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Some Speculations on the Mechanisms of the Vitamins E and K Starting from Origin of Life Considerations and the Antioxidant Theory

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Origin of life considerations can be useful for understanding function and mechanism in metabolism of present cofactors. An important role is postulated for chromanols such as tocopherol in the transition from anaerobic to aerobic metabolism, this transition being a critical point in evolution. The antioxidant and amphipathic properties of such molecules make them plausible candidates for dioxygen detoxification within membranes of the earliest respiratory systems. In the light of this, the possibility is examined that tocopherol still plays such a role of a detoxicating dioxygen carrier within membranes. In addition, a mechanism for vitamin K-dependent carboxylation is suggested in line with the proposal for vitamin E and in accord with the present state of experimental knowledge.

More than 50 years after the recognition of vitamin E as a required nutrient (*1*) and more than 30 years after the first postulation of an antioxidant role for vitamin E in biological membranes (*2*) its function is still controversial (*3–7*) in spite of numerous publications on the subject. Most authors seem to favor a role involving some sort of stabilization of membranes, especially those containing relatively many polyunsaturated fatty acids (mitochondrial inner membrane and endoplasmatic reticulum). There is less agreement about the way vitamin E fulfills this function. Many authors describe vitamin E as a (nonenzymatic) biological antioxidant (*8–10*), which inhibits the peroxidation of unsaturated fatty acids by molecular oxygen (autoxidation) acting in the same way as antioxidants *in vitro*, namely, by quenching initiation reactions. Others emphasize its role as a lipid-soluble autoxidation radical chain terminator (*6, 7*) (radical scavenger), but several other hypotheses exist in which enzymes play a role (*3, 5*). Still others claim a structural role for tocopherol (*11*) in membranes.

In this article the global position is taken that vitamin E by a variety of mechanisms minimizes directly or indirectly the deleterious effect of molecular oxygen (*12*) on polyunsaturated lipid membranes, especially those through which a continuous flow of dioxygen exists such as the inner mitochondrial and microsomal membranes. However, for the further development of this theme, it will be necessary also to consider vitamin E within the context of evolution.

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ORIGIN OF LIFE

Within the framework of origin of life considerations, one can conceptually divide present day enzymes into two categories as far as concerns the principles they use for catalysis.

The first category bears a prosthetic group or makes use of coenzymes (mostly nucleotides or nucleotide-like substances) that account for an important part of the lowering of the transition state free energy. These enzymes show often a fairly broad substrate specificity. Good examples of this type are found under the adenosylcobalamin-dependent enzymes, some of which catalyze for instance the isomerization of derivatives of the normal substrate substituted with methyl or other substituents on the carbon atoms directly involved in the reaction (*13*). Some will even catalyze the (irreversible) isomerization of the unnatural enantiomer of the normal substrate *with equal rate* (*14*). In this type of catalysis bonding of substrates—and especially transition states—to the chiral peptide part of the enzyme must be of lesser importance. These enzymes can be rationalized as being descended from primordial catalysts upon which, after the development of the genetic code, the polypeptide apoenzyme evolved in the beginning primarily to regulate the catalyst. Transition metals and complexes thereof, sugars and especially (poly)nucleotides (also sugar derivatives), together with solid minerals and noncoded polypeptides must have played important roles in primordial catalysis. These pre-genetic code "biocatalysts" are viewed as being the result of a relatively short but decisive evolutionary process in which ribonucleic acid-like molecules still combined the functions of regulator/catalyst and genetic information stores (*15*).

The most extreme forms of the second category of enzymes do not require nucleotide cofactors for activation and are highly specific, accepting often only one conformation of one enantiomer of a particular substance. Catalysis and specificity displayed by these types of enzymes are most easily understood with the concept of complementarity of the polypeptide enzyme surface to the transition state of the reaction to be catalyzed (*16*). The high substrate and reaction specificity which these enzymes are capable of is in fact also their limitation. Examples of this type can be found in many hydrolytic enzymes and in the biotin-dependent enzymes (*17*). These enzymes can be rationalized as being post-genetic code biocatalysts.

In the reductive primordial environment triplet dioxygen—continuously supplied by photolysis of water—had presumably an appreciable lifetime thanks to the spin inversion barrier (*18*), which makes most reactions with oxidizable compounds (which have almost always singlet ground states) forbidden. Special catalysts, capable of bypassing this barrier, are required for the reduction back to water, which is thermodynamically by far the most stable situation. There are two obvious ways to achieve such a bypass: either by providing one-electron reaction pathways or by complexing dioxygen in such a fashion that the spin restrictions are lowered or removed in the complex. Candidates for the first possibility are, among others, flavinoid compounds and for the second transition metal ions with complex spin states such as Cu(I) and Fe(II) with or without ligands like hemes,

tetrahydrobiopterins, and ascorbate. In the presumed ribonucleic acid evolution these catalysts assumed the function of dioxygen detoxicators, thus making possible the process of self-organization of oxygen-sensitive molecules. In present day metabolism these ancient, water-soluble, fairly highly reduced catalysts are partially maintained in the prosthetic groups of the polypeptide dioxygen-handling enzymes of the first category mentioned above. Superoxide dismutases can be considered to belong to this class (*19*); ascorbate/Fe^{2+}-dependent monooxygenases are other possible examples (*20*).

