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Liquid membrane phenomenon in vitamin E: studies on α-tocopherol

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Summary

The liquid membrane phenomenon in vitamin E has been studied. Hydraulic permeability data have been obtained to demonstrate the existence of the liquid membranes in series with a supporting membrane generated by α-tocopherol and also by the lecithin–cholesterol–α-tocopherol mixtures. Data on the transport of oestrogen, progesterone, cystine, methionine, creatinine and sodium, potassium and calcium ions in the presence of the liquid membrane generated by the lecithin–cholesterol–α-tocopherol mixture have been obtained and discussed in the light of the various syndromes caused by vitamin E deficiency. The data indicate that modification in the permeability of the various relevant permeants in the presence of liquid membranes is likely to play a significant role in causing and prevention of the various syndromes due to the deficiency of vitamin E.

Introduction

Recent studies (Bhise et al., 1982, 1983a and b, 1984a and b, 1985; Srivastava et al., 1984) on a variety of surface-active drugs belonging to different chemical and pharmacological categories, have revealed that modification in the transport of relevant permeants by the liquid membranes likely to be generated by them at the respective sites of action may be an important step common to the mechanism of action of all surface-active drugs.

α-Tocopherol is the most important tocopherol because it comprises about 90% of the tocopherols in animal tissues and exhibits maximum biological activity. It is distributed throughout the tissues of animals and man and its deficiency causes a variety of syndromes in the animal organism. Just by looking at the structure of α-tocopherol one suspects it to be surface-active in nature. Since according to Kesting's hypothesis (Kesting et al., 1968) surface-active agents, when added to aqueous phase, generate a surfactant layer liquid membrane at the interface, it is likely that the phenomenon of liquid membrane formation may play a role in the actions of α-tocopherol.

In the present study investigations were carried out to explore the role of liquid membrane phenomenon in the actions of α-tocopherol. Critical micelle concentration of α-tocopherol in water has been determined. The data on hydraulic permeability have been obtained to demonstrate: (1) the formation of a liquid membrane by α-tocopherol in series with the supporting membrane; and (ii) the incorporation of α-tocopherol in the lecithin–cholesterol liquid membrane existing in series with

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the supporting membrane. Transport of relevant permeants in the presence of the liquid membrane generated by the lecithin–cholesterol–α-tocopherol mixture has been studied and the data obtained have been discussed in the light of the various syndromes caused by vitamin E deficiency.

Materials and Methods

Lecithin (egg phosphatidyl choline) and progesterone (Patel Chest Institute, CSIR Centre for Biochemicals, Delhi), cholesterol (Centron Research Laboratories, Bombay), DL-α-tocopherol acetate (Sigma T3376), ethinyl oestradiol (Roussel Pharmaceuticals (India) Bombay), cystine, methionine and creatinine (all from Loba Chemie), sodium, potassium and calcium chlorides (all Analar grade) and distilled water distilled twice in an all-pyrex glass still were used in the present experiments.

Aqueous solutions of desired concentration of α-tocopherol, lecithin–cholesterol–α-tocopherol mixtures, ethinyl oestradiol and progesterone which are not so easily soluble in water were prepared by adding necessary volume of ethanolic stock solution of known concentration to aqueous phase with constant stirring. In the aqueous solutions thus prepared the final concentration of ethanol was never allowed to exceed 0.1% by volume because it was experimentally shown that a 0.1% solution of ethanol in water did not lower the surface tension of water to any measurable extent. The aqueous solutions of cystine, methionine, creatinine and chlorides of sodium, potassium and calcium were prepared in the usual way.

The critical micelle concentration (CMC) of aqueous α-tocopherol, aqueous lecithin and aqueous cholesterol were determined from the variation of their surface tension with concentration and were found to be 5.0×10^{-8} M, 1.599×10^{-5} M and 30.08×10^{-9} M, respectively. Surface tensions were measured using a Fisher tensiomat model 21.

