Interactions of Tocopherols and Ubiquinones with Monolayers of Phospholipids

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1. The penetration of α -tocopherol and seven of its derivatives, and five compounds in the ubiquinone series, having differing chain lengths, into monolayers at the air/water interface of 11 different synthetic phospholipids and cholesterol was investigated; the properties of mixed monolayers of the tocopherols and of ubiquinones with phospholipids were also studied. 2. Penetration of α -tocopherol into diarachidonylglycerolphosphorylcholine was approximately constant for molar ratios of tocopherol/phospholipid ranging from 0.4:1.0 to 2.0:1.0. 3. Tocopherols with shorter or longer side chains than α -tocopherol had a lesser ability to penetrate monolayers of phospholipid molecules with 16 or more carbon atoms in their acyl chains. 4. All the tocopherols penetrated more readily as unsaturation in the phospholipids was increased, and their penetration into mixed monolayers of phospholipids was greatly facilitated by the presence of relatively small quantities of unsaturated phospholipid molecules. 5. There was relatively little interaction between the tocopherols and cholesterol, or between the ubiquinones and phospholipids. 6. The possible significance of the observed interactions between α -tocopherol and polyunsaturated phospholipids is discussed in relation to the biochemical actions of α -tocopherol *in vivo*. 7. It is suggested that fluidity of the lipid bilayer in membranes containing polyunsaturated phospholipids may allow α -tocopherol to interact in a dynamic manner with a number of phospholipid molecules.

According to the antioxidant hypothesis, the primary function of α -tocopherol *in vivo* is prevention of the destructive peroxidation of polyunsaturated lipids (Tappel, 1962, 1972). Green & Bunyan (1969) have, however, drawn attention to a number of observations on vitamin E and selenium that led them to question the validity of this hypothesis. Some of these objections have now been resolved by the work of Hoekstra (1973), which has shown that the enzyme glutathione peroxidase contains selenium (cf. Diplock, 1976). Further, the studies by Little & O'Brien (1968) showed that lipid peroxides are substrates for this enzyme.

Nevertheless, the fact that the rate of destruction of trace quantities of α -[^{14}C]tocopherol in the tissues of vitamin E-deficient animals is not increased by dietary polyunsaturated fatty acids (Green *et al.*, 1967) indicates that the nutritional interaction between α -tocopherol and unsaturated lipids cannot be ascribed solely to an antioxidant mechanism. On the basis of molecular-model building, it was there-

fore proposed that α -tocopherol may physically stabilize biological membranes that are rich in polyunsaturated phospholipids: this stabilization might arise from interactions between the phytol side chain of α -tocopherol and the polyunsaturated fatty acyl residues of phospholipid molecules in the hydrophobic regions of biological membranes (Lucy, 1972; Diplock & Lucy, 1973).

Monolayers of phospholipids at the air/water interface provide a suitable experimental system with which to investigate molecular interactions occurring in an oriented molecular array. This model system has been used in the present paper to investigate interactions of different phospholipid molecules with α -tocopherol and seven of its derivatives, and with compounds in the ubiquinone series. The results of these studies are consistent with the hypothesis that α -tocopherol may play a role in the stability of biological membranes containing polyunsaturated phospholipids, and they indicate that the molecular interactions concerned depend both on the nature of the fatty acyl chains of the phospholipid molecules and on the lengths of the side chains of the tocopherols.

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Materials and Methods

Synthetic derivatives of α -tocopherol and ubiquinone (Table 1) were a gift from Roche Products Ltd. (Welwyn Garden City, Herts., U.K.). Synthetic phospholipids from commercial sources were of the highest purity available and were used without further purification; dioleoyl-, dilinoleoyl-, dilinolenoyl- and diarachidonoyl-glycerolphosphorylcholine were from Serdary Research Laboratories (London, Ont., Canada); dipalmitoylglycerolphosphorylcholine and dipalmitoylglycerolphosphorylethanolamine were from Koch-Light Laboratories (Colnbrook, Bucks., U.K.). Dioleoylglycerolphosphorylethanolamine was from Supelco Inc. (Bellefonte, PA, U.S.A.). Distearoyl- and 1-stearoyl-2-oleoylglycerolphosphoryl-

choline were a gift from Dr. R. A. Demel (University of Utrecht). Dilauroyl- and dimyristoyl-glycerolphosphorylcholine were from Sigma (London) Chemical Co. (London S.W.6., U.K.). The phospholipid preparations were divided into small portions which were stored under N_2 in sealed ampoules. Before using a sample of phospholipid, its u.v.-absorption spectrum (100 μ g/ml in ethanol) was determined. The oxidation indexes ($A_{233}:A_{215}$), as defined by Klein (1970), for the unsaturated phospholipids used were 0.06, 0.08, 0.1 and 0.08 for dioleoyl-, dilinoleoyl-, dilinolenoyl- and diarachidonoyl-glycerolphosphorylcholine respectively.

