VITAMIN E DEFICIENCY IN THE MONKEY

I. Muscular Dystrophy, Hematologic Changes, and the Excretion of Urinary Nitrogenous Constituents*

By JAMES S. DINNING,‡ Ph.D., and PAUL L. DAY, Ph.D.

(From the Department of Biochemistry, School of Medicine, University of Arkansas, Little Rock)

(Received for publication, January 12, 1957)

Vitamin E has been shown to be required by a large number of species of animals (1). The most common deficiency signs are reproductive difficulties and degeneration of the skeletal muscle. The latter condition, referred to as nutritional muscular dystrophy (2), is especially prominent in vitamin E-deficient herbivorous animals.

Prior to our recent brief papers (3–5) there had appeared no report of the development of clear cut vitamin E deficiency in the monkey. Mason and associates (6, 7) have reported the results of long term feeding of diets low in tocopherol to monkeys. Three animals, sacrificed after more than 2 years of feeding, exhibited skeletal muscle pathology which was considered to indicate vitamin E deficiency. Electrocardiographic changes were also observed in other monkeys fed the diet low in vitamin E but no other deficiency signs were observed.

The present report describes experiments in which monkeys were fed a purified diet devoid of vitamin E. After from 6 to 13 months all the animals developed acute vitamin E deficiency. The signs of vitamin E deficiency in the monkey include muscular dystrophy; elevated urinary excretion of creatine, allantoin, and free amino acids; decreased urinary excretion of creatinine; anemia and granulocytosis. All these signs are reversed by treatment with alpha tocopherol.

Methods

Young *rhesus* monkeys, weighing approximately 2 kg. each, were obtained from an animal dealer. They were housed in steel cages equipped with a sloping stainless steel pan for the collection of urine. The animals were given a basal diet consisting of: casein, 18 gm.; lard, 8 gm.; salt mix (8), 4 gm.; choline, 0.1 gm.; inositol, 0.1 gm.; corn starch, 46.2 gm.; sucrose, 21.6 gm.; baking powder, 1.5 gm.; cod liver oil, 3.0 gm.; riboflavin,

* This investigation was supported by Research Grant No. A-741 *et seq.* from the National Institutes of Health, Public Health Service.

‡ Studies carried out during the tenure of a Lederle Medical Faculty Award.

0.5 mg.; pyridoxine, 0.5 mg.; calcium pantothenate, 2 mg.; nicotinic acid, 2 mg.; menadione, 0.44 mg.; folic acid, 0.5 mg.; thiamine hydrochloride, 0.5 mg.; and ascorbic acid, 20 mg. The diet components, with the exception of the cod liver oil, thiamine hydrochloride, and ascorbic acid, were mixed and a small amount of water was stirred in to make a thick paste. The diet was then cooked in an electric oven at 375°F. for approximately 30 minutes. Cod liver oil, ascorbic acid, and thiamine hydrochloride were added to the diet just before feeding. Four monkeys were given this basal diet and 4 other monkeys were given this diet with pyridoxine (vitamin B_6) omitted. In the rat it has been shown that vitamin B_6 deprivation hastens the appearance of muscular dystrophy when the animals are also deficient in vitamin E (9).

TABLE I

The Influence of Nutritional Muscular Dystrophy in the Monkey on the Excretion of Urinary Nitrogenous Compounds

Animals	Vitamin deficiency	Monkey No.	Days on diet	Mg. excreted per kg. of body weight per day			
				Creatine	Creatinine	Allantoin	Amino acid nitrogen
Normal	None	—	—	1.9 ± 0.4*	29.4 ± 2.0	7.2 ± 0.5	19.7 ± 3.4
Dystrophic	E	1–89	167	63.3	3.4	6.0	32.4
	"	1–94	203	40.7	5.9	18.7	8.9
	"	1–91	391	28.3	25.7	15.7	30.5
	"	1–97	250	16.0	22.1	19.9	9.0
	E and B_6	1–85	347	35.3	12.9	18.1	45.3
	" " "	1–86	203	85.8	12.7	22.4	43.0
	" " "	1–81	269	125.5	27.1	17.6	88.0
	" " "	1–88	325	69.2	6.0	6.2	20.6

* Standard error.

