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Effect of Antioxidants on Muscle and Plasma Lipids of Vit. E-deficient Guinea Pigs.*† (24045)

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Evidence has been accumulating showing that addition of certain antioxidants to Vit. E-deficient diets either delays or prevents occurrence of Vit. E-deficiency lesions in skeletal muscle of animals. DPPD (N, N'-diphenyl-p-phenylenediamine) delays the onset of muscular dystrophy in lambs fed a Vit. E-deficient diet(1) and also prevents muscular degeneration in chicks fed diets low in both Vit. E and sulfur(2). DPPD, DBH (2,5-di-*t* - butylhydroquinone) and Santoquin (6-ethoxy-2,2,4-trimethyl - 1,2-dihydroquinoline) delay but do not prevent occurrence of muscular dystrophy in guinea pigs fed a highly purified diet deficient in Vit. E(3). Accumulation of cholesterol and total lipid in the skeletal muscle in animals deficient in Vit. E (4-9) and the hypercholesteremia observed in Vit. E-deficient rabbits(4) suggests that this vitamin may have a role in controlling lipid metabolism of skeletal muscle and plasma. Since certain antioxidants can replace Vit. E in one of its functions, it seemed desirable to determine whether the ability of Vit. E to function as antioxidant is responsible for its role in regulation of cholesterol and lipid metabolism. Accordingly an investigation was undertaken in which groups of guinea pigs were fed diets free of Vit. E and diets supplemented with either α-tocopherol acetate or various other antioxidants. Progress of guinea pigs was closely followed to determine incidence and frequency of dystrophy.

Procedure. Female guinea pigs were fed a purified diet consisting of casein, 30 g; cornstarch, 19.5 g; dextrose, 11 g; sucrose, 10 g; cellulose, 10 g; Wesson salt mix, 6 g; stripped lard, 5 g; agar, 5 g; potassium acetate, 2.5 g; magnesium oxide, 0.5 g; inositol, 0.2 g; choline chloride, 0.2 g; niacin, 20 mg; p-amino benzoic acid, 10 mg; calcium pantothenate, 4 mg; thiamine chloride, 1.6 mg; riboflavin, 1.6 mg; pyridoxine hydrochloride, 1.6 mg; folic acid, 1 mg; 2-methyl-1,4-naphthoquinone, 0.5 mg; biotin, 0.1 mg; Vit. B_{12}, 10 μg; Vit. A, 500 U.S.P. units and Vit. D_2, 50 U.S.P. units; the tocopherol content of this diet is less than 25 μg. In addition, each animal received daily (6 times/week) an oral

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TABLE I. Cholesterol Content of Blood Plasma.

Dietary supplement	Condition of animals	No. of animals	Plasma cholesterol (mg %) Free	Total
α-tocopherol acetate	Normal	3	17.6 ± 1.12*	48.5 ± 1.40*
None	Non-dystrophic	3	34.2 ± 13.9	75.1 ± 15.4
"	Dystrophic	3	57.9 ± 10.7	119 ± 20.4
BHT	Non-dystrophic	5	25.7 ± 7.03	68.8 ± 7.71
Santoquin	"	6	24.1 ± 3.68	77.0 ± 8.90
DPPD	"	6	24.9 ± 2.73	91.3 ± 9.65
DBH	"	6	33.6 ± 12.9	91.1 ± 10.8

* ± S.E.

supplement of 10 mg ascorbic acid in 0.1 ml water. One group of guinea pigs received this basal diet, a second group received the basal diet plus additional oral supplement of α-tocopherol acetate (5 mg/day, 6 times/week) and each of the remaining groups received one of the following antioxidants at a level of 0.025% of the diet: DPPD, Santoquin, BHT (butylated hydroxytoluene) or DBH. After 6 months of feeding, non-dystrophic animals from each group were autopsied; in these animals the creatine content of the gastrocnemius muscle as determined by the Jaffe reaction(10,11) was not depressed. The remaining animals in each group were maintained on experimental rations until they became dystrophic; at this time they were autopsied. All animals were sacrificed under Nembutal anesthesia. Blood samples were obtained by heart puncture. Individual bloods were centrifuged and the lipids extracted from the plasmas with ethanol-acetone (1:1). Skeletal muscle was removed from thigh, ground with acid-washed sand and anhydrous sodium sulfate and then extracted with boiling ethanol-petroleum ether (skellysolve B) (3:20). The supernatant was decanted, and the residue was extracted twice more with fresh portions of boiling petroleum ether, which were added to the first extract. All extracts were analyzed for free and total cholesterol by the method of Nieft and Deuel (12). Total lipids were determined gravimetrically on petroleum ether extracts of muscle.

