Relation of Vitamin E to Lipid Metabolism

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A REQUIREMENT for vitamin E by various species of animals, excluding man, has long been recognized and the various ill effects observed as a result of a deficiency of vitamin E have been well documented. Our interest in the role that vitamin E exerts in some phases of lipid metabolism has arisen from three areas of our research: (1) the protection afforded polyunsaturated fatty acids in the body by the ingestion of vitamin E; (2) the production of experimental muscular dystrophy in certain species of animals when fed a vitamin E-deficient diet; and (3) the alleviation by vitamin E of interferences with reproductive performance in rats fed certain heat treated oils as the sole source of fat in the diet.

Reports concerning the relations between vitamin E and various phases of cholesterol metabolism are relatively few and are controversial in nature. The action of vitamin E in the maintenance of normal cholesterol levels in skeletal muscle is well substantiated. An elevation of the cholesterol levels in skeletal muscle has been observed in vitamin E-deficient rabbits, guinea pigs, rats, calves and chicks.[1–8] It has also been reported that vitamin E deficiency in rabbits and guinea pigs results in elevated plasma cholesterol levels,[4,7–9] although Gray and Loh[10] have reported an increased plasma cholesterol value in healthy subjects after tocopherol administration. Cholesterol levels in the liver are supposedly unaffected in vitamin E deficiency.[4,7] In experiments in which cholesterol was fed to both chicks and rabbits,[4,11] the marked increase of cholesterol in the liver which resulted was independent of the tocopherol content of the diet.

The activity which α-tocopherol exerts on tissue and plasma cholesterol levels may be mediated through its antioxidant effect on the protection of the polyunsaturated fatty acids, which are now known to be important in the regulation of some phases of cholesterol metabolism.[12] Several years ago in a study of the effect of a saturated animal fat, i.e., lard and an unsaturated vegetable fat, i.e., cottonseed oil on cholesterol levels of tissues of the rat, it was concluded that the type of dietary fat fed was an important factor, not only for its fatty acid composition but also with respect to the tocopherol content of the fat.[13] In feeding studies with rats, no significant differences were observed in plasma cholesterol levels until after twenty-four weeks on the diets when the plasma cholesterol levels in the rats fed cottonseed oil were markedly lower when compared with those fed lard. The differences in cholesterol levels in the liver were apparent as early as twelve weeks after the initiation of the two dietary regimens (Table I).

These changes were not due to dietary deficiency of essential fatty acids for two reasons: (1) An analysis of the fatty acid composition of the fats used in the feedings (Table II) revealed that the presence of 12 per cent dienoic (linoleic) acid in the lard (which corresponds to approximately 180 mg. of linoleic acid per 10 gm. of diet) should certainly have been adequate even in the presence of saturated fatty acids which increase the requirement for essential fatty acids.[14] (2) In the typical picture of the rat with an essential fatty acid

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TABLE I

Effect of Feeding Lard or Cottonseed Oil at a Level of 15 Per Cent in the Diet on Plasma and Liver Cholesterol Levels in Male Rats

Weeks on Diet	Lard		Cottonseed Oil	
	Plasma Cholesterol Level (mg. %)	Liver Cholesterol Level (mg./gm.)	Plasma Cholesterol Level (mg. %)	Liver Cholesterol Level (mg./gm.)
6	80.5	2.77	84.1	2.76
12	75.4	3.25	79.6	2.66
24	77.9	3.18	61.9	2.48

TABLE II

Fatty Acid Composition of Lard and Cottonseed Oil*

Composition	Lard	Cottonseed Oil
Iodine value	68	107
Saturated fatty acids	39	31
Monoenoic fatty acids	48	20
Dienoic fatty acids	12	49
Polyenoic fatty acids	1	..

* Per cent.

deficiency, the increase in liver cholesterol is accompanied by a decrease in plasma cholesterol and this was not the case in the animals fed lard. The lard used in this experiment was stripped of vitamin E but was protected by the addition of certain antioxidants (0.01 per cent propyl gallate and 0.02 per cent butylated hydroxy anisole) with 0.005 per cent citric acid in propylene glycol added as preservative.

