Vitamin E as a Food Additive[1]

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ABSTRACT

Vitamin E is a cheap, nontoxic food additive or dietary supplement. The vitamin is such a poor antioxidant that very little, if any, increase in stability is attained by its addition to any food product containing linoleic acid or other more highly unsaturated fatty acid. Increased ingestion of vitamin E results in decreased absorption. Since α-tocopherol is present in tissues largely in subcellular membranes, it is not surprising that incorporation and storage in such sites is severely restricted. On the basis of the kinetics of autoxidation in vitro, it does not seem reasonable to expect massive ingestion of vitamin E to significantly ameliorate slow deteriorative processes, such as those associated with the generalized phenomenon of aging.

INTRODUCTION

On the basis of newspaper columns, women's magazines, and the outpourings of the food faddists, vitamin E appears to be the most popular micronutrient not supported by a Nobel prize winner (1). In view of this popularity, the question arises as to the desirability and practicality of adding vitamin E to foodstuffs to either improve product stability or to confer superior nutritional properties. The answers to such questions must be balanced in terms of toxicity, undesirable side effects, and the growing tendency of the Food and Drug Administration to request proof of claims of beneficial action or superior properties.

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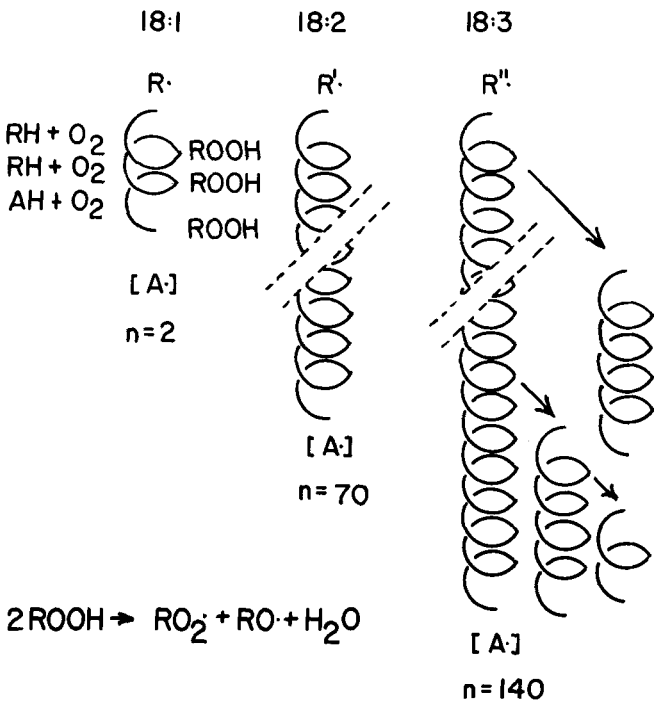

FIG. 1. Effect of increasing the degree of unsaturation upon the length of the cyclic chain reaction and quantity of hydroperoxide produced at optimal concentrations of R-α-tocopherol. Breakdown of product hydroperoxides to initiate new cyclic chain reactions is illustrated by the branching of the autoxidation of linolenate.

PRODUCT STABILITY

Food products which contain fats and oils may develop undesirable organoleptic properties as the result of autoxidation reactions (2). Oxidative rancidity is minimized or delayed by natural antioxidants, such as the tocopherols, or by synthetic lipid antioxidants, such as butylated hydroxytoluene or butylated hydroxyanisole. The use of these synthetic antioxidants has become so widespread that they have incurred the particular wrath of the health food faddists (3).

The tocopherols are rather poor antioxidants, particularly in products containing linoleic acid or other more highly unsaturated fatty acids (4,5). Despite an enormous amount of work on product stabilization, the reasons why the tocopherols are such poor antioxidants in items containing polyunsaturated fats do not seem to be well understood by the members of the food industry. A 1969 review by Mahoney (6), while not specifically dealing with foods per se, is particularly helpful in this area.

