HYPOTHESIS

THE BIOCHEMICAL MODES OF ACTION OF VITAMIN E AND SELENIUM: A HYPOTHESIS

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1. Introduction

It has previously been suggested that a biochemically active form of selenium may be selenide and that the selenide may comprise part of the active centre of an, as yet, uncharacterised class of catalytically active non-haem iron proteins that are protected from oxidation *in vivo* by vitamin E [1]. Ideally, any theory about the biochemical mode of action of vitamin E should explain its relationships with sulphur-containing amino acids and with polyunsaturated fatty acids, as well as with selenium, and the fact that certain synthetic antioxidants can mimic many of the nutritional effects of vitamin E. An attempt is therefore made in the present paper to develop the initial suggestions into a more comprehensive general theory.

2. Interactions of vitamin E with selenium

In a recent study of isotopically-labelled selenium in the liver organelles of rats receiving a limiting quantity of this element, it was found that the quantity of radioactive selenium behaving as acid-labile, protein-bound selenide was dependent on the presence of vitamin E in the animals' diet. Significantly more selenium was present as selenide when vitamin E was included in the diet than when it was absent [2].

The major excretory product found in selenium toxicity is a volatile substance which has been identified as dimethyl selenide [3, 4]. In this laboratory it has been found that this compound is trapped quantitatively by passage through 8 N HNO_3 in agreement with the work of Ganther [4], but that it is not trapped at all in 0.1 N $AgNO_3$. Conversely, hydrogen selenide is trapped quantitatively in 0.1 N $AgNO_3$ solution, but only to a small extent when it is passed through 8 N HNO_3. The acid-volatile selenium found in rat liver homogenates, and in isolated subcellular organelles, behaves similarly to hydrogen selenide and not like dimethyl selenide [5].

In further experiments on the selenium of rat liver, a zonal centrifugation technique was employed to achieve an improved separation of the subcellular organelles [6]. It was then observed that acid-labile selenide was particularly associated with the mitochondria, and with the smooth (and to a lesser extent the rough) endoplasmic reticulum in the livers of adequately-fed rats. This association was absent in vitamin E-deficient rats, but re-feeding of vitamin E to deficient rats for 5 days allowed the pattern of selenide distribution to return to normal. It was also found that there was a large increase in the total amount of selenium, and also in the proportion of selenide, in the smooth endoplasmic reticulum of the livers of vitamin E-deficient rats that were re-fed with vitamin E and given phenobarbitone simultaneously [7]. When vitamin E was not re-fed, this specific effect of phenobarbitone on the smooth endoplasmic reticulum was not seen. These various results appear to support the hypothesis that selenide may form part of the active centre of certain non-haem iron proteins [1].

In the absence of dietary vitamin E, the turnover of selenide-containing proteins would be expected to be increased because these demonstrably oxidation-sensitive proteins would not benefit from the protective, antioxidant action of vitamin E. It is relevant to note in relation to this suggestion that the quantity of

selenide recovered in the liver organelles of rats given ^{75}Se was greater when the organelles were isolated in the presence of both α-tocopherol and mercaptoethanol than when antioxidants were not used [2]. In unpublished experiments it has been found that α-tocopherol alone is capable of protecting the selenide from oxidation *in vitro*, although the protection afforded is somewhat less than when the two antioxidants were used together. It is suggested that α-tocopherol may exert a similar antioxidant function towards selenide *in vivo*. Lesions of the liver would then be expected to develop in vitamin E-deficient animals when the dietary supply of selenium becomes limiting. Metabolic failure resulting from the inadequacy of a selenide-depleted mitochondrial electron transfer chain may lead to the occurrence of liver lesions, and this effect might be exacerbated by an abnormality of some component of the microsomal electron-transfer chain that also depends on selenide. Since a major function of the latter system is to provide reducing equivalents for the cleavage of O_2 required for drug metabolism, faulty detoxication resulting from the absence of selenium would be expected to contribute to cell death. Whether the microsomal system actually contains a non-haem iron protein remains a matter for speculation [6], but the experiments with phenobarbitone [7] indicate the existence of a specific, vitamin E-dependent involvement of selenium in the smooth endoplasmic reticulum. When the demethylation of aminopyrine was recently investigated in microsomes prepared from the livers of vitamin E-deficient rats, it was found that this system exhibited altered kinetics as compared to normal [8]. This result is similar to those reported by Carpenter [9, 10] who has also observed abnormal kinetics for microsomal drug metabolism in vitamin E-deficient rats.