The all-glass cell described earlier (Srivastava and Jakhar, 1981; Bhise et al., 1982) was used for the transport studies (Fig. 1). A Sartorius cellulose

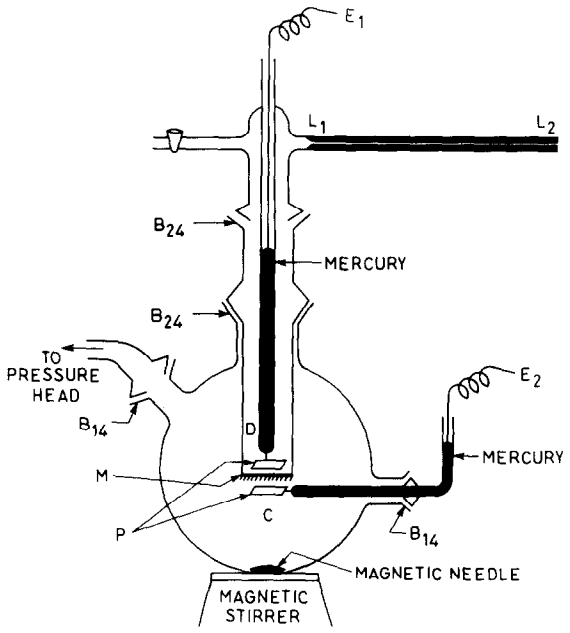

Fig. 1. Transport cell. M, supporting membrane; P, bright, platinum electrodes; E_1 and E_2, electrode terminals; L_1 and L_2, capillary.

acetate microfiltration membrane (Cat No. 11107, pore size 0.2 μm) of thickness 1×10^{-4} m and area 2.55×10^{-5} m^2 which in fact acted as a supporting membrane for the liquid membranes, divided the transport cell into two compartments C and D (Fig. 1).

To obtain the hydraulic permeability data, solutions of varying concentrations of α-tocopherol in water or in the aqueous solution of the lecithin–cholsterol mixtures of fixed composition were filled in compartment C of the transport cell (Fig. 1) and the compartment D was filled with water. The details of the method used for the hydraulic permeability measurements have been described earlier (Srivastava and Jakhar, 1981, 1982; Bhise et al., 1982, 1983b).

For solute permeability (σ) measurements for various permeants, namely oestrogen, progesterone, cystine, methionine, creatinine and sodium, potassium and calcium ions, the procedure described earlier (Bhise et al., 1982, 1983a and b, 1984a and b, 1985; Srivastava and Jakhar, 1982) was followed. Compartment C of the transport cell (Fig. 1) was filled with the solution of known

concentration of the permeant prepared in the aqueous solution of lecithin, cholesterol and α-tocopherol mixture of composition 1.919×10^{-5} M with respect to lecithin, 1.175×10^{-6} M with respect to cholesterol and 3.75×10^{-8} M with respect to α-tocopherol and compartment D was filled with distilled water. In control experiments, however, no α-tocopherol was used.

This particular composition, of the aqueous solution of lecithin–cholesterol–α-tocopherol mixture, used in the experiments for solute permeability measurements, which was derived from our earlier study (Srivastava and Jakhar, 1982) and the present data on hydraulic permeability, is the composition at which the lecithin liquid membrane compeletely covers the supporting membrane and is saturated with both cholesterol and α-tocopherol.

Since lecithin, cholesterol and α-tocopherol are all surface-active in nature and have both hydrophobic and hydrophilic parts in their structure, it is obvious that in the liquid membranes generated in these experiments the hydrophobic tails of these molecules will be preferentially oriented towards the hydrophobic supporting membrane and the hydrophilic moieties would be drawn outwards away from it.

The values of the solute permeability (ω) were estimated using the equation (Katchalsky and Kedem, 1962; Katchalsky and Curran, 1967):

$$\left(\frac{J_s}{\Delta\pi}\right)_{J_v=0} = \omega \qquad (1)$$

where J_s is the solute flux, J_v is the volume flux per unit area of the membrane and $\Delta\pi$ is the osmotic pressure difference. The value of $\Delta\pi$ used in the calculation of ω from Eqn. 1 was the average of its value at the beginning of the experiment (t = 0) and at the end of the experiment.