In the reaction sequence:

$$RH \xrightarrow{r_i} R\cdot \qquad \text{initiation}$$

$$R\cdot + O_2 \xrightarrow{k_2} RO_2\cdot$$

$$RO_2\cdot + R'H \xrightarrow{k_3} ROOH + R'\cdot \qquad \text{propagation}$$

$$RO_2\cdot + AH \xrightarrow{k_4} ROOH + A\cdot \qquad \text{termination}$$

$$ROOH \xrightarrow{k_5} RO\cdot + \cdot OH \qquad \text{branching}$$

$$2ROOH \xrightarrow{k_6} RO_2\cdot + RO\cdot + H_2O$$

a fatty acid free-radical (R·) is formed in an initiation reaction catalyzed by trace metal contaminants. The fatty acid free-radical reacts with oxygen to form a peroxy free-radical ($RO_2\cdot$), which, in turn, reacts with another molecule of fatty acid (R'H) to form a hydroperoxide (ROOH) and a new free radical (R'·), thus setting off a cyclic chain reaction (7). An antioxidant (AH) competitively terminates the cyclic chain reaction by withdrawing free-radicals from the system via dimerization or through the formation of tocopherol quinone.

Initiation of lipid autoxidation is a relatively complex process. The term, autoxidation denotes as apparently uncatalyzed reaction, whereas it is well known that the reaction is, in fact, catalyzed by metal contaminants present at very low levels.

The initiation reaction is written in a generalized form above to indicate that free-radical initiation occurs by several routes. It is inappropriate to specify a single specific rate constant, k_i, and a more general constant r_i is used. A potential source of free-radical initiations is from the cleavage of hydroperoxides. It is important to recognize that the concentration of these peroxides increases as the autoxidation reaction procedes. As long as hydroperoxides are present at minimal levels, they do not effect r_i greatly.

The propagation rate k_3 increases in the order k_3 oleate $< k_3$ linoleate $< k_3$ linolenate in ca. the ratios 0.025:1:2, whereas the termination rate k_4 is related to the nature of the antioxidant and remains essentially constant. The quantity of hydroperoxide formed/cyclic chain reaction

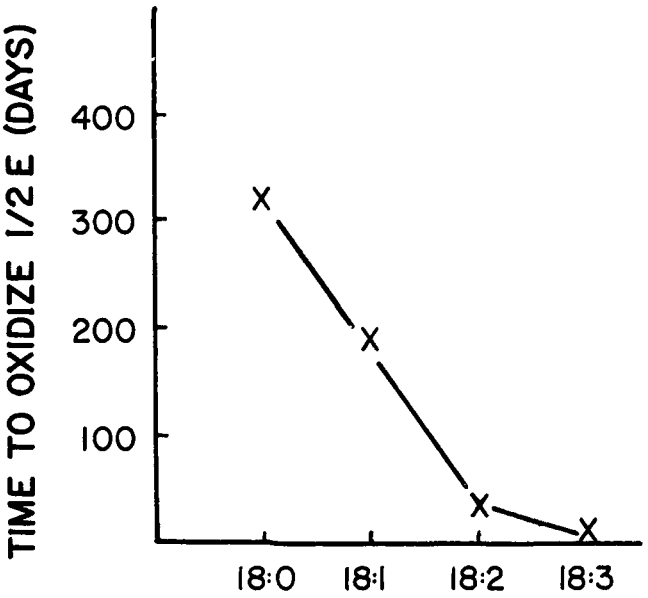

FIG. 2. Rate of destruction of one-half the initial concentration of R-α-tocopherol in various esters at 40 C (from Table II, ref. 4).

