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Vitamin E: molecular and biological function

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Vitamin E, the generic term for a group of eight lipid-soluble substances, four tocopherols and four tocotrienols, was discovered by Evans & Bishop (1922) during a course of studies examining the relationship between fertility and nutrition. Female rats fed on a vitamin E-deficient diet for several months suffered a loss of fertility through resorption of the fetus which was prevented by supplementing the diet with small amounts of fresh lettuce (*Lactuca sativa*), wheat germ, or dried lucerne (*Medicago sativa*) leaves. Subsequently, in the early 1930s, vitamin E was found to be required not only for normal gestation in female rats but also for the prevention of sterility in male rats (Mason, 1933) and chickens (Adamstone & Card, 1934), encephalomalacia in chicks (Pappenheimer & Goettsch, 1931) and nutritional muscular dystrophy in rabbits and guinea-pigs (Goettsch & Pappenheimer, 1931). Eventually, studies carried out with a wide variety of experimental animals revealed that vitamin E deficiency can cause many different pathological conditions (Scott, 1978; Diplock, 1985).

The death and subsequent resorption of the fetus observed in vitamin E-deficient rats has become the basis of the now classical assay (rat fetal resorption assay) for vitamin E activity. Use of this assay has established (Bunyan *et al.* 1961; Century & Horwitt, 1965) that the relative order of bioactivity of the tocopherols is $\alpha>\beta>\gamma>\delta$. The tocotrienols are much less abundant and, of these, only the α- and β-tocotrienols have been tested. Again, the α-form is most active, but it is substantially less active than its tocopherol counterpart.

The connection between vitamin E activity and its molecular function as an antioxidant appears to have been made first by Olcott (Olcott & Emerson, 1937; Diplock, 1985) who found that an unsaponifiable fraction from lettuce oil inhibited the autoxidation of lard (Olcott & Mattill, 1931). Subsequently, after vitamin E was isolated, there were innumerable *in vitro* demonstrations of its ability to inhibit effectively the autoxidation of fat. Not surprisingly, it has been concluded that the biological function of vitamin E is the same, i.e. to prevent the oxidative conversion (lipid peroxidation) of membrane and lipoprotein polyunsaturated fat (LH) into lipid hydroperoxides (LOOH; reaction 1).

$$L\text{–}H + O_2 \rightarrow LOOH. \quad (1)$$

FREE RADICALS, ANTIOXIDANTS AND LIPID PEROXIDATION

The chemistry of autoxidation in general, and of lipids in particular (lipid peroxidation), is now well-understood. O_2 reacts with oxidizable substrates, such as polyunsaturated

lipids, via the intermediacy of transient free radical species that may be formed in many different ways. A potentially significant source of free radicals, and an example of how free radical reactions are initiated, is the transition-metal (e.g. Fe or Cu)-catalysed decomposition of the hydroperoxide products of lipid oxidation (i.e. H_2O_2 or lipid hydroperoxides; reaction 2).

$$\text{LOOH (HOOH)} + Fe^{2+} \rightarrow \text{LO}\bullet\ (\text{HO}\bullet) + HO^- + Fe^{3+}. \quad (2)$$

The presence of an unpaired electron imparts to a free radical a reactivity that is characterized by a strong tendency to unite the unpaired electron with another electron, of opposite spin, from another molecule. This can occur between two free radicals, leading to the formation of non-radical products. However, the probability of this reaction is limited by the usually very low concentration of free radicals. More probable is the reaction between a free radical and the much more abundant non-radical molecules from the surrounding medium. The driving force for this reaction is the creation of a new bond which is stronger than the bond that is destroyed. However, this reaction occurs at the expense of creating a new radical from the parent, non-radical molecule. This is the essence of a chain reaction, which, in effect, is a series of propagative reactions that do not stop until the chain-carrying radical eventually meets and reacts with another radical, forming stable, non-radical products (termination reaction).

