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Abstract

Vitamin E was one of the last fat-soluble vitamins to be discovered. We provide here an historical review of the discovery and the increasingly more detailed understanding of the role of α-tocopherol both as an antioxidant and as a structural component of phospholipid bilayer membranes. Despite the detailed descriptions now available of the orientation, location, and dynamics of α-tocopherol in lipid bilayers, there are still gaps in our knowledge of the effect of α-tocopherol and its potential receptors than control gene transcription.

The discovery of vitamin E and the trouble with diets

When first observed by Evans and Bishop [1] and Mattill [2] in 1922 (and re-described shortly after by Barnett Sure [3]), it was already clear that the anti-sterility substance later known as vitamin E, was a hydrophobic compound. The animals fed the synthetic diets lacking "substance X" could have their fertility restored by increasing the proportion of butterfat in the diet from 9 % to 24% by weight. The addition of ground whole wheat also restored fertility, and this would be made clear three years later when a vitamin E rich fraction was obtained from wheat germ by extraction with various organic solvents. Evans and Burr [4] noted that the vitamin is "almost completely miscible with solvents representing such a range as methyl alcohol, ethyl alcohol, ether, pentane, benzene, acetone, ethyl acetate, carbon disulfide, etc.". Thus, vitamin E would join vitamins A and D as fat soluble nutrients. The same researchers would later correctly deduce the molecular formula of tocopherol as $C_{29}H_{50}O_2$ from allophanate derivatives (addition products

of tocopherol with isocyanic acid) [5]. The same derivatives would later be separated and denoted as β- and ɣ-tocopherol.

The correct chemical structure of vitamin E (α-tocopherol) was soon after announced by Fernholz in 1938 [6] and supported by others [7]. From the methods of isolation and structural identification, vitamin E was noted to be resistant to heat, to treatment with acids or bases, but marginally susceptible to oxidation when treatment with base did not exclude oxygen.

Researchers who first investigated the relation of vitamin E to animal sterility were well aware that rescuing infertile animals with synthetic diets was dependent on how the diet was prepared. Using vitamin E from wheat germ oil, Evans and Burr [8] noted that "When lard is increased from 7 per cent to 22 per cent, other constituents being the same though changed in proportions…initial fertility practically disappears." However, "If fats containing Vitamin E (*e.g.*, butter) are fed in the same high proportion (22 percent) perfect fertility remains throughout life." Mattill also noted that when diets contained portions of lard or cod liver oil "vitamin E…was made less effective by the presence of these two unsaturated fats." [9]. And further that, "It was not a case of critical level of intake of vitamin E but of apparent destruction or inactivation of this accessory by the lard, a phenomenon for which we had no explanation." Anderegg and Nelson [10] also noted that synthetic diets made from skim milk powder and containing added fat in the form of cod liver oil frequently could not rescue infertile animals. If, however, the fish oil was mixed with the wheat oil (the source of vitamin E) not at the beginning, but rather the fish oil was fed separately, the animals experienced reproductive success. The fish oil was noted to go rancid rather quickly. Similar results were reported by Supplee and Dow [11]. Later work focusing on a muscular dystrophy arising from vitamin E deficiency would confirm the loss of vitamin E when premixed with cod liver oil [12-14] and that this could be alleviated if the oil was first hydrogenated [15, 16]. The manner by which this caused confusion in the early work on vitamin E was reviewed by Mason [17].

The link between dietary fats and fertility, nutritional muscular dystrophy, and colored fatty-tissue pigments (ceroid), was soon understood to be dependent on the degree of fat unsaturation, which was known to accelerate rancidity. Burr and co-workers [18-21] would show that

tocopherol stabilized body fats of rats and pigs, and Mason and Filer [17] opined that this should likely extend to phospholipids. The chemistry of lipid autooxidation was known to produce peroxides [22, 23], but the mechanism of how vitamin E might prevent their formation was unknown.

