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Protective action of cholesterol against changes in membrane fluidity induced by methylparathion

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Cholesterol is an important component of many biological membranes in which its fraction may reach as much as 50% of the total lipid content [1 - 4]. Cholesterol can lower the degree of ordering of phospholipid bilayer in the crystalline state by perturbing the hexagonal packing of the lipids, while the ordering of the fluid state is increased because the rigid ring structure of the sterol limits the possibility *of cis-trans* isomerizations in the neighboring lipid chains [1].

Antunes-Madeira & Madeira [5 - 7] showed that cholesterol could modulate the interaction between insecticides and biological membranes. This statement also concerns organophosphorus insecticide, whose binding to the membrane can be inhibited by cholesterol [8, 9]. In the present work the influence of the organophosphorus insecticide methylparathion (*O,O*-dimethyl *O*-4-nitrophenyl phosphorothioate) on the fluidity of both native and model membranes differing in cholesterol content has been investigated.

Pig erythrocyte membranes were obtained according to Dodge *et al.* [10] and unilamellar liposomes were formed either of phosphatidylcholine or from equimolar amounts of phosphatidylcholine and cholesterol. Protein was determined according to Bradford [11]. A suspension of liposomes or erythrocyte membranes was incubated for 1 h at 37°C in the presence of the insecticide. Fluorescence spectra were measured in a Perkin-Elmer spectrofluorometer, Model LS5-B. Steady-state anisotropy of 1,6-diphenyl-1,3,5-hexatriene (DPH), a probe located in the center of the lipid bilayer, and 8-anilino-1-napthalene sulfonate (ANS), a probe located in the outer region of the bilayer, was calculated from the relationship [12, 13]

$$r = \frac{I_V - GI_H}{I_V + 2GI_H} \qquad (1)$$

where I_V and I_H are the observed fluorescence intensities measured with polarizers parallel or perpendicular to the vertically polarized excitation beam, respectively. G is a factor used to correct for the inability of the instrument to transmit differently polarized light equally. A high value of fluorescence anisotropy reflects a high structural order in the environment of a probe (low fluidity) and *vice-versa.* All the measurements were corrected for light scattering [14]. For DPH[1] the excitation was set at 350 nm, emission at 429 nm; for ANS these wavelengths were 388 and 478 nm, respectively. Fluorescence anisotropy can be related to the rotational diffusion (microviscosity) of the lipids surrounding a probe [12, 13]. Lateral diffusion of lipids can be evaluated in terms of the lateral diffusion coefficient with pyrene as a probe, by measuring the ratio of the intensity of the excimer (I_D) to the intensity of monomer state (I_M) because [15]

$$\frac{I_D}{I_M} = \text{const} \times D_{AQ} \qquad (2)$$

where D_{AQ} is the lateral diffusion coefficient. The greater the ratio I_D/I_M, the greater is the fluidity of the membrane lipids in the sense of lateral diffusion. The excitation was set at 334

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[1]Abbreviations: ANS, 8-anilino-1-naphthalene sulfonate; DPH, 1,6-diphenyl-1,3,5-hexatriene