The monkeys were each given approximately 80 gm. of the diet per day. Both males and females were used and no sex differences were observed. 24-hour urine collections were analyzed for creatine and creatinine (10), allantoin (11), and free amino acids (12). Complete blood counts were made at frequent intervals on blood obtained from the ear veins.

RESULTS AND DISCUSSION

All the animals fed the vitamin E-deficient diet developed deficiency signs. The absence of vitamin B_6 from the diet did not affect the development of vitamin E deficiency, hence all the animals will be considered together.

The physical signs of vitamin E deficiency, muscular dystrophy, were observed after from 167 to 391 days of feeding (Table I), and they were quite consistent. The earliest manifestation was a slowness of movement. The monkey would move slowly around in the cage and when taken from the cage would have difficulty climbing back in. The animal would tire quickly and after a few minutes of activity would be unable to climb at all. The course of the dystrophy

progressed rapidly from this point and within a few days the monkey would exhibit advanced stages of the disease. At this time it would be unable to right itself when placed on its side. There was an obvious loss of muscle tissue, particularly noticeable in the hind legs. Also, at this time there was usually evidence of difficulty in breathing. The monkey would breathe in gasps particu-

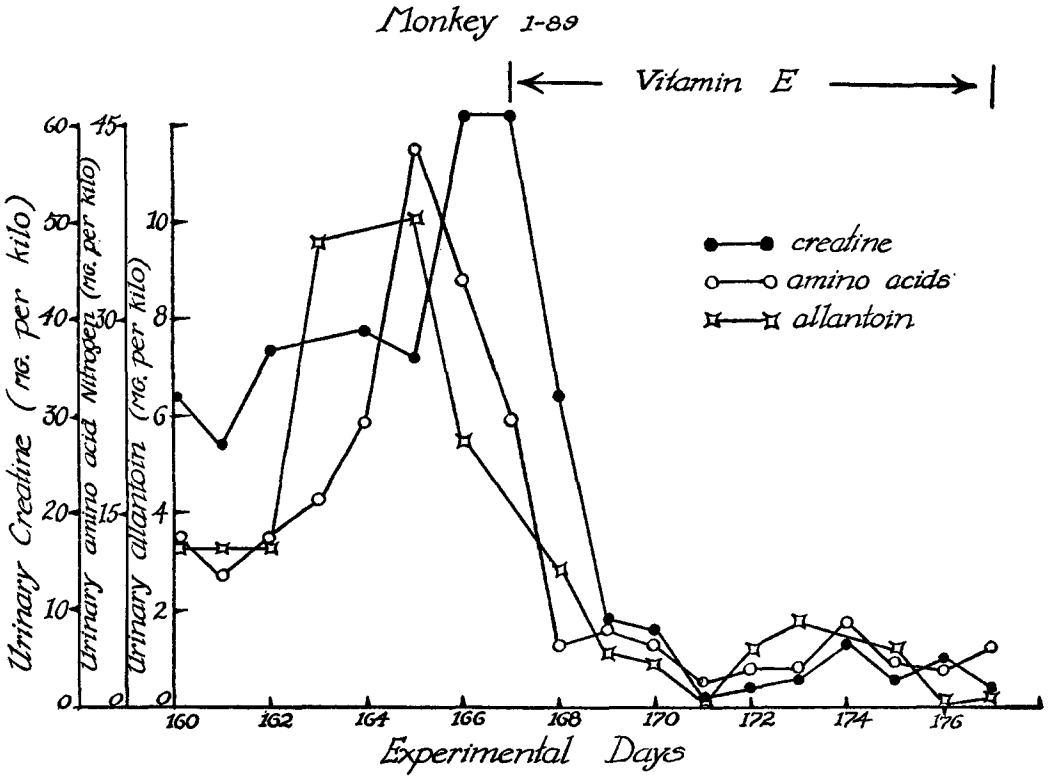

FIG. 1. The response of a vitamin E–deficient monkey to therapy with α-tocopherol. The effect on the excretion of urinary nitrogenous constituents.

larly noticeable when it was placed on its side. When the animal was held in an upright or sitting position it appeared to breathe more easily. Unless the animal was treated it would usually die within a week or 2 after the first signs of dystrophy were observed.