After the genetic code had evolved and polypeptide enzymes coded by nucleic acids had taken over (for the most part) the catalytic function from those nucleic acids, evolution toward the first organisms went on with progressing speed in a practically anaerobic environment. Only thereafter, in a time of extremely slow raising of the dioxygen concentration, abiogenic photolysis of water still being the source of dioxygen, the first primitive dioxygen-consuming respiratory systems could have been developed, organized in membranes. Present day peroxisomes are sometimes interpreted as being relicts of such systems (*21*). In this way dioxygen changed slowly from a general biocidal compound into one that is advantageous for life. Evolution to dioxygen-producing photosynthesis and dioxygen-consuming oxidative phosphorylation became subsequently feasible, thereby giving rise to a relatively steep increase in dioxygen concentration in the atmosphere. However, in the "peroxisome-like" stage, dioxygen-handling peptides for the first time became organized in complexes in membranes. In this stage there arose a need for a new type of dioxygen-handling molecular structure, again capable of bypassing the spin inversion barrier, but now fat soluble and functioning only as a carrier for a detoxicated form of dioxygen between separated active sites. A catalytic function was not necessary because the problem of specific catalysis had already been solved in this stage in the form of the endlessly variable polypeptide surfaces of the enzymes of the second category. Such a carrier would make possible the evolution of a dioxygen-based respiration system within membranes that had evolved in a practically anaerobic environment and so must have been vulnerable to dioxygen.

The goal of this article is—starting from the antioxidant theory for vitamin E—to trace the indications from chemistry and biochemistry whether this vitamin could (and still can) fulfill such a role, namely, that of being a carrier (between several active sites) for a detoxicated dioxygen species within membranes. Mechanistically such a vitamin E–dioxygen adduct would be also analogous to carboxybiotin, another prosthetic group of the second category (*17*), which transfers CO_2 within an enzyme complex to another active site. In both cases there is a stable intermediate and the cofactors can still not be viewed as catalysts in themselves.

TOCOPHEROL AS AN ANTIOXIDANT

It is generally accepted that autoxidation of polyunsaturated fatty acids proceeds by a radical chain, leading to hydroperoxides via end-on addition of

dioxygen to the pentadienyl radical R· formed as an intermediate (*22;* for a recent review see Ref. (*23*)):

$$\begin{aligned} RH &\rightarrow R\cdot + [H\cdot] && \text{initiation} \\ R\cdot + O_2 &\rightarrow ROO\cdot \\ ROO\cdot + RH &\rightarrow ROOH + R\cdot \end{aligned} \Big\} \text{propagation}$$

Antioxidants, typically phenols and aromatic amines, can retard this process by scavenging initiators and interrupting the propagation chain; this occurs through the formation of aroxyl radicals that are more stabilized by resonance than the pentadienyl radical. Possibilities for initiation of membrane peroxidation are manifold. Much recent work has been reported on initiators derived from the various dioxygen species (superoxide, singlet oxygen, hydrogen peroxide) like hydroxyl and alkoxyl radicals (*19, 24, 25*).

Besides these known processes there is of course another means of stopping uncontrolled peroxidation, namely, by excluding free dioxygen—the root of all evil—from vulnerable membranes. The chromanoxyl radical, formed by hydrogen abstraction from tocopherol, belongs to the general class of aroxyl radicals (*26*). In contrast to reactive radicals which are scavenged irreversibly by dioxygen or very stable ones like semiquinones and ascorbate radical anion, which fail to react with oxygen, the aroxyl radicals of moderate stability react quantitatively but reversibly with dioxygen via peroxy radicals forming peroxides, predominantly at the *para* position. This reaction is used for quantitative estimation of small amounts of dioxygen or of aroxyl radicals (*26*). In membranes with a relatively high amount of polyunsaturated fatty acids and of tocopherol, and with a steady flow of dioxygen such as for instance mitochondrial and microsomal membranes, the following reactions can be expected to occur (EH = tocopherol):

$$EH \rightarrow E\cdot + [H\cdot] \qquad \text{(tocopherol as an antioxidant)}$$

