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EFFECT OF TRANS-RETINOL ON THE PERMEABILITY OF EGG LECITHIN LIPOSOMES

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Summary: trans-Retinol is shown to significantly increase the permeability of egg lecithin liposomes to K^+, Cl^-, Br^-, I^-, glycine, lysine and glucose. A relationship is demonstrated between the amount of trans-retinol required to induce permeability and the size of the diffusing species. From this data it is concluded that trans-retinol at levels only slightly above those found normally could increase the cristae permeability to protons enough to cause uncoupling of oxidative phosphorylation.

Despite intense effort, the molecular role of vitamin A in the maintenance of an organism's normal health remains a mystery (1,2). Since this vitamin is so lipid-soluble, suggestions have been made that it might exert its initial effect at the cell membrane (3). Some time ago Seward (4) proposed that vitamin A in excess may somehow be uncoupling oxidative phosphorylation. This attractive hypothesis has never been experimentally established and has been criticized for employing very large, unphysiological levels of vitamin A. However, the effect of low levels of vitamin A on membranes has yet to be clearly defined. Recently we have been testing the uncoupling hypothesis by measuring the effect vitamin A has on lipid bilayer permeability.

Our previous experiments have demonstrated that trans-retinal disrupts normal bilayer structure enough to significantly increase the membrane permeability to protons, glucose (5) and K^+(6). Since larger amounts of trans-retinal were required to increase the permeability of the large solute, glucose compared to protons and K^+, we concluded that it should be possible to define for the first time the extent of membrane perturbation caused by the vitamin by measuring the permeability of liposomes to a series of different sized solutes. In unrelated experiments, Kataoka and Kinsky (7) probed the size of the pore formed during

Abbreviations: CFCCP, Carbonyl Cyanide p-triflioromethoxyphenyl-hydrazone; DNP, 2, 4 dinitrophenol.

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complement mediated lysis by measuring the efflux rate from liposomes for a series of different sized proteins. Since it was anticipated that vitamin A's effect on membranes would be far more subtle than that of complement, we decided to measure the efflux rates from vitamin A-liposomes for a series of small water-soluble ions and molecules including K^+, Cl^-, Br^-, I^-, glycine, lysine and glucose.

From these experiments it was concluded that trans-retinol in levels less than 10 times that found naturally in membranes can increase the permeability of lipid bilayers to protons enough to cause uncoupling of oxidative phosphorylation. This implies that hypervitaminosis A could possibly lead to uncoupling of oxidative phosphorylation by disrupting normal bilayer structure and dissipating energetically essential gradients.

Materials and Methods: Liposomes were made from 300 mg of egg lecithin (Sigma, Type IX-E) and 0 to 7 membrane mole % trans-retinol (Sigma, Type X) in 15 ml of appropriate aqueous buffer via the ether evaporation method (8) or sonication method (9). The aqueous buffer contained the solute to be sequestered (either 250 mM KCl, NaBr, NaI, lysine, glycine or 500 mM glucose) as well as 10 mM Tris buffered at pH 9.5. The untrapped solute was removed from the liposomes by three preliminary dialysis steps, followed by chromatography on Sephadex G-50 (Pharmacia) all done with osmotically balanced buffers to prevent liposome breakage (5). Fifteen ml of the loaded liposomes were then transferred to cellophane dialysis tubing (Carolina Biological Supply Co.) and dialysized against 50 ml of the same buffer used in the preliminary dialysis step. Appearance of the diffusing species was detected outside the dialysis bag via ion selective electrodes (K^+-Markson Scientific; Br^-, I^- - Lazar Research Labs; Cl^--homemade) or colorimetric tests (glycine, lysine-ninhydrin reaction (10); glucose- glucose oxidase test (11)). The ion selective electrodes were used in conjunction with a Beckman Model 3500 Digital pH Meter and readings were taken every 5 minutes for several hours. One ml samples of glycine, lysine or glucose were withdrawn every 30 minutes and analyzed by the colorimetric tests. A final determination was made after dialysis had ceased (3 days). From this long term dialysis the total amount of solute initially trapped was determined. The initial diffusion rate per minute was determined for each experiment (eg. for every solute, at each concentration of trans-retinol). The data, represented as the % diffused/min is the amount of material diffused for each time period divided by the total amount trapped at time zero.