The unpaired electron in a free radical usually is located either on a C atom (a carbon-centred radical) or on an O atom (an oxy radical), although in proteins and peptides the unpaired electron also may be located on a S atom (e.g. glutathione). Under aerobic conditions, a carbon-centred radical (L•) undergoes a very rapid propagative reaction with O_2, itself a radical with two unpaired electrons, yielding a peroxyl radical (LOO•; reaction 3).

$$\text{L}\bullet + \bullet\text{O}_2\bullet \rightarrow \text{LOO}\bullet. \quad (3)$$

Oxy radicals include superoxide radical anion ($O_2^-\bullet$), hydroxyl radical (HO•), peroxyl radical (LOO•), alkoxyl radical (LO•), phenoxyl radical (PhO•) and nitric oxide (NO•). Although superoxide radical is almost certainly the most abundantly formed oxy radical, it is unreactive towards most organic substrates. However, it can spontaneously dismutate to H_2O_2 which, in the presence of certain transition metal ions, can lead, through Fenton chemistry, to the generation of powerful and destructive oxidizing species that attack a wide range of organic substrates (reaction 2). Alkoxyl radicals (LO•) are very reactive radicals that can be generated by transition-metal-catalysed decomposition of lipid hydroperoxides (reaction 2). Hydroxyl radicals (HO•) are extremely reactive, reacting with any organic substrate within their immediate vicinity. They are generated, for example, in tissues by γ-irradiation. Hydroxyl radicals, also, are thought to be formed through Fenton chemistry (reaction 2). However, recent work indicates that Fenton chemistry is effected by some other molecular species (Sawyer *et al.* 1993).

Although peroxyl radicals (LOO•) are less reactive than hydroxyl radicals, they pose a special risk because they can participate in chain reactions that can greatly amplify damage to lipid environments (e.g. membranes, lipoproteins). Typically, peroxyl (or alkoxyl) radicals attack the vulnerable C-H bonds of methylene (CH_2) groups sandwiched between the double bonds of polyunsaturated fatty acid moieties, generating

carbon-centred, fatty acid radicals and a hydroperoxide (or alcohol) as products (reaction 4).

$$\text{LOO}\bullet\ (\text{LO}\bullet) + \text{L–H} \rightarrow \text{LOOH}\ (\text{LOH}) + \text{L}\bullet. \quad (4)$$

The radical L• continues the propagation reaction sequence by reacting with O_2 (reaction 3). The sequence of reactions 3 and 4 represents a lipid peroxidation chain reaction which continues until the chain-carrying peroxyl radical eventually reacts with another radical in a self-termination reaction, yielding stable, non-radical products (reaction 5).

$$\text{LOO}\bullet + \text{LOO}\bullet\ (\text{LO}\bullet, \text{L}\bullet) \rightarrow \text{non-radical products}. \quad (5)$$

Although polyunsaturated lipids are the most vulnerable and most abundant target susceptible to free radical attack, the free radical damage may spread beyond the immediate lipid environment to include structural and functional damage to components containing proteins, carbohydrates and DNA. The damage may be effected directly by the radicals themselves or through the generation of labile lipid peroxidation products, e.g. aldehydes.

Free radical-mediated peroxidative damage can be prevented or at least inhibited by the intervention of molecules called antioxidants. Preventive antioxidants inhibit the formation of free radicals. These may function, for example, by the enzymic removal of free radical precursors, such as removal of H_2O_2 by catalase (*EC* 1.11.1.6) or glutathione peroxidase (*EC* 1.11.1.9), or by chelation of transition metal ion catalysts (Fe, Cu) of hydroperoxide decomposition. In the event that free radicals are formed, potential chain reactions are curtailed by substances called chain-breaking antioxidants. These substances limit the length of the chain reaction by reacting directly with radicals, converting them to more stable products. Vitamins E and C, respectively, are examples of lipid- and water-soluble, chain-breaking antioxidants.

Vitamin E has been found to account, at least quantitatively, for most of the lipid-soluble antioxidant present in mammalian tissues and plasma (Burton *et al.* 1983; Cheeseman *et al.* 1984). *In vitro* studies, conducted in the 1980s (Burton *et al.* 1985), showed that α-tocopherol (α-T) in low concentration in bulk solution is one of the best chain-breaking antioxidants for peroxyl radicals, not only among the tocopherols but also among all the known phenolic antioxidants.