Purest spectroscopic-grade light petroleum (b.p. 60–80°C) and chloroform, which were used as spreading solvents, and AnalaR cholesterol were from

Table 1. *Chemical structures of tocopherols and ubiquinones*

Compound*	Polar head group	Hydrophobic side chain
α -T-0	2,2,5,7,8-Pentamethylchroman-6-ol	None
α -T-2	2,5,7,8-Tetramethylchroman-6-ol	$\begin{array}{c} \text{CH}_3 \\ \\ (-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}-)_2-\text{CH}_3 \end{array}$
α -T-3 (α -tocopherol)	2,5,7,8-Tetramethylchroman-6-ol	$\begin{array}{c} \text{CH}_3 \\ \\ (-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}-)_3-\text{CH}_3 \end{array}$
α -T-4	2,5,7,8-Tetramethylchroman-6-ol	$\begin{array}{c} \text{CH}_3 \\ \\ (-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}-)_4-\text{CH}_3 \end{array}$
α -T-5	2,5,7,8-Tetramethylchroman-6-ol	$\begin{array}{c} \text{CH}_3 \\ \\ (-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}-)_5-\text{CH}_3 \end{array}$
α -T-6	2,5,7,8-Tetramethylchroman-6-ol	$\begin{array}{c} \text{CH}_3 \\ \\ (-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}-)_6-\text{CH}_3 \end{array}$
α -T-7	2,5,7,8-Tetramethylchroman-6-ol	$\begin{array}{c} \text{CH}_3 \\ \\ (-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}-)_7-\text{CH}_3 \end{array}$
α -T-9	2,5,7,8-Tetramethylchroman-6-ol	$\begin{array}{c} \text{CH}_3 \\ \\ (-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}-)_9-\text{CH}_3 \end{array}$
Q-0	2,3-Dimethoxy-5-methyl-1,4-benzoquinone	None
Q-3	2,3-Dimethoxy-5-methyl-1,4-benzoquinone	$\begin{array}{c} \text{CH}_3 \\ \\ (-\text{CH}_2-\text{CH}=\text{C}-\text{CH}_2)_3-\text{H} \end{array}$
Q-Phy	2,3-Dimethoxy-5-methyl-1,4-benzoquinone	$\begin{array}{c} \text{CH}_3 \\ \\ (-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}-)_3-\text{CH}_3 \end{array}$
Q-7	2,3-Dimethoxy-5-methyl-1,4-benzoquinone	$\begin{array}{c} \text{CH}_3 \\ \\ (-\text{CH}_2-\text{CH}=\text{C}-\text{CH}_2)_7-\text{H} \end{array}$
Q-9	2,3-Dimethoxy-5-methyl-1,4-benzoquinone	$\begin{array}{c} \text{CH}_3 \\ \\ (-\text{CH}_2-\text{CH}=\text{C}-\text{CH}_2)_9-\text{H} \end{array}$

* The terminology used is based on that given in *Biochem. J.* (1975) **147**, 11–14, 15–21.

BDH Chemicals (Poole, Dorset, U.K.); the solvents were further purified through alumina. All water used was double-distilled in an all-glass apparatus (final distillation over alkaline KMnO_4). Measurements of surface pressure and surface potential were recorded simultaneously with the aid of automated equipment that has been described previously (Maggio & Lucy, 1975, 1976).

All experiments were performed in duplicate or triplicate on a subphase of water at $27 \pm 1^\circ\text{C}$. Reproducibility in the force-area isotherms was within $\pm 1 \text{ mN} \cdot \text{m}^{-1}$ ($\pm 1 \text{ dyn} \cdot \text{cm}^{-1}$) for surface pressure, and $\pm 0.03 \text{ nm}^2$ per molecule for surface area. Measurements of surface potential were reproducible within $\pm 10 \text{ mV}$.

Interactions between the derivatives of α -tocopherol or ubiquinone and phosphatidylcholine were studied in two ways. In one type of experiment, we measured the increase in surface pressure that occurred at constant area when a tocopherol was injected into the subphase below a monolayer of phospholipid, which was spread at a surface pressure that was greater than the collapse pressure of the tocopherol. For these studies a quantity of phospholipid, which was usually enough to cover about half of the total surface area (96 cm^2) of the subphase in the Teflon trough after compression (see below), was spread on the subphase. About 2 min was allowed for evaporation of the solvent. The monolayer was then compressed (at a constant rate of $18.4 \text{ cm}^2/\text{min}$) with a Teflon barrier to the required value of surface pressure. A tocopherol or ubiquinone, dissolved in ethanol (final concentration 1 mM), was injected below the compressed phospholipid monolayer while the subphase was briefly stirred with a magnetic stirrer. Subsequent changes in surface pressure and surface potential were recorded on a chart recorder, without stirring, as described earlier (Maggio & Lucy, 1976). Penetration of α -tocopherol and related compounds into monolayers of phospholipid was very rapid, and equilibrium values of surface pressure were usually reached within 1 or 2 min. Final readings were taken after 5 min. Ethanol injected into the subphase did not produce any variation in the surface properties studied.

