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Alteration of α-Tocopherol Content in the Developing and Aging Peripheral Nervous System: Persistence of High Correlations with Total and Specific (n-6) Polyunsaturated Fatty Acids

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Abstract: In contrast to brain, the sciatic nerve concentration of vitamin E in rats increased rapidly during the postnatal period (approximately fivefold between days 1 and 8), then decreased dramatically (about twofold between days 8 and 30), and further decreased slowly between days 30 and 60 and remained constant up to 2 years. Although the sciatic nerve concentration of vitamin E decreased by 58% between days 8 and 30, the concentration of vitamin E in serum presented a marked decrease (~75%). The vitamin E concentrations varied in a similar pattern in whole sciatic nerve and in endoneurium and showed a very close correlation (r = 0.94). The age-related changes in fatty acid concentration of the endoneurial fraction of the sciatic nerve were characterized by a large increase in content of saturated and monounsaturated fatty acids up to 6 months (twofold for saturated and fourfold for monounsaturated fatty acids). Then, up to 24 months, the amount of these fatty acids decreased very slowly. The content of (n-6) polyunsaturated fatty acids (PUFAs) decreased rapidly up to 1 year and slowly afterward. In contrast, during development the amount of (n-3) PUFA was relatively stable and decreased during aging. A highly significant correlation between vitamin E and (n-6) PUFA [18:2(n-6), 20:4(n-6), and total (n-6)] was observed but not between (n-3) PUFA and vitamin E. It is suggested that there may be a relationship between vitamin E and (n-6) PUFA in the PNS membranes during development and aging. **Key Words:** Vitamin E—Polyunsaturated fatty acids—PNS—Development—Aging. **Clément M. and Bourre J. M.** Alteration of α-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 (1990).

Under basal conditions, all aerobic cells form reactive oxygen species during respiration, phagocytosis, oxidative metabolism of arachidonic acid, aging, and xenobiotic metabolism. Each cell is protected by a complex network of antioxidant mechanisms, including enzymes such as superoxide dismutase, catalase, and glutathione peroxidase and scavengers of free radicals such as vitamin E, vitamin C, and thiol-containing compounds.

Vitamin E, as an integral part of membranes, is seen as a biological antioxidant, which, by sequestering free radicals, functions to terminate the propagation of autooxidation processes such as lipid peroxidation. In addition, specific effects of α-tocopherol that do not involve its antioxidant function and that act on the architecture of membranes by controlling the profiles of their unsaturated phospholipid and cholesterol components have been suggested (Diplock, 1983). Vitamin E stabilizes the membrane (Erin et al., 1986), changes the membrane order and fluidity (Patel and Edwards, 1988), regulates phospholipid metabolism (Cao et al., 1987) and phospholipase activity (Douglas et al., 1986), and controls some enzymatic activities (Kawai et al., 1974; Reddana et al., 1985).

In recent years, many authors have emphasized the role of free radicals as important causative agents in aging (Walton and Packer, 1980; Harman, 1981, 1984). Evidence for this is the presence of fluorescent pigments in the nerve cells. These pigments are formed from peroxidized lipids and proteins, and they accumulate in brain (specially in neurons) and other organs (Reichel, 1968; Brizzee et al., 1975).

It is known that vitamin E deficiency increases the production of fluorescent pigments (Moore and Wang,

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Abbreviation used: PUFA, polyunsaturated fatty acid.

1974; Noda et al., 1982), whereas a reduction in content of fluorescent pigments has been demonstrated in animals fed a vitamin E-supplemented diet (Reddy et al., 1973; Kruk and Enesco, 1981). Vitamin E plays an important role in neurological function (Muller et al., 1983; Satya-Murty et al., 1986; Sokol, 1988) and in the neurobiology of aging, possibly by protecting, among others, the polyunsaturated fatty acids (PUFAs) of the membranes against the deleterious effect of free radicals. It has been demonstrated that protection of membranes against free radicals by vitamin E is a function of its concentration in the membranes (Fukuzawa et al., 1985). This concentration varies according to the needs of the membranes for antioxidant protection, i.e., the most oxygenated tissues or the most unsaturated membranes contain the highest levels of vitamin E (Kornbrust and Mavis, 1980). Because brain contains large amounts of phospholipids rich in PUFAs sensitive to peroxidation, Vatassery et al. (1984) studied the concentration of vitamin E in various regions of the rat brain at 3, 14, and 30 months of age. They have shown that aging induces an increase of vitamin E concentrations in liver, adipose tissue, medulla, and spinal cord, whereas no significant changes are observed in other brain areas. This is in disagreement with early reports showing that in rat brain, vitamin E concentrations decrease slightly with aging (Weglicki et al., 1969).

