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Membrane fluidity as affected by the organochlorine insecticide DDT

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Fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene (DPH) was used to study the interaction of DDT with model and native membranes. DDT decreases the phase transition midpoint temperature (T_m) of liposomes reconstituted with dimyristoyl-, dipalmitoyl- and distearoylphosphatidylcholines (DMPC, DPPC and DSPC), and broadens the thermotropic profile of the transition. The effects of DDT are concentration dependent and are more pronounced in bilayers of short-chain lipids, e.g., DMPC. The insecticide fails to alter DPH polarization in the fluid phase of the above lipids. Similar effects were observed in binary mixtures of DMPC plus DPPC. Furthermore, DDT alters the single broad transition of the equimolar mixture of DMPC plus DSPC into a biphasic transition. The lower temperature component has a midpoint at 25 °C, i.e., a value close to the T_m of DMPC. DDT inhibits to some extent the cholesterol-induced ordering in DMPC bilayers and high cholesterol concentrations ($\geq$ 30 mol%) do not prevent insecticide interaction, conversely to the effect observed for lindane (Antunes-Madeira, M.C. and Madeira, V.M.C. (1989) Biochim. Biophys. Acta 982, 161–166). Apparently, the bilayer order is not disturbed by DDT in fluid native membranes of mitochondria and sarcoplasmic reticulum, but moderate disordering effects are noticed in membranes enriched in cholesterol, namely, brain microsomes and erythrocytes.

Introduction

DDT, a persistent and lipophilic insecticide, has been widely used in the control of insect pests. However, the persistence and lipophilicity of the insecticide induces progressive accumulation in tissues of man and other species and toxic concentration may be reached. Lethal doses of the insecticide disrupt the transmission of impulses along the nervous system producing loss of coordination followed by tremors, convulsions, paralysis and death [2–4]. On the other hand, resistance to DDT has developed in many species [3]. Therefore, this compound has been largely banned from agriculture use, although it is still in use to combat insect vectors of disease in several countries. Therefore, the precise knowledge of DDT effects is an imperative task since it may help in the development of analogs with reduced environmental persistence but with increased biological activity.

The efforts, in the past two decades, to define the molecular basis of DDT toxicity have been relatively unsuccessful. However, the strong lipophilic character of this insecticide suggests that it may act at the level of biomembranes as indicated by several studies. DDT alters specific properties of ion channels in axon membranes responsible for delayed repolarization of the action potential and repetitive activity of the nerve [3–6]. Furthermore, DDT affects membrane ATPases associated with transport and energy transduction [7–12]. On the other hand, DDT alters the permeability of liposomes and native membranes to K^+ [13–15] as well as to other electrolytes and non-electrolytes [16,17]. Also, it has been shown that DDT alters the thermotropic behaviour of lipid bilayers [18–22]. The relationship of the above effects to the toxic action of DDT in vivo is still unclear. However, the overall data suggest that biomembranes are good candidates for target sites, either for acute or delayed DDT action. In order to relate the perturbation effects of the insecticide upon membrane mechanisms to the actual bilayer concentrations, partition coefficients have been determined in

Abbreviations: DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DSPC, distearoylphosphatidylcholine; DDT, 2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane; T_m, midpoint temperature of thermotropic phase transition; DPH, 1,6-diphenyl-1,3,5-hexatriene.

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several model and native membranes [23]. Data indicate that membrane fluidity * is an important parameter affecting DDT incorporation and, probably, its toxicity. Therefore, the effects of DDT on the fluidity of well defined model and native membranes are here under study.

Materials and Methods

Preparation of liposomes and native membranes

For preparing multilayered liposomes, solutions of pure phospholipids were taken into round-bottom flasks and the solvent was evaporated to dryness. The lipid film was hydrated with an appropriate volume of 50 mM KCl, 10 mM Tris-maleate (pH 7.0), and dispersed under N_2 atmosphere by hand shaking in a water bath, 7–10 Cdeg above the transition temperature of the phospholipids. The sample was then shaken vigorously by vortex for 1 min and briefly sonicated (5 bursts of 30 s each). Heterogeneous phospholipid bilayers were obtained with equimolar amount of single components and phospholipid-cholesterol bilayers by supplementing original phospholipid solutions with appropriate amounts of cholesterol. The final lipid concentration was nominally 345 μM in all cases.