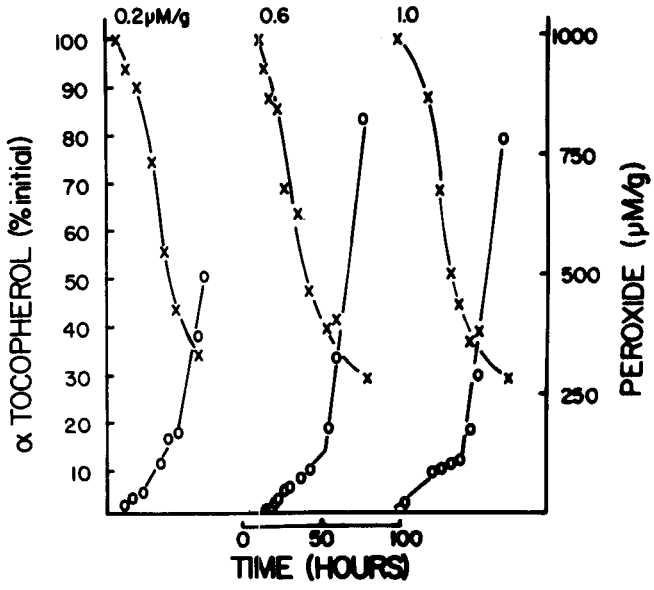

FIG. 3. Tocopherol oxidations during the autoxidation of ethyl linolenate. Peroxide formation (o) and tocopherol oxidation (x). Initial concentration of R-α-tocopherol in moles/g as indicated at top of figure (5).

will be governed by the compeition between the fatty acid and the antioxidant for the reactive peroxy free-radical common to both the propagation and termination reactions. That is to say that the average number of revolutions of the cyclic chain reaction (Fig. 1) which will occur/free-radical initiation prior to termination is governed by the ratio k_3 [RH]:k_4 [AH]. At optimum concentrations of RRR-α-tocopherol, the yield of peroxide is in the ratios 2-3:70:140 for oleate linoleate and linolenate, respectively. Starting from the same rate of initiation, the progressively greater build-up of peroxide in the progressively more unsaturated fatty acids permits more "branching" of the cycle chain reaction (Fig. 1) via free-radical multiplication, $R\cdot + O_2 + R'H \rightarrow RO\cdot + R'\cdot + \cdot OH$.

In stearate or oleate containing RRR-α-tocopherol, branching or free-radical multiplication does not become significant until most of the antioxidant has been destroyed. In any fat containing linoleate or other more highly unsaturated fatty acid, it is not possible to prevent rapid free-radical multiplication with RRR-α-tocopherol. If twice as much peroxide is produced/initiation in linolenate as in linoleate, twice as many branchings of the cycle chain reaction also may occur in linolenate, each of which also will produce twice as much peroxide.

While the rate of tocopherol destruction is not strictly equal to the rate of initiation of new cyclic chain reactions, the effects of degree of fatty acid unsaturation is quite apparent, as shown in Figure 2. Upon addition of equal quantities of α-tocopherol to linoleate and linolenate, the tocopherol is destroyed five times as rapidly in the linolenate as the linoleate (4,5). For reasons discussed in detail elsewhere (5), the rate of tocopherol destruction becomes independent of fatty acid structure as relatively low (0.1 μmole 1 g fat) tocopherol concentrations.

As the unsaturation of the fatty acids in food product increases, two serious problems arise. First, the induction period decreases, and, second, the antioxidant α-tocopherol allows the build up of relatively high levels of hydroperoxides during the induction period. These hydroperoxides are precursors to compounds causing off-flavors and odors, and the oil becomes rancid despite the presence of the antioxidant.

Logically one might expect that increasing the tocopherol content of a product would decrease oxygen uptake and increase the stability of the food. It is quite well known that such results are not obtained with the tocopherols. The