Action as an antioxidant

The observation that vitamin E was acting as an antioxidant was made quite soon after its discovery in the fertility assays [9, 24, 25] but it was some years before the explanation of the oxidative chemical processes involved in fat rancidity would be made in any chemical detail [26-31]. Even with the acknowledgment of tocopherol's antioxidant action and its protection of the more easily oxidized polyunsaturated groups of free fatty acids, fats, and lipids, researchers made no distinction between the protection of bulk fats and vegetable oils and the tissues/adipose of animals, and certainly not the membranes of cells which was still a developing concept. Interestingly, the protection of bulk lipids by antioxidants (including tocopherol) would later be studied in great detail by food chemists who discovered the so called "polar paradox". Generally, lipophilic antioxidants like tocopherols are more efficient in emulsions, whereas hydrophilic antioxidants like ascorbic acid or Trolox are better in bulk oil systems. At issue are the supramolecular structures such as micelles and the placement of polar antioxidants at the interface of bulk lipid and air [32-36]. Today we know that the observations of tocopherol loss in animal diets made with bulk lard and fish oils are due to the presence of lipid hydroperoxides and their ability to form radicals that would destroy the added tocopherol. The process of lipid peroxidation (or the breakdown of already present hydroperoxides) eventually consumes all of the vitamin E present in the synthetic diets, and none would remain for the animals to ingest.

The discovery of the antioxidant behavior of tocopherol by Olcott, Mattill, and co-workers through the 1920s and 1930s [9, 24, 25] would be followed some 20 years later by more thorough chemical explanations of its possible in vivo role as an antioxidant [26-31]. The idea that phospholipid bilayer membranes might be the more important in vivo location for the protective effect of tocopherol, while discussed previously [17, 37] would follow shortly thereafter [38-41].

Tocopherol in membranes: the early years

Opinions about the molecular behaviour of tocopherol in lipid membranes have evolved alongside our understanding of the structure of the lipid bilayer itself. Indeed, tocopherol played an important part in the development of the modern notion of cell membrane structure, even as its physiological role was being debated.

From the latter half of the 19th century, there emerged the idea of a diffusion barrier bordering cells [42], later postulated as a bilayer lipid membrane [43]. Danielli and Davson [44] constructed the first hypothetical model of the membrane architecture in 1935, where a cellular membrane was composed of a bimolecular sheet of phospholipids with their hydrocarbons towards the interior, with both faces of the bilayer covered and strengthened with a molecular layer of electrostatically bound protein. "Boundary lipids" could mean lipids thought to be bound to the protein layer for enzymatic or other reactive purposes [45, 46].

The 1960s was still the era of the "unit membrane" theory of the cell boundary [45]. The resolving power of the electron microscope was of little use as the fixative requirements of membranes introduced unknown perturbations, and most micrographs were still interpreted in light of this theory.

As for tocopherol's location in this model, Silber et al. wrote in 1969, "whereas the phenolic group is responsible for the vitamin's reactivity with free radicals, the fatty acid component may account for its solubility in the lipid-rich membrane of the erythrocyte. There is evidence suggesting that cell membranes are formed from repeating lipoprotein units having hydrophobic lipid-protein interactions. It is likely that α-tocopherol would have a much greater affinity for the membrane than for the polar aqueous interior of the erythrocytes." [47].

In 1962 Mueller reported the formation of the first black lipid film (BLF) [48]. Analogous to the black soap films of Isaac Newton, Mueller used ox-brain lipid extract to form a stable, bimolecular lipid membranes under water, approximately 10 nm thick membrane across a 10 mm^2 opening. The term "black" referred to their dark optical properties when viewed stretched across the opening. By introducing conductance promoting proteins, they were able to measure

membrane electrical resistance and capacitance of this "excitable lipid membrane", opening whole new areas for model membrane research to probe the properties of the lipid bilayer itself [49, 50].

Interestingly, what made the BLF possible was the addition of copious amounts of tocopherol [50, 51]. The first stable lipid BLF required the use of a "plasticizing solvent" in the typical chloroform-methanol solvent system; liquid hydrocarbons such as tetradecane, mineral oil, squalene, or α-tocopherol to prevent solidification and allow for drying of the films without rupture. The terminology of "plasticizer" implies that tocopherol increases the flexibility or fluidity of the membrane, also thought to be a function of cholesterol. Mueller found that tocopherol was the best, in quantities of 20-40% of the lipids [51]. In such quantities, it's difficult to know the true significance of the many bilayer conductance experiments of the era [52].