3. Interactions of vitamin E with polyunsaturated lipids

The explanation of the mode of action of vitamin E that has received the widest measure of support for many years is the antioxidant hypothesis [11]. This hypothesis has two important features: that the *only* function of vitamin E in biological systems is that of an antioxidant, and that the molecules protected from oxidation are *unsaturated lipids*. Support for the antioxidant hypothesis is provided by: i) the ability of synthetic antioxidants, which are otherwise unrelated to vitamin E, to substitute for the vitamin in preventing certain deficiency symptoms, ii) the inhibition by vitamin E of the autooxidation of unsaturated lipids *in vitro*. However, even though polyunsaturated lipids exacerbate many of the symptoms of vitamin E deficiency, there is nevertheless much accumulated evidence that the vitamin does not prevent the autoxidation of unsaturated lipids *in vivo* [12, 13]. By contrast, as outlined above, experimental work undertaken in this laboratory indicates that the antioxidant function of vitamin E *in vivo* may be to protect a reduced form of protein-bound selenium from oxidation. If the antioxidant function of vitamin E is indeed this, rather than the inhibition of the autoxidation of polyunsaturated lipids, why do increased dietary levels of polyunsaturated fatty acids undoubtedly increase the dietary requirement for vitamin E?

One possible explanation of this apparent paradox is that α-tocopherol may play a physico-chemical role in the stabilization of biological membranes that contain high levels of polyunsaturated fatty acids, by virtue of lipid–lipid interactions between the vitamin and the unsaturated fatty acids. It is relevant in this connection to note that α-tocopherol can be used as the hydrocarbon solvent for the preparation of bilayer membranes of phospholipid [14], and that α-tocopherol is itself capable of forming a thin membrane separating two aqueous phases [15]. Vitamin E, like squalene, phytol, vitamin K_1 and ubiquinone-30, inhibits the haemolysis of rabbit erythrocytes *in vitro* by retinol, while hydroquinone and *N,N'*-diphenyl-*p*-phenylene diamine are completely without effect [16]; long chain compounds, including vitamin E, also apparently modify the structure of colloidal aggregates of retinol in saline solution in such a way as to inhibit the autoxidation of retinol in the system [17]. Recently, Levander and Morris [18] have reported a stimulation of transport of rubidium ions in liver slices obtained from animals fed a diet containing vitamin E, or selenium, as compared with liver slices from vitamin E-deficient animals. Since they found no correlation between total lipid peroxidation and transport of Rb^+ ions, these workers proposed that vitamin E and selenium may help to maintain transcellular cation gradients by stabilizing membrane structures.

Molecular model building studies prompt us to suggest that vitamin E may stabilize membrane structure