In contrast to brain, the PNS has rarely been examined for protection against peroxidation. It has been shown that sciatic nerve exhibits enzymatic defense against peroxidation in dogs (Chvapil et al., 1982) as well as in mice (Cloëz et al., 1989) and contains measurable amounts of vitamin E (Vatassery et al., 1986; Gross-Sampson et al., 1988). It is interesting that in a neurological dysmyelinating mutant with an altered PNS, the trembler mouse, we found that enzymatic protection (Cloëz et al., 1989) and vitamin E levels (Bourre et al., 1987*a*) are altered.

Moreover, vitamin E changes related to (n-3) and (n-6) PUFAs were not parallel.

Thus, the aim of this study was to determine the age-related changes in vitamin E concentrations in relation to fatty acid composition of sciatic endoneurial tissue during the neonatal period and aging.

MATERIALS AND METHODS

For the experiments with growing rats, the animals used were unfasted Sprague–Dawley pups. Dams were purchased from Iffa Credo (l'Arbresle, France) and were fed a standard laboratory diet ad libitum containing 47 mg of vitamin E/kg, 50 μg of selenium/kg, and 5% lipids (UAR, France). The fatty acid composition of commercial chow was as follows: 16:0, 21%; 16:1, 1.5%; 18:0, 7.2%; 18:1(n-9), 28.6%; 18:1(n-7), 1.4%; 18:2(n-6), 37.6%; and 18:3(n-3), 2.7%. Aged rats were fed the same diet. To eliminate blood contamination, animals were anesthetized with diethyl ether and were infused with ice-cold isotonic saline containing 10 μg/ml of heparin and 0.5% pyrogallol through the left ventricle of the heart. The sciatic nerves were dissected out, and adhering connective tissues were removed. The samples were pooled and weighed. The endoneurial fraction was separated by microdissection of the sciatic nerve on a cold plate under a dissecting microscope. Endoneurial tissue and sciatic nerves were lyophilized and stored at −20°C until lipid analysis.

Analytical procedure for vitamin E

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 and Smith (1987), tocopheryl quinone was not detected after dessication of the tissue. The retention time of tocopheryl quinone was determined using a standard synthesized according to the technique of Nair and Machiz (1967).

Vitamin E content was determined by HPLC according to the method of Katsui (1981) with slight modifications. In brief, dry samples of sciatic nerves or endoneurial fractions were homogenized in an all-glass homogenizer with 1 ml of ice-cold water. The samples (0.8 ml of homogenate, 0.1 ml of serum, and 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 μg/ml in ethanol) as an internal standard. Then, 5 ml of hexane was added to the tube, which was vigorously shaken. The mixture was centrifuged at 3,000 *g* for 10 min, the hexane layer was evaporated off, and the residue was redissolved in methanol/water (95:5 vol/vol) and filtered.

For HPLC determination, a 12.5-cm-long Lichosphere RP 18 column containing particles 4 μm in diameter was used (Merck Clevenot, France). The eluant, methanol/water (95:5 vol/vol), was pumped at a rate of 1 ml/min with an LKB 2150 pump. Vitamin E was detected by its fluorescence (excitation of vitamin, 295 nm; emission, 320 nm) using a Schoffel FS 970 fluorometer to eliminate the UV-absorbing compounds that migrated close to vitamin E. The concentration of vitamin E was calculated with an integrator (Delsi, France). Results were expressed as micrograms per gram fresh weight, microgram per milligram of protein, or microgram per milligram of total lipid fatty acid. Protein contents were determined according to the technique of Lowry et al. (1951). The reproducibility of the α-tocopherol assay was 6%, and the day-to-day accuracy was 6.4%. The smallest amount of α-tocopherol that could be detected was <30 ng/injected sample.