Against this backdrop of the early 1960's, the antioxidant theory of tocopherol was still establishing itself, although the details of exactly what molecules needed reducing by the vitamin were still being debated. The Swiss chemist Paul Karrer wrote in 1960 that "We are fairly well informed about the physiological functions of the water-soluble vitamins because they are parts of enzymes, the reactions of which are pretty well known. On the other hand, we have very little information about the biological role of the fat-soluble vitamins." [53].

It was understood from nutritional studies that the site of cellular damage in vitamin E deficiency was at the membrane, and researchers were forced to recognize some connection with the degree of poly-unsaturated lipids found in the organism's diet. A. L. Tappel in 1962 wrote "[t]his chemical knowledge (of free-radical olefin peroxidation chain reaction) compels us to accept the fact that animals that contain the very oxygen-labile polyunsaturated lipids require lipid anti-oxidants. This biological essential of a lipid antioxidant is mainly filled by vitamin E." [30]. However, Tappel took the idea that "the loss of some fatty acids, vitamin A, carotene, etc. would appear to be of minor significance compared to lipid peroxidation damage to structural and functional components of the cell." [30]. Despite this acknowledgement of the change of structural integrity of the membrane, there was the view, and an abundance of evidence, that the

antioxidant property of tocopherol was to reduce sulphur and selenium containing proteins and enzymes [30].

In 1964, Jack Lucy looked more closely at the role of the fat-soluble vitamins, A, E, and K_1, and we find perhaps one of the earliest considerations of how molecular structure relates to antioxidant mechanisms. The "physiological actions of fat-soluble vitamins may be concerned with the 'membrane- active' properties of these substances"; that "inhibition (of haemolysis) is a feature of compounds having a long isoprenoid chain" [54].

We find that tocopherol has from the beginning played a role alongside cholesterol in the development of theories of how small molecule inclusions alter the bilayer's thickness-area relationship. By the later part of the 1960's, biophysics research was rapidly formulating new theories of lipid organization in the bilayer. Experimentally, monolayers at the air-water interface allowed for studying the molecular interactions of tocopherol with various lipids, from which the famous condensing effect of cholesterol was first discovered [55]. Viewed from the perspective of these fragile mono-molecular films, we first find the idea that "tocopherol may play an important role in regulating the stability of these membranes" in a very analogous way to cholesterol [56].

Tocopherol in membranes: The later years

By 1970, there were an estimated 8000 papers on vitamin E published in the previous 20 years, with no clear insight to its biological function [57]. In 1972, the evidence for tocopherol was summarized by Molenaar *et al.* as leading to four distinct theories: as a nonenzymatic biological antioxidant, as a factor in enzyme-dependent lipid peroxidation, as a factor in biological oxidations or oxidative phosphorylation, or as a membrane stabilizer with redox capacities [57].

This very influential last view was put forward the previous year by Diplock, Baum, and Lucy. Diplock and Lucy had set out to argue for the selenium and sulphur redox activity of tocopherol and noted that "[t]he redox function of the molecule, being localized in the hydrophilic chromanol ring structure, would be expected to be associated with polar rather than non-polar

residues of membrane proteins; in a region of membrane having a bilayer structure, the redox function would presumably be capable of acting at or near the membrane surface." [58]

Lucy and Diplock followed this idea with the first molecular-level biophysical model of tocopherol in a lipid bilayer. Drawing together the evidence of the liquid-like properties of tocopherol's hydrocarbon tail, its "filler" solvent properties in BLM, and the comparison with cholesterol's disruption of the ordered organization of the acyl chains of saturated lipids (without implying that cholesterol's hydroxyl group must have a specific chemical reaction), Lucy proposed that tocopherol stabilizes membranes though "specific physicochemical interactions between its phytyl side chain and the fatty acyl chains of polyunsaturated phospholipids, particularly those derived from arachidonic acid." [59, 60]

