97.
22. Bieri, J. G., Thorp, S. L. & Tolliver, T. J. (1978) Effect of dietary polyunsaturated fatty acids on tissue vitamin E status. J. Nutr. 108: 392–393.
23. Lehmann, J., Marshall, M. W., Slover, H. T. & Locono, J. M. (1977) Influence of dietary fat level and dietary tocopherols on plasma tocopherols of human subjects. J. Nutr. 107: 1006–1015.
24. Subramanian, C. S. & Mead, J. F. (1986) A relationship between essential fatty acid and vitamin E deficiency. Lipids 21: 603–607.
25. Meydani, S. N., Shapiro, A. C., Meydani, M., Macauley, J. B. & Blumberg, F. B. (1987) Effect of age dietary fat (fish, corn and coconut oils) on tocopherol status of C57BL/6Nia mice. Lipids 22: 345–350.
26. Poukka Evarts, R. & Bieri, J. G. (1974) Ratios of polyunsaturated fatty acids to alpha-tocopherol in tissues of rats fed corn or soybean oils. Lipids 9: 860–864.
27. Buckingham, K. W. (1985) Effect of dietary polyunsaturated/saturated fatty acid ratio and dietary vitamin E on lipid peroxidation in the rat. J. Nutr. 115: 1425–1435.