Results. A marked increase obtains in both free and total cholesterol levels of plasma (Table I) of dystrophic animals fed the diet deficient in Vit. E as compared with animals fed the complete diet. This is in agreement with our previous results in Vit. E-deficient guinea pigs and rabbits(6) and with findings of Morgulis and Spencer in Vit. E-deficient rabbits(4). In animals fed the Vit. E-deficient diet and sacrificed before onset of paralysis in what may be considered an early stage of the disease, there was also evidence of an increased plasma cholesterol concentration although less marked than in dystrophic animals fed the Vit. E-deficient ration. These elevations of plasma cholesterol are similar to those observed in the remaining groups where Vit. E-deficient diet has been supplemented with antioxidants BHT, Santoquin, DPPD and DBH.

Elevation of skeletal muscle cholesterol (Table II) in dystrophic animals fed the Vit. E-deficient diet also agrees with earlier findings(4-9); these increases also precede the onset of dystrophy. Both prior to dystrophy and after paralysis a similar increase obtains in cholesterol of skeletal muscle of guinea pigs receiving BHT, Santoquin, DPPD, and DBH.

The muscle total lipid levels (Table II) of all groups except the dystrophic animals receiving DBH are elevated over those of animals receiving Vit. E; moreover, the levels of all groups receiving antioxidants are below those of the animals receiving the Vit. E-deficient diet. However, due to the great variation in these values within the groups, it is not possible to conclude that antioxidants prevent the rise of muscle total lipid in Vit. E-deficiency.‡

‡ It is possible that the slightly beneficial effects of antioxidants other than Vit. E, which have been observed, may be due in part to the fact that these antioxidants spare traces of Vit. E in the diet.

TABLE II. Free and Total Cholesterol and Total Lipid Content of Skeletal Muscle.

Dietary supplement	Condition of animals	No. of animals	Cholesterol, mg/g wet wt Free	Cholesterol, mg/g wet wt Total	Total lipid, g/100 g wet wt
α-tocopherol acetate	Non-dystrophic	6	.66 ± .05*	.72 ± .06*	2.13 ± .02*
None	Non-dystrophic	7	1.07 ± .05	1.16 ± .06	4.11 ± .24
	Dystrophic	7	1.39 ± .13	1.44 ± .13	5.10 ±1.12
BHT	Non-dystrophic	5	1.01 ± .04	1.05 ± .04	3.84 ± .49
	Dystrophic	3	1.37 ± .25	1.40 ± .26	3.13 ± .58
Santoquin	Non-dystrophic	6	.98 ± .16	.98 ± .14	2.69 ± .90
	Dystrophic	4	.87 ± .05	.90 ± .06	2.68 ± .38
DPPD	Non-dystrophic	6	1.11 ± .07	1.13 ± .07	3.66 ± .54
	Dystrophic	3	1.03 ± .11	1.01 ± .13	4.05 ±1.20
DBH	Non-dystrophic	6	1.15 ± .05	1.17 ± .04	2.89 ± .18
	Dystrophic	3	1.64 ± .14	1.72 ± .11	1.98 ± .11

* ± S.E.

These results indicate that the effect of Vit. E on cholesterol and lipid levels of skeletal muscle and plasma is not that of a nonspecific *in vivo* lipid antioxidant as evidenced by inability of antioxidants tested to maintain normal cholesterol and total lipid levels in the absence of Vit. E. Vit. E probably exerts its effect on cholesterol and lipid metabolism through some enzymatic mechanism in which non-tocopherol antioxidants are inactive; in this connection, it is of interest that the tocopherols but not DPPD and Santoquin stimulate isooctane-extracted, digitonin-solubilized DPN-cytochrome c reductase(13).

Summary. In guinea pigs with muscular dystrophy induced by feeding of Vit. E-deficient ration, there is a rise in cholesterol levels of plasma and skeletal muscle; these rises precede onset of dystrophy. Supplementation of Vit. E-deficient ration with antioxidants DPPD, BHT, DBH and Santoquin, although delaying onset of dystrophy symptoms, does not prevent the rise in plasma and muscle cholesterol and muscle total lipid in Vit. E-deficiency.

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