Quantification of total fatty acids

The endoneurial concentrations of total fatty acids were measured according to the method of Lepage and Roy (1986), providing direct transmethylation and thus fatty acid methyl esters without extraction of lipids. Endoneurial tissue homogenate (0.1 ml, corresponding to 0.1–0.2 mg of proteins) was introduced into a test tube containing 2 ml of methanol/benzene (4:1 vol/vol) and 10 or 50 μg of heptadecanoic acid as the internal standard. Then, 0.2 ml of acetyl chloride was carefully added. The tube was filled with nitrogen gas, sealed with a Teflon-lined cap, and heated for 1 h in a boiling water bath. After cooling, 5 ml of 6% potassium carbonate was added. The tube was centrifuged for 10 min at 3,000 *g* and the upper phase, containing the fatty acid methyl esters, was collected. The methyl esters were analyzed by GLC on a silica capillary column in a Girdel 3 300 chromatograph with flame ionization detection (length, 50 m; internal diameter, 0.32 mm; stationary phase, Carbowax 20M), with helium as the carrier gas, and the following temperature of injector and detector: 250°C oven, from 200 to 235°C at 2°C/min after

10 min of isotherm. Identification of fatty acids was performed with commercial standards.

Areas were calculated with an integrator (Delsi, France), and fatty acid concentrations were expressed as micrograms per 100 μg of fatty acids or micrograms per milligram of proteins. Results are presented as mean ± SD values. Statistical analysis was performed using Student's t test. The accuracy of the total fatty acid content determination was estimated to be ≤2% for the major fatty acids and ≤4% for the minor fatty acids. The smallest amount of fatty acid that could be detected was <1 ng/injected sample.

RESULTS

Figure 1 shows the time course of the concentration of α-tocopherol in the rat sciatic nerve and in serum. During the first 8 days of life, there was a rapid increase in the level of α-tocopherol in the nerve: The content of vitamin E increased approximately fivefold during this period. Then, there was a marked decrease up to 1 month (approximately twofold), followed by a smaller decrease. From 2 months, the concentration of vitamin E in rat nerve remained approximately constant up to 2 years. The serum levels of α-tocopherol decreased markedly by ~75% between day 8 and 1 month. After a small rise between 1 and 2 months, no marked changes were observed up to 2 years.

The relative fatty acid composition (expressed as a percentage of total fatty acid) of total endoneurial lipids is shown in Table 1. The age-related changes in saturated fatty acid composition was characterized by a marked decrease in 16:0 and 18:0 species up to 6 months; then, between 6 and 24 months the percentage of these fatty acids remained quite stable. This decrease was compensated for by a significant rise in 20:0, 22:0, and 24:0 species. During development and aging, the saturated fatty acids represent nearly 50% of the total endoneurial fatty acid.

In contrast to saturated fatty acids, the monounsaturated fatty acids increased considerably in content during development. After 6 months, the amount of monounsaturated fatty acid was approximately constant. It is interesting to note that 18:1(n-9) and 24:1(n-9) accounted for ~80–90% of the total monounsaturated fatty acids.

The percentage of PUFA decreased rapidly during development and adulthood. After 6 months, no marked changes were observed in the percentage of PUFA.

Table 2 lists the changes in the α-tocopherol concentration in the endoneurial fraction of sciatic nerve and shows development and aging changes of the endoneurial concentration of (n-6) and (n-3) PUFAs per milligram of protein. The general pattern observed in the α-tocopherol concentration in endoneurium (data not shown) was basically the same as that seen in the whole sciatic nerve (Fig. 1).

The amount of vitamin E related to total lipid fatty acid decreased dramatically up to 6 months and remained nearly stable afterward.

As shown in Table 2, up to 1 month the content of 18:2(n-6) and 22:4(n-6) decreased, respectively, by 55 and 25%. During the same period, that of 20:4(n-6) remained approximately constant, whereas, after a small rise by day 14, that of 20:3(n-6) returned to normal values. After 1 month, there was a slow decrease in concentration of each (n-6) fatty acid. During the first month of life, there was a small rise in 22:6(n-3) content, followed by a gradual decrease up to 2 years. The amounts of 22:5(n-3) remained approximately constant for 2 years.