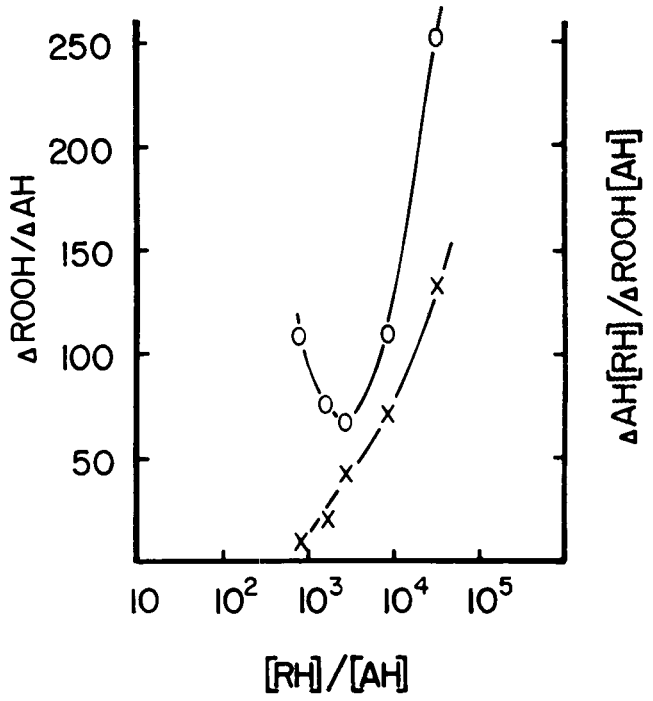

FIG. 4. Effect of concentration upon the efficiency of R-α-tocopherol as an antioxidant in ethyl linoleate. Moles peroxide formed/mole tocopherol oxidized (o) and efficiency of R-α-tocopherol as an antioxidant (x) (5).

termination reaction, $RO_2\cdot + AH \leftrightharpoons ROOH + [A\cdot]$, is reversible with k_4 forward/k_4 reverse ranging from ca. 30,000 for a hindered phenolic antioxidant, such as 2,4,6-tri-*tert*-butyl phenol, to as little as 0.05 for an unhindered phenolic antioxidant (6). Unhindered phenolic antioxidants, such as α-tocopherol, have an optimum concentration for a minimum rate of oxygen uptake, and increasing the antioxidant beyond this optimum results in an increased rate of oxygen uptake. It can be shown easily that addition of preformed peroxide to a system containing a low level of an unhindered phenolic antioxidant reverses the equilibrium of the termination reaction and the rate of oxygen uptake increases despite the presence of antioxidant.

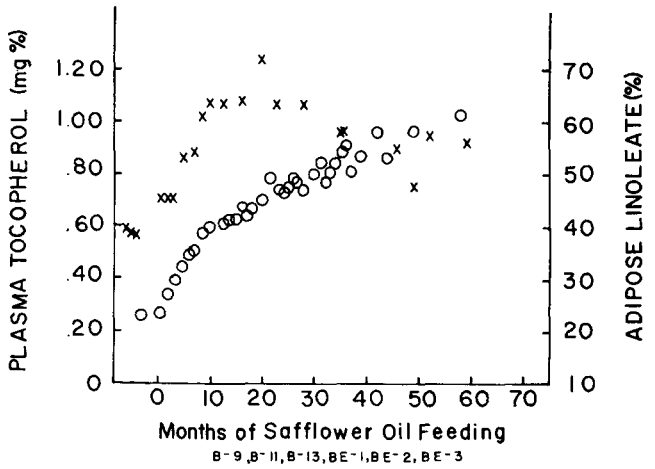

FIG. 5. Adipose tissue linoleate (o) and plasma tocopherol (x) in a male adult human fed a diet containing 60 g/day safflower oil after having been fed a diet containing 60 g/day coconut oil for 30 months (11,13).

A comparable effect is noted with peroxide accumulation during the course of the autoxidation process. As a typical example, in ethyl linolenate (5), hydroperoxide formation and α-tocopherol destruction (Fig. 3) occur in the relationship of ca. 140-300:1 until the molar ratio of peroxide to residual antioxidant is ca. 320:1. At this stage, the rate of oxygen uptake increases rapidly, and hydroperoxide formation excedes tocopherol oxidation by a factor of at least several thousand to one. The critical ratio of peroxide to residual antioxidant for transition from slow to rapid autoxidation is proportional to the degree of unsaturation of the fatty acids. Ca. 30-50% of the original level of α-tocopherol remains unoxidized at the transition.

The older literature states incorrectly that, at low concentrations, α-tocopherol is an antioxidant and at high concentration it is a prooxidant (8). In actuality (5), the efficiency of a α-tocopherol as an antioxidant expressed as $\Delta AH[RH]/\Delta ROOH[AH]$ decreased markedly with increasing concentration, [RH] / [AH]. The minimum formation of peroxide/free-radical initiation $\Delta ROOH/\Delta AH$ occurs at a concentration of ca. 1-3 μmole of α-tocopherol/g fat, (Fig. 4). Since tocopherol concentration is presented on a logarithmic scale, the optimum activity of the antioxidant appears to be relatively constant through one order of magnitude. An increase in tocopherol concentration results in increased peroxide formation/free-radical initiation and, therefore, results in more autocatalytic initiations and, thus, increases the rate of tocopherol destruction. This is not to say categorically that addition of tocopherol may not increase product stability slightly, but rather makes the point that only rather small beneficial effects, less than an order of magnitude, are to be anticipated.