Lucy used a space filling molecular model to show hypothetical "complexes" formed when the methyl groups of the phytyl tail of tocopherol fit into pockets formed from *cis* double bonds of the lipid acyl chains. The greater the degree of unsaturation, the more pockets for the four methyl groups; good "complex" formation may be anticipated with acids containing three methylene-interrupted double bonds, and optimum "complex" formation with four double bonds, since these can provide pockets for two of the methyl groups of the phytol chain of tocopherol." [59]

At this same time, the classification of membrane proteins as peripheral or extrinsic versus integral or intrinsic was initially proposed by Singer and Nicolson [61] in their "fluid mosaic" model which forms the basis of the modern theory of the membrane [45, 46]. Lucy and Diplock do not make this distinction, however, their diagrams fit in both systems. Indeed, for the next several decades, Lucy and Diplock's molecular models had a profound influence on the thinking of subsequent research.

Using lysosome leakage and monolayer films, Fukuzawa *et al.* tested Lucy and Diplock's idea that it was the "isoprenoid side chains of α-tocopherol, not the property of hydroxyl group, may play an important role in the stabilization of membranes." [62] Monolayer films expanded due to dispersion in the molecular orientation of the lipid acyl chains; and the full length of tocopherol's isoprenoid side chain was needed to reduce lysozyme leakage. These observations seem to

support the model of important steric interactions between the hydrophobic tails of tocopherol and lipid. However, removal of the 6-hydroxyl group 'destabilized' all the same systems, indicating the chroman ring was equally essential.

A counter example would be Cushley *et al.* where α-tocopherol was described as a "*membrane destabilizer"* (italics in original). [63] The presumed cause is the large area requirements of the branched phytyl chain incorporated in the bilayer; the decrease in London-Van der Waals dispersion forces between lipid chains "is proportional to the inverse fifth power of the intermolecular distance. Thus, even a small lateral expansion of the lipid bilayer would result in a large decrease in the interaction energies of adjacent lipid molecules." Vitamin E "simply decreases the attraction of one lecithin molecule for an adjacent".

Fukuzawa disagreed and continued to compare the "condensing and fluidizing effects" of tocopherol, upholding the analogy with cholesterol [64]. Expanding the fatty acyl chains "stabilized" lysosomes (less permeable to small molecules) and that "cholesterol may function as a partial substitute for tocopherol." The discrepancy could be caused "by a difference in depth of the polar head groups", an idea we'll return to below in later years.

Meanwhile, researchers were still searching for molecular mechanisms of tocopherol's antioxidant activity, although now discussion incorporates both the OH group and the phytyl chain in how the vitamin is distributed throughout the membrane. Molecular diagrams of the process begin to incorporate pictures placing tocopherol in the structural context of the bilayer [65]. Nakano *et al.* (1980) noted that "fatty acid radicals may be protected from destructive peroxidation either by rapid chain termination … fortified by the molecular packing effect of the side chain in vitamin E within polyunsaturated phospholipid membrane." [66]

Diplock and Lucy's model was firmly in mind when Burton and Ingold wrote in 1981 "The major, and possibly the only, role of the phytyl moiety of the tocopherols would therefore seem to be to increase the solubility of the hydroxychroman moiety in those regions of biological systems which require protection against autoxidation, e.g., biomembranes." [67].

As far back as 1967 the notion that vitamin E has a membrane presence and that its presence could have a structural influence on a membrane system [56]. However, it would take the next 55+ years to achieve the picture, albeit a murky one, of vitamin E's membrane presence. We know that the majority of the non-adipose pool of vitamin E is found in cellular membranes [68] thus directing much biophysical efforts towards the understanding of tocopherol in phospholipid bilayer membranes. The fluidizing properties of vitamin E continued to develop with an emphasis on lipid-specific interactions. In the 1980's, differential scanning calorimetry investigations of tocopherol in saturated lipid membranes by Massey et al. and Ortiz et al. largely supported some of the previously hypothesized behaviours [69, 70]. Specifically, added vitamin E depressed the gel to fluid transition enthalpy, broadened the transition, and eliminated the pre-transition – all features characteristic of the behaviour of cholesterol, but with a greater effect. The transition was completely obliterated by concentrations as low as 20 mol % [70]. Moreover, these works provided evidence that vitamin E orientates parallel to the lipid moieties and prefers to associate with more disordered phases, with all these trends driven by lipid packing limitations induced by the phytyl chain.