The present study shows a highly significant correlation between α-tocopherol content and (n-6) fatty acid concentration in the endoneurial fraction of sciatic nerve both during development (up to 1 month) and during development and aging (up to 2 years) (Fig. 2). The values obtained for the correlation coefficient were 0.73 for all (n-6) PUFAs up to 1 month. Over a 2-year period, the correlation coefficients were, respectively, 0.84, 0.83, and 0.90 for 18:2(n-6), 20:4(n-6), and all (n-6) PUFAs ($p < 0.01$).

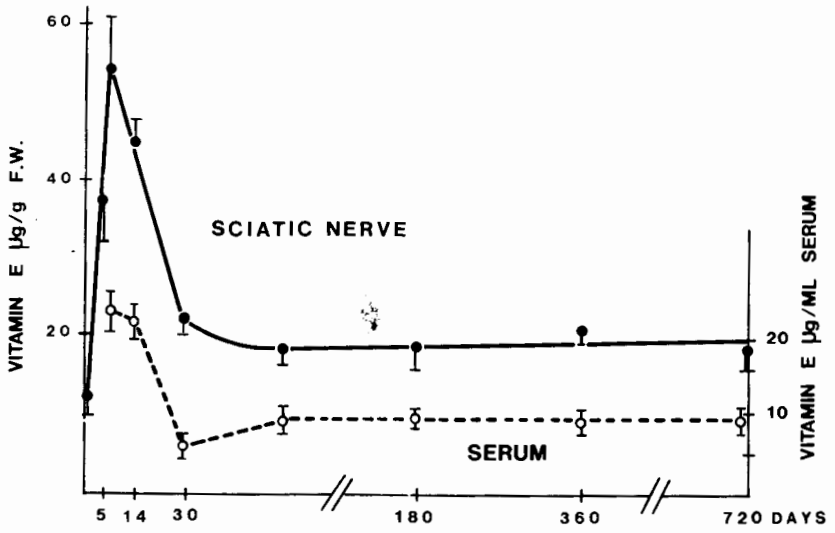

FIG. 1. Alteration in α-tocopherol concentration during development and aging in sciatic nerve and serum. F.W., fresh weight.

TABLE 1. *Total fatty acid percent distribution (mg%) of rat sciatic endoneurium*