All measurements were carried out at constant temperature using a thermostat set at 37 ± 0.1°C.

Estimations

The amounts of the various permeants transported to compartment D were estimated as follows:

(1) *Amino acids.* The amounts of cystine and methionine were estimated from the amount of their reaction products with ninhydrin measured at 570 nm (Moore and Stein, 1954) using a Bausch & Lomb Spectronic-20 spectrophotometer.

(2) *Creatinine.* The amount of creatinine was estimated using the method described in literature (Oser, 1965) in which creatinine was subjected to react with alkaline picrate to form an orange-coloured 'Jaffe complex', the intensity of which was measured spectrophotometrically at 520 nm.

(3) *Oestrogen.* The amount of ethinyl oestradiol was estimated by a chemical method (Brown, 1955) using quinol-H_2SO_4 as a colouring reagent and the colour developed was measured at 538 nm using a spectrophotometer.

(4) *Progesterone.* The amount of progesterone was estimated using a Cary 17-D Spectrophotometer at 242 nm the absorption maxima for progesterone (Heller, 1980).

(5) *Cations.* The amounts of sodium, potassium and calcium ions were determined using a flame-photometer (Model CL-22, Elico, India).

Results and Discussion

The hydraulic permeability data at various concentrations of α-tocopherol were, in all cases, found to be represented by the equation:

$$J_v = L_P \cdot \Delta P \qquad (2)$$

where J_v represents the volume flux per unit area of the membrane, ΔP the applied pressure difference and L_P stands for the hydraulic conductivity coefficient. The values of L_P at various concentrations of α-tocopherol, estimated from the slopes of the J_v versus ΔP plots show a decreasing trend with the increase in concentration of α-tocopherol up to its CMC beyond which they become more or less constant (Table 1). This trend indicates progressive coverage of the supporting membrane with the liquid membrane generated by α-tocopherol in accordance with the Kesting's hypothesis (1968). At the CMC the α-tocopherol liquid membrane completely covers the supporting membrane. Analysis of the transport data (Table 1) in light of mosaic membrane model

TABLE 1

VALUES OF L_P AT VARIOUS CONCENTRATIONS OF α-TOCOPHEROL

Concentration $\times 10^8$ M	0.00	1.25 (0.25 CMC)	2.50 (0.5 CMC)	3.75 (0.75 CMC)	5.00 (1 CMC)	10.00	15.00
$L_P{}^a \times 10^8$ $(m^3 \cdot s^{-1} \cdot N^{-1})$	4.891 ± 0.137	4.473 ± 0.189	4.078 ± 0.121	3.669 ± 0.113	3.170 ± 0.077	3.112 ± 0.141	3.098 ± 0.042
$L_P{}^b \times 10^8$ $(m^3 \cdot s^{-1} \cdot N^{-1})$	–	4.461 ± 0.122	4.031 ± 0.107	3.600 ± 0.092	–	–	–

[a] Experimental values.

[b] Calculated values using mosaic model.

(Spiegler and Kedem, 1966; Sherwood et al., 1967; Harris et al., 1976) furnishes further support in favour of the formation of the liquid membrane in series with the supporting membrane. Following the argument given in earlier publications (Srivastava and Jakhar, 1981, 1982; Bhise et al., 1982, 1983a and b, 1984a and b, 1985) it follows that if concentration of the surfactant is n times its CMC, $n \leqslant 1$, the value of L_P should be equal to $[(1 - n)L_P^c + n\, L_P^s]$ where the superscripts c and s, respectively, represent the bare supporting membrane and the supporting membrane completely covered with the surfactant layer liquid membrane. Functionally, in the present case, the values of L_P^c and L_P^s would respectively represent the values of L_P for O and the CMC of α-tocopherol. The values of L_P thus computed at various concentrations of α-tocopherol below its CMC match with the experimentally determined values (Table 1).