Results and Discussion: The efflux rates for a series of small spherical ions (K^+, Cl^-, Br^- and I^-) initially sequestered inside retinol-egg lecithin liposomes is reported in Figure 1. Figure 2 presents the efflux rates for larger, nonspherical solutes (glycine, lysine and glucose). For all cases, a large increase in diffusion was measured as a function of trans-retinol concentration. The smaller ions reached maximum efflux rates at lower levels of trans-retinol than did the larger solutes. When the initial efflux rates were plotted against the diameter of the diffusing species (12), a straight line was obtained for the smaller ions (Figure 3). I^- and the other nonspherical solutes tested (glycine, lysine and glucose) did not

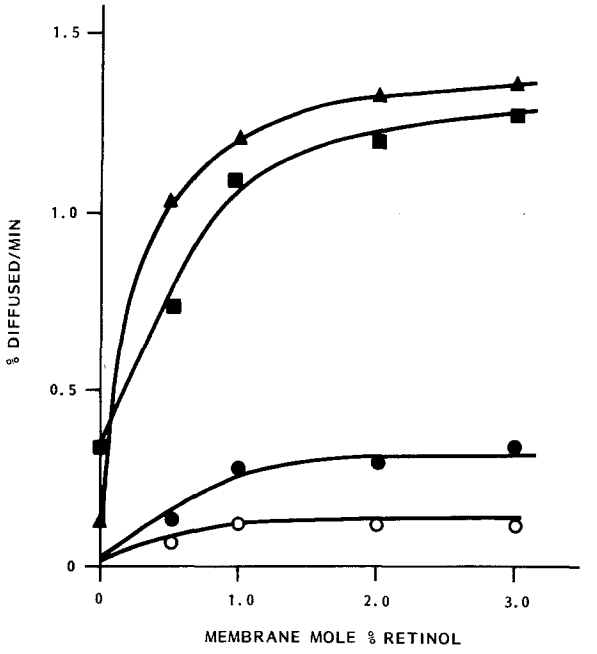
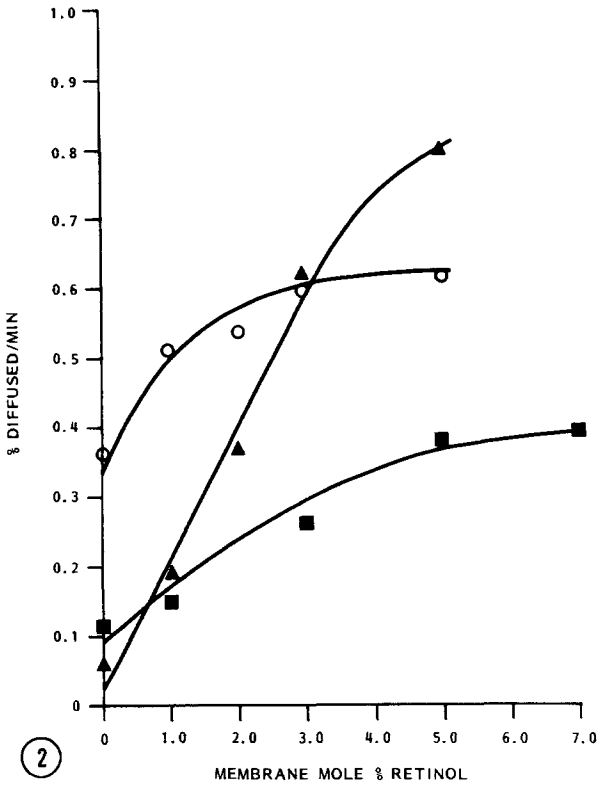


Figure 1. Efflux rates from trans-retinol-egg lecithin liposomes for a series of spherical ions vs the membrane mole % trans-retinol incorporated into the liposomes. (K^+,-▲-; Cl^-,-■-; Br^-, -●-' I^-, -○-)

fit on this line but, as expected, did display diffusion rates much slower than those of the small ions. The initial efflux rates expressed as the % diffused/ min/ membrane mole % trans-retinol for all of the solutes are listed in Table 1.