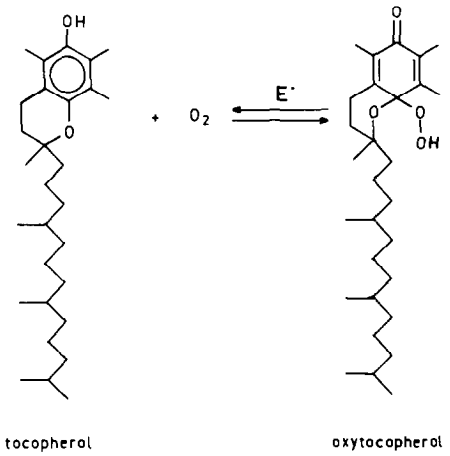
$$E\cdot + O_2 \rightleftarrows EO_2\cdot \qquad \text{(general reaction for aroxyl radicals)}$$

$$EO_2\cdot + EH \rightleftarrows EO_2H + E\cdot$$

The last reaction is a dehydrogenation of a phenol by a peroxide radical and is known to be exothermic by roughly 5 kcal/mol (*27*). Because there is no spin barrier in this scheme and activation energies are expected to be small, the reactions can safely be added to give the equilibrium of Eq. [1], in which it is assumed that dioxygen attacks the phenol ring *para* to the hydroxyl group. Although there is no direct evidence for reversible formation of such an oxytocopherol species, conclusive evidence for reversibility of oxygen addition at 40°C has recently been reported for the analogous case of methyl linoleate hydroperoxides (*28*). Whereas dioxygen exchange in that case must proceed via the somewhat less stable pentadienyl radical, reversible formation of an oxytocopherol species can be assumed a fortiori.

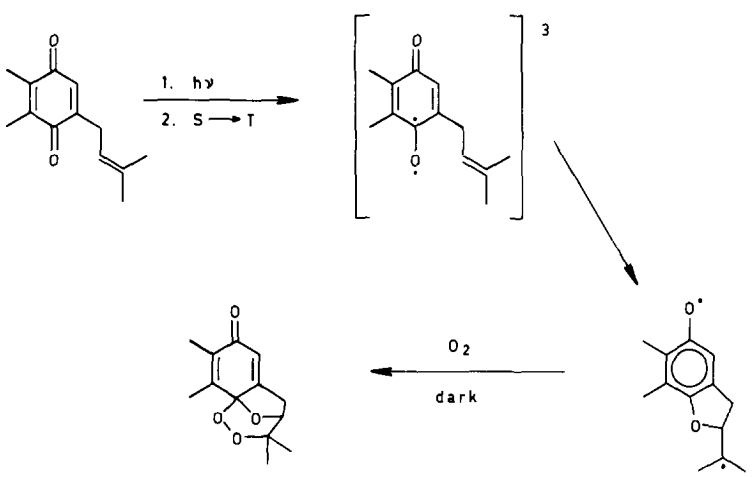
In this context a product from photochemistry of plastoquinone-1 is of interest.

(*29, 30*). After irradiation in isopropanol a triplet biradical is formed which closes into an aromatic biradical. In a subsequent dark reaction this biradical combines



[1]

with triplet oxygen (the singlet was excluded) to a product akin to oxytocopherol. Equation [2] shows the proposed reaction.



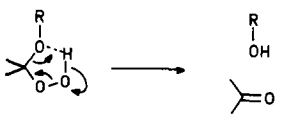
[2]

The form in which dioxygen would be found in membranes would depend to a first approximation on the ΔG of the equilibrium for Eq. [1] *in the ordered environment of a biomembrane*. An a priori estimation of this ΔG value is not easily made. On the one hand the loss of a certain amount of resonance energy is unfavorable but inspection of space-filling models suggests a compensating factor in that the hydroperoxide function fits in nicely, resulting in a molecule that very well can insert itself as a structural constituent of biomembranes, perhaps even better than

vitamin E itself. If oxytocopherol under natural circumstances were to be only a couple of kilocalories more stable than tocopherol plus free dioxygen then this would *automatically* mean that tocopherol in fact acts as a dynamic dioxygen carrier within the membrane[1] in addition to its role as a nonenzymatic antioxidant.

TOCOPHEROL AS A POSSIBLE CARRIER FOR A DETOXIFIED DIOXYGEN SPECIES

It seems to be becoming a rule that in cases where oxidation with dioxygen can proceed via a relatively stable radical (1,4-dienes, phenols, flavinoids), photooxidation and autoxidation give the same initial products. The autoxidation product of tocopherol postulated in Eq. [1] is also identical to the initial photooxidation product recently isolated by Foote and co-workers (*31*). Most of the $^1O_2$ is quenched by tocopherol (a possible additional biological role) but some $^1O_2$ reacts with it to form oxytocopherol as proposed in Eq. [1]. This compound is reported to be thermally stable for a period of hours to days before it decomposes to tocopherylquinone and tocopherylquinone epoxide (*31*). These products are those expected if oxytocopherol behaves the same as other α-hydroperoxyethers, which are very effective oxenoids (*32*), and which presumably react in a manner analogous to peroxyacids, Eq. [3].