Three of the vitamin E–deficient animals exhibiting the signs of muscular dystrophy were treated with vitamin E. One was given two 30 mg. doses of α-tocopherol acetate by stomach tube on alternate days and two were each given two 20 mg. doses of α-tocopherol phosphate intraperitoneally on alternate days. The diet of all three was then supplemented with 20 mg. of α-tocopherol

acetate daily. Improvement was noticeable within 2 or 3 days and after 2 to 4 weeks the animals appeared to be fully recovered. Thus the muscular dystrophy observed appears to be a specific manifestation of vitamin E deficiency. One of the dystrophic monkeys which was recovered with vitamin E was deficient in both vitamin E and B_6. This animal responded as well to tocopherol therapy as did the monkeys deficient only in vitamin E. It is rather surprising that no signs of vitamin B_6 deficiency were observed in any of the monkeys fed diets devoid of this vitamin since vitamin B_6 deficiency has been readily produced in the monkey (13).

TABLE II

The Influence of Vitamin E Deficiency on the Hemogram of the Monkey

Vitamin deficiency	Monkey No.	Erythrocytes	Hemoglobin	Hematocrit value	Neutrophils	Lymphocytes
		millions/μl.	*gm/100 ml.*	*per cent*	*thousands/μl.*	*thousands/μl.*
None	—	4.8 ± 0.2*	12.5 ± 0.4	44 ± 2	4.7 ± 0.5	10.7 ± 1.2
E	1-89	2.9	6.4	30	25.7	1.1
"	1-94	1.6	4.0	15	6.0	4.4
"	1-91	3.3	9.2	32	11.7	3.6
"	1-97	0.7	2.9	12	42.8	11.9
E and B_6	1-85	2.6	8.1	28	10.7	4.2
" " "	1-86	3.2	9.5	34	18.6	3.8
" " "	1-81	2.5	6.7	26	20.0	1.6
" " "	1-88	3.4	9.0	31	18.0	3.9

* Standard error.

Vitamin E deficiency in rabbits is characterized by a greatly increased urinary excretion of creatine (14), allantoin (15), and free amino acids (16). It was of interest to determine whether similar metabolic aberrations accompanied vitamin E deficiency in the monkey. The data in Table I show that all the vitamin E-deficient monkeys excreted rather large quantities of creatine. The values for normal animals which are given in this table are average data from 6 monkeys fed the basal diet supplemented with 20 mg. of α-tocopherol acetate daily. The urinary excretion of allantoin and free amino acids was elevated in most of the deficient animals although this was not as consistent a change as the increased creatinuria.

Creatinine excretion is reduced in vitamin E-deficient rats (17, 18) owing to the loss of skeletal muscle and to decreased concentration of creatine in the remaining muscle. The data in Table I show that vitamin E deficiency in the monkey led to a reduction in creatinine excretion to exceedingly low values in some cases.

The influence of vitamin E therapy on the excretion of creatine, allantoin, and free amino acids by an initially dystrophic monkey is illustrated in Fig. 1. This animal was given 20 mg. of α-tocopherol acetate daily and in addition 2 doses of 20 mg. of α-tocopherol phosphate intraperitoneally. The response was very prompt and the excretion of these nitrogenous constituents was restored to normal after a few days of treatment.