Through the 1990s, preference of vitamin E for disordered environments was endorsed by spectroscopic methods [71]. Deuterium NMR investigations by Wassall *et al*. and fluorescence by Stillwell *et al.* demonstrated the cholesterol-like nature of vitamin E to fluidize ordered phases, while decreasing fluidity and increasing order of liquid crystalline states [72, 73]. In addition, oxidation studies suggested a tighter association exists between tocopherol and unsaturated lipid environments; a characteristic of oxidation-sensitive environments. Despite their findings, Stillwell et al. advise that "[t]he effects of α-tocopherol on membranes are complicated and very phospholipid dependent" [73].

Sánchez-Migallón and colleagues demonstrated non-uniform mixing behaviour of tocopherol with variously unsaturated heteroacyl lipids of biological relevance [74]. In their work, they allude that "[recent studies] suggested an inhomogeneous distribution in membranes with a 'lateral clustering' of α-tocopherol. If the distribution of α-tocopherol is really not homogeneous, this may have important consequences not only in order to interpret the results obtained when studying its interaction with membranes, but also with respect to the biodispersion of the vitamin

in biomembranes, where it may concentrate mainly in particular domains" [74-77]. Of course, this proposition aligns well with the idea of cell membranes functionalized by cholesterol-rich lipid rafts that was proposed around the same time by Simons and Ikonen [78].

At the turn of the century, as researchers continued to explore these trends, it became increasingly evident that vitamin E has clear biases for lipid interactions that may stem from its molecular shape. Theories that were proposed over a decade earlier were corroborated with high-resolution X-ray diffraction studies. As a case in point, Wang and Quinn identified a strong aversion of tocopherol for lipids with phosphatidylethanolamine headgroups, regardless of the lipid tail structure [79, 80]. This was followed by studies by Bradford et al., that measured a large negative spontaneous curvature for α-tocopherol (-13.7 Å) which would induce local curvature stressed when paired with similarly negative curved lipids, such as phosphatidylethanolamines [81].

Decades of progression in the understanding of interactions between vitamin E and lipid membranes fueled new propositions of nonantioxidant biological roles of tocopherol which may emanate from its physical influence on membrane properties. Tocopherols were hypothesized to be involved in protein regulation, gene regulation, and cellular trafficking with many articles referencing the lack of conclusive biological evidence to support an antioxidant role [82-84]. The level of speculation lead Azzi to claim in 2007: "A number of lines of evidence, evolutionary, genetic, biochemical, and functional, have indicated that the natural function of α-tocopherol is that of cell signaling. Such a property is not shared by any other antioxidant molecule" [82].

Research continues to uncover interesting facets of tocopherol's membrane presence. With many historical parallels to cholesterol, studies emerged to understand how they may coexist in biological membranes. Tocopherol was found to be able to destabilize cholesterol-rich lipid domains by acting in a linactant-like fashion at domain interfaces [85]. DiPasquale et al. demonstrated that at lower concentrations (albeit significantly higher than physiological) this effect is much less pronounced and likely isn't a factor in the complex milieu of living systems [86]. However, as with most effects, if higher local concentrations can be achieved through clustering these effects may serve as a compelling compliment to an antioxidant mechanism.

As our comprehension of biomembranes structure and behaviour develops, biophysicists have become more so appreciative of the intricacies that manifest at the physiological and mechanistic level. It's enticing to anticipate if the biophysical characteristics of vitamin E can overcome its limitations as an antioxidant, or if these properties may incite new roles that are critical to cellular homeostasis.

As the debate of vitamin E's in vivo antioxidant action continues in the literature, emphasis on the location of tocopherol has emerged as a key question to understanding its biological role. As far back as the 1980's efforts were being employed to understand the location of tocopherol in simple lipid bilayers. Using ^{13}C enriched α-tocopherol and employing ^{13}C-NMR Ingold and co-workers determined that tocopherol resides in both leaflets of egg PC bilayers and orients its hydrophilic chromanol group near the lipid-water interface [68]. With the use of NMR shift reagents, they were able to assign a minimal depth where the tocopherol head group resides, noting that the C5-methyl group located in the inner leaflet was at a minimum 40 Å from the lipid-water interface of the outer leaflet.