Information about the incorporation of α-tocopherol in the liquid membranes generated at the interface by lecithin–cholesterol mixture can be gathered from the hydraulic permeability data for solutions of various concentrations of α-tocopherol prepared in the aqueous solutions of lecithin–cholesterol mixtures of fixed composition, i.e. 1.919×10^{-5} M with respect to lecithin and 1.175×10^{-6} M with respect to cholesterol, which in fact is the composition at which the liquid membrane generated by lecithin completely covers the interface and is saturated with cholesterol (Srivastava and Jakhar, 1982). The values of L_P at various concentrations of α-tocopherol estimated, from the J_v vs ΔP plots, which in this case also were found to be in accordance with Eqn. 2 are recorded in Table 2. The decreasing trend in the values of L_P which continues up to the α-tocopherol concentration equal to 3.75×10^{-8} M (Table 2) indicates that more and more of α-tocopherol is incorporated in the lecithin–cholesterol liquid membrane generated at the interface and at concentration equal to 3.75×10^{-8} M the lecithin–cholesterol liquid membrane is saturated with α-tocopherol. In order to ascertain whether the added α-tocopherol reaches straight up to the interface or not, surface tensions of solutions of various concentrations of α-tocopherol prepared in the aqueous solution of the lecithin–cholesterol mixture of composition 1.919×10^{-5} M with respect to lecithin and 1.175×10^{-6} M with respect to cholesterol, were measured. The surface tension of the aqueous solution of the lecithin–cholesterol mixture showed a further de-

TABLE 2

VALUES OF L_P AT VARIOUS CONCENTRATIONS OF α-TOCOPHEROL IN LECITHIN–CHOLESTEROL–α-TOCOPHEROL MIXTURES [a]

Concentration $\times 10^8$ M	0.00	1.25	2.50	3.75	5.00	10.00
$L_P \times 10^8$ $(m^3 \cdot s^{-1} \cdot N^{-1})$	1.575 ± 0.084	1.402 ± 0.045	1.296 ± 0.012	1.178 ± 0.011	1.185 ± 0.031	1.164 ± 0.033

[a] Lecithin and cholesterol concentrations kept constant at 1.919×10^{-5} M and 1.175×10^{-6} M, respectively.

crease on addition of α-tocopherol and the decreasing trend continued up to the α-tocopherol concentration equal to 3.75×10^{-8} M. This trend indicates that the added α-tocopherol reaches deep up to the interface in the liquid membrane generated by the lecithin–cholesterol mixture in series with the supporting membrane.

Solute permeability data

Data on the solute permeability (σ) of several permeants, namely oestrogen, progesterone, cystine, methionine, creatinine and cations (Na^+, K^+ and Ca^{2+} ions), in the presence of the liquid membranes generated by the mixture of lecithin, cholesterol and α-tocopherol in series with the supporting membrane are recorded in Table 3. The data appear to be relevant to causation of various syndromes in animal organisms due to deficiency of vitamin E, i.e. α-tocopherol.

TABLE 3

SOLUTE PERMEABILITY (σ) OF VARIOUS PERMEANTS IN PRESENCE OF LECITHIN–CHOLESTEROL–α-TOCOPHEROL MIXTURE [a]

	σ [b] $\times 10^9$ (mol·s^{-1}·N^{-1})	σ [c] $\times 10^9$ (mol·s^{-1}·N^{-1})
Methionine [d]	8.79 ± 0.27	6.25 ± 0.24
Cystine [e]	2.62 ± 0.09	4.66 ± 0.56
Creatinine [f]	0.27 ± 0.03	0.29 ± 0.02
Ethinyl oestradiol [g]	5.50 ± 0.40	4.14 ± 0.24
Progesterone [h]	4.90 ± 0.38	4.11 ± 0.20
Sodium (chloride) [i]	0.12 ± 0.01	0.12 ± 0.01
Potassium (chloride) [j]	0.13 ± 0.01	0.14 ± 0.01
Calcium (chloride) [k]	0.16 ± 0.03	0.18 ± 0.01

[a] Lecithin concentration, 1.919×10^{-5} M; cholesterol concentration, 1.175×10^{-6} M; α-tocopherol concentration, 3.75×10^{-8} M.
[b] Control value when no α-tocopherol was used.
[c] Lecithin–cholesterol–α-tocopherol mixture in compartment C together with the permeant.
[d] Initial concentration 100 mg/l.
[e] Initial concentration 100 mg/l.
[f] Initial concentration 1 g/l.
[g] Initial concentration 50 mg/l.
[h] Initial concentration 100 mg/l.
[i] Initial concentration 5.382 g/l.
[j] Initial concentration 10.430 g/l.
[k] Initial concentration 0.222 g/l.