RECOMMENDED DIETARY ALLOWANCE FOR VITAMIN E

Nutritionists insist that, in our affluent society, consumption of a normal mixed diet ensures a more than adequate intake of all essential nutrients. Our major nutritional problems are those, such as obesity and perhaps atherosclerosis, associated with excessive consumption. Despite the truth of these statements, it is, for all practical purposes, impossible to design a normal mixed diet which meets the current "recommended dietary allowance" (RDA) for vitamin E.

The text of the 1968 revision of the RDA (9) states quite correctly that: "The adult needs about 10 IU [international unit] or less of (of vitamin E) when PUFA [polyunsaturated fatty acid] intake is low (less than 7 g daily) and about 30 IU when PUFA intake is high (over 35 g/day) and totla fat consumption is at the level prevalent in the United States (about 40% of calories)." Unfortunately, the generally used master tabulation of RDAs listed only the higher value. This value was reduced in the 1973 revision, but the tabulated values still exceed the normal requirement (10).

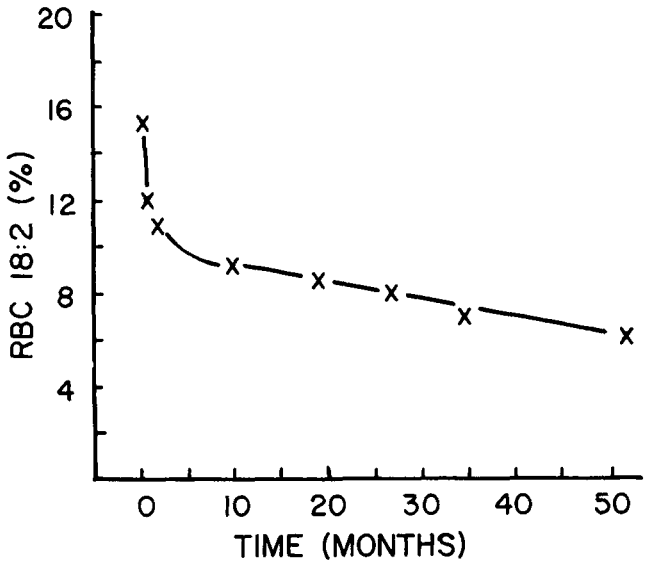

FIG. 6. Average level of linoleate in erythrocyte (RBC) lipids of 5 male adult humans fed a diet containing 60 g/day beef fat after having been fed a diet containing 60 g/day corn oil for 59 months. L.A. Witting, C.C. Harvey, and M.K. Horwitt, unpublished results).

In general the tocopherol content of most fats and oils parallels their PUFA content (11), and most foods are inherently nutritionally balanced in this regard. Since fats and oils are our principal sources of vitamin E, a diet low in PUFA tends to be low in vitamin E, and a diet high in PUFA tends to be high in vitamin E. It, therefore, follows that a normal mixed diet, not only cannot meet the RDA for this vitamin, but does not generate this high requirement for this vitamin.

DIET AND TISSUE LEVELS OF PUFA

When experiments are conducted with young, rapidly growing animals, the fatty acid composition of the tissues lipids is altered readily by dietary lipid (12). In such a situation, we have a relatively direct relationship between dietary lipid composition and the requirement for vitamin E. After a drastic shift in dietary lipid composition (13,14), more than 5-6 years may be required for the tissue lipids of an adult human, who maintains a constant wt, to reach equilibrium with the new diet (Figs. 5 and 6). When an adult male subject was transferred (Fig. 5) from a diet which had furnished 60 g/day coconut oil for 30 months to a diet which provided 60 g/day of safflower oil, adipose tissue linoleate levels had not come into equilibrium with the diet within 58 months. When 5 adult male subjects (B-9, B-11, B-13, BE-1, BE-2, and BE-3) were transferred (Fig. 6) from a diet which had furnished 60 g/day of corn oil to a diet which furnished 60 g/day of beef fat, erythrocyte linoleate levels initially decreased very rapidly but had not come into equilibrium with the new diet after 52 months. Ca. 33% of the observed decrease in linoleate occurred between 10-52 months after the change in diet. An individual's vitamin E requirement is not determined by today's, yesterday's, or last week's diet but is, instead, a

composite of the requirements generated by the intake of PUFA over a period of months or years.