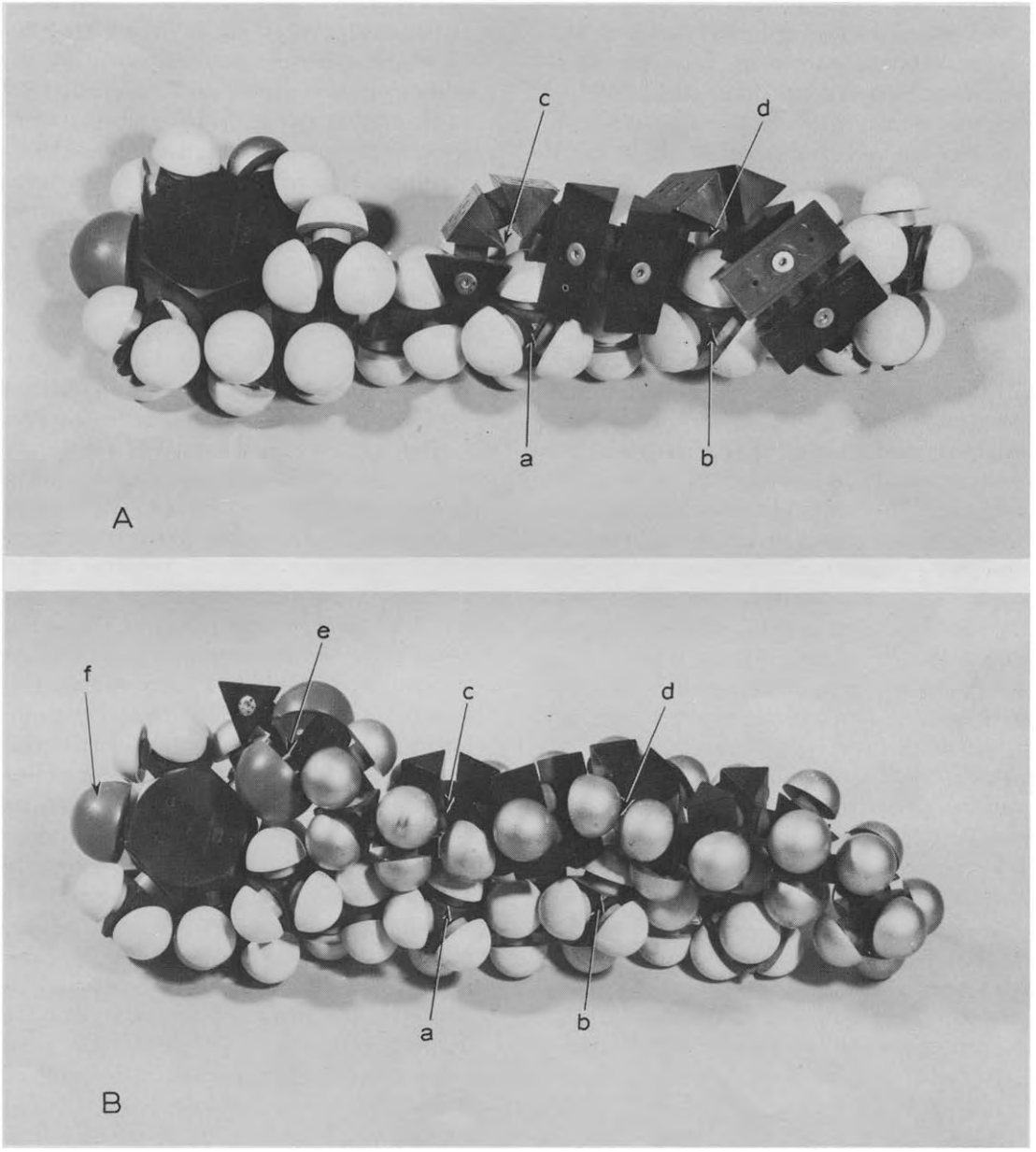

Fig. 1. Space-filling molecular models illustrating the way in which it is proposed that a phospholipid molecule containing an arachidonyl residue may interact with α-tocopherol in a biological membrane. (A) A model of part of the carbon skeleton of arachidonic acid (from C_4–C_{16}) aligned with a model of α-tocopherol to allow the methyl groups at $C_{4'}$ and $C_{8'}$ of the vitamin (arrowed: a and b) to fit into pockets created respectively by the Δ^5 and Δ^{11} *cis* double bonds of the fatty acid (arrowed: c and d). The quasi-helical conformation of the unsaturated arachidonyl chain that is required for the formation of the "complex" between the two molecules is clearly apparent. (B) A similar model (reproduced at a slightly lower magnification) in which the complete unsaturated chain of an arachidonyl phospholipid, with its hydrogen atoms, is shown interacting with α-tocopherol. The methyl groups of α-tocopherol, and the arachidonyl double bonds are labelled as before. The carbon skeleton of the glycerol moiety of the phospholipid (arrowed; e), and the hydroxyl group of α-tocopherol (arrowed; f) lie at the same end of the complex.