However, in a determination of possible oxidation of the fats in the diets (by the thiobarbituric acid test), the amount of oxidized material present in the diet containing lard was approximately ten times that present in the cottonseed oil diet despite the fact that antioxidants were present in the lard, and that the lard contained much less potentially oxidizable material than did the cottonseed oil. On this basis it is possible that less dienoic acid than was originally present in the lard was ingested. However, tests of liver function performed on these animals did not reveal any impairment or damage as a result of the ingestion of oxidized fat.

TABLE III

Percentage Distribution of Fatty Acids of Cholesterol Esters and Phospholipids of Pooled Liver Extracts of Animals Fed a Lard Diet with and without Vitamin E

Composition	Cholesterol Esters		Phospholipids	
	Lard	Lard with Vitamin E	Lard	Lard with Vitamin E
Iodine value	78	79	96	147
Saturated fatty acids	30	27	68	49
Monoenoic fatty acids	59	64	..	11
Dienoic fatty acids	9	5	12	7
Trienoic fatty acids	..	1	4	5
Polyenoic fatty acids	2	3	16	28

The addition of vitamin E to the diet of the animals fed lard caused a decrease in cholesterol levels in the liver without affecting the plasma cholesterol levels. Analysis of the fatty acid composition of the cholesterol esters and phospholipids in the liver lipids of animals in these groups revealed that the presence of large amounts of tocopherol resulted in higher unsaturation of the fatty acids in the phospholipid fraction, although the fatty acid composition of the cholesterol esters is essentially unchanged (Table III). The action of vitamin E in this case would seem to be that of an *in vivo* antioxidant. Similar observations on changes in fatty acid composition resulting from vitamin E deficiency have been reported by Hove and Seibold,[15] who observed that lipids of the muscle and liver of vitamin E-deficient hogs had lower concentrations of dienoic, tetraenoic and pentaenoic acids than did those of vitamin E-supplemented control animals.

It is well known that a deficiency of vitamin E will produce an experimental muscular dystrophy in various species of animals. Attempts to determine whether or not this activity is linked to the antioxidant properties of this vitamin have been made by many investigators using a series of antioxidants; here again the literature is confusing and controversial. It is possible to explain the contradictory findings by variation in such factors as type of antioxidant used, size of dose, level and type of fat in the diet, and

TABLE IV

Comparative Effect of α-Tocopherol Acetate, DPPD and Other Antioxidants on the Incidence and Time of Onset of Muscular Dystrophy in Guinea Pigs Fed a Vitamin E-Deficient Ration*

Supplement	Incidence of Muscular Dystrophy (%)	Average Time of Onset of Muscular Dystrophy (days)
α-Tocopherol acetate	0	..
None	100	139
DPPD	62.5	235
Santoquin	87.5	182
DBH	100	231
BHT	75	240

* Eight animals per group.

most important, the duration of the experiment. Results of an investigation in which we compared the activity of several antioxidants with that of tocopherol in feeding studies with guinea pigs have been published.[8,16]

MATERIAL AND METHODS

Groups of guinea pigs which had been placed on a vitamin E free ration containing 30 per cent protein and 5 per cent stripped lard had the following antioxidants incorporated into their diet: (1) 0.025 per cent N,N′ diphenyl *p*-phenylenediamine (DPPD); (2) 0.025 per cent 6-ethoxy-2,2,4-trimethyl - 1,2 - dihydroquinoline (Santoquin®); (3) 0.025 per cent 2,5-di-tert-butyl hydroquinone (DBH); and (4) 0.025 per cent butylated hydroxytoluene (BHT). Two other groups of guinea pigs were included. One group was fed a diet supplemented with α-tocopherol acetate and the other group was given an unsupplemented diet. The results are shown in Table IV.

RESULTS

For the first twelve weeks of the feeding period there were no significant differences in weight or gross appearance among the various groups of guinea pigs. All animals survived and were indistinguishable from the guinea pigs on the control, tocopherol-supplemented ration. The average body weight at this time is shown in Table V.