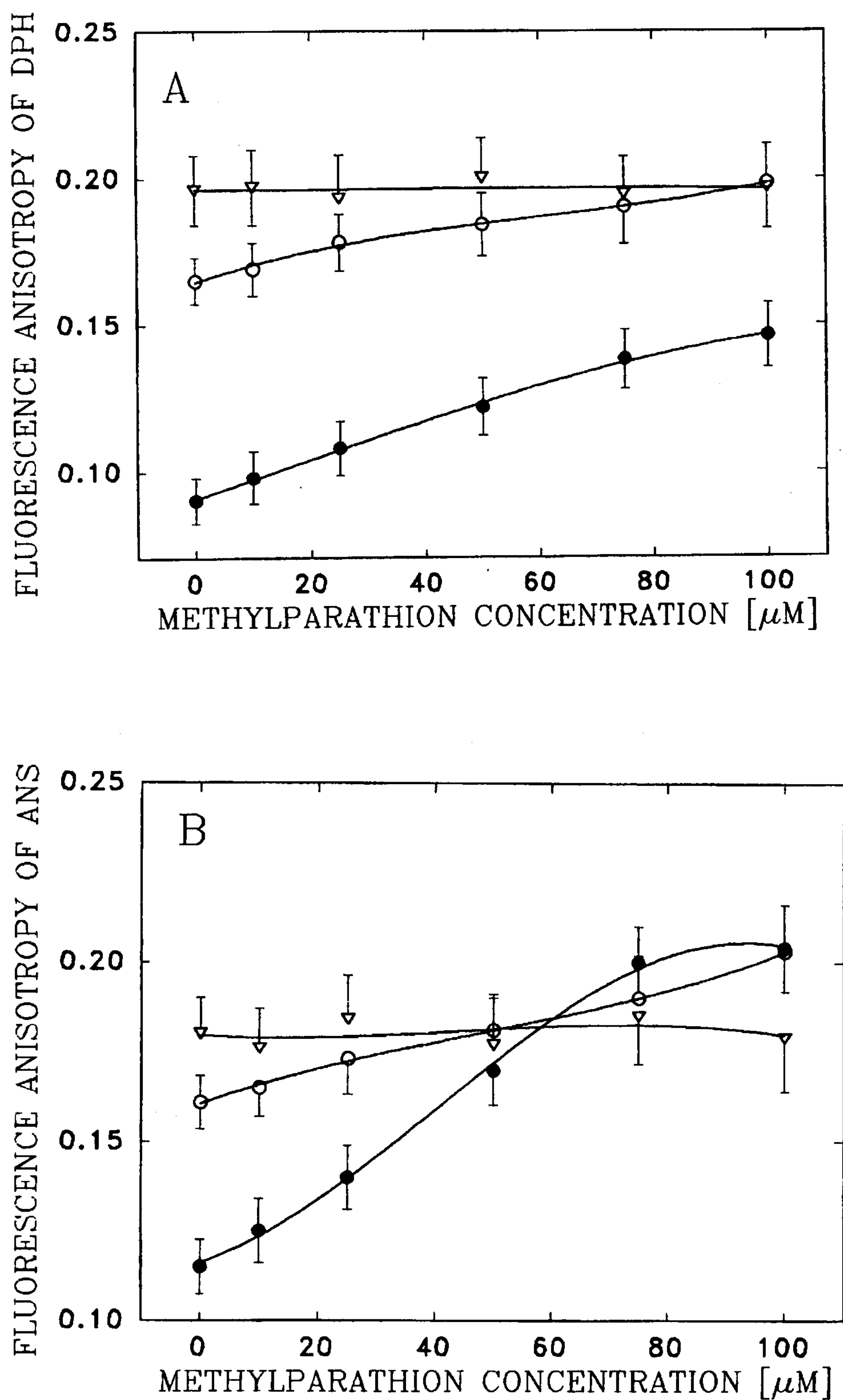

Fig. 1. Fluorescence anisotropy of DPH (A) and ANS (B) for: erythrocyte membranes (○), liposomes prepared from phosphatidylcholine (●), and liposomes made from phosphatidylcholine and cholesterol mixed in equimolar amounts (∇), as a function of methylparathion concentration.
Error bars denote ± S.E., each experimental point is the mean of five replications