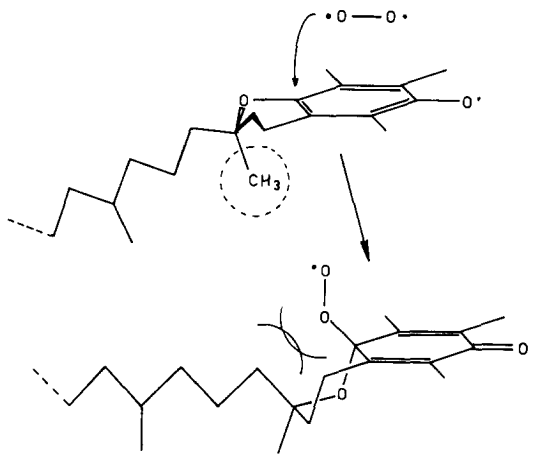


[3]

In fact, oxytocopherol appears surprisingly stable for an α-hydroperoxyether capable of self-epoxidation. This stability can be rationalized by conformational analysis of the proposed molecule in the membrane. The addition of dioxygen to the vitamin E chromanoxyl radical involves an important conformational change. In the membrane the preferred conformation of tocopherol and its radical should be that with the dihydropyran ring in the half-chain conformation with the saturated tail in a pseudoequatorial position. This conformation leads to the best shape for the molecule to fit in the membrane parallel to other fat molecules. Addition, which is readily reversible, of dioxygen can in principle lead to two diastereomeric forms for the oxytocopherol radical. However, as the lower side of the vitamin E radical (see Eq. [4]) is effectively shielded by the pseudoaxial methyl group addition is anticipated to occur exclusively from the less hindered top side. Because the dienone ring in the product can only assume an equatorial position, the incoming dioxygen stays in an axial position. In consequence, the saturated tail is forced toward an axial position and is pressed against the peroxy radical substituent. Similar reasoning applies for the oxytocopherol molecule

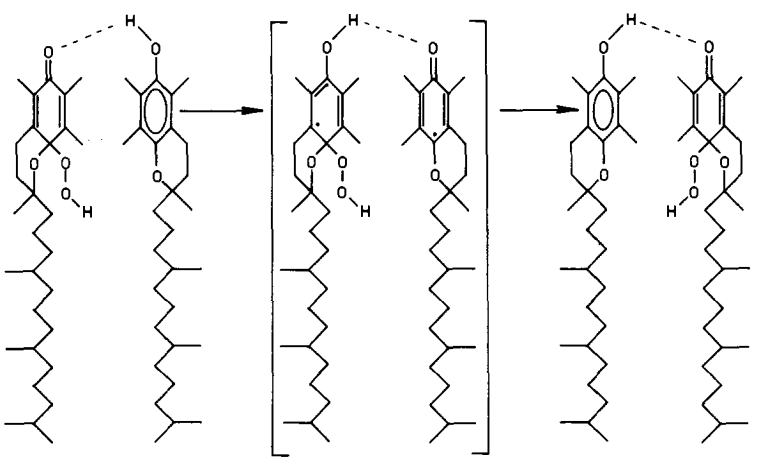
[1] By "carrier" in this paper is thus not meant an entity that accelerates transport of a substance *across* a membrane, but one that carries a substance and transports it *within* membranes via *lateral* diffusion.

which means that the hydroperoxy function cannot attain easily the



[4]

intramolecular five-membered transition state (Eq. [3]) for the oxenoid reaction, which would result in destruction of the molecule. The demonstrated stability of oxytocopherol makes vitamin E a plausible candidate for dioxygen detoxification within membrances in the course of the evolution of the first respiratory systems. As an integral part of the membrane it can participate in the free lateral diffusion process. In such a way a continuous dioxygen flow in membranes containing polyunsaturated fatty acids can be maintained. The tocopherol concentration in membranes varies greatly but runs to a certain extent parallel to that of the polyunsaturated fats and to the need of dioxygen transport (*3, 5*). At places where the tocopherol concentration is high, especially in the direct neighborhood of dioxygen-consuming enzymes, an additional transport mechanism might be important as pictured in Eq. [5], in which the hydroperoxide function directly is transferred via a quinhydrone-like complex or as a pair of



[5]

radicals. Such a reaction would be greatly promoted by the parallel position of the reactants in the membrane. The general scheme is reminiscent of other "bucket-brigade mechanisms" such as those of plastoquinone and ubiquinone for electrons (*32*), of lipoyl residues on pyruvate dehydrogenase complex for acyl groups and electrons (*34–38*), and of hemoglobin for dioxygen transfer (*39*). Although via an entirely different molecular mechanism and in a different environment, the last case is particularly interesting since hemoglobin might have had the same function in the origin of life (dioxygen transport and detoxification). Because of the low solubility of dioxygen in water relative to hydrophobic solvents, hemoglobin has become primarily a dioxygen transport molecule whereas for tocopherol dioxygen detoxication is of primary importance.