Except for the work cited by Wagner and Folkers (1963) there is enough evidence to indicate that vitamin E is essential for normal reproduction in several mammalian species (Marks, 1962; Mandel and Cohn, 1980) and its deficiency is known to cause habitual abortions. The fundamental mechanism by which vitamin E deficiency interferes with reproduction is obscure (Mandel and Cohn, 1980). The present data (Table 3) on the impedimenting in the transport of oestrogen and progesterone in the presence or α-tocopherol may offer an explanation for occurrence of habitual abortions caused by vitamin E deficiency.

It is not only the high concentrations of oestrogen and progesterone but also a proper ratio of their concentrations which is essential for the maintenance of pregnancy (Kincl, 1971). As the present data indicate, the deficiency of vitamin E in the membranes of the uterus would enhance the outflow of oestrogen and progesterone to an unequal extent. This outflow would disturb the oestrogen–progesterone ratio resulting in the failure of pregnancy.

In many species, deficiency of vitamin E leads to the development of muscular dystrophy. Metabolic disturbances during muscular dystrophy include increased water content of the tissues, changes in electrolyte pattern and increased excretion of creatine in urine–creatinurea (Mason, 1944). The values of solute permeability, σ, for the cations and also for creatinine in the presence of α-tocopherol do not show any significant difference in comparison to the values obtained from the control experiment where no α-tocopherol was used. The data on hydraulic permeability (Table 2), however, appear relevant to causation of increased water content of the tissues and creatinurea. The data in Table 2 imply that the cell membranes deficient in vitamin E are likely to be more permeable to water which may be one of the factors responsible for the increased water content of the tissue. It has been suggested (Beard, 1941; Mason, 1944) that creatinurea in nutritional muscular dystrophy might be due to hydration of creatinine to creatine due to increased water content of the tissues–creatinine is formed inside the cells as a result of creatine metabolism. The alteration in the normal water balance of tissues is a

consistent finding in the biochemical and histological examinations of tissues affected by vitamin E depletion (Mason, 1944). Nitowsky et al. (1962) have shown that tocopherol can decrease the elevated creatine excretions of children with cystic fibrosis.

Dam and associates (1952) have shown that muscular dystrophy in chicks could be prevented by supplementing the diet with either vitamin E or cystine. Later Machlin and Shalkop (1956) showed that cystine and methionine were equally effective in prevention of dystropy. However, Scott (1962) and Scott and Calvert (1962) have reported that cystine is more effective than methionine. The present data (Table 3) show that the permeability of cystine is enhanced and that the amount of methionine was reduced in the presence of α-tocopherol. This observation is consistent with the inference drawn by Scott (1962) and Scott and Calvert (1962) that cystine is more effective than methionine in prevention of dystrophy.

Certain diets low in protein and especially in the sulfur-containing amino acids, particularly cystine, have been found (Harper, 1975) to produce an acute massive hepatic necrosis in experimental animals. A vitamin E deficiency is reported to enhance the effects of such diets, whereas added vitamin E exerts a preventive action upon the necrosis (Harper, 1975). The enhanced permeability of cystine in the presence of α-tocopherol (Table 3) could be a plausible explanation for these observations on the causation and prevention of hepatic necrosis.

Thus the model studies reported in this paper indicate that the phenomenon of liquid membrane formation may also play a notable role in the causation and prevention of various syndromes due to vitamin E deficiency. It may be emphasized that since the supporting membrane chosen in this study was a non-specific, non-living membrane, the present study highlights the role of passive transport in biological action.

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