Vitamin E Nutrition in the Rhesus Monkey (36632)

J. G. Bieri and R. H. Poukka Evarts

Laboratory of Nutrition and Endocrinology, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland 20014

Experimental vitamin E deficiency in the rhesus monkey (*Macaca mulatta*) has been reported (1, 2) but only limited information is available on the dietary requirement for this vitamin (3). The National Research Council bulletin on nutritional requirements of monkeys (4) gives a range of 0.33–0.83 mg α-tocopherol/g of dietary polyunsaturated fatty acids, based on the study of Fitch and Dinning (3) and the human study by Horwitt (5). Since the only estimation with monkeys (3) was made from a curative experiment in which the diet contained 3% cod liver oil and 8% lard, it appeared desirable to reassess the requirement with a diet lower in total fat and polyunsaturated fatty acids. In the present study, we report (a) the early changes in plasma α-tocopherol when the vitamin is removed from the diet; (b) the amount of dietary α-tocopherol required to restore plasma concentration in partially depleted animals; and (c) the apparent intestinal absorption of α-tocopherol.

Experimental Methods. Two female monkeys when received from the importer were fed restricted amounts of a canned, commercial primate diet[1] for 3 weeks and then given for 8 weeks a semipurified type diet containing 59 mg (80 IU) of *d*-α-tocopheryl acetate/kg. This latter diet was similar to the experimental diet described below but was orange flavored and did not contain agar. During this preliminary period the monkeys were permitted to gain weight slowly. At the start of the experimental period weights were 4.5 kg (no. I) and 4.0 kg (no. II). Weights were then kept constant by feeding 170 g of the wet diet daily, half in the morning and half in the afternoon.

The dry portion of the vitamin E-deficient diet contained (%): vitamin-free casein, 18; stripped lard,[2] 2; stripped corn oil,[2] 2; Fox-Briggs salt mixture, 4; cellulose, 12; vitamin mixture[3] without vitamin E, 2; sucrose, 20; dextrin, 40; ascorbic acid, 0.1. To this dry mixture was added an equal weight of boiling 2% agar solution containing 1.2% cocoa[4] for flavor. The cold, solid diet was fed in 2–3 cm cubes and was consumed without wastage.

Supplements of *d*-α-tocopherol[2] in ethanol were added while mixing the dry diet. For the absorption study, small batches of diet were prepared containing cold *d*-α-tocopherol and *d*-[5-^{14}C]-α-tocopherol[5] (sp act = 2 μCi/mg) previously purified by thin layer chromatography. Weighed portions (5–10 g) of this labeled diet were fed to the monkeys in the morning, followed by their regular ration of deficient diet.

The monkeys were fasted overnight and heparinized blood samples were taken from a femoral vein while the animals were immobilized with Sernylin.[6] One milliliter of plasma was mixed with 2 ml redistilled absolute ethanol and extracted twice with 3 ml

[1] Science Diet, Primate; Institutional Products, Division of Hill Packing Co., Topeka, KS.

[2] Eastman Organic Chemicals, Rochester, NY. An antioxidant, BHT, was added to the fats at 0.02%.

[3] The vitamin mixture provided the following per kg diet: thiamine HCl, 15 mg; riboflavin, 15 mg; pyridoxine HCl, 15 mg; calcium pantothenate, 45 mg; niacin, 50 mg; choline chloride, 1 g; folic acid, 2 mg; biotin, 1 mg; menadione, 1 mg; vitamin B_{12}, 30 μg; vitamin A palmitate, 4 mg; vitamin D, 75 μg. Selenium, 0.05 mg/kg diet, was added as Na_2SeO_3.

[4] Hershey's Cocoa, Hershey Company, Hershey, PA.

[5] Prepared by hydrolysis from *d*-[5-^{14}C]-α-tocopheryl succinate, Distillation Products Industries, Rochester, NY.

[6] Bioceutic Labs, St. Joseph, MO.