Various native membranes, namely, erythrocytes, brain microsomes, myelin, sarcoplasmic reticulum and mitochondria were prepared as described elsewhere [24]. Protein concentrations were determined by the biuret method [25] calibrated with serum albumin. Membrane suspensions were rapidly frozen in liquid nitrogen and kept at $-80\,^{\circ}$C.

DPH and DDT incorporation into membranes

Membranes were labeled with DPH in the following way: the probe (2 mM) in tetrahydrofuran was injected, while vortexing, into vesicle suspensions (345 μM in lipid) to give a phospholipid/probe molar ratio of about 200. The complete uptake of the probe was accomplished letting the membrane suspensions to stand at room temperature for 18–20 h in the dark. After this period, DDT was added from concentrated ethanolic solutions (50 mM). The period of equilibration with DDT varied from 1 to 2 h, according to the concentration used. Control samples received equal amounts of tetrahydrofuran and ethanol. It should be stressed that added concentrations of DDT are within its solubility range in our experimental conditions. The solubility was monitored by recording the Raleigh scattering (360 nm) in a spectrofluorometer at high sensitivity.

* The term 'fluidity' is used here in an operational sense as being related to the order and kinetics of phospholipid acyl chains affecting the rate of rotational diffusion of DPH. This fluidity is related but not identical with the physical definition of fluidity that assumes isotropical probe motion in an isotropic liquid.

Fluorescence polarization measurements

Fluorescence spectra were measured as described for lindane [1] with appropriate modifications. The excitation slit was 4 nm in all cases. The emission slit was 8 nm for mitochondrial and erythrocyte membranes and 6 nm for the other preparations. The degree of polarization (P) was calculated according to Refs. 26 and 27, from the equation

$$P = \frac{I_{\parallel} - I_{\perp} G}{I_{\parallel} + I_{\perp} G}$$

as previously described for lindane [1]. The correction factor G for instrument polarization is given by the ratio of vertical to the horizontal components when the excitation light is polarized in the horizontal direction [28]. A high degree of fluorescence polarization (P) represents a high structural order or low membrane fluidity, and vice-versa.

For the determination of phase transition midpoints (T_m), the polarization curves were decomposed into three linear segments and T_m was taken as the midpoint of the vertical projection of the segment with the steepest slope.

Reagents

Cholesterol, dimyristoyl-, dipalmitoyl- and distearoylphosphatidylcholines, at least 98% pure, and DPH were obtained from Sigma. DDT (chromatographic grade) was obtained from Supelco, Inc.

Results and Discussion

Model membranes of single lipid species

The effects of DDT on the thermotropic properties of single phospholipid bilayers of DMPC, DPPC and DSPC were investigated (Fig. 1). The insecticide decreases the phase transition temperature midpoint (T_m) of the above phospholipid bilayers and broadens the temperature range of the transition profile. These concentration dependent effects are more pronounced in DMPC as compared with DPPC and DSPC (Fig. 1). Thus, for 50 μM DDT, T_m of DMPC, DPPC and DSPC are shifted by 0.9, 0.4 and 0.3 Cdeg, respectively. These differences parallel the degree of incorporation of the insecticide since its partition coefficient in DMPC is higher than that observed in DPPC and DSPC [23]. Conversely to the organochlorine insecticide lindane (Fig. 1 and Table I of Ref. 1), DDT does not extensively perturb the cooperativity of the above bilayers, since only relative small effects in T_m accompanied by slight broadening effects are observed (Fig. 1), despite the high partition coefficient of DDT into DMPC, DPPC and DSPC (Table I of Ref. 29). Therefore, the small effect of DDT is certainly related with its molecular structure and its localization across the thickness of the