DIET AND TISSUE LEVELS OF VITAMIN E

Vitamin E functions as a lipid antioxidant in vivo and is responsible for minimizing peroxidative damage to subcellular membranes (15,16). Since the tocopherols are destroyed in the process of terminating the free-radical initiated cyclic chain reactions, a constant need for replacement occurs. Under normal conditions the rate of free-radical initiation is relatively low, and systems exist, such as glutathione peroxidase, which will destroy lipid peroxides (Fig 7). RRR-α-tocopherol, to use the latest nomenclature, is preferred in vivo to other synthetic lipid antioxidants which are more effective in vitro because of their superior absorption, preferential distribution in subcellular membranes, and relatively slow turnover. Other vitamers, such as β, γ, δ-tocopherol, turn-over faster in the tissues; and the synthetic lipid antioxidants are absorbed poorly do not concentrate in the right subcellular areas, and are not retained well in the tissues.

Attempts to increase tissue levels of vitamin E are complicated by the decreased absorption from the digestive tract noted with increased intakes. Losowsky, et al., (17) noted that as the dosage of α-tocopherol was increased from 0.04 mg, to 20 mg, and then to 200 mg; absorption decreased from 50-80% to 20-50% and then to as little as 5%. Prolonged ingestion of 500 times the normal requirement of vitamin E (18) resulted in as much as a 6-8-fold increase in plasme tocopherol (8.6 mg/100 ml). Withdrawal of this excessive dosage for only 64 hr, however, produced a 50% decrease in plasma tocopherol levels. This is consistant with similar observations at lower dosage levels in the Elgin study (19).

The level of vitamin E found in the various organs and tissues of man (20) and experimental animals (21) consuming normal diets is relatively constant, 1-3 μmoles/g fatty acid, when stated in terms of tissue lipid. This corresponds quite nicely to the concentration of vitamin E having optimum antioxidant activity in vitro. Vitamin E is found associated with phospholipids and occurs largely in subcellular membranes. There appears to be practical limits to the amount of vitamin E that can be accomodated within a normal membrane. Using techniques that currently would not be considered adequate, Mason (22), in 1942, demonstrated that, in the rat, the liver was the only organ which appeared capable of appreciable storage of α-tocopherol. This storage was observed at 10,000 times the minimum daily requirement which would correspond to ca. 60 g α-tocopherol/day in man.

REASONS FOR ADDING VITAMIN E TO DIET

Natural foodstuffs contain vitamin E and PUFA in balanced quantities. The inverse relationship between dosage and absorption tends to ensure that human tissues will contain an optimum level of α-tocopherol. The various proposals for massive addition of vitamin E to the diet are derived from considerations other than simple nutritional statues. Vitamin E and other synthetic lipid antioxidants have beneficial effects in certain acute or chronic intoxications. The possible erroneous belief that increasing antioxidant concentration will decrease the yield of lipid peroxide/free-radical initiation has led to suggestions that vitamin E might ameliorate the deteriorative processes associated with the general phenomenon of aging.

As shown by Recknagel (23), Diluzio (24) and others (25-27), the metabolism of such hepatotoxins as carbon tetrachloride and ethanol induces lipid peroxidation. The hepatic microsomal mixed function oxidase system which metabolizes and detoxifies various drugs also can initiate