The successful functioning of α-T as a chain-breaking antioxidant requires that it possess two properties. First, it must be able to transfer rapidly its phenolic H atom to a lipid peroxyl radical, converting it to a lipid hydroperoxide (which subsequently must be removed before it becomes a source of free radicals via reaction 2) and an α-tocopheroxyl radical (i.e. a phenoxyl radical; reaction 6).

$$\text{LOO}\bullet + \text{PhOH}\ (\alpha\text{-T}) \rightarrow \text{LOOH} + \text{PhO}\bullet\ (\alpha\text{-T}\bullet). \quad (6)$$

Ideally, the reaction of α-T with a peroxyl radical is sufficiently fast compared with reaction of a peroxyl radical with another polyunsaturated fatty acid group so that a small amount of α-T can protect a large amount of polyunsaturated lipid. Second, the tocopheroxyl radical must be unreactive towards either O_2 or polyunsaturated lipid. Eventually the tocopheroxyl radical may react with another tocopheroxyl radical or a peroxyl radical to give non-radical products (reaction 7).

$$\text{PhO}\bullet + \text{PhO}\bullet\ (\text{LOO}\bullet, \text{L}\bullet) \rightarrow \text{non-radical products}. \quad (7)$$

This is the usual outcome in simple oxidations carried out in homogeneous bulk solution.

Very recently, however, Bowry and co-workers (Bowry *et al.* 1992; Bowry & Stocker, 1993; Ingold *et al.* 1993) have shown that the α-tocopheroxyl radical actually can react with polyunsaturated lipid in the heterogeneous micro-environments typical of lipoproteins and lipid membranes. This occurs because the phytyl tail anchors the tocopheroxyl radical within a lipid particle and when there is only one radical in a particle the rate of reaction 7 is lowered to such an extent that reaction 8 becomes a competitive alternative.

$$PhO\bullet + LH + O_2 \rightarrow PhOH + LOO\bullet. \quad (8)$$

Under these conditions a chain reaction is established (reactions 8 and 6) in which the tocopheroxyl radical becomes a chain-carrying radical. Furthermore, the presence of α-T facilitates the entry of radicals into the low-density lipoprotein (LDL) particle, causing lipid peroxidation to occur more rapidly than in the absence of vitamin E (Bowry & Stocker, 1993). That is, vitamin E can act as a pro-oxidant! However, when ubiquinol (reduced coenzyme Q), vitamin C, or both are present also (a closer reflection of normal physiological conditions), there is a dramatic reversal from pro-oxidation to anti-oxidation. The reversal occurs because vitamin C or ubiquinol is able to react with the tocopheroxyl radical (e.g. reaction 9, where $AscH^-$ is ascorbate), regenerating α-T and effecting the transfer of the radical centre from within the lipid particle to the relative safety of the surrounding aqueous medium.

$$PhO\bullet + AscH^- \rightarrow PhOH + Asc\bullet^-. \quad (9)$$

Normally, it would be uncommon to encounter physiological conditions in which the depletion of vitamin C and ubiquinol (and any other natural substance able to function in a similar manner) render vitamin E a pro-oxidant. The known beneficial effects of vitamin E support this assertion. However, the findings from these carefully conducted, *in vitro* model studies provide clear indications of the limitations that may arise in real biological systems in which free radical-mediated initiation and onset of disease states and medical conditions occur. The expanded knowledge of the behaviour of vitamin E provides a better framework upon which to base rational therapeutic approaches to medical conditions and diseases that appear susceptible to antioxidant intervention.

BIOAVAILABILITY OF VITAMIN E: MECHANISMS OF ABSORPTION, TRANSPORT AND TISSUE UPTAKE

In view of the antioxidant potential of vitamin E *in vivo*, it is desirable to know some details about its absorption, transport and distribution in tissues. *In vivo* studies of the intestinal absorption, transport and tissue uptake of oral vitamin E have been carried out using: (1) radiolabelled tocopherol; (2) large doses of the unlabelled vitamin; and (3) deuterium-labelled vitamin E with mass spectrometric determination (Burton & Ingold, 1993).