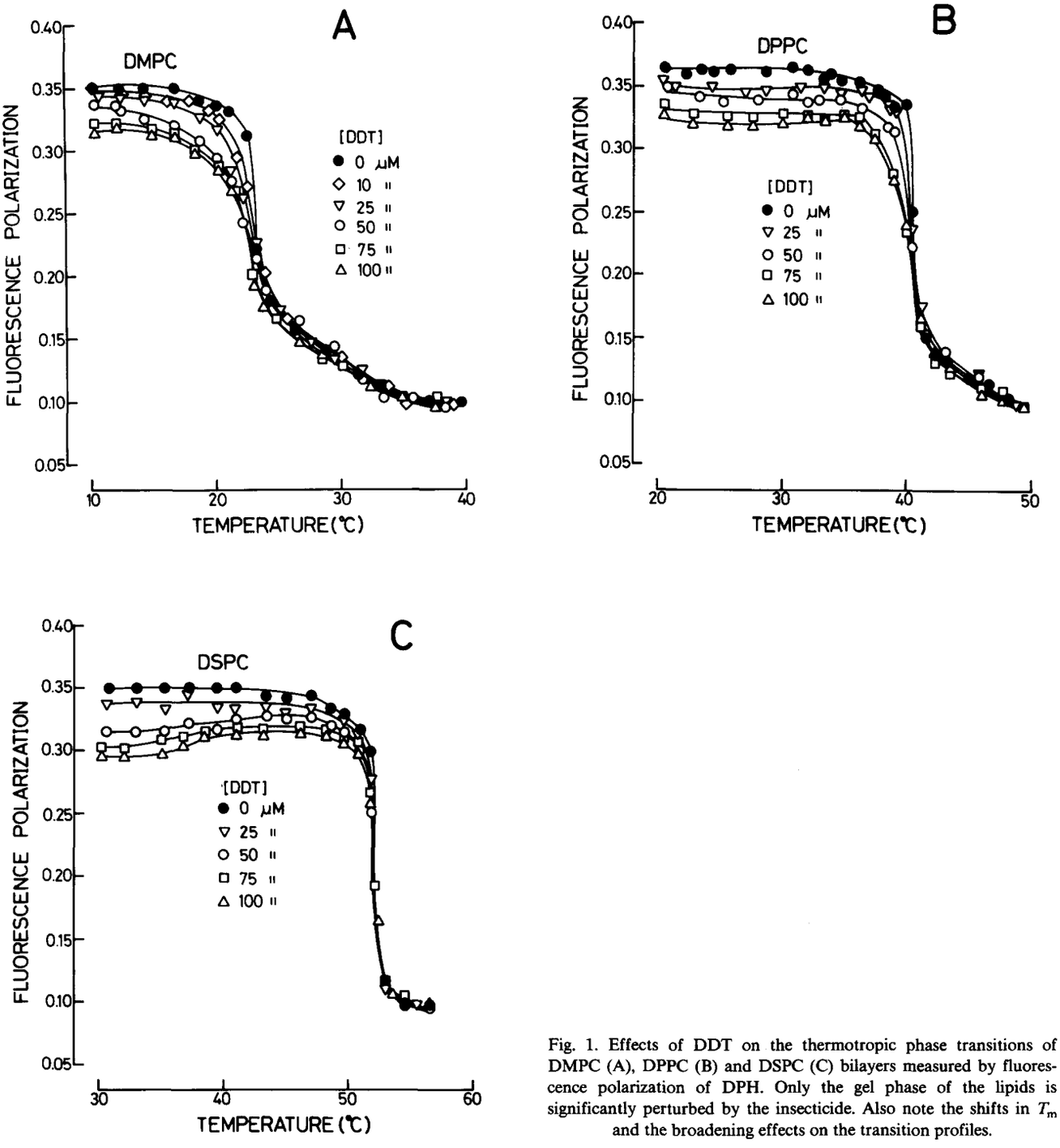

Fig. 1. Effects of DDT on the thermotropic phase transitions of DMPC (A), DPPC (B) and DSPC (C) bilayers measured by fluorescence polarization of DPH. Only the gel phase of the lipids is significantly perturbed by the insecticide. Also note the shifts in T_m and the broadening effects on the transition profiles.

bilayer. Consequently, DDT is accommodated in the bilayer without significant perturbation of the molecular order and/or distortion of lipid phase geometry in regions where DPH is distributed.