In other experiments, the mean molecular area per molecule and mean surface potential per molecule were plotted as functions of the molar composition of the mixed monolayers. These plots were compared with those obtained by using theoretical values for the two surface properties, which were calculated by using the additivity rule for ideally mixed films (Gaines, 1966; Shah, 1970); the formulae used were those given previously (Maggio & Lucy, 1976). To prepare mixed monolayers for these studies, solutions of the individual components were pre-mixed in the appropriate volumetric ratios before spreading in a monolayer.

Results

α -Tocopherol and its derivatives

The isotherms for surface pressure-area and surface potential-area for α -tocopherol (α -T-3) and four related compounds are shown in Fig. 1(a), which shows that the force-area curves have an increasingly liquid-expanded character (greater areas per molecule for a given surface pressure) as the number of isopentane units in the hydrophobic chain is increased. The biggest change in this respect was found with the attachment of the first three isopentane units to the chromanol ring system, relatively small changes then occurring on increasing the number of isopentane units to nine. Fig. 1(b) shows that the area per molecule remained approximately constant (0.65 nm^2 per molecule at $5 \text{ mN} \cdot \text{m}^{-1}$), for molecules containing from four to nine isopentane units in the side chain.

The collapse pressures and surface potentials of compounds in the tocopherol series showed similar variations with the length of the hydrophobic chain, although these variables continued to decrease slightly with increasing chain length for the higher-molecular-weight compounds (Fig. 1b).

Fig. 1. Surface behaviour of tocopherols
 (a) Surface pressure-area (—) and surface potential-area (---) curves for α -T-0 ($\blacktriangle$), α -T-2 ($\triangle$), α -T-3 ($\bullet$), α -T-5 ($\square$) and α -T-9 ($\circ$). (b) Surface behaviour of tocopherols as a function of the number of isopentane units in the hydrophobic side chain. Collapse pressure ($\circ$); molecular area at $5 \text{ mN} \cdot \text{m}^{-1}$ ($\bullet$); surface potential at $5 \text{ mN} \cdot \text{m}^{-1}$ ($\square$).

Penetration of phospholipid films by tocopherol compounds

Eleven different synthetic phospholipids, and cholesterol, were investigated. The surface pressure-area isotherms of these compounds were determined and found to be individually similar to corresponding isotherms reported in the literature (Shah, 1970; Phillips, 1972).

Experiments were undertaken to test the ability of tocopherols to penetrate monolayers of phospholipid which were spread at a surface pressure that was greater than the collapse pressure of the tocopherol. α -Tocopherol (α -T-3) penetrated and increased the surface pressure (at constant area) of diarachidonylglycerolphosphorylcholine spread in a monolayer at an initial surface pressure of $30 \text{ mN} \cdot \text{m}^{-1}$, and Fig. 2 shows the effect of varying the quantity of α -tocopherol injected into the subphase below the monolayer of phospholipid. The extent of penetration, as measured by the increase in surface pressure of the monolayer, was approximately constant for values of molar ratio of 0.5–2.0 (mol of α -tocopherol/mol of phospholipid). Interestingly, penetration was greatly decreased only when the quantity of α -tocopherol present was less than 0.3 mol/mol of diarachidonylglycerolphosphorylcholine. In all the experiments on the penetration of phospholipid monolayers by tocopherols and ubiquinones that are described below, equimolar ratios of phospholipid and penetrating molecule were used.

The abilities of a number of tocopherols (Table 1) to penetrate and increase the surface pressure of dioleoylglycerolphosphorylcholine, at differing initial surface pressures in the phospholipid monolayer,

Fig. 2. Penetration of α -tocopherol (α -T-3) into a monolayer of diarachidonylglycerolphosphorylcholine for different molar ratios of tocopherol/phospholipid

The initial surface pressure of the monolayer of phospholipid was $30 \text{ mN} \cdot \text{m}^{-1}$. Final equilibrium values for the increases in surface pressure, when the molar ratio of α -T-3 to the phospholipid in the monolayer was less than 1:1, were the same at 5, 10 and 15 min after injection of the tocopherol into the subphase.