	8 days (n = 4)	14 days (n = 6)	1 month (n = 8)	6 months (n = 8)	12 months (n = 5)	24 months (n = 6)
Saturated						
14:0	1.3 ± 0.2[a]	1.4 ± 0.5[a]	0.5 ± 0.1	0.4 ± 0.04	0.4 ± 0.05	0.3 ± 0.1
16:0	25.4 ± 3.3[a]	24.2 ± 2.0[a]	18.2 ± 0.9	14.2 ± 0.7[a]	13.4 ± 0.4[a]	14.2 ± 0.7[a]
18:0	16.2 ± 1.8[a]	14.2 ± 0.4[a]	11.9 ± 0.2	10.3 ± 0.3[a]	9.5 ± 0.7[a]	10.6 ± 0.2[a]
20:0	1.3 ± 0.1[a]	1.4 ± 0.1[a]	1.9 ± 0.05	2.4 ± 0.1[a]	2.3 ± 0.1[a]	1.7 ± 0.1[a]
22:0	1.6 ± 0.2[a]	3.0 ± 0.3[a]	5.2 ± 0.2	8.8 ± 0.3[a]	8.6 ± 0.3[a]	7.9 ± 0.5[a]
24:0	3.0 ± 0.4[a]	4.1 ± 0.6[a]	5.9 ± 0.3	9.9 ± 0.8[a]	11.6 ± 0.2[a]	13.9 ± 1.1[a]
Total	48.8	48.3	43.6	46.0	45.8	48.8
Monounsaturated						
16:1 (n-9)	0.7 ± 0.1	1.2 ± 0.4	0.9 ± 0.1	0.7 ± 0.1	0.6 ± 0.1[a]	0.3 ± 0.04[a]
16:1 (n-7)	1.2 ± 0.1	1.0 ± 0.1	0.8 ± 0.1	0.8 ± 0.04	0.9 ± 0.1	0.8 ± 0.1
18:1 (n-9)	20.8 ± 4.1[a]	24.5 ± 0.9[a]	33.7 ± 0.6	38.0 ± 1.5[a]	38.9 ± 0.6[a]	34.3 ± 0.8
18:1 (n-7)	2.1 ± 0.3	1.3 ± 0.1[a]	1.9 ± 0.05	0.6 ± 0.1[a]	0.5 ± 0.1[a]	0.6 ± 0.1[a]
20:1 (n-9)	0.3 ± 0.08	0.5 ± 0.06	0.6 ± 0.08	0.7 ± 0.01	0.5 ± 0.1	0.3 ± 0.03[a]
22:1 (n-9)	0.8 ± 0.2	0.4 ± 0.06[a]	0.9 ± 0.1	0.5 ± 0.06[a]	0.4 ± 0.03[a]	0.5 ± 0.05[a]
24:1 (n-9)	2.8 ± 0.6[a]	5.1 ± 0.5[a]	6.5 ± 0.5	6.3 ± 0.2	6.7 ± 0.4	7.7 ± 0.4
Total	28.7	34.0	45.3	47.6	48.5	45.3
Polyunsaturated						
18:2 (n-6)	3.5 ± 0.4[a]	2.4 ± 0.2[a]	0.9 ± 0.08	0.7 ± 0.07	0.6 ± 0.1	0.9 ± 0.1
18:3 (n-6)	0.2 ± 0.03	0.2 ± 0.03	—	—	—	—
20:3 (n-6)	1.8 ± 0.2[a]	2.0 ± 0.1[a]	1.2 ± 0.02	0.6 ± 0.06[a]	0.4 ± 0.1	0.5 ± 0.05[a]
20:4 (n-6)	9.8 ± 1.0	7.7 ± 0.3	5.2 ± 0.4	3.3 ± 0.2	3.0 ± 0.1	3.3 ± 0.2[a]
22:4 (n-6)	3.2 ± 0.7	2.0 ± 0.1	1.4 ± 0.1	0.5 ± 0.05	0.5 ± 0.1	0.4 ± 0.1[a]
22:5 (n-6)	0.4 ± 0.1	—	—	—	—	—
Total	19.0	14.30	8.70	5.10	4.5	5.1
18:3 (n-3)	0.8 ± 0.3	0.4 ± 0.1	—	—	—	—
22:5 (n-3)	0.6 ± 0.2	0.9 ± 0.2	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.03	0.6 ± 0.1
22:6 (n-3)	2.2 ± 0.3[a]	1.9 ± 0.1	1.7 ± 0.1	0.7 ± 0.05[a]	0.6 ± 0.07[a]	0.6 ± 0.07
Total	3.6	3.2	2.2	1.1	1.0	1.2
(n-3)/(n-6)	0.18	0.22	0.25	0.21	0.22	0.23

Data are mean ± SD values from the indicated number of measurements (n). Three, two, one, one, one, and one animals were used for each measurement at 8 and 14 days and 1, 6, 12, and 24 months, respectively.

[a] Significantly different from 1-month-old animals ($p < 0.01$).

TABLE 2. *Age-related changes in vitamin E and fatty acid content of endoneurium*