by virtue of a specific physico-chemical interaction between its phytyl side-chain and the fatty acyl chains of polyunsaturated phospholipids, particularly those derived from arachidonic acid. From our studies it would seem that significant interactions of α-tocopherol with unsaturated fatty acids are less likely to occur when the acyl chains of the latter are curved than when they are straight. Curving a *cis*-polyunsaturated acyl chain allows the carbon atoms to remain coplanar [19]. By contrast, when the overall configuration of the chain is straight, the constituent carbon atoms are twisted out of a common plane, and the chain assumes a quasi-helical appearance. Rosenberg [20, 21] has previously pointed out, in the case of the galactosyl diglycerides of chloroplasts of *Euglena*, that twisted chains may provide "pockets" for the accomodation of the protruding methyl groups of the phytyl chains of chlorophyll. In our model, comparable interactions between the methyl groups of the phytyl chain of α-tocopherol and the *cis* double bonds of the arachidonyl residues of membrane phospholipids are proposed. Thus, the methyl group at C_4, of α-tocopherol can fit into a pocket provided by the *cis* double bond nearest the carboxyl group. The methyl group at C_8, of tocopherol is then in register and able to interact similarly with the third *cis* double bond. In the "complex" formed, the hydroxyl group of the chromanol ring of α-tocopherol, and the polar groups of the phospholipid lie together at one end, where they would be expected to participate in polar interactions at the surface of any region of membrane having a lipid bilayer structure (fig. 1). The fit of the methyl groups in the pockets created by the *cis* double bonds permits the methylene groups in the backbones of both the phytyl and fatty acyl chains to associate closely, further promoting the stability of the complex through London–Van der Waals dispersion–attraction forces [20, 21].

Oleic acid, with only one *cis* double bond interacts little, if at all, in the way described because two methylene-interrupted *cis* double bonds are needed to form a "pocket". Linoleic acid may be expected to provide one interaction of the type envisaged. Good "complex" formation would be anticipated with acids containing three methylene-interrupted double bonds, and optimum "complex" formation with four double bonds as these can provide pockets for two of the methyl groups of the phytyl chain of tocopherol.

Complex formation may have the following functional consequences. First, an inhibition of the oxidative destruction of polyunsaturated fatty acids in cells and in cellular membranes occurring either *in vivo*, cf. Molenaar et al. [22], or more particularly for example as a result of treatment of erythrocytes with dialuric acid *in vitro*. Secondly, a reduction in the permeability of biological membranes containing relatively high levels of polyunsaturated fatty acids, particularly arachidonic acid.

Thirdly, the prevention of the degradation of membrane phospholipids *in vivo* by membrane-bound phospholipases [23–26]. If vitamin E facilitates the packing of polyunsaturated lipids in the way suggested, it would seem highly probable that membranes containing high levels of polyunsaturated fatty acids would suffer from breakdown by endogenous phospholipases in the absence of vitamin E. This would correspond to the degradation that occurs when normal mitochondria are treated with an excess of exogenous oleic or linoleic acid: mitochondrial swelling is then observed owing to the "activation" of an endogenous phospholipase A as a result of the added, unsaturated fatty acid disturbing the structure of mitochondrial membranes [23]. Breakdown of the vitamin E-deficient membranes by an endogenous phospholipase A may result in non-haem iron proteins located at the membrane surface becoming exposed to molecular oxygen. A spontaneous reaction between oxygen and the non-haem iron proteins might then produce oxygen radicals (O_2^-), in a manner similar to that known to occur in certain flavin-containing iron–sulphur proteins [27]. Once initiated, such radical reactions would probably cause damage among membrane-localized proteins, which could be prevented by small quantities of α-tocopherol or synthetic antioxidants.

We have calculated that the ratio of arachidonic acid residues to α-tocopherol is about 500:1 for erythrocyte membranes. Clearly, if vitamin E is to have a structural role in membranes, as well as behaving as an antioxidant, some explanation of the effectiveness of such low levels of α-tocopherol is required. It is therefore suggested that the majority of the unsaturated fatty acyl residues of membranes are most probably associated with cholesterol [28], and that only relatively few, structurally-differentiated, polyunsaturated fatty acids are associated with α-tocopherol. This differentiation may conceivably arise from asymmetry

in lipid bilayer membranes. For example, there is evidence that most of the cholesterol of myelin membranes is on one side only of the lipid bilayer [29], while Bretscher [30] has proposed that phosphatidyl choline and sphingomyelin are located chiefly in the outer half of the lipid bilayer in erythrocytes, with phosphatidylserine and phosphotidylethanolamine in the inner (cytoplasmic) half.