Intestinal absorption

In mammals the absorption of vitamin E is dependent on the ability to absorb fat from the small intestine. The subsequent transport of the vitamin within the body and its

uptake by tissues (Kayden & Traber, 1993; Traber *et al.* 1993*a*) appear largely to follow the paths available to other, relatively non-polar, lipids such as triacylglycerols and cholesterol (Gotto *et al.* 1986).

Lipoprotein transport: lipoprotein lipase (EC 3.1.1.34) and LDL receptor-mediated uptake

Dietary vitamin E is absorbed from the small intestine and secreted into the lymph in intestinally-derived chylomicrons that are rapidly catabolized in the circulation by lipoprotein lipase bound to the endothelial wall. During lipoprotein lipase-catalysed hydrolysis of triacylglycerols to free fatty acids and monoacylglycerols, fatty acids and tocopherols are transferred to tissues. Concomitantly, chylomicron components, including apolipoprotein C, cholesterol, phospholipids, and tocopherols, are transferred to plasma high-density lipoproteins (HDL), while apolipoprotein E is transferred in the reverse direction, i.e. from HDL to the chylomicrons. The binding of apolipoprotein E to chylomicron remnants leads to a rapid clearance of the chylomicron remnant particles from the plasma by the liver via specific apolipoprotein E receptors.

Vitamin E is secreted from the liver into plasma in triacylglycerol-rich, very-low-density lipoproteins (VLDL). Hydrolysis of VLDL by lipoprotein lipase delivers fatty acids and vitamin E to tissues and yields LDL. LDL, which carry the major portion of plasma vitamin E and appear to exchange vitamin E readily with HDL, provide a further route for delivery of vitamin E to tissues via the LDL receptor-mediated uptake pathway.

Much of the information about absorption and uptake has been obtained by studying competitive absorption and uptake of tocopherols from mixtures of known composition (Burton *et al.* 1993). For example, administration of mixtures of α-T and γ-tocopherol (γ-T) (1:1, w/w) to rats, pigs or humans initially results in the uptake of approximately equal amounts of the two tocopherols. However, the concentrations of γ-T in plasma and tissues subsequently decline much more rapidly than those of α-T. Thus, α-T and γ-T are absorbed equally well into lymph from the small intestine: therefore, a postabsorptive mechanism must come into play to account for the higher levels of α-T in plasma.

Liver cytosolic α-T-binding protein

The liver is most likely to be responsible for the higher level of plasma α-T. The work of Behrens & Madère (1982, 1983) strongly supports the involvement of a low-molecular-weight (about 32 kDa) tocopherol-binding protein (TBP; Catignani, 1975), because it binds substantially more α-T *in vitro*.

Studies of the competitive uptake into human and primate plasma lipoprotein fractions of two deuterium-labelled stereoisomers of α-T (*RRR*-α-T and *SRR*-α-T) have shown that both isomers are incorporated equally well into intestinally-derived chylomicrons (Traber *et al.* 1990*a*,*b*). However, the natural stereoisomer (*RRR*) is incorporated in much higher concentration into the VLDL fraction that subsequently is secreted into the plasma from the liver. A study of the competitive uptake of γ-T, *RRR*-α-T and *SRR*-α-T into human plasma (Traber *et al.* 1992) has shown also that all three tocopherols are taken up equally well into chylomicrons, but neither γ-T nor *SRR*-α-T are incorporated into VLDL to the same extent as *RRR*-α-T. This finding suggests that all forms of

tocopherol are subject to a discrimination mechanism in the liver that favours secretion of α-T into plasma.

The cytosolic liver TBP may function as an intracellular transfer protein (Mowri *et al.* 1981; Sato *et al.* 1991), shuttling α-T between subcellular components, particularly to nascent VLDL. A lack of or a defect in the TBP is believed to be the cause of impaired discrimination between *RRR*-α-T and *SRR*-α-T and γ-T in patients with familial-isolated vitamin E deficiency (Traber *et al.* 1993*b*). There is evidence that the liver TBP may play a role in maintaining plasma α-T levels and controlling excretion of vitamin E via the bile (Kayden & Traber, 1993).