The transition from anaerobic to aerobic metabolism has caused drastic changes in metabolism. If tocopherol indeed played the role sketched above in the origin of life, then it would be very unlikely that the reaction of Eq. [1] in the further course of evolution would not have come under the catalyzing and regulating influence of enzymes. Perhaps the plant enzyme tocopherol oxidase, which catalyzes the destruction of tocopherol by O_2 into a variety of unknown products (*40*), is in fact such a tocopherol dioxygenase. In addition one would expect oxytocopherol to have evolved into a normal group-transferring coenzyme for a number of oxidases, oxygenases, lipoxygenases, cyclooxygenases, and peroxidases, making it unnecessary to releave dioxygen before it is ultimately used. Perhaps the synergistic effect between tocopherol and the selenoenzyme glutathione peroxidase stems from such a relation (*41*). From an evolutionary point of view it would also seem more likely that the important peroxide tone in membranes (*42*) is under enzymatic control via tocopherol rather than that peroxidation is only prohibited by the nonenzymatic antioxidant action of tocopherol.

Finally, the chemically closely related chromanols of ubiquinone and plastoquinone can have a similar function in mitochondria and in chloroplast membranes, respectively.

MECHANISM OF VITAMIN K-DEPENDENT CARBOXYLATION

For the last several years it has been clear that vitamin K_1 (2-methyl-3-phytyl-1,4-naphthoquinone), **1**, is directly involved in the post-translational modification of a number of proteins by carboxylation of peptide-bound glutamate (Glu) residues. The so-formed γ-carboxyglutamate (Gla) residues are active in Ca^{2+} binding in an increasing number of proteins found to be involved in blood clotting and bone formation. The subject has recently been reviewed extensively (*43–46*). Gla residues have also been found in kidney and renal stones (*45*), in ribosomal proteins (*47*), and in blood plasma proteins which have an unknown function (*43*). Although the mechanism of these carboxylation reactions is not known, several aspects have now become clear (*43*):

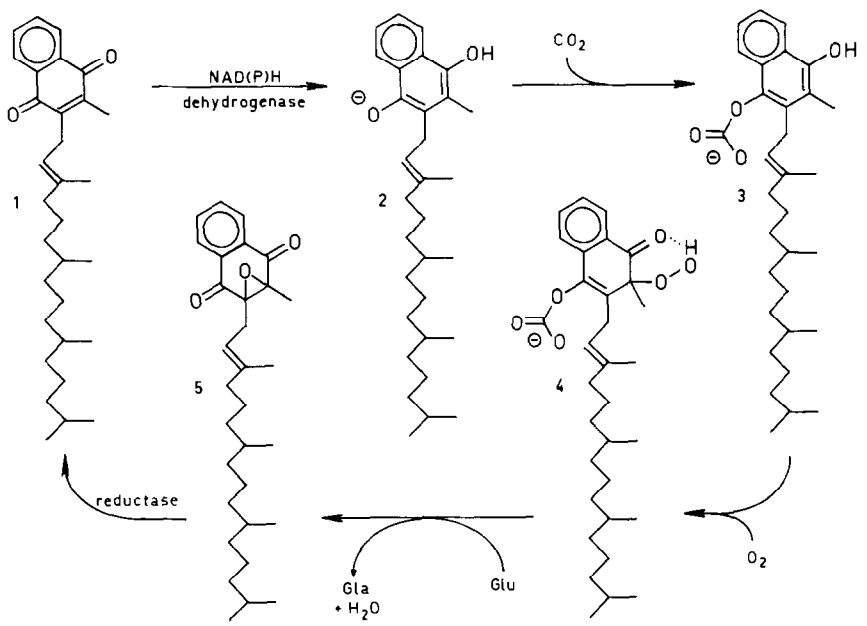
—The active form of the vitamin is dihydrovitamin K_1, **1**, synthesized by a NAD(P) dehydrogenase (EC 1.6.99.2) (*48*).

—The system requires CO_2 (not $HOCO_2^-$) and O_2 (*49*).