Fig. 3. Penetration of tocopherols into dioleoylglycerolphosphorylcholine

The initial surface pressure of the monolayer of phospholipid was that shown on the abscissa (a), or was in excess of the collapse pressure of the relevant tocopherol by the value shown on the abscissa (b). (The collapse pressures of the individual tocopherols are given in Fig. 1.) The molar ratio of tocopherol, injected into the subphase, to the phospholipid in the monolayer was 1:1. α -T-0 (Δ); α -T-2 ($\triangle$); α -T-3 ($\bullet$); α -T-4 ($\blacksquare$); α -T-5 ($\square$); α -T-6 (∇); α -T-7 ($\blacktriangledown$); α -T-9 ($\circ$).

are shown in Fig. 3(a). Similar experiments (not shown) were undertaken with all the other phospholipids studied. In Fig. 3(a) some of the smaller tocopherols, e.g. α -T-2 to α -T-4, appear to penetrate the phospholipid monolayers at high surface pressures more effectively, as judged by the measured increase in surface pressure occurring on penetration, than the higher-molecular-weight compounds of the series (α -T-5 to α -T-9). However, Fig. 1(b) shows that the difference between the surface pressure of a monolayer of phospholipid spread at $20 \text{ mN} \cdot \text{m}^{-1}$ and the collapse pressure of α -T-2 is only $2 \text{ mN} \cdot \text{m}^{-1}$, whereas the corresponding value for α -T-9 is $13 \text{ mN} \cdot \text{m}^{-1}$. It is perhaps not surprising therefore that α -T-2 and other small tocopherols seem to be able to penetrate phospholipid monolayers the most effectively under the conditions of the experiments that are illustrated in Fig. 3(a).

To obtain comparable measures of penetration, we also studied the ability of tocopherols to penetrate phospholipid monolayers which were spread at initial surface pressures that were higher, by known increments, than the collapse pressures of the individual tocopherols. The results of these experiments are given in Fig. 3(b), which shows that, under these conditions, α -T-0 penetrates the phospholipid films least effectively. It is noteworthy that, in both types of experiments, the naturally occurring tocopherol (α -T-3) penetrated dioleoylglycerolphosphorylcholine most effectively and gave the largest increases in surface pressure (Figs. 3a and 3b).

Effects of unsaturation in phospholipid molecules

Of particular interest, in relation to possible mechanisms of action of α -tocopherol at the molecular level in biological membranes, is the effect of unsaturation in the fatty acyl chains of phospholipid molecules on the interaction of phospholipids with α -tocopherol. Fig. 4(a) shows the effects of changing the hydrophobic chains of the phospholipid, and of varying the length of the chain of the tocopherols, on the penetration by tocopherols of phospholipid monolayers, which were spread at a surface pressure that was $15 \text{ mN} \cdot \text{m}^{-1}$ above the collapse pressure of the individual tocopherols. For each monolayer of phospholipid studied, there was a sharp increase in the ability of tocopherols to penetrate the monolayer on increasing the length of the phytol side chain from α -T-0 to α -T-3. With α -T-4, penetration was slightly less than or comparable with that found with α -tocopherol (α -T-3). Thereafter, higher-molecular-weight derivatives penetrated less effectively, as in experiments with dioleoylglycerolphosphorylcholine that are described above (cf. Figs. 3a and 3b).

Fig. 4(a) also shows that the magnitude of the increase in surface pressure occurring on penetration of tocopherols into monolayers of phospholipids was related to the unsaturation of the phospholipid molecules. Introduction of one or two double bonds into one or both acyl chains of the phospholipids had a marked effect, for example, on penetration by α -tocopherol (α -T-3). There were, by contrast, only negligible differences in the behaviour of α -tocopherol (α -T-3) with phospholipids containing a total of four, six or eight double bonds. This is further illustrated by Fig. 5, which shows that the capacity of α -T-2, α -T-3 and α -T-7 to penetrate monolayers of unsaturated phospholipid molecules increased with increasing unsaturation of the acyl chains up to two double bonds per chain (dilinoleoylglycerolphosphorylcholine), but remained almost constant thereafter. Increases in surface pressure observed on the penetration of α -tocopherol (α -T-3), under similar conditions, into monolayers prepared from aged (oxidized) samples of diarachidonylglycerolphosphorylcholine were less than $2 \text{ mN} \cdot \text{m}^{-1}$, as compared

Fig. 4. Penetration of tocopherols into saturated and unsaturated phospholipids

Effect of unsaturation of the phospholipid (a): distearoylglycerolphosphorylcholine (Δ); 1-stearoyl-2-oleoylglycerolphosphorylcholine ($\blacktriangle$); dioleoylglycerolphosphorylcholine ($\square$); dilinoleoylglycerolphosphorylcholine ($\blacksquare$); dilinolenoylglycerolphosphorylcholine ($\circ$); diarachidonylglycerolphosphorylcholine ($\bullet$). Effect of the length of phospholipid acyl chains (b): distearoylglycerolphosphorylcholine (Δ); dipalmitoylglycerolphosphorylcholine ($\blacktriangle$); dimyristoylglycerolphosphorylcholine ($\square$); dilauroylglycerolphosphorylcholine ($\blacksquare$). The initial surface pressures of the phospholipid monolayers were $15 \text{ mN} \cdot \text{m}^{-1}$ higher than the collapse pressure of each tocopherol. The molar ratio of tocopherol, injected into the sub-phase, to the phospholipid in the monolayer was 1:1.

with $14 \text{ mN} \cdot \text{m}^{-1}$ found with the unoxidized phospholipid. The aged phospholipid exhibited u.v.-absorption bands at 230 and 280 nm that are characteristic of oxidized phospholipids, and gave an oxidation index of 0.66 (see the Materials and Methods section).