Data of Fig. 1 also indicate that DDT significantly decrease the fluorescence polarization of DPH in the temperature region below the phase transition, i.e., in the gel phase of the above lipid bilayers. However, no apparent effect was detected in the liquid-crystalline phase despite the high partition coefficient of DDT in the fluid phase of DMPC, DPPC and DSPC bilayers [23] in agreement with the results of Buff et al. [20] for DPPC bilayers, where they found that only the gel phase is affected by the insecticide, but not the fluid phase. However, the incorporated insecticide must in some way perturb the physical behaviour of lipids in the fluid phase, since DDT significantly increases the permeability of fluid egg-PC membranes to non-electrolytes and to ion-ionophore complexes [16]. Presumably the perturbation occurs in domains close to the

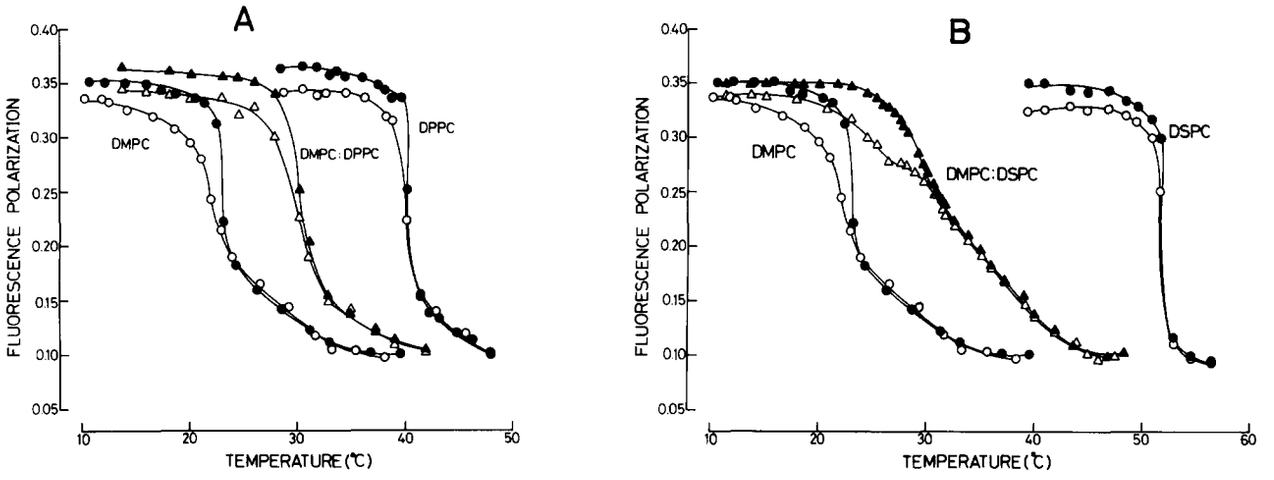

Fig. 2. Thermotropic transitions of bilayers formed with equimolar mixtures of DMPC/DPPC (A) and DMPC/DSPC (B) determined by fluorescence polarization of DPH, in absence (solid symbols) and presence (open symbols) of 50 μM DDT. In mixtures of DMPC/DSPC, a biphasic transition is induced by the insecticide suggesting phase separation.

headgroups of phospholipids not reached by DPH, although most of the DDT molecules would probably distribute in the central core of the bilayer.