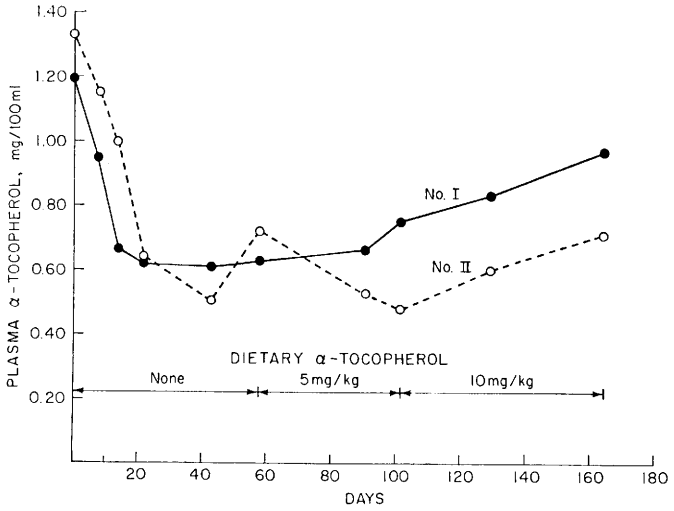

FIG. 1. Plasma α-tocopherol concentration of monkeys depleted of vitamin E for 60 days and then repleted as indicated.

hexane. The extract was evaporated, the lipid was dissolved in 0.1 ml chloroform and the α-tocopherol was determined colorimetrically with $FeCl_3$-bathophenanthroline, using a 1-ml Beckman cuvette (6). Since the diet was free of carotenoids, no correction for these pigments was necessary. Analyses in which the α-tocopherol was first separated by TLC (6) gave values 20% lower than the direct method. Most of this difference was due to loss of α-tocopherol during TLC. Plasma total lipids were determined by the method of Bragdon (7).

Feces were collected each morning and afternoon, combined and refrigerated. One day's collection (30–60 g) was homogenized in a Virtis apparatus with 250 ml acetone, followed by refluxing for 1 hr. The suspension was transferred to a graduated cylinder and the volume made to 300 ml. After the solids settled, a 50 ml aliquot of the acetone was added to 50 ml water and extracted three times with hexane. The hexane extracts were evaporated in a counting vial, Liquifluor[7] was added and the samples were counted in a Packard Model 3003 scintillation counter. Corrections for quenching were made by addition of an internal standard. Recovery of radioactive α-tocopherol added to fecal samples was > 98%.

[7] New England Nuclear, Boston, MA.

Results. Changes in plasma concentration of α-tocopherol when the monkeys were fed the deficient diet and then resupplemented are shown in Fig. 1. At the start of the depletion period, plasma vitamin E levels were high, 1.19 and 1.32 mg/100 ml. (Two monkeys fed commercial stock pellets for 6 months had values of 0.53 and 0.73 mg/100 ml.) There was an immediate, marked decline in concentration upon withdrawal of the vitamin and by 21 days the levels were about one-half those initially (0.61 and 0.62 mg/100 ml). This concentration remained stationary in monkey no. I for 40 days when resupplementation began. In monkey no. II, a further decrease to 0.51 mg/100 ml in the next 20 days was followed by a rebound to 0.72 mg/100 ml for unknown reasons.

The addition of *d*-α-tocopherol (5 mg/kg diet) produced after 40 days a slight elevation to 0.75 mg/100 ml in monkey no. I, but in no. II the concentration (0.48 mg/100 ml) remained at about the same level as existed 2 weeks prior to supplementation (0.51 mg/100 ml). When the supplement was increased to 10 mg/kg for 60 days the concentrations in both monkeys rose steadily, one to 0.71 and the other to 0.96 mg/100 ml. Occasional determinations of plasma total lipids during the repletion period showed that monkey no. I, which had the higher

TABLE I. Fecal Excretion of *d*-α-Tocopherol by Monkeys.

Monkey no.	Dose of α-tocopherol[a] (mg)	Percentage of dose excreted				
		Day 1	Day 2	Day 3	Day 4	Total
I	0.2	27.3	8.2	8.2	1.0	43.7
	0.2	26.6	12.4	3.7	1.0	42.7
II	0.2	14.4	22.3	4.2	1.0	40.9
	1.0	1.0	37.3	11.68	1.91	50.8

[a] Each dose contained ca. 8000 cpm of *d*-[5-^{14}C]-α-tocopherol. Experiments were made at 7-day intervals.

α-tocopherol level, consistently had higher total lipids (3.3 vs 2.8 mg/ml, respectively, at 130 days).

The fecal excretion of a radioactively labeled dose of α-tocopherol is shown in Table I. With a very low dose, 0.2 mg, about 42 to 44% was excreted, while with a larger dose of 1.0 mg (equiv to 10 mg/kg diet) only a slightly greater amount was excreted (51%).