Data on the penetration of the tocopherols into monolayers of fully saturated phospholipids, but with acyl chains of differing lengths, are given in Fig. 4(b). Decreasing the length of the saturated acyl chains facilitated penetration of phospholipid monolayers by the tocopherols. However, as shown by a comparison of Figs. 4(a) and 4(b), shortening the acyl

Fig. 5. Influence of unsaturation in phospholipids on the penetration of tocopherols

The initial surface pressures of the phospholipid monolayers were $15 \text{ mN} \cdot \text{m}^{-1}$ higher than the collapse pressure of each tocopherol. The molar ratio of tocopherol, injected into the subphase, to the phospholipid in the monolayer was 1:1. α -T-2 (Δ); α -T-3 ($\bullet$); α -T-7 ($\blacktriangledown$).

chain from 18 to 12 carbon atoms was less effective than introducing two double bonds into two C_{18} chains. Fig. 4(b) also shows that the maximum penetration, which occurred with monolayers of dilauroyl- and dimyristoylglycerolphosphorylcholine, was found with α -T-2, rather than with α -tocopherol (α -T-3) as in experiments with unsaturated phospholipids.

Changes in surface pressure observed on the penetration of derivatives of α -tocopherol (α -T-3) into monolayers of dipalmitoyl- and dioleoyl-glycerolphosphorylethanolamine were very similar, within $\pm 2 \text{ mN} \cdot \text{m}^{-1}$ (not shown), to those obtained with monolayers of phosphatidylcholines possessing corresponding fatty acyl chains. This finding, taken together with those of Figs. 4 and 5, indicates that the abilities of derivatives of α -tocopherol (α -T-3) to penetrate monolayers of phospholipids may be regulated more by the hydrophobic chains of the phospholipid, and by the length of the phytyl chain of the tocopherol derivative, than by the polar head group of the phospholipid.

Fig. 6(a) gives data obtained from experiments on the penetration of the tocopherols into a mixed monolayer of diarachidonyl- and distearoyl-glycerolphosphorylcholine (1:1 molar ratio). Interestingly, the behaviour of this mixed monolayer much more closely resembles that of the unsaturated phospho-

Fig. 6. Penetration of tocopherols into mixed monolayers of diarachidonyl- and distearoyl-glycerolphosphorylcholine

Penetration of tocopherols into phospholipids (a). Distearoylglycerolphosphorylcholine (Δ); diarachidonylglycerolphosphorylcholine ($\bullet$); equimolar mixture of diarachidonyl- and distearoyl-glycerolphosphorylcholine ($\circ$).

Penetration of α -T-3 into diarachidonyl- and distearoyl-glycerolphosphorylcholine present in different molar proportions in mixed monolayers (b). The molar ratio of α -T-3, injected into the subphase, to the mixed phospholipids in the monolayer was 1:1 in each case. In (b), final equilibrium values for the increases in surface pressure, when the molar ratio of α -T-3 to the phospholipid film was less than 1:1, were the same at 5, 10 and 15 min after injection of the tocopherol into the subphase. The initial surface pressures of the phospholipid monolayers were $15 \text{ mN} \cdot \text{m}^{-1}$ greater than the collapse pressure of each tocopherol.

lipid component, i.e. extensive penetration by the tocopherols, than that of distearoylglycerolphosphorylcholine. Fig. 6(b) shows observations on the penetration of α -tocopherol (α -T-3) into mixed monolayers containing differing proportions of diarachidonyl- and distearoyl-glycerolphosphorylcholine. Increases in surface pressure that were practically equal to those found with monolayers of the unsaturated phospholipid alone were obtained with mixed monolayers containing only about 20% of the unsaturated component. It is thus apparent that penetration by α -tocopherol (α -T-3) is greatly facilitated by the

presence of relatively small quantities of unsaturated phospholipid in the monolayer.

Unlike their behaviour with phospholipids, the tocopherols exhibited very little penetration of a monolayer of cholesterol which was spread at a surface pressure that was initially $15 \text{ mN} \cdot \text{m}^{-1}$ higher than the collapse pressures of the individual tocopherols (Fig. 7). Increases in surface pressure produced by the penetration of tocopherols into mixed films of dioleoylglycerolphosphorylcholine/cholesterol (1:1 molar ratio) were intermediate between those observed for monolayers of the individual components (Fig. 7).