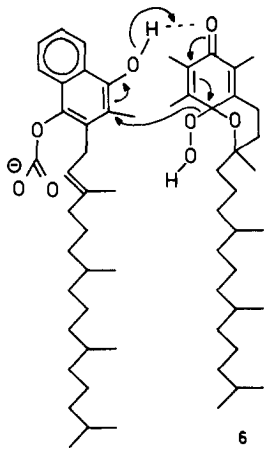
—There is evidence for the involvement of vitamin K_1 epoxide in the carboxylation scheme. This long-known metabolite can be recycled to vitamin K_1 by a special reductase (*50*).

This proposal is built in addition on the assumption that vitamin K_1 is an integrated part of the endoplasmatic reticulum membrane system, in which it has a dynamic position thanks to its phytyl tail, just like vitamin E. The oxygen dependence of the system strongly suggests that dihydrovitamin K_1 acts in its chromanol form just like vitamin E. However, neither dihydrovitamin K_1 chromanol or chromenol is active in the carboxylation reaction (*43*). A chromanol is a quinol compound with one of its OH groups bound as an ether. An analogous compound, however, can be an intermediate in the carboxylation scheme (quinol ester instead of quinol ether), which leads to a mechanism as pictured below.

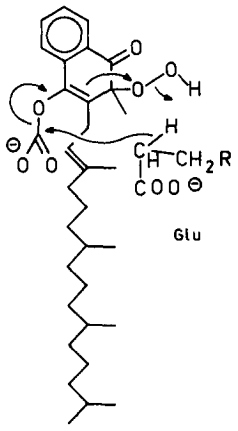
After reduction of vitamin K_1 to dihydrovitamin K_1 anion, **2**, the cofactor is ready to pick up a CO_2 molecule from the membrane, leading to carboxydihydrovitamin K_1, **3**, which is a chromanol derivative that can react with a molecule



of oxygen in two ways. Alternatively, carboxydihydrovitamin K_1 can get dioxygen directly from oxyvitamin E, in which case structure **4** becomes the more feasible one for oxycarboxydihydrovitamin K_1. When **3** and oxyvitamin E, **6**, are lined up next to each other in the lipid bilayer, transfer of dioxygen gives automatically **4** because the hydroquinonoid functional group of **3** has a skew orientation toward oxyvitamin E, **6**. The same intermediate oxycarboxydihydrovitamin K_1, **4**, is mentioned in Ref. (*43*) in a different scheme, in which it is formed from hydrogen peroxide. It is there considered untenable because catalase does not inhibit the reaction and hydrogen peroxide does not replace the oxygen requirement. With vitamin E as dioxygen carrier, however, there is no need for hydrogen peroxide as an intermediate.



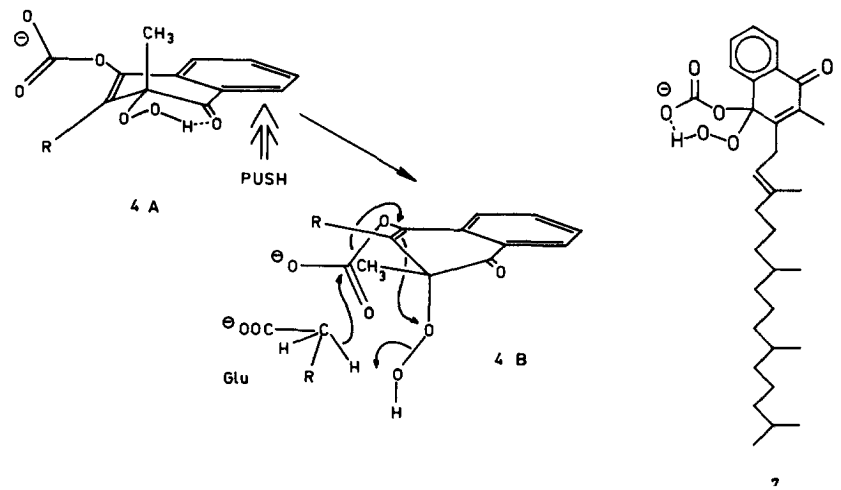
3



4

Reaction of 4 with the substrate, a glutamate residue of a protein, can then be pictured as above.