TABLE V

Average Body Weight of Guinea Pigs During the First Twelve Weeks of Feeding

Supplement	Weight (gm.)
None	506
α-Tocopherol acetate	537
DPPD	534
Santoquin	545
DBH	510
BHT	515

During the thirteenth week, clinical symptoms of muscular dystrophy developed in three of the animals on the basal unsupplemented vitamin E-free ration. The animals dragged their hind limbs and had difficulty in turning over when they were placed on their backs. In no other groups were there dystrophic animals. At this time, the three dystrophic animals, one non-dystrophic animal from this group and four animals from each of the other groups were sacrificed and autopsies were performed. Blood and tissues were extracted for cholesterol and lipid analyses and sections of the gastrocnemius muscle were prepared for histologic analysis. The dystrophic animals exhibited the typical Zenker degeneration observed by Pappenheimer[17] and others as characteristic of the dystrophic voluntary muscles of vitamin E-depleted animals. The musculature of the other animals appeared normal in all respects. In contrast to results obtained on the basal vitamin E-free ration, clinical symptoms of muscular dystrophy did not develop in animals fed diets containing DPPD, Santoquin, DBH or BHT until after the twenty-fifth week of feeding, whereas muscular dystrophy did not occur in any of the animals to which α-tocopherol acetate was administered.

It may therefore be said that DPPD, Santoquin, DBH and BHT delayed but did not prevent occurrence of muscular dystrophy in guinea pigs; however, α-tocopherol acetate gave full protection to the animals in this respect. Since the basal diet employed in these studies was free of vitamin E and contained no cod liver oil or other highly unsaturated oils, it would appear that the delay in onset of mus-

TABLE VI

Effect of α-Tocopherol and Other Antioxidants on Plasma Cholesterol Levels of Guinea Pigs

Supplement	Plasma Cholesterol (mg. %)	
	Free	Total
Non-dystrophic Animals		
None	34.2	75.1
α-Tocopherol acetate	17.6	48.5
DPPD	24.9	91.3
Santoquin	24.1	77.0
DBH	33.6	91.1
BHT	25.7	68.8
Dystrophic Animals		
None	57.3	119.0

cular dystrophy in animals on diets containing DPPD, Santoquin, DBH and BHT was due not to the sparing effect of these antioxidants on residual quantities of vitamin E in the diet (which is readily destroyed in the presence of unsaturated fats, particularly fish oils) but rather to the sparing effect of these antioxidants on vitamin E in the tissues. As long as tissue stores of vitamin E were adequate, muscular dystrophy did not develop. When tissue stores were depleted, the antioxidants were ineffective in preventing muscular dystrophy in contrast to the continued effectiveness of α-tocopherol.

The results of the lipid analyses are reported in Tables VI and VII. There is a marked increase in both free and total cholesterol levels in plasma of dystrophic animals fed the unsupplemented vitamin E-deficient diet as well as in those fed the vitamin E-free ration to which the antioxidants other than tocopherol have been added (Table VI). This is in agreement with our previous experiments with vitamin E-deficient guinea pigs and rabbits[7] and with those of Morgulis and Spencer[1] in vitamin E-deficient rabbits. In the group with the unsupplemented vitamin E-deficient diet in which animals were sacrificed before the onset of the symptoms of muscular dystrophy, in what might be considered an early stage of

TABLE VII

Effect of α-Tocopherol and Other Antioxidants on Cholesterol and Total Lipid Levels in the Muscle of Guinea Pigs

Supplement	Cholesterol (mg./gm.)		Total Lipid (gm./100 gm.)	
	Non-dystrophic	Dystrophic	Non-dystrophic	Dystrophic
None	1.16	1.44	4.11	5.10
α-Tocopherol acetate	0.72	..	2.13	..
DPPD	1.13	1.01	3.66	4.05
Santoquin	0.98	0.90	2.69	2.68
DBH	1.17	1.72	2.89	2.98
BHT	1.05	1.40	3.84	3.13

the disease, there is also evidence of an increased concentration of plasma cholesterol. Elevations in plasma cholesterol levels are also observed in the groups of animals fed the vitamin E-free ration supplemented with antioxidants other than α-tocopherol, i.e., DPPD, Santoquin, DBH and BHT.