Mixed monolayers of phospholipids and tocopherols

Fig. 8 gives the mean molecular areas and mean surface potentials per molecule, at a surface pressure of $5 \text{ mN} \cdot \text{m}^{-1}$, as functions of molar composition for mixed monolayers of α -T-2, α -T-3, α -T-4 and α -T-7 with distearoyl-, dioleoyl- and diarachidonyl-glycerolphosphorylcholine. Mixed monolayers of the tocopherols with distearoylglycerolphosphorylcholine followed the additivity rule with respect to mean molecular areas. Further, the mixed monolayers of α -T-2 and α -T-7 with this phospholipid, in molar proportions of 0.5:1.0 and 0.75:1.0 (tocopherol/phospholipid), showed collapse pressures that were almost identical with those of monolayers of the individual tocopherols alone; similar behaviour was found for mixed monolayers of α -T-3 and α -T-4 with this phospholipid (0.75:1.0 molar ratios). This behaviour is indicative of immiscibility of the com-

ponents in these proportions in the surface film (Gaines, 1966). For certain molar ratios (tocopherol/phospholipid; 0.25:1.0 for α -T-2 and α -T-7, and both 0.25:1.0 and 0.5:1.0 for α -T-3 and α -T-4), the tocopherols were miscible with distearoylglycerolphosphorylcholine, as indicated by an increase in the collapse pressure of the mixed monolayer above that of the tocopherol alone.

The four tocopherols in Fig. 8 were miscible, in all of the proportions studied, in surface monolayers with dioleoylglycerolphosphorylcholine, as indicated by a progressive increase in the collapse pressures of the mixed monolayers on increasing the proportion of phospholipid present. Negative deviations in mean molecular area and in mean surface potential per molecule were found when the molar ratios of tocopherol to phospholipid were 0.5:1.0 and 0.75:1.0. These four tocopherols were also fully miscible with diarachidonylglycerolphosphorylcholine. The negative deviations in mean molecular area and in mean surface potential per molecule observed were rather more marked with this phospholipid, depending on the molar ratios, than those observed for mixed monolayers containing dioleoylglycerolphosphorylcholine (Fig. 8).

Derivatives of ubiquinone

A series of ubiquinone compounds, which are related to the tocopherols but which mostly have isoprenyl side chains and a benzoquinone ring, was also studied (Table 1). Fig. 9 shows the surface pressure-area isotherms for the ubiquinones. Q-0 did not form a monolayer, and the force-area curves for the other ubiquinones were more expanded than those found for tocopherols of corresponding chain length, as might be expected for the more unsaturated ubiquinones.

Experiments were undertaken on the penetration of ubiquinones into monolayers of phospholipids. The increases in surface pressure produced in monolayers of phospholipids, which were spread at an initial surface pressure that was more than $5 \text{ mN} \cdot \text{m}^{-1}$ greater than the collapse pressure of the ubiquinones, was $2 \text{ mN} \cdot \text{m}^{-1}$ or less (indicating virtually no penetration). Penetration into phospholipid monolayers at an initial surface pressure that was $5 \text{ mN} \cdot \text{m}^{-1}$ greater than the collapse pressure of the ubiquinones (rather than $15 \text{ mN} \cdot \text{m}^{-1}$ as with the tocopherols) was also small (Fig. 9b). Increases in surface pressure on the penetration of ubiquinones into monolayers of distearoyl-, dioleoyl- and diarachidonyl-glycerolphosphorylcholine in these experiments were no greater than those found for the penetration of α -tocopherol (α -T-3) into monolayers of the saturated phospholipid dipalmitoylglycerolphosphorylcholine (cf. Fig. 4b). Similar findings to those shown in Fig. 9(b) were obtained for the penetration of ubiquinones into dipalmitoyl-, dilinoleoyl-, dilinolenoyl- and

Fig. 7. Penetration of tocopherols into a mixed monolayer of cholesterol and dioleoylglycerolphosphorylcholine. Penetration of tocopherols into cholesterol (○), dioleoylglycerolphosphorylcholine (□), or an equimolar mixture of cholesterol and dioleoylglycerolphosphorylcholine (●). The initial surface pressures of the cholesterol and phospholipid monolayers were $15 \text{ mN} \cdot \text{m}^{-1}$ higher than the collapse pressure of each tocopherol. The molar ratio of tocopherol injected into the subphase to the phospholipid or cholesterol in the monolayer was 1:1.

Fig. 8. Mean area per molecule and mean surface potential per molecule in mixed monolayers of tocopherols and phospholipids. The mean surface potential per molecule (a) and mean area per molecule (b) were obtained at $5 \text{ mN} \cdot \text{m}^{-1}$ for mixed monolayers of distearoyl- (Δ), dioleoyl- ($\square$) and diarachidonyl-glycerolphosphorylcholine ($\bullet$) with the tocopherol indicated. The broken lines represent values calculated by the additivity rule.

dimyristoyl-glycerolphosphorylcholine. In these experiments, there was no enhancement of the ability of ubiquinones to penetrate monolayers of phospholipid on introducing double bonds into the acyl chains of the phospholipid molecules or on shortening the length of the acyl chains. Overall, the be-

haviour of the ubiquinones was clearly very different from that found for α -tocopherol (α -T-3) and its derivatives.