4. Nutritional effects of vitamin E

The several aspects of our theory are summarised diagramatically in fig. 2. The effects of vitamin E deficiency that are seen at the nutritional level, and the interactions of the vitamin with selenium, sulphur amino acids and polyunsaturated fatty acids may, according to our hypothesis, be explained either by membrane instability caused by the absence of vitamin E, or by metabolic failure of pathways that involve selenide- or sulphide-containing proteins, or by a combination of both causes. Synthetic antioxidants are regarded as exerting a protective effect toward the membrane-localized proteins that would otherwise be oxidised in the absence of vitamin E.

Using this framework as a basis, it is possible to put forward consistent biochemical explanations for all of the nutritional diseases associated with vitamin E deficiency. Thus, haemolysis of vitamin E-deficient rat erythrocytes by dialuric acid [31] may be regarded as essentially a "membrane" phenomenon, although the recently demonstrated involvement of selenium in the active centre of glutathione peroxidase [32] may also be important. The foetal resorption and testicular degeneration that are the classical signs of vitamin E deficiency in the rat may perhaps be caused by a failure in the biosynthesis of a key steroid hormone, which in turn arises from malfunction in non-haem iron proteins. Alternatively recent work [33] indicates that, in the absence of vitamin E, membrane disorganisation might itself result in foetal resorption. More probably, however, these syndromes may result from a combination of both causes. Dietary liver necrosis has been discussed above and elsewhere [6], and the pathogenesis of silver-induced toxicity may be caused by the exceptional affinity of silver ions for selenide [34].

Similar considerations may be applied to deficiency diseases in the chick. Thus encephalomalacia [35] is

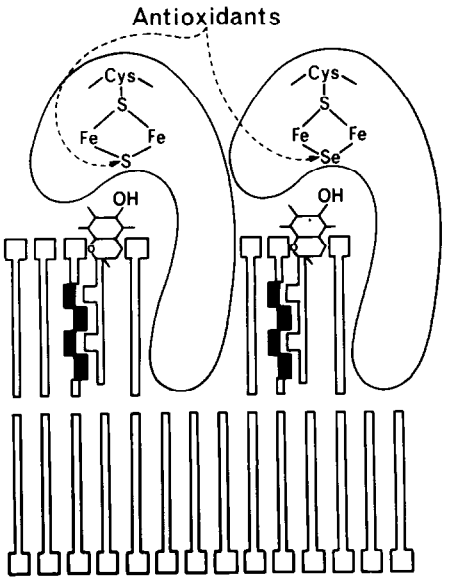

Fig. 2. A diagrammatic representation of the proposed interactions between vitamin E, synthetic antioxidants, selenide- and sulphide-containing proteins, and polyunsaturated phospholipids in a biological membrane. The suggested site of action of the synthetic antioxidants is shown by the arrows. For simplicity, the phospholipids are shown as rigid structures in a bilayer membrane, but this is not intended to imply that the membrane has a "crystalline" rather than a partially fluid character.

possibly caused in the first instance by the perturbing effects of linoleic acid on cerebellar membranes in the absence of vitamin E, while exudative diathesis [36] may be considered to be comparable in its biochemical aetiology to necrosis of the liver in rats. Muscular dystrophy [37] involves, we suggest, an accelerated turnover of sulphide-containing proteins that stems from the absence of the protective effect of α-tocopherol: the primary lesion in this desease may, however, be the disruption of membrane structure by polyunsaturated fatty acids.

5. Summary

In this paper recent experimental work on rat liver is discussed which is considered to indicate that a primary function of vitamin E *in vivo* may be to inhibit the oxidation of selenide-containing proteins present in mitochondria and in smooth endoplasmic

reticulum. On the basis of molecular model building studies, it is also suggested that the well-known nutritional relationships between vitamin E and dietary polyunsaturated lipids may be due to the occurrence within normal membranes of specific complexes between the vitamin and some of the molecules of polyunsaturated phospholipids. Without the vitamin, membranes may have an abnormally high permeability and they may be subject to degradation by endogenous phospholipases *in vivo*, as well as being abnormally susceptible to damage *in vitro* by dialuric acid and hydrogen peroxide.

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