	8 days (n = 4)	14 days (n = 6)	1 month (n = 8)	6 months (n = 8)	12 months (n = 5)	24 months (n = 6)
Vitamin E						
μg/mg of protein	0.455 ± 0.029[a]	0.439 ± 0.080[a]	0.306 ± 0.038	0.178 ± 0.013[a]	0.194 ± 0.017[a]	0.195 ± 0.032[a]
μg/mg of total lipid fatty acid	1.17 ± 0.15[a]	0.89 ± 0.13[a]	0.47 ± 0.06	0.21 ± 0.01[a]	0.25 ± 0.03[a]	0.27 ± 0.04[a]
PUFAs (μg/mg of protein)						
(n-6)						
18:2	13.6 ± 1.6[a]	11.6 ± 1.7[a]	6.1 ± 0.8	5.7 ± 0.6	5.10 ± 0.7	6.5 ± 0.0
18:3	0.8 ± 0.1	1.0 ± 0.2	—	—	—	—
20:3	7.1 ± 0.9	9.6 ± 1.3	8.3 ± 0.7	5.0 ± 0.5[a]	3.5 ± 0.5[a]	3.4 ± 0.3[a]
20:4	38.3 ± 3.9	37.2 ± 4.5	36.1 ± 3.7	26.6 ± 1.9[a]	22.6 ± 2.1[a]	22.1 ± 1.2[a]
22:4	12.4 ± 2.6	9.6 ± 0.9	9.2 ± 1.8	4.5 ± 0.8[a]	4.1 ± 0.7[a]	2.8 ± 0.6[a]
22:5	1.4 ± 0.4	—	—	—	—	—
Σ(n-6)	73.8 ± 7.4	69.2 ± 7.8	55.0 ± 5.7	42.0 ± 3.2	35.0 ± 3.5	35.0 ± 3.2
(n-3)						
18:3	3.3 ± 1.1	2.0 ± 0.5	—	—	—	—
22:5	2.3 ± 0.8	3.9 ± 0.7	3.5 ± 0.8	3.5 ± 0.7	3.6 ± 0.4	4.3 ± 0.9
22:6	8.6 ± 1.4[a]	9.4 ± 1.3[a]	12.0 ± 0.7	5.6 ± 0.9[a]	4.7 ± 0.6[a]	4.1 ± 0.4[a]
Σ(n-3)	14.2 ± 1.2	14.5 ± 1.8	14.9 ± 1.3	8.6 ± 1.8	6.9 ± 1.0	8.3 ± 1.1
Monounsaturated	104 ± 13[a]	163 ± 17[a]	288 ± 16	385 ± 31[a]	326 ± 6[a]	295 ± 11
Saturated	184 ± 23[a]	233 ± 15[a]	294 ± 26	375 ± 18[a]	333 ± 22	325 ± 17

Data are mean ± SD values from the indicated number of measurements (n). Three, two, one, one, one, and one animal were used for each measurement at 8 and 14 days and 1, 6, 12, and 24 months, respectively.

[a] Significantly different from 1-month-old animals ($p < 0.01$).

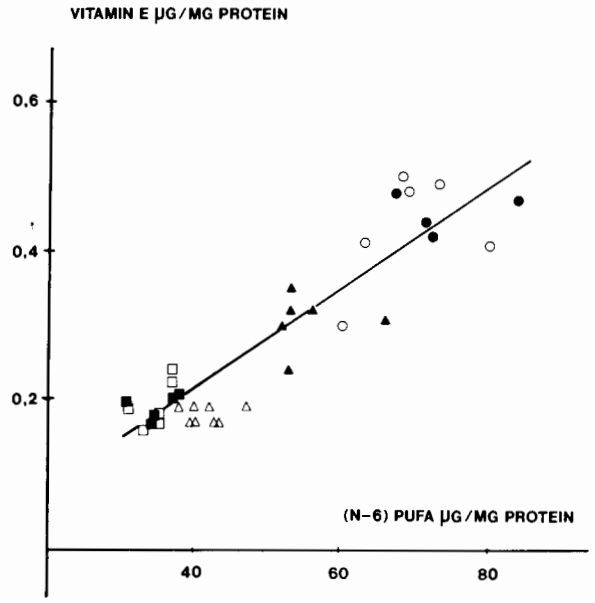

FIG. 2. Correlation between α-tocopherol and (n-6) PUFA content during development and aging: 8 days (●), 14 days (○), 1 month (▲), 6 months (△), 12 months (■), and 24 months (□).

In contrast, the correlation between total (n-3) PUFAs and vitamin E content of the endoneurial fraction of sciatic nerve was not significant during the first month of life (0.08) or between 1 month and 2 years (0.004).

DISCUSSION

It is commonly accepted that the principal function of vitamin E is to scavenge free radical species that arise during peroxidation of membrane PUFAs. To determine the role of vitamin E in aging, Vatassery et al. (1984) have provided some information on the concentration of vitamin E in different CNS and other tissues, but to our knowledge a time course of distribution of vitamin E in the sciatic nerve has not been previously determined during development and aging in animals fed a nondeficient diet. Recently, to study the role of cerebral antioxidant systems other than vitamin E during cerebral oxidative stress, we established the time course of alteration of vitamin E concentration in the brains of young rats fed a vitamin E-deficient diet (Chaudière et al., 1988). From these results, it appears that the brain is protected compared with other tissues, because it maintains its initial vitamin E concentration, and that antioxidant enzyme systems do not compensate for the lack of increase in concentration of vitamin E. Similar results have been found in the PNS (Gross-Sampson et al., 1988). Levels of vitamin E in rat tissues such as liver, heart, and adrenal glands have been shown to increase with age (Weglicki et al., 1969). Moreover, the level of vitamin E in the CNS has been a much debated question. Depending on the cerebral area, age of animals, and level of vitamin E in the diet, aging induces either no change or a decrease in concentration of vitamin E in the brain (Weglicki et al., 1969; Vatassery et al., 1984; Meydani et al., 1986). The present study shows, for the first time, the alteration in the distribution of vitamin E in rat sciatic nerve during development and aging.