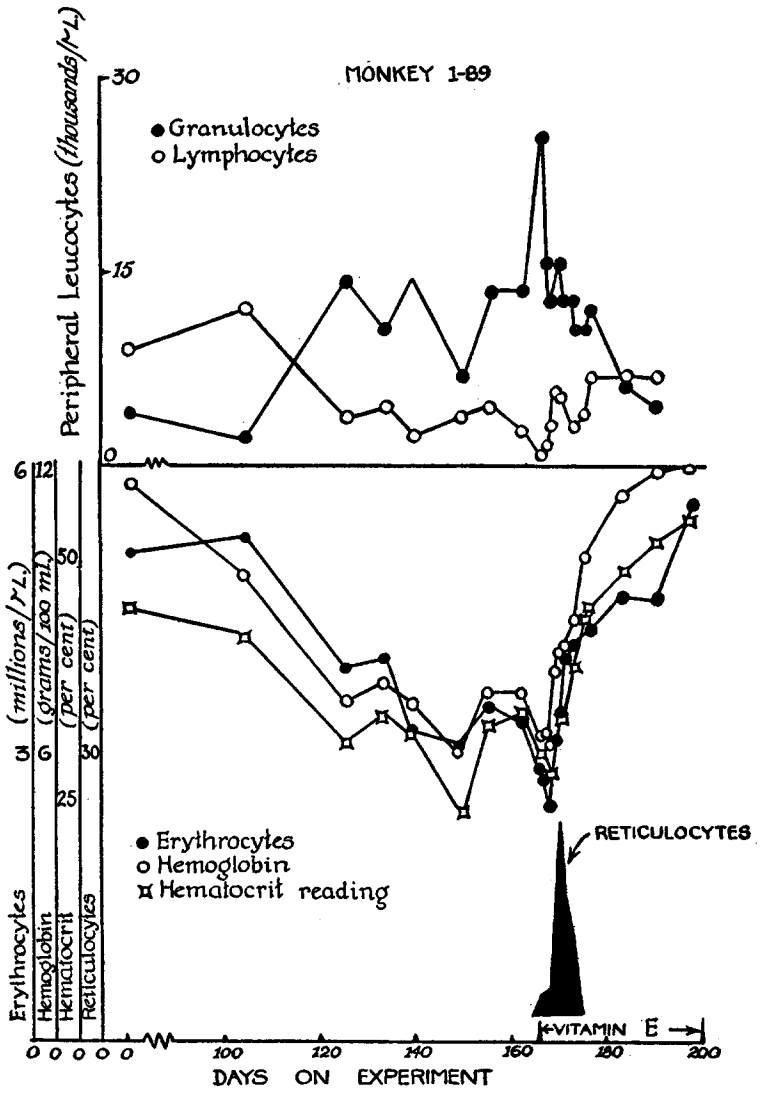

FIG. 2. The response of a vitamin E-deficient monkey to therapy with α-tocopherol. The effect on peripheral blood cells.

The hemogram of vitamin E–deficient monkeys is given in Table II. The normal values are taken from 6 monkeys receiving the basal diet supplemented with 20 mg. of α-tocopherol acetate daily. Vitamin E deficiency in the monkey resulted in anemia which was slightly macrocytic. The anemia was the first sign of vitamin E deficiency which was observed in these monkeys and occurred on an average of 83 days before physical signs of dystrophy were observed. There appears to be no report of anemia resulting from vitamin E deficiency in any other mammal.

The vitamin E–deficient monkeys exhibited granulocytosis and in most cases a lymphopenia. Granulocytosis has been shown to accompany vitamin E deficiency in the rabbit (19).

The hematologic response of a monkey to vitamin E therapy is shown in Fig. 2. This animal was given two 20 mg. doses of α-tocopherol phosphate intraperitoneally on alternate days and the diet was supplemented with 20 mg. of α-tocopherol acetate daily. The response was quite satisfactory with a reticulocyte peak occurring after 6 days of treatment. Data from a more dramatic experiment are given in Table III. This animal was severely anemic with less than 1 million erythrocytes per microliter of blood. After 17 days of treatment the peripheral erythrocytes were restored to normal levels. Likewise the initial granulocytosis was corrected. Of particular interest in this experiment was the initial high reticulocyte count which reached 48 per cent during therapy. The other deficient monkeys exhibited reticulocyte counts of from 5 to 7 per cent and there was always an increase in reticulocytes during therapy. It seems likely that the anemia of vitamin E–deficient monkeys is in part hemolytic and in part due to a block in maturation.

TABLE III

Response of a Vitamin E–Deficient Monkey to Therapy with Tocopherol

Days of treatment	Erythrocytes	Hemoglobin	Hematocrit value	Reticulocytes	Neutrophils	Lymphocytes
	millions/μl.	*gm./100 ml.*	*per cent*	*per cent*	*thousands/μl.*	*thousands/μl.*
0	0.68	3.0	12	26	39.9	11.9
3	0.78	3.9	14	41	38.6	25.4
6	2.63	7.6	30	48	8.1	6.9
10	4.45	8.8	35	44	8.0	12.0
17	5.24	11.8	42	8	4.6	9.6

These experiments demonstrate the consistent production of vitamin E deficiency in a primate. The signs of the deficiency are similar to those observed in other species. Of particular interest is the anemia which occurred quite early in vitamin E–deprived monkeys. The possibility should be considered that some cases of unexplained anemia in man may be the result of mild tocopherol deficiency.