Nothing is known about how vitamin E is returned from tissues to the circulation for reprocessing and recirculation or removal by the liver.

TURNOVER AND LEVELS OF VITAMIN E IN TISSUES

Much of the vitamin E in the body is stored in skeletal muscle, adipose tissue and the liver. Adipose and adrenal tissues have the highest concentrations of vitamin E, with levels that are tenfold greater than in other tissues (Diplock, 1985). Studies of uptake of dietary, deuterium-labelled α-T in young male rats and guinea-pigs have shown that turnover is highest in liver, spleen, lung and kidney and smallest in neural tissues (brain, spinal cord; Burton & Ingold, 1993).

Very little is known about the determinants of the levels and turnover of vitamin E in individual tissues, and the extent of bioconversion of vitamin E into metabolites. Some of the factors which may be relevant are: (1) the free radical burden imposed on the tissue vitamin E stores; (2) the extent to which vitamin E is spared or regenerated from the tocopheroxyl radical by other substances (e.g. vitamin C or ubiquinol); (3) the rate of cell turnover; (4) cellular and extracellular mechanisms that control the traffic of vitamin E to and from tissues. The importance of another factor, hormones, is highlighted by a very recent report indicating that the androgen, testosterone, and the oestrogen, oestradiol, have profound and opposing effects on levels of vitamin E in liver and adrenal tissues, without affecting plasma levels (Feingold *et al.* 1993).

HEALTH BENEFITS OF VITAMIN E: BIOLOGICAL FUNCTION

The antioxidant effect of vitamin E should provide long-term health benefits to the extent that (a) free radicals are a factor in the onset and progression of diseases, and (b) vitamin E is able to diminish effectively harmful levels of oxy radicals. The latter consideration reflects the fact that the localization of vitamin E in membranes makes it impossible for it to protect directly non-membrane components and that its antioxidant activity depends on the functional presence of other co-operative antioxidants, such as vitamin C or ubiquinol. Increasingly, attention is being drawn to the relationship between oxidants, including free radicals, antioxidants and the degenerative diseases of ageing, especially cancer, cardiovascular disease, immune system decline, brain dysfunction and cataracts (Ames *et al.* 1993).

There are four likely sources of oxidants and oxy radicals in cells (Ames *et al.* 1993). They are: (1) mitochondria, which produce $O_2^{-}\bullet$ and H_2O_2 as a consequence of normal respiration; (2) phagocytes, which produce nitric oxide (NO•) and hypochlorite (ClO^-), as well as $O_2^{-}\bullet$ and H_2O_2 during the 'respiratory burst'; (3) peroxisomes, which degrade

fatty acids and other substances to give H_2O_2; and (4) cytochrome P-450 enzymes, which are responsible for a very wide range of oxidation reactions of both endogenous substrates (e.g. production of steroid hormones, nitric oxide) and exogenous, foreign substrates ('detoxification').

Under normal metabolic conditions it is reasonable to suppose that there is a tolerable balance between the level of radical production and the antioxidant capacity of cells. It is not difficult, however, to envisage circumstances in which an increase in radical production from any one of the sources 1–4 (most likely 2 and 4) could overwhelm cellular antioxidant defences. For example, chronic inflammation (e.g. caused by infection) will lead to a prolonged exposure to enhanced levels of oxidants from phagocytes, which may deplete cellular antioxidant capacity (e.g. arthritis; Fairburn *et al.* 1992). This increases the probability of the occurrence of damage that has profound consequences beyond the cell itself, e.g. impaired protein function or heritable damage to DNA. Perhaps not surprisingly, chronic inflammation is a major risk factor for cancer (Ames *et al.* 1993).