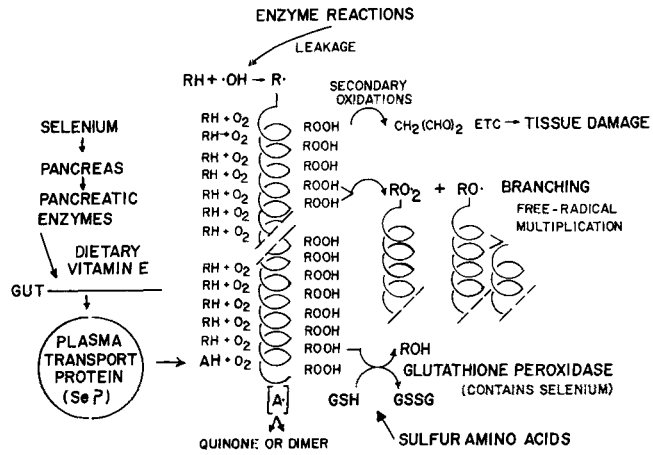

FIG. 7. Interaction of polyunsaturated fatty acids, vitamin E, biologically available selenium, sulfur amino acids, and several enzymes in lipid peroxidation.

lipid peroxidation (Fig. 7) (28,29). Lipid peroxidation also may be promoted by atmospheric contaminants, such as the oxides of nitrogen or ozone (30).

In an acute intoxication, a sufficient number of cyclic chain reactions may be initiated simultaneously so that the level of product peroxide precludes effective antioxidant action by shifting the equilibrium of the termination reaction, $RO_2\cdot + AH \rightleftharpoons ROOH + [A\cdot]$, to the left. Tissue damage may occur as the result of uninhibited lipid peroxidation despite the presence of an antioxidant, and antioxidant destruction may not be well related to the rate of lipid peroxidation or to tissue PUFA levels. Protection against tissue damage would only be afforded by antioxidant concentrations that permit the chain termination reaction to procede effectively.

Green and coworkers (31,32) have presented data relevant to this point. This group gave rats a massive, acute dose of carbon tetrachloride (2.0 ml/kg) which produced necrosis of the entire centrilobular region with an outer zone of hydropic degeneration in 24 hr. In vitamin E-deficient rats, carbon tetrachloride had no significant effect upon the distribution of a simultaneous oral dose of ^{14}C-α-tocopherol between unchanged ^{14}C-α-tocopherol and labeled tocopherol metabolites in the liver of rats sacrificed 24 hr later (31). The level of peroxidation required to produce such severe tissue damage would be expected to shift the equilibrium of the termination reaction to the left. No clear relation between the rate of lipid peroxidation and tocopherol oxidation would, therefore, be expected. A close relation between the mode of action of vitamin E in vitro and in vivo would seem to be indicated.

Chronic intoxications also would be expected to produce tissue damage by generally increasing the rate of production of new free-radicals. Those individuals who must work with certain industrial chemicals, attend cocktail parties, or live in smog areas conceivalby might benefit from increased intake of vitamin E.

VITAMIN E AND AGING

Since the natural biological lipid antioxidant, α-tocopherol is a very poor antioxidant, it is not surprising that lipopigments of the lipofucsin or ceroid type containing oxidized lipid and protein progressively accumulate in the tissues with age. An analysis of the kinetics of autoxidation in the presence of α-tocopherol suggests that development of reserve antioxidant capacity to protect against toxic compounds may be possible. Amerlioration of the deteriorative processes associated with the general phenomenon of aging would require minimization of hydroperoxide pro-

duction/free-radical initiation throughout the entire lifespan. The tissues levels of α-tocopherol normally present in individuals consuming normal diets appears to be the optimum for this purpose.

TOXICITY OF VITAMIN E

At the price of vitamin E in tonnage quantities, the human requirement for this micronutrient could be met for a little over $.08/year. The ready availability of vitamin E has resulted in the presence in our population of faddists who have ingested 3-5 g vitamin E/day for several years. Del Giudice (33), for instance, gave 2 g α-tocopherol/day to his children for years and reported no adverse side effects. Shute (34), however, recommends that high dose levels not be given initially because of possible adverse side effects. Convincing evidence of toxicity in man or animals is essentially nonexistant. The literature, however, is replete with reports which would seem to indicate that, as with most substances, experiments can be designed which will produce results which can be interpreted as evidence of toxicity (35-68). March and coworkers (69-72) have studied the effect of hypervitaminosis E upon reticulocytosis, mitochondrial respiration, thyroid function, bone calcification, and prothrombin time in the chick.

A commercial deodorant product containing vitamin E recently was withdrawn on the basis of ca. 50 reports of irritation, mostly the formation of a rash. At this time it has not been established specifically that vitamin E was the causative agent.

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