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MEMBRANE PERMEABILITY CHANGES WITH VITAMIN A/VITAMIN E MIXED BILAYERS

WILLIAM STILLWELL and LEAH BRYANT

Purdue University School of Science, Biology Department, 1125 E. 38th Street, P.O. Box 647, Indianapolis, IN 46223 (U.S.A.)

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Vitamin A (all *trans*-retinol) enhances the permeability of egg phosphatidylcholine liposomes to glucose, urea, and erythritol while vitamin E (α-tocopherol) decreases permeability to the same solutes. Egg phosphatidylcholine bilayers containing both vitamin A and vitamin E are shown to have an altered permeability more similar to that affected by vitamin E alone. The membrane stabilizing effect of vitamin E appears dominant over the membrane destabilizing effect of vitamin A.

The two fat-soluble vitamins A and E are believed to function independently and in concert in a number of as yet poorly defined biological processes [1,2]. Because both vitamins are amphipathic, possessing a polar head group and a very apolar tail, it was predicted sometime ago that either compound could partition into the lipid bilayer portion of membranes, and perhaps it is there that the initial site of action for the vitamins may be found [3,4].

Although it is known that both vitamin A and E can be isolated from the same biological membranes [5,6], these two vitamins often effect the membranes in opposite ways. Vitamin A has been shown to destabilize membranes resulting in increases in permeability of erythrocytes [7–9], lysosomes [10–12], and artificial lipid bilayers [13,14], while vitamin E has been implicated in preserving membrane integrity [15]. For example, vitamin E can prevent vitamin A-induced hemolysis of erythrocytes [4] and has been shown to enhance lysozome stability [16] and decrease permeability of unsaturated lipid bilayers to glucose and chromate [17,18]. Although the major function of vitamin E apparently is as an antioxidant and vitamin A has been linked to some non-membrane processes such as control of gene expression, both vitamins probably serve a number of important functions including some poorly defined roles in the membrane [1,2].

In ths communication we compare the effect of vitamin E (α-tocopherol) and vitamin A (all *trans*-retinol) on the permeability of egg phosphatidylcholine liposomes to urea, erythritol and [^{14}C]glucose. We also report the interaction of these two vitamins as they may effect membrane permeability.

Efflux rates for sequestered [^{14}C]glucose (D-[U-^{14}C]glucose, New England Nuclear) were measured from vitamin A (all *trans*-retinol, Type X, Sigma Chemical Co.) or vitamin E (α-D,L-tocopherol, Sigma) containing egg phosphatidylcholine (Type IX-E, Sigma) liposomes. Since the biological egg phosphatidylcholine contained a mixture of fatty acids (myristic, 0.1%; palmitic, 42.8%; stearic, 20.6%; oleic, 24.8%; linoleic, 7.9% and arachidonic, 1.8%) the bilayer membranes made from these lipids were well above the phase transition temperature of these experiments (25°C). Liposomes were made by the ether evaporation method [19] in [^{14}C]glucose, 20 mM potassium phosphate buffered at pH 7.0. After a brief

centrifugation to remove non-liposomal lipids, the non-sequestered [^{14}C]glucose was removed on a Sephadex G-50 column and 5.0 ml of the radio-labeled liposomes were added to dialysis bags and dialyzed against 10 ml of the phosphate buffer. 500-μl aliquots were taken at various times and counted on a Beckman LS 100-C Scintillation counter. Results are expressed as the percentage of initially tapped radioactivity leaking out after 3 h of dialysis as a function of vitamin A or vitamin E incorporated into the membranes.

Urea and erythritol permeability were measured by the method of De Gier et al. [20] in 40 mM glucose, 10 mM Tris-HCl buffered at pH 7.0. After centrifugation to remove the excess lipid, 100 μl of the liposomes were rapidly mixed in a cuvette with 2.0 ml of either 40 mM urea, 10 mM Tris-HCl, pH 7.0 or 40 mM erythritol, 10 mM Tris-HCl, pH 7.0. Liposome swelling was followed on a Beckman DU-8 Computing Spectrophotometer controlled at 25 ± 0.1°C. Results are expressed as vitamin induced changes in swelling rates ($\Delta d(1/A)dt$ %) [21], compared to the vitamin-free controls.