Cancer

Few controlled human studies have been conducted of the effect of vitamin E on cancer. However, epidemiological findings suggest, in accordance with findings from animal studies, that higher intakes of vitamin E (and other dietary antioxidants) may decrease the risk for certain cancers (Knekt, 1993). The most consistent associations have been reported for cancers of the lung, oesophagus, and colorectum. However, gynaecological cancers, with the possible exception of cervical cancer, have generally not been found to be associated with vitamin E status.

Animal studies provide stronger evidence for an anticancer effect of vitamin E. For example, experiments with mice have shown that diets supplemented with high levels of vitamin E suppress the promotion by ethanol of chemically-induced oesophageal cancer (Eskelson *et al.* 1993). Although alcohol is not a carcinogen, chronic alcohol intake is associated with increased incidence of cancers of the pharynx, larynx, oesophagus, stomach, lungs, liver and rectum. Alcohol may promote cancer development through increased free radical generation. Ethanol is oxidized to acetaldehyde by alcohol dehydrogenase (*EC* 1.1.1.1), catalase and the microsomal ethanol-oxidizing system. It is believed that free radicals are released during the oxidation of ethanol by the latter system. Vitamin E also retards the incidence and growth of chemically-induced oesophageal tumours in mice immunocompromised by AIDS retroviral infection (Watson *et al.* 1992). The effect of vitamin E may result from its known ability to enhance immune response (Meydani & Tengerdy, 1993) in addition to its action as an antioxidant combating the effects of infection-induced lipid peroxidation (Odeleye *et al.* 1992).

It is important to recognize the possibility that the effects of vitamin E may not be uniformly beneficial, and sometimes may even be harmful. In this regard, a cautionary note is sounded by two recent studies of chemically-induced (Mitchel & McCann, 1993) and u.v.-B-induced (Gerrish & Gensler, 1993) skin cancers in mice. In one study it was found that *all-racemic* α-T (a mixture of eight stereoisomers) was a promoter of chemically-induced skin cancer (Mitchel & McCann, 1993). The authors suggested that reduction of cellular oxidant levels may trigger tumour promotion. On the other hand,

α-T can inhibit proliferation of cultured smooth muscle cells in a dose-dependent manner that also is paralleled by its inhibition of protein kinase C (*EC* 2.7.1.37), a major cell signalling agent (Chatelain *et al.* 1993). The second study showed that, although vitamin E can significantly reduce the incidence of photocarcinogenesis in mice, the level of dietary *RRR*-α-tocopheryl acetate necessary to achieve this effect ultimately proved fatal to the animals (Gerrish & Gensler, 1993)!

Clearly, there is much to learn and understand about the behaviour of vitamin E at the cellular level. It is possible that free radicals are fundamentally involved in the regulation of cell growth and development (Rice-Evans & Burdon, 1993). If this is so, it suggests that high levels of antioxidants might be detrimental to cells, in which case cellular antioxidant levels would have to be controlled.

Cardiovascular disease

Epidemiological studies suggest that populations with high plasma levels of vitamin E have a lower risk of fatal heart disease (Gey *et al.* 1991). Very recent studies indicate that higher intakes of vitamin E may reduce the risk of coronary heart disease in both women (Stampfer *et al.* 1993) and men (Rimm *et al.* 1993), prompting serious consideration of recommending the use of supplemental vitamin E and other, so-called 'antioxidant vitamins' (vitamin C and β-carotene) as a therapeutic strategy for prevention of atherosclerosis (Steinberg *et al.* 1992).

Strong support for the potential of 'antioxidant drugs' comes from the discovery of the involvement of oxidized LDL in the pathogenesis of atherosclerosis (Carew *et al.* 1987; Steinberg *et al.* 1989). Although it is not known how LDL becomes oxidized, it has been shown in animal studies that orally available, synthetic chain-breaking antioxidants confer significant protection against the development of atherosclerotic lesions (Carew *et al.* 1987; Sparrow *et al.* 1992). Protection by dietary vitamin E against atherogenesis has been demonstrated in studies carried out on monkeys (Verlangieri & Bush, 1992) and rabbits (Williams *et al.* 1992). Vitamin E may confer protection against atherosclerosis through multiple mechanisms that operate at multiple sites at different stages of disease development. For example, vitamin E regulates platelet aggregation by inhibiting platelet cyclooxygenase (*EC* 1.14.99.1) activity and, thus, decreases prostaglandin (thromboxane) production (Meydani *et al.* 1993). This effect would be important in the latter stages of heart disease, when constriction of arterial blood flow increases the probability of thrombosis.