One issue biophysicists have faced when studying the location of tocopherol in lipid bilayers over the subsequent decades has been inconsistencies in the literature. These discrepancies seem to arise from the choice of lipid system and the technique being used to identify the location. Throughout the 1990's reports on the location of tocopherol in lipid bilayers made their way into the growing body of literature. With the exception of some one-off experiments [87], the majority of the work revolved around strategies employing bulky probes such as spin labels and fluorescence probes. These experiments yielded inconsistent and often contradictory results depending on the nature of the probe and lipid system being investigated. Atkinson, Epand and Epand produced a comprehensive review on tocopherol in membranes which highlights these inconsistencies [83].

In the late 2000's and early 2010's a series of studies aimed unambiguously and without the use of probes determine the location of tocopherol in lipid membranes were undertaken. Using a deuterium labelled C5-methyl on tocopherol (α-[5-$^{2}H_3$]-tocopherol) and employing the non-

traditional, yet powerful, technique of neutron diffraction, the location tocopherol was determined in a survey of lipid membranes. The first report of this endeavour came in 2010 [76] and presented preliminary data for monounsaturated systems and subsequent work reporting on a collection of saturated, mono-unsaturated, and polyunsaturated PC lipids (2013 and 2015) [88, 89], and 14:0 PC (2014) [90], as well as different head groups and lipid backbones (2015) [91]. This broad survey showed that the C5-methyl is relatively invariant to the phospholipid environment and located at or near the lipid-water interface (with the exception of DMPC), a location largely consistent with the first reports from ^{13}C-NMR in 1985 [68]. Ultimately, an antioxidant mechanism for Vitamin E that correlates strongly with its physical depth in the lipid bilayers was demonstrated [88].

Despite the revealing results from neutron diffraction experiments, efforts continued to locate tocopherol. In 2017 and 2018 Ausili *et al.* [92, 93] used phospholipids labeled with the fluorescent quenching doxyl moiety and X-ray diffraction, to qualitatively reproduce the probe-free observations by Marquardt *et al.* [91] Notably, Ausili's and Marquardt's work demonstrate that the location of tocopherol is largely independent of the bilayer fatty acid chain unsaturation. As with any study, systematic errors are present and some discrepancies, on the order of angstroms, arise. Nevertheless, these recent publications utilizing very different approaches to determining the location of vitamin E in lipid membranes seem to arrive at similar conclusions. The same cannot be said for the similar efforts of the 1990's.

Some effort has been made to study tocopherol using molecular dynamics simulations. However, the development of MD force fields depends on the recapitulation of experimental data, and with decades of inconsistencies progress has largely been slowed. Many of these computational studies aim to address experimentally convoluted facets of the behaviour of vitamin E in membranes, such as the dynamic processes of lateral diffusion and flip flop [94].