Discussion. The very rapid fall in plasma α-tocopherol concentration in the first 7 to 14 days after the vitamin was removed from the diet was unexpected. The past dietary history of these monkeys made it certain that they were well fortified with the vitamin, and the high initial blood concentrations confirmed this. Fitch and Dinning (3) determined plasma levels periodically in monkeys during depletion but did not give values before 50 days. They noted, as did we, that after the initial decline there was a plateau for a considerable period. In the study by Horwitt (8) with men, a rapid initial decline in plasma α-tocopherol was not noted, but a second depletion after long repletion gave a marked drop in several subjects in only 8 days. In rats well nourished with vitamin E, a marked initial plasma decrease followed by a slower rate of loss was found (9).

Depletion studies with rats have shown that most of the tissue α-tocopherol is labile and disappears within a few weeks, with adipose tissue being depleted the slowest (9). In the present study, during the 60-day depletion period labile reserves of all tissues would very probably have been lost. The supplement of 5 mg/kg diet for 40 days was sufficient to stabilize the plasma level in one monkey (no. II) and to give a slight elevation in the other. Whether this supplement would have produced an eventual rise in both monkeys if continued longer can only be speculated upon. The 10 mg/kg supplement, however, gave a progressive increase in both animals. These results indicate that the minimum requirement under these conditions is slightly more than 5 mg/kg diet, and that 10 mg/kg is an adequate level. This assumes that sustained plasma concentrations above 0.5 mg/100 ml produce sufficient levels in tissues to prevent deficiency symptoms. To our knowledge, evidence of deficiency in experimental animals has never been reported when plasma concentrations were above 0.5 mg/100 ml, regardless of the amount of linoleic acid. Our diet contained 1.38% of linoleic acid (dry diet basis) equivalent to 3.6% of calories. The α-tocopherol supplement of 5 mg/kg diet provided 0.36 mg of α-tocopherol/g linoleic acid while 10 mg/kg diet provided a ratio of 0.72. These values are close to those suggested in the National Research Council bulletin, 0.33–0.83, arrived at by more indirect methods (4).

The fecal excretion of labeled α-tocopherol indicated that about one-half of the dose was absorbed. This compares with 59–79% absorption reported recently in normal human subjects (10, 11). Measurement of fecal lipid-soluble radioactivity probably gives high values for total absorption, since some degradative products could be water soluble. McMahon and Neale (11), however, found negligible water-soluble radioactivity in human feces and only 8% of the dose was excreted in the bile.

Summary. Two normal monkeys were fed a vitamin E-free diet containing 3.6% calories as linoleic acid. Plasma α-tocopherol concentrations fell to 50% of initial values by 21 days and then remained essentially stable for another 39 days. *d*-α-Tocopherol was then added to the diet, 5 mg/kg for 40 days followed by 10 mg/kg for 60 days. The lower supplement stabilized the plasma level in one animal and gave a slight elevation in the other. The higher supplement produced progressive increases in both monkeys. It was estimated that the minimum requirement was slightly more than 0.36 mg *d*-α-tocopherol/g linoleic acid and that 0.72 mg was nutritionally adequate. Fecal excretion studies of normal doses of radioactive *d*-α-tocopherol gave an apparent intestinal absorption of about 50%.

1. Filer, L. J., Rumery, R. E., Yu, P. N. G., and Mason, D. E., Ann. N.Y. Acad. Sci. **52**, 284 (1949).

2. Dinning, J. S., and Day, P. L., J. Exp. Med. **105**, 395 (1957).

3. Fitch, C. D., and Dinning, J. S., J. Nutr. **79**, 69 (1963).

4. Portman, O. W., "Nutrient Requirements of Laboratory Animals." Nat. Acad. Sci. Nat. Res. Council, Washington, D.C. (1972).

5. Horwitt, M. K., *in* "Vitamins and Hormones" (R. S. Harris and I. G. Wool, eds.), Vol. 20, p. 541. Academic Press, New York (1962).

6. Bieri, J. G., "Lipid Chromatographic Analysis" (G. V. Marinetti, ed.), Vol. 2, p. 459. Dekker, New York (1969).

7. Bragdon, J. H., J. Biol. Chem. **190**, 513 (1951).

8. Horwitt, M. K., Amer. J. Clin. Nutr. **8**, 451 (1960).

9. Bieri, J. G., Ann. N.Y. Acad. Sci., in press.

10. Kelleher, J., and Losowsky, M. S., Brit. J. Nutr. **24**, 1033 (1970).

11. MacMahon, M. T., and Neale, G., Clin. Sci. **38**, 197 (1970).

Received Apr. 10, 1972. P.S.E.B.M., 1972, Vol. 140.