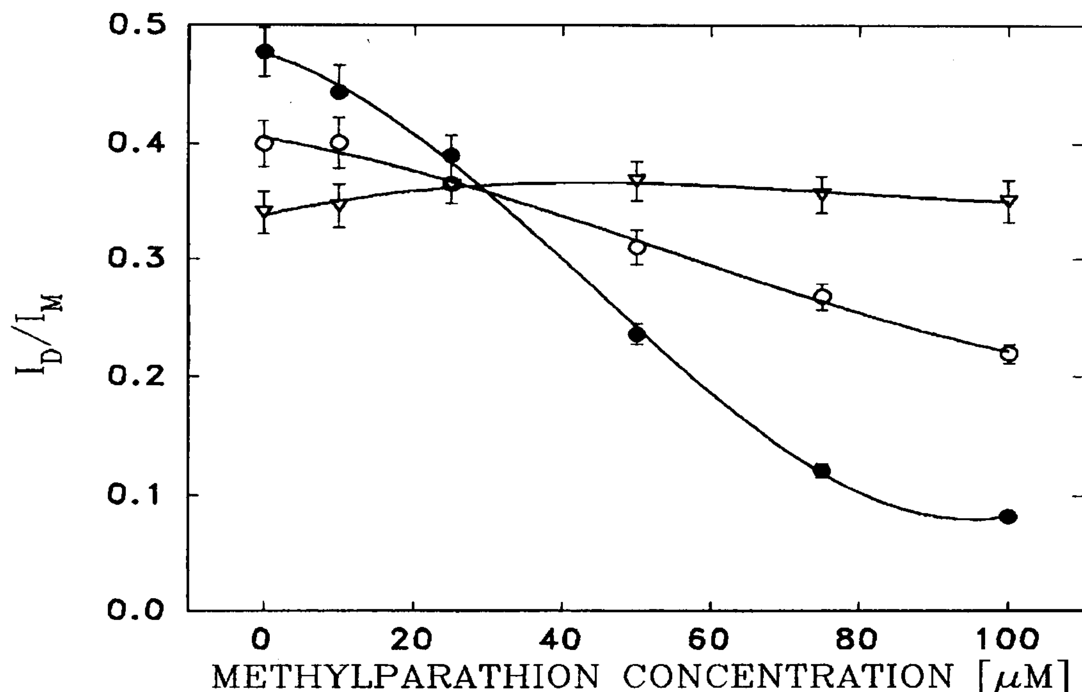

Fig. 2. The ratio of intensity of fluorescence of excimer (I_D) to monomer (I_M) state of pyrene for: erythrocyte membranes (○), liposomes prepared from phosphatidylcholine (●), and liposomes made from phosphatidylcholine and cholesterol mixed in equimolar amounts (∇), as a function of methylparathion concentration.
Error bars denote ± S.E., each experimental point is the mean of five replications

nm and the emission spectra were taken in the range of 350 - 500 nm. The spectra were corrected for light scattering. The differences between mean values were compared by the Scheffe's multiple comparison test [16].

Methylparathion significantly altered anisotropy in the erythrocyte membranes and liposomes of pure phosphatidylcholine measured with DPH (Fig. 1) and ANS (Fig. 2). The presence of the insecticide, either DPH or ANS, caused an increase in anisotropy of the two probes (decrease of fluidity) in erythrocyte membrane and liposomes of pure phosphatidylcholine, in a dose-dependent manner. This increase was approximately three times higher in liposomes than in erythrocyte membranes, whereas in the liposomes enriched in cholesterol no changes in anisotropy were observed.

The insecticide also evoked a decrease in the fluidity of erythrocyte membranes and liposomes of pure phosphatidylcholine, as evaluated by the ratio I_D/I_M for pyrene (Fig. 3).

The decrease was approximately twice as large for liposomes than for native membranes. There were no changes in the I_D/I_M ratio for liposomes enriched in cholesterol.

From the obtained results it follows that the effect of methylparathion on the fluidity of the membrane preparations used decreased with the increase of the relative cholesterol content in the membranes. It was the strongest in the case of pure phosphatidylcholine liposomes, weaker with erythrocyte membranes and almost nil with 1:1 phosphatidylcholine-cholesterol membranes. In this sense some protective action of cholesterol against exposition to the insecticide can be considered. It has been established that cholesterol can evoke a decrease in fluidity of biological membranes in their fluid state [1, 17], so it seems reasonable to assume that the 50 mol% content of cholesterol leads to a specific saturation preventing the further "solidifying" action of the insecticide, observed for erythrocyte membrane and liposomes without added cholesterol.

The second difference in the action of methylparathion was observed in its effect on anisotropy. The changes were more pronounced for

ANS, a probe located in the outer region of the bilayer. This could be due to the insecticide binding preferentially to this region of membrane. Since it is the same region in which cholesterol can be localized, it follows that methylparathion can compete with the sterol for the same binding sites. Antunes-Madeira & Madeira [8, 9] showed that the partition coefficient for malathion and parathion in egg phosphatidylcholine bilayers decreased linearly with temperature in the range at which the lipid was in the liquid-crystalline state; addition of 50 mol% cholesterol strongly decreased incorporation of the insecticides and abolished the temperature dependence. Partition values in native membranes decreased sequentially as follows: rabbit sarcoplasmic reticulum, rat liver mitochondria, pig brain microsomes, myelin and erythrocytes; this dependence reflects the relative content of cholesterol in these membranes.

The protective action of cholesterol against malathion was also reported by Błasiak & Walter [18]. In another work [19] it was demonstrated that changes in the fluidity of liposomes formed from synthetic phosphatidylcholines, evoked by parathion and methylparathion were less pronounced when the liposomes were enriched in cholesterol.

From the results obtained in this work as well as from those referred in the text it follows that the presence of cholesterol should be taken into account in considering the interaction between organophosphorus insecticides and biological membranes.

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