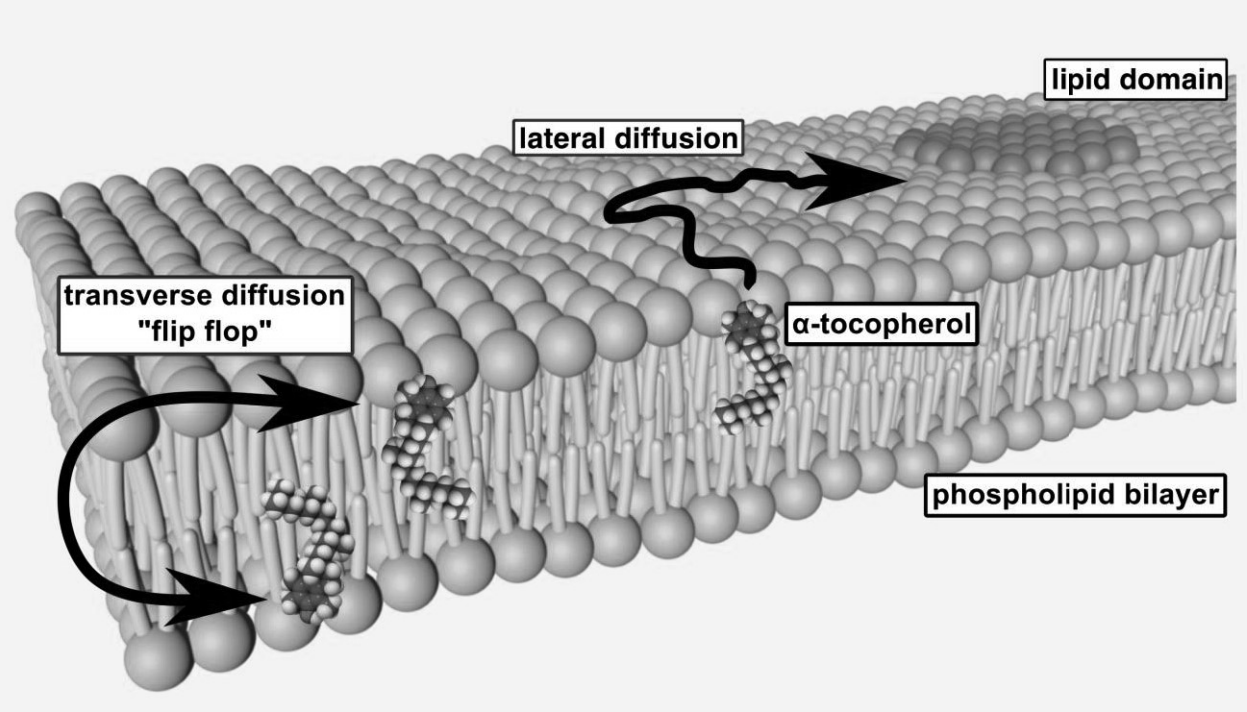

Figure 1. Tocopherol played an important role in developing the fluid model of the lipid bilayer. Within the membrane, individual molecules can move in several ways; they may diffuse laterally within a leaflet, or flip from one leaflet to another. The rates of these motions are influenced by integral proteins and lipid domains.

Tocopherol makes the news

Owing to the lipophilic and in vitro antioxidant properties of vitamin E, along with its global approval by consumer safety authorities, tocopherols have become the choice preservative in a vast array of consumer products, from supplements to foods and cosmetics. As our understanding of the role of tocopherols develops, so does our understanding of risks associated with overconsumption of vitamin E [95]. In fact, the upper intake limit has been dramatically decreased in recent years, with new evidence supporting a further decrease [96].

In the 21st century uses of more stable analogs of vitamin E, such as α-tocopherol acetate, have contributed to a resurgence of health concerns. Decades of research have illuminated a stark contrast in the membrane behaviour of α-tocopherol acetate compared to its natural counterpart [69, 97-100], Most notably, inclusion of vitamin E acetate in vape products has been correlated to the epidemic of e-cigarette/vaping associated lung injury (EVALI) that came into the limelight

in late 2019 [101, 102]. The lung condition EVALI, which largely affected the young adult population, remains somewhat of a medical enigma. Current belief is that one component of the disease stems from a physical interaction of vitamin E with the lipid monolayer system of the pulmonary surfactant [101, 102]. The pulmonary surfactant that lines alveoli in the lungs is responsible for reducing the high surface tension of respiration to minimize the work of breathing and prevent airspace collapse during exhalation. One such explanation that has emerged is the destruction of membrane elasticity in the presence of even low doses of vitamin E acetate [103]. Using neutron spin-echo spectroscopy on lipid mimics of pulmonary surfactant, DiPasquale and team described a decrease in membrane elasticity upon the addition of vitamin E acetate that can make the monolayer more prone to collapse, consistent with the symptoms of EVALI.

Conclusion

Despite all the progress made in a century's worth of research, the biophysical behavior of vitamin E remains a topic of much interest. We have advanced from isolating an unknown nutrient required for reproduction in rats, to pin-pointing the exact location of vitamin E in a model phospholipid membrane. However, it seems that much remains to be explained, including the possible non-antioxidant role of α-tocopherol and other members of the tocol family, and the identification of receptors that could govern the effects observed on gene transcription. We trust this will not require another century to explain.

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