Mixed monolayers of derivatives of ubiquinone with distearoyl-, dioleoyl- and diarachidonyl-glycerolphosphorylcholine at $5 \text{ mN} \cdot \text{m}^{-1}$ showed ideal be-

Fig. 9. Surface behaviour of derivatives of ubiquinone. Surface pressure–area and surface potential–area curves (a). Q-0 (○); Q-3 (●); phytilybiquinone (Q-Phy) (□); Q-7 (▲); Q-9 (△). Penetration of ubiquinones into phospholipids (b). Distearoyl-glycerolphosphorylcholine (▲); dioleoyl-glycerolphosphorylcholine (□); diarachidonyl-glycerolphosphorylcholine (○). The initial surface pressures of the phospholipid monolayers were $5 \text{ mN} \cdot \text{m}^{-1}$ greater than the collapse pressure of each ubiquinone. The molar ratio of ubiquinone injected into the sub-phase, to the phospholipid in the monolayer was 1:1.

haviour for mean molecular area and mean surface potential per molecule. However, since the collapse pressures of the mixed monolayers studied (0.25:1.0, 0.5:1.0 and 0.75:1.0; molar ratios, ubiquinone/phospholipid) differed by no more than $2 \text{ mN} \cdot \text{m}^{-1}$ from the collapse pressures of monolayers of the ubiquinones alone, it would seem that these ubiquinones are practically immiscible with the phospholipids in monolayers under the conditions studied.

Discussion

Our experiments indicate that, of all the tocopherols studied, the naturally occurring α -tocopherol (α -T-3) may best be able to function in biological membranes, since the molecular structure of α -tocopherol enables it to undergo a maximum physical interaction with polyunsaturated phospholipids. α -Tocopherol (α -T-3), and to a lesser extent α -T-4, appear to have an optimum length of side chain for interacting with phospholipids with 16–20 carbon atoms in their acyl chains independently of the degree of unsaturation in

the phospholipid (Figs. 4a and 4b). However, a decrease in the length of the acyl chains of phospholipids, as in dimyristoylglycerolphosphorylcholine and dilauroylglycerolphosphorylcholine, enables α -T-2 to interact with them at least as well as α -tocopherol (α -T-3) itself interacts (Fig. 4b). This observation presumably indicates that optimal interactions between tocopherols and phospholipid molecules occur when the lengths of their hydrophobic moieties are approximately equal.

It might be suggested that the specificity of the observed interactions between polyunsaturated phospholipids and α -tocopherol (α -T-3) (Fig. 4a) is more apparent than real, and that our findings simply reflect the liquid-expanded character of unsaturated and shorter-chain phospholipids. According to such a view, 'holes' in the liquid-expanded monolayers will facilitate penetration into the monolayer of small (Rosano & La Mer, 1956; Blank, 1962) and large molecules (Phillips *et al.*, 1975). Non-specific 'cavity-filling' (Shah, 1970) may indeed be concerned in the interactions found. Nevertheless, although it is not possible to interpret these interactions in precise molecular terms, it appears unlikely that cavity-filling is the only phenomenon involved, because it seems unable to account for the quite marked differences in properties between α -T-2 and α -T-3 in their ability to penetrate unsaturated phospholipids. The similarity between the penetration of tocopherols into choline-containing and ethanolamine-containing phospholipids (despite the latter having a more condensed type of isotherm), and the failure of molecules in the ubiquinone series to interact with the phospholipids (Fig. 9b), also indicate that specific molecular interactions are important.

A number of our findings are of particular interest in relation to the possible behaviour of α -tocopherol (α -T-3) in biological membranes. For example, the observations illustrated in Fig. 6(b) show that the penetration of α -tocopherol (α -T-3) into a mixed monolayer containing both distearoyl- and diarachidonyl-glycerolphosphorylcholine was very dependent on the quantity of unsaturated phospholipid in the monolayer. When the proportion of diarachidonyl-glycerolphosphorylcholine present was between 5 and 10% of the total phospholipid, a relatively small change in the molar ratio of the two phospholipids markedly displaced the behaviour of the mixed monolayer towards that given by one or other of the individual phospholipid components. Thus the penetration of α -tocopherol (α -T-3) into a mixed monolayer containing more than 15% of diarachidonyl-glycerolphosphorylcholine was essentially the same as that for a monolayer of the unsaturated phospholipid alone. Since the mixed phospholipid monolayer used in this experiment was spread at a high initial surface pressure ($30 \text{ mN} \cdot \text{m}^{-1}$), it is likely that separate domains of saturated and polyunsaturated phospho-