The effect of retinol or α-tocopherol on the trans-membrane diffusion of [^{14}C]glucose is strik-

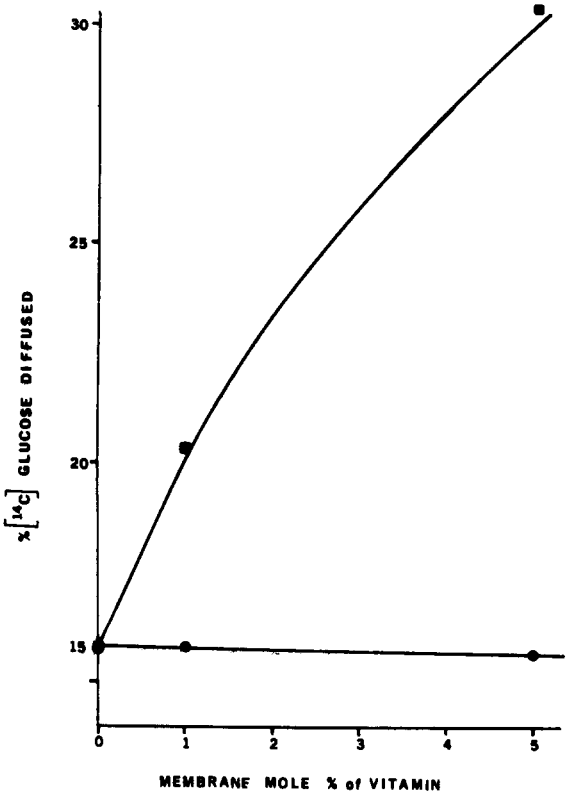

Fig. 1. Permeability of [^{14}C]glucose to egg phosphatidylcholine liposomes as a function of vitamin A (retinol, ■) or vitamin E (α-tocopherol, ●) incorporated into the bilayers.

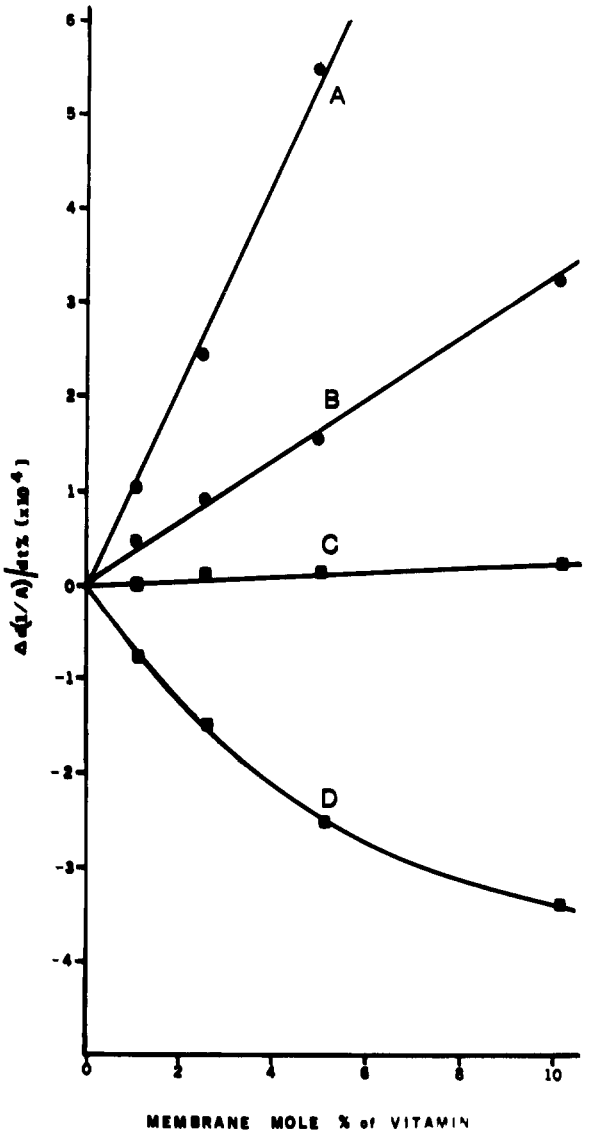

Fig. 2. Effect of vitamin A (retinol) and vitamin E (α-tocopherol) on the permeability of egg phosphatidylcholine liposomes to urea and erythritol. A, Effect of retinol on urea permeability; B, effect of retinol on erythritol permeability; C, effect of α-tocopherol on erythritol permeability; and D, effect of α-tocopherol on urea permeability.

ingly different. Retinol enhances glucose diffusion when incorporated over the range of 0 to 5 membrane mole percent while α-tocopherol is shown to exert no effect when incorporated over the same range (Fig. 1). Previously we have demonstrated that vitamin A can enhance the membrane permeability to a number of charged solutes including cations, anions and zwitterions [13,14]. Here we report the retinol-enhanced diffusion of three neutral solutes – glucose, urea, and erythritol.

Liposome swelling in isotonic solutions clearly demonstrated a retinol-dependent enhancement in both urea and erythritol permeability while α-tocopherol produced a large decrease in the permeability of urea and had little effect on the membrane permeability of erythritol (Fig. 2). The effect of either vitamin is apparently solute size dependent. The largest vitamin A enhancement was noted for the smallest solute, urea, while the largest vitamin E-induced decrease in permeability was also reported for urea. For the larger solutes, erythritol and glucose, smaller enhancements in permeability were noted for vitamin A while little or no change in permeability was measured for vitamin E. These experiments support the prior reports that vitamin A disrupts lipid bilayers resulting in a general increase in membrane permeability [3,13,14] while vitamin E appears to enhance membrane stability while decreasing permeability [15–18].