In the carboxylation the leaving group OH^- is used as a base to create an enolate ion which is subsequently carboxylated by the enol carboxylate, leaving K_1 as epoxide, 5. Reduction of vitamin K_1 epoxide to 1 closes the cycle. The proposed intermediate 4 is specially suited for a shuttle function between several active sites, not only thanks to its tail, but also through the conformational mobility in the partly saturated six-membered ring. Two half-chair conformations are possible, 4A and 4B. Conformation A is the lowest in energy because there is the least steric hindrance between the tail (R) and the methyl group, and because of the intramolecular six-membered ring hydrogen bond between the hydrogen peroxide function and the keto group. In this conformation the molecule is presumed to travel between the active sites. The orientation of the hydroperoxy



function (pseudoequatorial) is not proper for epoxide formation in **4A**. The enzyme (or a part of the peptide substrate) can, however, easily activate **4A** by pushing against the benzene ring in the right direction. Because the direction of the tail is presumed to be fixed in the membrane, the molecule flips into the other possible conformation of higher energy. In this conformation there is steric interaction between the methyl group and the tail and this eclipsing is also present in the epoxide. The intramolecular hydrogen bond is broken and the hydroperoxy group has now the right orientation (pseudoaxial) for a stereoelectronically feasible epoxide formation.

Of course, reaction of carboxydihydrovitamin K_1 with molecular oxygen cannot be excluded and thus the second possible oxycarboxydihydrovitamin K_1, **7**, cannot be excluded either. But **7** seems less apt to react with the voluminous substrate (a polypeptide) than **4**; moreover, **7** can be expected to be a reactive oxenoid (*32*) and decarboxylation of **7** would lead directly to vitamin K_1 (and not the epoxide) and hydrogen peroxide, which thus far has not been found as a product.

The mechanism proposed here is in accord with the recently found inhibition of glutathione peroxidase (*51*), a nonspecific reductase for organic hydroperoxides. Presumably, **4** or **6** or both are substrates for that enzyme. In addition, the mechanism places vitamin K_1 just like vitamin E in the group of cofactors which by virtue of their tails function as shuttles between several active sites. In speculations around the origin of life they belong to the group of nonribonucleotide cofactors that arose in metabolism after the evolution of the genetic code and the first polypeptide enzymes, at a moment that there was already life on earth (*17, 52*).

The above proposals concerning the relation between the vitamins E and K and dioxygen, based as they are for the most part on speculations and circumstantial evidence, are only put forward to raise some new questions on the mechanisms of action of these important metabolites. Such questions are: What is the initial product of autoxidation of tocopherol in membrane-like conditions? What is the position of the equilibrium between tocopherol, dioxygen, and that product? Is this equilibrium catalyzed and regulated by enzymes? Are there other enzymes for which "oxytocopherol" acts as a coenzyme? Is there a relation between tocopherol and the vitamin K-dependent carboxylation system?

REFERENCES

1. H. M. Evans and K. S. Bishop, *Science* **56,** 650 (1922).
2. H. Dam and H. Granados, *Acta Physiol. Scand.* **10,** 162 (1945).
3. I. Molenaar, J. Vos, and F. A. Hommes, *Vitam. Horm.* **30,** 45 (1972).
4. R. H. Wasserman and A. N. Taylor, *Annu. Rev. Biochem.* **41,** 179 (1972).
5. J. G. Bieri and P. M. Farrell, *Vitam. Horm.* **34,** 31 (1976).
6. W. A. Pryor (Ed.), "Free Radicals in Biology," Vol. I, pp. 1–49. Academic Press, New York, 1976.
7. J. F. Mead, "Free Radicals in Biology" (W. A. Pryor, Ed.), Vol. I, pp. 51–68. Academic Press, New York, 1976.