Comparison of the time course of vitamin E levels in brain and sciatic nerve of suckling rats showed that they were different and that vitamin E concentrations in sciatic nerve were always higher than in brain.

During the first week of life, the vitamin E level in brain increased from 3.3 ± 0.6 to 5.8 ± 0.6 μg fresh weight (Chaudière et al., 1988), whereas the vitamin E level in sciatic nerve increased sharply from 12.1 ± 2.6 to 54.4 ± 7.5 μg/g fresh weight. These results indicate that during this period the uptake of vitamin E by sciatic nerve was much more rapid than by the brain. It is tempting to speculate that the increase in vitamin E concentration in sciatic nerve at the peak of myelin formation could be closely related to the protection of newly synthesized PUFAs in Schwann cell membranes. In agreement with this proposal, Dinesen and Clausen (1976) have shown that vitamin E deficiency lowers the amount of myelin in the CNS. More recently, Ogunleye and Odutuga (1986) have reported a pronounced reduction in content of myelin lipids, cerebrosides, and sphingomyelin in rats fed a vitamin E-deficient diet. These findings support the hypothesis of a possible relationship between the increase in vitamin E concentration and the synthesis of myelin in sciatic nerve.

The results of the present study also show that the concentration of vitamin E remains approximately constant from 2 months to 2 years. Vatassery et al. (1984) have reported similar results only for a few brain areas during aging. These data lead us to conclude that CNS and PNS vitamin E concentrations are not affected by aging, in contrast to certain tissues such as liver and adipose tissue. The serum vitamin E concentrations reported here are in agreement with other results (Yoshioka et al., 1987). It has been shown in humans that the vitamin E level is significantly higher in early milk than in mature milk (Jansson et al., 1981). It can be expected that in rats the same phenomenon occurs and may explain the high levels of vitamin E in serum and therefore the transient increase in vitamin E levels in sciatic nerve. It is widely accepted that vitamin E serves as an antioxidant protecting PUFAs against deleterious effects of free radicals. Therefore, it seemed useful to quantify its presence in tissues as a function of PUFA content. We chose to determine the total fatty acid composition of endoneurium for several reasons: (a) Possible contamination by fat cells could be eliminated, (b) differences exist in fatty acid composition between endoneurium and perineurium in rat sciatic nerve (Fressinaud et al., 1986), and (c) analysis of endoneurium is an efficient approach to myelin lipids.

Although the fatty acid profiles were obtained in the endoneurium without lipid extraction, our results are in agreement with those of Fressinaud et al. (1986) for adult rat except for 24:0 and 24:1. It is curious that these authors found low values for 24:0 and trace amounts for 24:1 in total fatty acids of endoneurium, which is rich in myelin.

In addition, results observed with young rats (14 days old) were quite similar to our previously published results (Bourre et al., 1987*b*). Fatty acid composition of peripheral nerve myelin has been studied by Yao (1982) in developing and regenerating rat sciatic nerve, by measuring the fatty acid composition of endoneurial phosphatidylethanolamine. This study showed that development is characterized by a marked increase in content of monounsaturated fatty acids and a significant decrease in content of saturated fatty acids and PUFAs. Similar results were obtained on whole endoneural sciatic nerve except for saturated fatty acid. During development, the level of saturated fatty acid as a percentage of total fatty acid remained constant (~50%), although the amount of saturated fatty acids per milligram of protein increased up to 6 months. This increase was due mainly to 22:0 and 24:0, which represent >37% of total fatty acids in sphingolipids (Yao et al., 1981).