Since vitamin A and E are thought to interact in some fashion at a physiological level [21,22], perhaps at the membrane surface [4,23,24], we decided to measure permeabilities with bilayers containing both vitamins simultaneously to determine which, if either, of the vitamins exerts the dominant effect. Fig. 3 presents the results of these experiments. The mixed vitamin permeability curves follow those of vitamin E more closely than those of vitamin A. For example, with constant vitamin A (2.5 membrane mole percent) a substantial vitamin E-induced decrease in urea permeability was measured, similar to that of the vitamin A-free control reported in Fig. 2. Vitamin E is also shown to prevent the expected large vitamin A-induced increase in urea permeability. For the large solute, erythritol, small permeability changes were attributed to the presence of either vitamin. Vitamin A-induced erythritol permeability was completely suppressed by only 2.5 membrane mole percent vitamin E.

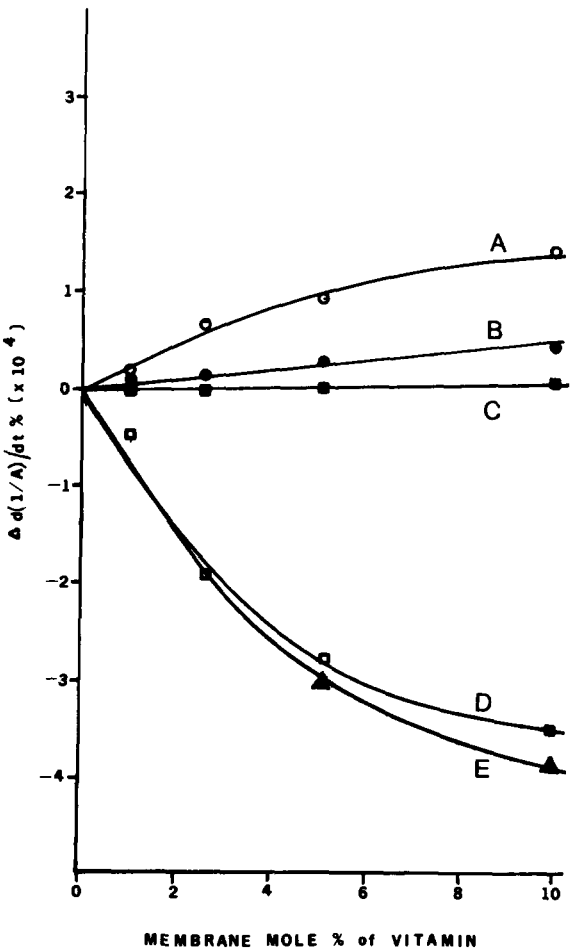

Fig. 3. Urea and erythritol permeability with vitamin A (retinol)/vitamin E (α-tocopherol) mixed egg phosphatidylcholine liposomes. A, Effect of increasing retinol on urea permeability with membranes containing 10 membrane mole percent α-tocopherol; B, effect of increasing retinol or erythritol permeability with membranes containing 2.5 membrane mole percent α-tocopherol; C, effect of increasing α-tocopherol on erythritol permeability with membranes containing 10 membrane mole percent retinol; D, effect of increasing α-tocopherol on urea permeability with membranes containing 2.5 membrane mole percent retinol; E, experiment identical to D except the vitamins were incorporated into lipid bilayers during liposome formation.

For most of these experiments the vitamins, dissolved in ethanol, were added to preformed egg phosphatidylcholine liposomes. Controls demonstrated that the carrier ethanol had no measurable

effect on permeability at the levels tested (1.0%). Upon incubation for 30 min, it was assumed that these very hydrophobic vitamins were incorporated into the bilayers. To test this hypothesis we compared results from experiments in which the vitamins A and E were added to preformed liposomes with experiments in which the vitamins were directly incorporated into the bilayers during liposome preparation. With these later experiments the vitamins are known to be homogeneously mixed with the phosphatidylcholine. Since both sets of experiments produced very similar results (Fig. 3, curves D and E), we conclude that vitamin A and E are absorbed homogeneously into preformed egg phosphatidylcholine liposomes.

These experiments further support the notion that one of vitamin A's functions may be to destabilize lipid bilayers, enhancing membrane permeability while vitamin E may counter the effect of vitamin A by stabilizing membranes and decreasing permeability. These results on egg phosphatidylcholine liposomes are in agreement with prior reports on erythrocytes and phosphatidylcholine/cholesterol monolayers [4,15,25]. Perhaps the proper balance of vitamin A and E is required to maintain necessary membrane stability and permeability. Vitamin E may also have a role in preventing poisoning due to excessive amounts of vitamin A.

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