How might vitamin E act to prevent the early events of atherosclerosis, for example, the events that lead to oxidized LDL? One possibility relates to the fact that high dietary fat intake (including cholesterol) predisposes to heart disease. Surplus lipid delivered via the LDL receptor and lipoprotein lipase routes to the endothelial and smooth muscle cells in the arterial wall probably causes increased activity of the peroxisomes (i.e. free radical source 3; see p. 256) as a means of degrading and eliminating unwanted lipid. The resultant increase in oxidants, including oxy radicals and the free radical precursor H_2O_2, would lead to a decrease of cellular vitamin E and increased lipid peroxidation (rat liver peroxisomes have been reported to have low levels of vitamin E; Drevon, 1993). It is conceivable that the intimate physical association of LDL with these cells could lead to oxidation of LDL, either directly because of locally high levels of oxy radicals produced by the lipid-loaded cells, or by exchange of cellular lipid hydroperoxides into the LDL.

Deficiency diseases

In humans, chronic deficiency of vitamin E results in progressive neurological degeneration. In animals for which vitamin E tissue kinetics are known (Ingold *et al.* 1987; Burton *et al.* 1990), the tissues most affected by chronic vitamin E deficiency are those with longer-lived cells and with slower turnover of vitamin E.

The specific mechanistic details responsible for the various abnormalities caused by vitamin E deficiency are unknown. Generally speaking, deficiency symptoms almost certainly derive from a developing inadequacy of antioxidant protection, but the question remains: why are some tissues more susceptible to deficiency than others?

Given the long period of time it takes for deficiency symptoms to appear, free radical-mediated cell damage must occur at a rate that is too slow to affect tissues whose cells turn over rapidly. However, in some tissues with sufficiently long-lived cells, the progressive imbalance between free radical generation and antioxidant protection exists long enough for an accumulation of cellular damage to occur (e.g. to proteins). The developing deficiency reveals the tissues in which the imbalance is most marked. Factors affecting the imbalance include the rate at which free radicals are generated within the tissues and the rate at which vitamin E is lost. In humans, neural tissue is most prominently affected by vitamin E deficiency, although symptoms can take years to show. Most other human tissues appear to be well-protected.

The discovery that NO functions as an important cellular signalling agent warrants further study to determine if the cytochrome P-450-catalysed generation of the radical results in significant consumption of neural vitamin E.

The testicular degeneration observed in vitamin E-deficient male rats (Mason, 1933) may result from inadequate protection against free radicals generated unavoidably during cytochrome P-450-catalysed synthesis of testosterone (i.e. source 4; see p. 257) in Leydig cells. If this proves to be correct, then an important function of vitamin E in the testis is to protect tissues against the harmful effects of free radicals generated during a normal metabolic process, i.e. the synthesis of a steroid hormone.

SUMMARY

There is a growing body of evidence implicating free radicals in a wide variety of medical diseases and conditions, especially the diseases of ageing, such as cancer and cardiovascular disease, which appear to be ultimate expressions of long-term, cumulative and sustained cellular damage. Vitamin E is an excellent lipid-soluble, chain-breaking antioxidant in the presence of other co-operative antioxidants such as vitamin C or ubiquinol, but it can act as a pro-oxidant in their absence. Epidemiological findings and animal studies support the belief that vitamin E is protective against cardiovascular disease and possibly cancer.

The wide range of symptoms associated with vitamin E deficiency is consistent with a loss of antioxidant protection in those long-lived cells in which there is sufficient opportunity for accumulation of free radical damage. The cellular damage is proposed to arise from the generation of free radicals during normal aerobic metabolism. Some susceptible tissues may have enhanced levels of radicals that are produced, for example, by the action of cytochrome P-450 enzymes in steroidogenic tissues, or by the generation of NO in neural tissues.

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