Model membranes of binary mixtures

In addition to single lipid species systems, the studies were extended to more complex models of mixed composition, namely, DMPC/DPPC and DMPC/DSPC, at equimolar concentrations. Effects similar to those described above for single bilayers of DMPC or DPPC were detected in mixed DMPC/DPPC bilayers (Fig. 2A). DDT (50 μM) lowers T_m of the mixture by about 0.7 Cdeg and broadens the transition profile (Fig. 2A). Furthermore, DDT changes the single transition of the binary mixture DMPC/DSPC into a biphasic profile (Fig. 2B). Apparently, DDT separates the bilayer lipids into two main domains, one containing DMPC presumably associated with most of the insecticide and the other containing a mixture of DMPC plus DSPC relatively free from insecticide interaction. The increased membrane fluidity with the promotion of phase separations may have physiological consequences if the insecticide could have similar behaviour in native membranes [30–33]. Interesting is also the fact that other insecticides, namely lindane and the organophosphorus compounds parathion and azinphos, likewise promote phase separations [1,18].

Bilayers enriched with cholesterol

As previously shown [34], the fluidity of DMPC bilayers markedly decreases with the cholesterol/phospholipid ratio (Fig. 3). The ordering effects of cholesterol in DMPC bilayers are partially inhibited by DDT, and high cholesterol contents ($\geq$ 30 mol%) do not prevent disordering effects induced by the insecticide (Fig. 3).

These observations concur with previous studies showing significant DDT partitioning in egg-PC membranes ($1.5 \cdot 10^5$) at high cholesterol concentrations ranging from 30 to 50 mol% [23], i.e., a range where other insecticides (parathion, lindane and malathion) are almost completely excluded [24,35,36]. Furthermore, the disordering effects induced by incorporated DDT are consubstantiated by the fact that the insecticide increases non-electrolyte and ion-ionophore permeability in egg-PC membranes enriched with cholesterol [16]. Consequently, native membranes with high cholesterol contents are expected to be disordered by DDT and basic membrane mechanisms dependent on membrane lipid will be accordingly affected.

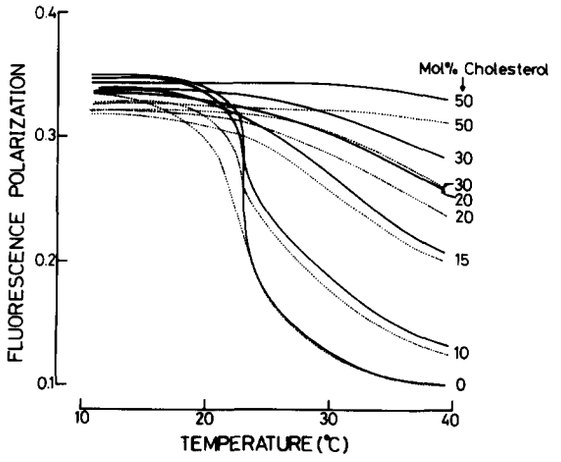

Fig. 3. Thermotropic properties of DMPC/cholesterol bilayers determined by fluorescence polarization of DPH, in the absence (solid lines) and presence (dotted lines) of 50 μM DDT. The numbers adjacent to the curves represent mol% of cholesterol incorporated into DMPC bilayers. Each curve was drawn across 15–17 experimental points subsequently removed for the sake of clarity.