8. A. L. TAPPEL, *Fed. Proc. Fed. Amer. Soc. Exp. Biol.* **24,** 73 (1965).
9. M. K. HORWITT, *Fed. Proc. Fed. Amer. Soc. Exp. Biol.* **24,** 68 (1965).
10. A. L. TAPPEL, "The Fat-Soluble Vitamins" (H. F. DeLuca and J. W. Suttie, Eds.), pp. 369–74. Univ. of Wisconsin Press, Madison, 1970.
11. J. A. LUCY, "Tocopherol, Oxygen and Biomembranes" (C. de Duve and O. Hayaishi, Eds.), pp. 109–120. Elsevier, Amsterdam, 1978.
12. C. DE DUVE, "Tocopherol, Oxygen and Biomembranes" (C. de Duve and O. Hayaishi, Eds), pp. 351–361. Elsevier, Amsterdam, 1978.
13. T. TORAYA, T. SHIRAKASHI, T. KOSUGA, AND S. FUKUI, *Biochem. Biophys. Res. Commun.* **69,** 475 (1976).
14. J. RÉTEY, A. UMANI-RONCHI, AND D. ARIGONI, *Experientia* **22,** 72 (1966).
15. L. E. ORGEL AND J. E. SULSTON, "Prebiotic and Biochemical Evolution" (A. P. Kimball and J. Oró, Eds.), p. 89. North-Holland, Amsterdam, 1971.
16. L. PAULING, *Chem. Eng. News. nr.* **10,** 1375 (1946).
17. C. M. VISSER AND R. M. KELLOGG, *J. Mol. Evol.* **11,** 171 (1978).
18. H. TAUBE, "Oxygen," Proc. Symp. N.Y. Heart Assoc., p. 29. Little, Brown, Boston, 1965.
19. I. FRIDOVICH, *Science* **201,** 875 (1978).
20. C. M. VISSER, *Bioorg. Chem.* **9,** 261 (1980).
21. C. DE DUVE, "Tocopherol, Oxygen and Biomembranes" (C. de Duve and O. Hayaishi, Eds.), pp. 351–361. Elsevier, Amsterdam, 1978.
22. G. SCOTT, "Atmospheric Oxidation and Antioxidants." Elsevier, Amsterdam, 1965.
23. J. F. G. VLIEGENTHART, *Chem. Ind.*, 241 (April 1979).
24. J. WILSHIRE AND D. T. SAWYER, *Accounts Chem. Res.* **12,** 105 (1979).
25. C. DE DUVE AND O. HAYAISHI (Eds.), "Tocopherol, Oxygen and Biomembranes." Elsevier, Amsterdam, 1978.
26. A. R. FORRESTER, J. M. HAY, AND R. H. THOMPSON, "Organic Chemistry of Stable Free Radicals," pp. 281–341. Academic Press, London/New York, 1968.
27. S. W. BENSON, *J. Chem. Educ.* **42,** 502 (1965).
28. H. W.-S. CHAN, G. LEVETT, AND J. A. MATTHEW, *Chem. Commun.* **1978,** 756.
29. D. CREED, H. WERBIN, AND E. T. STROM, *Chem. Commun.* **1970,** 47.
30. D. CREED, H. WERBIN, AND E. T. STROM, *J. Amer. Chem. Soc.* **93,** 502 (1971).
31. R. L. CLOUGH, B. G. YEE, AND C. S. FOOTE, *J. Amer. Chem. Soc.* **101,** 683 (1979).
32. J. REBEK, JR., AND R. MCCREADY, *Tetrahedron Lett.* **1979,** 1001.
33. R. BENTLEY AND I. M. CAMPBELL, "The Chemistry of the Quinonoid Compounds" (S. Patai, Ed.), Vol. 2, pp. 683–736. Wiley, London, 1974.
34. M. J. DANSON AND R. N. PERHAM, *Biochem. J.* **159,** 677 (1976).
35. G. B. SHEPHERD AND G. G. HAMMES, *Biochemistry* **16,** 5234 (1977).
36. D. C. SPECKHARD, B. H. IKEDA, S. S. WONG, AND P. A. FREY, *Biochem. Biophys. Res. Commun.* **77,** 708 (1977).
37. J. H. COLLINS AND L. J. REED, *Proc. Nat. Acad. Sci. USA* **74,** 4223 (1977).
38. D. L. BATES, M. J. DANSON, G. HALE, E. A. HOOPER, AND R. N. PERHAM, *Nature (London)* **268,** 313 (1977).
39. P. F. SCHOLANDER, *Science* **131,** 585 (1960).
40. E. MURILLO, S. E. PLUMPTON, AND J. K. GAUNT, *Biochem. Soc. Trans.* **4,** 486 (1976).
41. W. G. HOEKSTRA, *Fed. Proc.* **34,** 2083 (1975).
42. M. E. HEMLER, H. W. COOK, AND W. E. LANDS, *Arch. Biochem. Biophys.* **193,** 340 (1979).
43. R. E. OLSON AND J. W. SUTTIE, *Vitam. Horm.* **35,** 59 (1977).
44. J. STENFLO AND J. W. SUTTIE, *Annu. Rev. Biochem.* **46,** 157 (1977).
45. P. V. HAUSCHKA, J. B. LIAN, AND P. M. GALLOP, *Trends Biochem. Sci.* **3,** 75 (1978).
46. J. STENFLO, *Advan. Enzymol.* **46,** 1 (1978).
47. J. J. BUSKIRK AND W. M. KIRSCH, *Biochem. Biophys. Res. Commun.* **80,** 1033 (1978).
48. R. WALLIN, O. GEBHART, AND H. PRYDZ, *Biochem. J.* **169,** 95 (1978).
49. J. P. JONES, E. J. GARDNER, T. C. COOPER, AND R. E. OLSON, *J. Biol. Chem.* **252,** 7738 (1977).
50. D. S. WHITLON, J. A. SADOWSKI, AND J. W. SUTTIE, *Biochemistry* **17,** 1371 (1978).
51. A. E. LARSON AND J. W. SUTTIE, *Fed. Proc.* **37,** 1588 (abstr. 1754) (1978).
52. C. M. VISSER AND R. M. KELLOGG, *J. Mol. Evol.* **11,** 163 (1978).