The elevations of cholesterol in skeletal muscle observed in dystrophic animals fed the vitamin E-free ration also confirm the earlier findings.[1-3,5-7] However, it can be seen in Table VII that these changes also precede the onset of muscular dystrophy since in the non-dystrophic animals either receiving diets with no supplement or supplemented with non-tocopherol antioxidants, an increase in skeletal muscle cholesterol is observed.

The total lipid content of skeletal muscle of animals on vitamin E-free unsupplemented diets is markedly higher than that of animals fed α-tocopherol both before and after the onset of paralysis. The lack of tocopherol activity of the other antioxidants is also evident here although not to the same extent; in fact, the total lipid levels in skeletal muscle of animals whose diet was supplemented with DBH and Santoquin are only slightly elevated over those in the tocopherol-fed animals.

The results indicate that the effect of vitamin E on cholesterol and lipid levels of skeletal muscle and plasma is not that of a non-specific *in vivo* antioxidant but rather that either vitamin E is a highly specific antioxidant or that

TABLE VIII

Reproductive Performance of Female Rats Fed Unheated Soybean Oil and Heated Soybean Oil with and without Supplements of Linoleate or α-Tocopherol

Category	Females Bred (no.)	Litters Cast (%)	Litters (at birth)		Litters (at 3 days)		Litters (at 21 days)*		Mortality (%)	
			Total (no.)	Litters (no.)	Total (no.)	Average Weight (gm./rat)	Total (no.)	Average Weight (gm./rat)	0–3 Days	4–21 Days
Soybean oil	15	100	159	10.6	141	7.3	96	33.4	11	2
Soybean oil (LP)†	15	93	141	10.1	121	6.9	88	32.9	16	15
Soybean oil (HP)‡	15	80	110	9.2	72	6.8	59	34.6	34	0
Soybean oil (HP) plus linoleate	15	87	131	10.1	120	6.7	69	38.4	8	18
Soybean oil (HP) plus α-tocopherol	15	100	176	11.7	157	7.3	95	36.5	11	10

* Litters were cut to seven at the three-day period.
† Heated at 610°F. for seventy minutes.
‡ Heated at 610°F. for one hundred minutes.

it exerts its effect through some enzymatic mechanism in which non-tocopherol antioxidants are inactive.

It is generally accepted that the toxicity resulting from drastic heat treatment of unsaturated oils is a function of their polyunsaturated fatty acid content.[18–20] Interferences with nutrition which have resulted from the ingestion of these oils by various species of animals have been alleviated by administering fresh oil[21] and pyridoxine[22] supplements. In recent experiments[23] with fats and oils heated to 610°F. for periods of seventy and one hundred minutes, the only evidence of nutritional insufficiency was observed in a soybean oil which had been heated for the longer period of time. In animals fed this heated soybean oil, interference with reproductive performance was observed (Table VIII). The use of tocopherol as a supplement to the diet of these animals resulted in improved breeding, gestation and lactation. No changes in cholesterol metabolism were observed or were there any interferences with other nutritional indices studied such as growth, consumption of food, ability to digest the food or longevity. It was concluded that the amount of vitamin E necessary to regulate cholesterol levels in tissue is much less than that required for satisfactory reproductive performance in the rat, and that the vitamin E originally present in the oils was able to protect the polyunsaturated fatty acids from destruction during the heating process.

CONCLUSIONS

Although the requirement for vitamin E based on its protection against sterility and development of muscular dystrophy in animals has not been proved in man, the tocopherols have been accepted as essential for human nutrition. Tocopherols are the principal antioxidants in the body. Although there are some types of metabolic reactions in which tocopherol can be replaced by other antioxidants, there is no question that in some instances the requirement for tocopherol is specific. Antioxidants other than tocopherol may act to spare vitamin E for the metabolic processes for which it is essential. The activity of vitamin E in regulating cholesterol levels in various tissues of the body is probably due in part to its activity as an *in vivo* antioxidant but probably is due also to its activity in certain enzymatic processes.

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