The linearity for efflux rate vs solute size obtained for the small spherical ions afforded us the opportunity to extrapolate this line down to the size of the proton. The extrapolated number of 6.6 % diffused/ min/ membrane mole % trans-retinol was obtained. This number was then compared to published data on the proton permeability for the cristae to determine what level of trans-retinol would be required to produce a known level of uncoupling of oxidative phosphorylation caused entirely by proton permeability. Using the known proton permeability rate for the cristae (13) without uncouplers (0.11 μg H^+/sec/ Δ pH) and with uncoupler (0.5 μM carbonyl cyanide ρ- trifluoromethoxyphenylhydrazone (CFCCP), 1.76 μg H^+/sec/ Δ pH and 0.1 mM 2,4 dinitrophenol (DNP), 1.21 μg H^+/ sec/ Δ pH), and assuming a pH gradient across the cristae of 0.8 units (14), and a lipid to protein ratio of 1:3 (15), we have calculated a required trans-retinol concentration of 0.0153 membrane mole % based on phospholipids for uncoupling equivalent to 0.5 μM CFCCP. The normal retinol



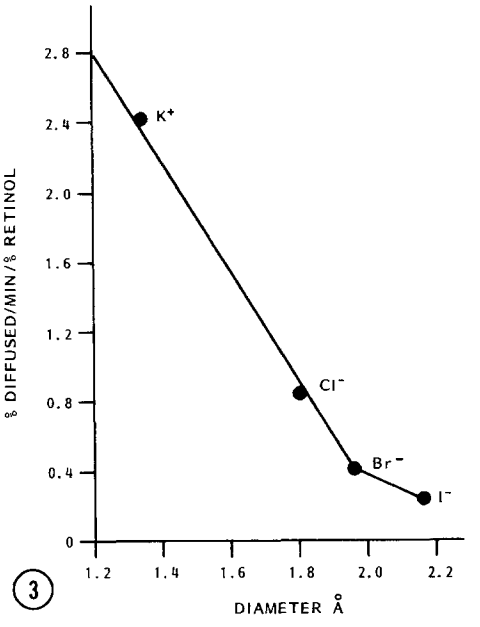


Figure 2. Efflux rates from trans-retinol-egg lecithin liposomes for some nonspherical solutes vs the membrane mole % trans-retinol incorporated into the liposomes. (glycine, -O-; lysine, -▲-; glucose, -■-)

Figure 3. Efflux rates from egg lecithin liposomes per membrane mole % trans-retinol for spherical ions vs the molecular diameter of the diffusing species.

Table 1. Efflux rates for egg lecithin liposomes per membrane mole % trans-retinol for spherical ions (K^+, Cl^-, Br^- and I^-) and nonspherical solutes (glycine, lysine and glucose).

Diffusing Species	% Diffused/Min/Membrane Mole % trans-Retinol
spherical ions	
K^+	2.46
Cl^-	0.87
Br^-	0.34
I^-	0.12
nonspherical solutes	
glycine	0.13
lysine	0.20
glucose	0.50

concentration in membranes has been reported as 0.004 μg retinol/mg N (16) which is the equivalent to 0.00216 membrane mole % retinol, again based on phospholipids. Uncoupling equivalent to 0.5 μM CFCCP would therefore require an increase in retinol of some 7.1 times over the normal level. A lower level of uncoupling caused by 0.1 mM DNP would require only an increase of 4.9 times normal (0.01056 membrane mole % retinol based on phospholipids).

Since the amount of trans-retinol required to induce proton permeability equivalent to that caused by 0.5 μM CFCCP or 0.1 mM DNP in mitochondria is not far from the physiological levels of vitamin A normally found in the cristae, it seems possible that elevated vitamin A levels resulting from hypervitaminosis A could increase proton permeability enough to affect oxidative phosphorylation.

The method presented here is a unique way to indirectly determine the extent of proton permeability with liposomes. Since bimolecular lipid membranes are known to be poorly permeable to ions (17), glucose (18) and amino acids (19), it is much easier to measure increases in their permeability rates than to directly measure the rates of the more readily permeable and more difficult to measure proton. This method is, however, based on the assumption that the diffusion rates can be linearly extrapolated down to proton size. Regardless of the validity of this assumption, these experiments provide a further indication that trans-retinol in levels of 1/2 membrane mole % or less increase significantly membrane permeability and also provide a method to quantitatively estimate the extent of membrane perturbation caused by low levels of vitamin A.

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