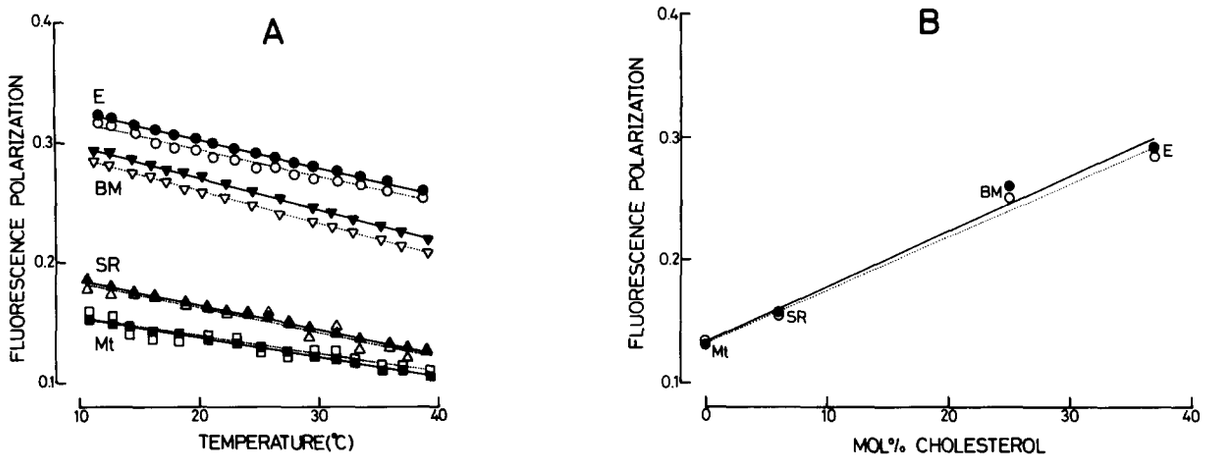

Fig. 4. Fluorescence polarization of DPH in native membranes as a function of temperature (A) and intrinsic cholesterol content (B), in absence (solid symbols) and presence (open symbols) of 50 μM DDT. Data of B were taken from A (fluorescence polarization data at 24°C). Regression lines were calculated by the least-squares method. Cholesterol/phospholipid molar ratios for mitochondria (Mt), sarcoplasmic reticulum (SR), brain microsomes (BM) and erythrocytes (E) are 0, 6, 25 and 37 mol%, respectively. The small statistical errors for most experimental points fall within the size of the symbols.

Native membranes

Well defined and representative membranes, namely, mitochondria, sarcoplasmic reticulum, brain microsomes and erythrocytes, differing in intrinsic cholesterol content, were chosen to study DDT induced fluidity changes as a function of temperature (Fig. 4). Membrane fluidity decreases linearly (correlation coefficient 0.992) with the contents of intrinsic cholesterol. Thus, membranes of mitochondria and sarcoplasmic reticulum with low cholesterol contents (0 and 6 mol%, respectively), are significantly more fluid than those of brain microsomes and erythrocytes where cholesterol accounts for 25 and 37 mol%, respectively. Therefore, according to the results summarized in Fig. 4B, the degree of fluidity follows the sequence: mitochondria > sarcoplasmic reticulum ≫ brain microsomes > erythrocytes. Additionally, Fig. 4 also shows that the effects of DDT (dotted lines) in native membranes are similar to those described for models. Thus, DDT does not exert apparent disordering effects into relatively fluid native membranes of mitochondria and sarcoplasmic reticulum, in close agreement with the results obtained in single and mixed phospholipid bilayers in the fluid state. Again, as described for model membranes enriched with cholesterol, DDT also induces moderate disordering effects in membranes of brain microsomes and erythrocytes, i.e., membranes with high cholesterol content.

Clearly, the fluidizing effects of DDT do not correlate with the partition coefficients, ranging from $6 \cdot 10^5$ to $12 \cdot 10^5$, with the following sequence in the membranes under study: sarcoplasmic reticulum > mitochondria > brain microsomes > erythrocytes [23]. Although the incorporation of DDT in sarcoplasmic reticulum and mitochondria is significantly higher than in the other membranes, fluidizing effects are only noticed in the late, but not in the former. Therefore, the structure and/or geometry of the lipid bilayer of sarcoplasmic reticulum and mitochondria membranes accommodates DDT with a minimum of distortion. However, the fluidizing effects of DDT into ordered domains of the model systems, either imposed by the temperature of cholesterol (Figs. 1 and 3) suggest that especially ordered domains surrounding integral proteins [37–40] can be affected by the insecticide, without perturbation of the general membrane fluidity. Actually, DDT affects the Ca^{2+} pump activity of sarcoplasmic reticulum [12] presumably by interacting with the boundary lipid of the pump. The moderate fluidizing effects of DDT noticed in erythrocytes by fluorescence polarization of DPH may, however, reflect bilayer perturbation since the insecticide increases glycerol permeability in pig erythrocytes [17], where glycerol translocation essentially occurs across the lipid continuum of the bilayer [41].

It can be concluded that the structural perturbation of lipid bilayers may partially explain the alterations of membrane mechanisms and, thus, the toxicity of DDT.

Acknowledgements

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