lipids were present (cf. Phillips, 1972), and that α -tocopherol (α -T-3) intercalated preferentially into regions of the unsaturated phospholipid for which it shows the greater affinity. Such a mixed monolayer may thus behave, with respect to α -tocopherol (α -T-3), as if it is composed only of diarachidonylglycerolphosphorylcholine. It is also important to note that α -tocopherol (α -T-3) is not miscible with long-chain saturated phospholipids in certain molar ratios, and it would probably therefore not be localized in regions of saturated phospholipids. Similar considerations might apply to natural bilayer membranes. It should not be overlooked, however, that the phospholipids of biological membranes normally have one unsaturated and one saturated fatty acyl chain; phospholipids of this type, having a polyunsaturated 2-acyl chain, were not available for our investigations.

Cholesterol molecules interact more strongly with phospholipid molecules containing oleoyl residues than with those containing more highly unsaturated acyl residues, such as linoleoyl and linolenoyl (Demel *et al.*, 1967). In the hypothesis put forward earlier (Lucy, 1972; Diplock & Lucy, 1973), it was proposed that α -tocopherol (α -T-3) plays a structural role in membranes that is comparable with aspects of the behaviour of cholesterol, but with the difference that α -tocopherol (α -T-3) interacts primarily with polyunsaturated phospholipids, rather than with phospholipids containing monoene acyl residues. It is relevant to note that Albarracin *et al.* (1974) have found that skeletal muscles of vitamin E-deficient rabbits have an increased content of cholesterol, which may perhaps compensate in part for the lack of α -tocopherol (α -T-3). The results of the present model experiments (Figs. 4a, 5, and 8) show that, at least in lipid monolayers, α -tocopherol (α -T-3) interacts more strongly with polyunsaturated phospholipids than with dioleoylglycerolphosphorylcholine. If α -tocopherol (α -T-3) has a structural function in plasma membranes, it might therefore be expected that the vitamin would be primarily important in the cytoplasmic half of the lipid bilayer, which probably contains a high proportion of the polyunsaturated phospholipids of plasma membranes (Zwaal *et al.*, 1973; Emmelot & Van Hoesen, 1975). It also seems likely that structural aspects of the functioning of α -tocopherol (α -T-3) may be particularly important in membranes that contain a relatively high proportion of polyunsaturated phospholipids and little or no cholesterol, such as the inner mitochondrial membrane.

Fig. 2 shows that α -tocopherol (α -T-3) interacted with diarachidonylglycerolphosphorylcholine even when the tocopherol was present at a molar ratio of only 0.25:1.0 [α -tocopherol (α -T-3)/phospholipid]. Further, an interaction, which was almost equivalent to that found for a 1:1 molar ratio, occurred when

α -tocopherol (α -T-3) was present at a ratio of 0.3:1.0. This implies that each molecule of α -tocopherol (α -T-3) is able to interact with more than one molecule of phospholipid in the monolayer, presumably as a result of the fluid nature of the phospholipid. On the basis of our present studies it is therefore suggested that the interactions which were previously proposed between projecting methyl groups of the phytol chain of α -tocopherol (α -T-3) and 'pockets' present in the polyunsaturated phospholipids of membranes (in regions of methylene-interrupted *cis* double bonds) may in fact occur on a dynamic basis between one molecule of α -tocopherol (α -T-3) and a number of molecules of polyunsaturated phospholipid. A strict 1:1 molar ratio of α -tocopherol (α -T-3) to polyunsaturated phospholipid may thus not be necessary in order to allow α -tocopherol (α -T-3) to play a structural role in biological membranes.

In conclusion, it is important to consider what bearing our observations may have on the biochemical functions of α -tocopherol (α -T-3) *in vivo*. If α -tocopherol (α -T-3) is involved in the protection of the polyunsaturated phospholipids of biological membranes against destructive peroxidation, it appears from our studies that α -tocopherol (α -T-3) is likely to be located in close association with polyunsaturated phospholipid molecules concerned. α -Tocopherol (α -T-3) may therefore be in an optimum position to inhibit lipid peroxidation by an antioxidant mechanism. On the other hand, our observations are also consistent with the hypothesis that α -tocopherol (α -T-3) may facilitate the molecular packing within biological membranes of polyunsaturated phospholipid molecules, and hence the vitamin may stabilize biological membranes containing high proportions of polyunsaturated phospholipid molecules (cf. the disorder in the interior of bilayers of egg phosphatidylcholine discussed by Birrell & Griffith, 1976). Recent experiments have shown that α -tocopherol (α -T-3) influences the permeability properties of liposomes containing polyunsaturated phospholipids (A. T. Diplock & J. A. Lucy, unpublished work). It is not impossible therefore that α -tocopherol (α -T-3) may have both antioxidant and structural functions in biological membranes *in vivo*.

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