SUMMARY

Eight young *rhesus* monkeys were fed a purified diet devoid of vitamin E. After from 6 to 13 months of feeding, all the animals developed signs of vitamin E deficiency. The signs of vitamin E deficiency in the monkey include muscular dystrophy, elevated excretion of creatine, allantoin, and free amino acids and decreased excretion of creatinine. The vitamin E–deficient monkeys all developed anemia and granulocytosis. Anemia was the first sign of vitamin E deficiency which was observed. All of these signs of vitamin E deficiency were reversed by treatment with α-tocopherol.

The authors gratefully acknowledge the technical assistance of Mr. J. M. Young, Jr., Mrs. Mary R. Simmons, Mr. Coy D. Fitch, and Miss Patricia Parker.

BIBLIOGRAPHY

1. Mason, K. E., Physiological action of vitamin E and its homologues, Vitamins and Hormones, 1944, **2,** 107.
2. Goettsch, M., and Pappenheimer, A. M., Nutritional muscular dystrophy in the guinea pig and rabbit, *J. Exp. Med.*, 1931, **54,** 145.
3. Day, P. L. and Dinning, J. S., Anemia in vitamin E–deficient monkeys, *Fed. Proc.*, 1956, **15,** 548.
4. Day, P. L., and Dinning, J. S., Vitamin E deficiency in the young *rhesus* monkey, *Proc. 3rd Internat. Congr. Biochem.*, Brussels, Belgium, 1955, 116.
5. Day, P. L., and Dinning, J. S., Nutritional muscular dystrophy in monkeys receiving a diet deficient in both vitamins B_6 and E, *Am. J. Clin. Nutr.*, 1956, **4,** 386.
6. Mason, K. E., and Telford, I. R., Some manifestations of vitamin E deficiency in the monkey, *Arch. Path.*, 1947, **43,** 363.
7. Filer, L. J., Rumery, R. E., Yu, P. N. G., and Mason, K. E., Studies on vitamin E deficiency in the monkey, *Ann. N. Y. Acad. Sc.*, 1949, **52,** 284.
8. Hubbell, R. B., Mendel, L. B., and Wakeman, A. J., A new salt mixture for use in experimental diets, *J. Nutrition*, 1937, **14,** 273.
9. Young, J. M., Dinning, J. S., and Day, P. L., Metabolic interrelationships of vitamin E and B_6, *Proc. Soc. Exp. Biol. and Med.*, 1955, **89,** 216.
10. Folin, O., On the determination of creatinine and creatine in urine, *J. Biol. Chem.*, 1914, **17,** 469.
11. Young, E. G., and Conway, C. F., On the estimation of allantoin by the Rimini-Schryver reaction, *J. Biol. Chem.*, 1942, **142,** 839.
12. Albanese, A. A., and Irby, V., Determination of urinary amino nitrogen by the copper method, *J. Biol. Chem.*, 1944, **153,** 583.
13. Rinehart, J. F., and Greenberg, L. D., Arteriosclerotic lesions in pyridoxine-deficient monkeys, *Am. J. Path.*, 1949, **25,** 481.
14. Mackenzie, C. G., and McCollum, E. V., The cure of nutritional muscular dystrophy in the rabbit by alpha tocopherol and its effect on creatine metabolism, *J. Nutrition*, 1940, **19,** 345.

15. Young, J. M., and Dinning, J. S., A relationship of vitamin E to nucleic acid metabolism, *J. Biol. Chem.*, 1951, **193**, 743.
16. Dinning, J. S., Cosgrove, K. W., Jr., Fitch, C. D., and Day, P. L., Influence of vitamin E deficiency in rabbit on urinary excretion of free amino acids, *Proc. Soc. Exp. Biol. and Med.*, 1956, **91**, 632.
17. Hove, E. L., and Hardin, J. O., Diminished urinary creatinine in vitamin E deficient rats, *J. Nutrition*, 1952, **48**, 193.
18. Dinning, J. S., The nature of the creatinuria of nutritional muscular dystrophy in the rat, *J. Nutrition*, 1955, **55**, 209.
19. Dinning, J. S., Leucocytosis in vitamin E deficient rabbits, *Proc. Soc. Exp. Biol. and Med.*, 1952, **79**, 231.