In brief, in agreement with others, the major changes in sciatic nerve fatty acids up to 1 month were as follows: reduction of 16:0 and 18:0 content, increase of 22:0, 24:0, 18:1, 22:1, and 24:1 content, and reduction of 20:4(n-6), 22:4(n-6), and 22:6(n-3) content. The increased amount of saturated and monounsaturated very-long-chain fatty acids is probably due to the synthesis of myelin. Moreover, the main alterations during aging are an increased amount of 24:0 and 24:1, probably related to continuous enrichment of the nerve in myelin. The amount of 20:3(n-6) expressed as a percentage of total fatty acids or per milligram of protein decreased during aging; this could be related to reduced Δ^6-desaturase. It is unlikely that the presence of 22:5(n-3) and 22:6(n-3) in the PNS originates from the diet; more probably, 18:3(n-3) is immediately converted into more elongated and desaturated (n-3) derivatives. The essential quality of α-linolenic acid is linked to its role as precursor, with no tissue deposition; its presence in sciatic nerve from young animals could be due to an increased uptake by nervous tissue during development. In agreement with this hypothesis, 18:3(n-6) was only detected in young animals, and 20:4(n-6) and 22:4(n-6) were reduced in content after 1 month. The presence of 22:5(n-6) in 8-day-old sciatic nerve suggests that the animals received a diet rich in 18:2(n-6) or deficient in α-linolenic acid at this age.

It is interesting that it has been shown in human milk that the most abundant essential fatty acid is linoleic acid [18:2(n-6)] (Jansson et al., 1981).

Data in humans (Jansson et al., 1981; Harzer and Haug, 1985; Jaffar et al., 1986) show that α-tocopherol milk content is stabilized from day 8 (after a dramatic fourfold decrease). This value has not been determined in the rat, but it probably follows the same pattern from day 2. Thus, decreased amounts of vitamin E in plasma are probably not related to vitamin E content in milk. Jansson et al. (1981) have shown that there is a significant correlation between the vitamin E content and the linoleic acid [18:2(n-6)] content of human milk. It seems probable that a similar correlation occurs in rat milk and may explain the significant correlation that we found in sciatic nerve during development.

As the requirement for vitamin E in tissues is related to the PUFA content of the membranes, Poukka Evarts and Bieri (1974) determined the ratio of PUFAs to α-tocopherol for several tissues but not for nervous tissues. They reported values between 810:1 for heart to 2,620:1 for liver. The PNS PUFA/α-tocopherol ratios calculated in this study ranged from 270:1 to 400:1 at the different ages and are lower than those reported by Poukka Evarts and Bieri (1974) for lung and heart. These latter tissues contain high levels of PUFAs and are more susceptible to peroxidative attack than PNS membranes.

The relatively low concentration of PUFAs in PNS membranes suggests that vitamin E may have a structural role besides its antioxidant properties.

Attempts to correlate the changing levels of PUFAs in endoneurial sciatic nerve with vitamin E concentration showed an apparently close relationship with (n-6) PUFAs but not with (n-3) PUFAs during development. It is interesting that Diplock (1983) has hypothesized a specific interaction of vitamin E and arachidonyl residues and suggested specific localization of tocopherol in membranes particularly liable to attack (Buttriss and Diplock, 1988*a*). In this respect, the very high ratio of (n-6) PUFA to vitamin E in PNS suggests an important structural role.

It is known that the activity of vitamin E depends on the bioavailability of selenium; moreover, there is a large increase in the vitamin E content in liver organelles of rats deprived of selenium but given adequate amounts of vitamin E, a finding suggesting an increased uptake or mobilization of vitamine E to compensate for the detrimental effects of selenium deficiency (Buttriss and Diplock, 1988*b*). On the contrary, in mice maintained on a selenium-deficient diet but well balanced in vitamin E, it was found that selenium deficiency did not affect significantly the brain vitamin E content (Vatassery et al., 1984).

In addition, Meydani et al. (1986) have shown that the increase of brain vitamin E content induced by vitamin E supplementation was reduced by a selenium-deficient diet in young and old rats. These results suggest that selenium may be partly responsible for maintaining the vitamin E concentration of the brain.

Thus, it would be interesting to measure the concentrations of vitamin E and selenium in both serum and the PNS during aging, because it has been shown that brain selenium concentrations decrease by 48% between 2 months and 2 years of age (Lai et al., 1985).

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