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THE CHANGES OF OSMOTIC FRAGILITY OF PIG ERYTHROCYTES INDUCED BY ORGANOPHOSPHORUS INSECTICIDES

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Organophosphorus insecticides: methylbromphenvinphos, dichlorvos, malathion and methylparathion exert antilytic effects on pig erythrocytes by preventing osmotic disruption of membranes in hypotonic saline media. The order of effectiveness is the following: methylbromphenvinphos, methylparathion, malathion, dichlorvos.

Organophosphorus insecticides (OI) which are poorly soluble in water, can bind with structures rich in lipids. Biological membranes seem, therefore, to be the site of the interaction between OI and the cell, especially as the membrane is a barrier to penetrate of these compounds into the cell. Therefore in considering the biological action of OI, their effect on the physical and chemical properties of biological membranes should be taken into account [1, 2]. The primary sites of OI activity causing toxic effects for mammals are body fluids. Blood can be regarded both as the OI interaction site and as a carrier of OI to different parts of the organism. The properties of biological membrane as the barriers separating the intracellular from extracellular environment are regulated by the physical state of their components affecting the lipid-lipid, lipid-protein and protein-protein interaction [3, 4]. The permeability of erythrocyte membranes and their osmotic fragility are also determined by these interactions [5]. Many insecticides increase the permeability of lipid membranes for non-electrolytes and ion-ionophore complexes [6] and their effectiveness is positively correlated with the degree of their toxicity for mammals. The changes of membrane permeability may result from the disturbance by OI of the lipid bilayer arrangement since OI are able to shift thermotropic transitions to lower temperature ranges [7]. The aim of the present work was to study the effect of some OI on the osmotic fragility of pig erythrocytes.

MATERIALS AND METHODS

Organophosphorus insecticides: methylbromphenvinphos [2-bromo-1 (dichlorophenyl)vinyl dimethyl phosphate], dichlorvos (2,2-dichlorovinyl dimethyl phosphate), malathion [*S*-1,2-bis-(etoxycarbonyl)ethyl *O*-*O*-

-dimethyl phosphorodithioate] and methylparathion (*O,O*-dimethyl *O*-4--nitrophenyl phosphorothioate) were obtained from the Institute of Organic Industrial Chemistry in Warsaw. The insecticides were derived from concentrated (about 60 mM) solutions in ethanol. Pig erythrocyte suspension, in such an amount as to give the final hematocrit value of 0.005, was added to one of the media containing NaCl at concentrations ranging from 17 to 163 mM in order to measure osmotic fragility. Then OI was added to the cells and incubated either for 2 h or 13 h at 37°C. The experiment was carried out in three series: control, and series containing OI at the concentrations of 10^{-5} and 10^{-4} M. The control series contained ethanol in the amount corresponding to the highest OI concentration. After incubation the samples were centrifuged for 10 min at $2000 \times g$ and the absorbance at 542 nm was determined in the supernatant. Osmotic fragility was measured by the degree of hemolysis. The absorbance obtained when the erythrocyte suspension was diluted with water was assumed as 100%. Each experiments was performed in five replications.

RESULTS AND DISCUSSION

The used insecticides protect erythrocytes against hypotonic hemolysis, as seen in Figs 1 and 2. To quantitatively estimate erythrocyte fragility, we introduced, following Antunes-Madeira *et al.* [5], the osmotic fragility index

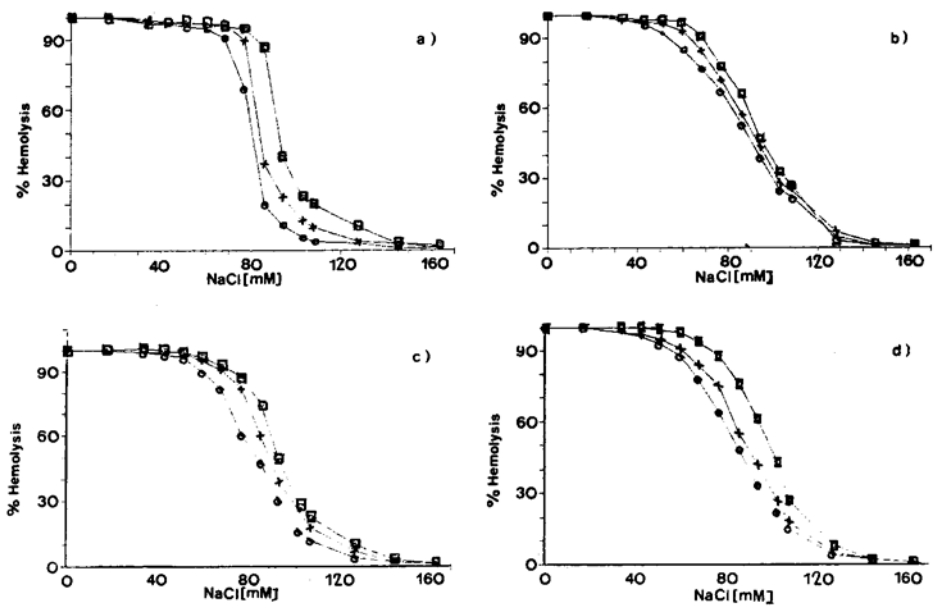

Fig. 1. Degree of hemolysis of erythrocytes after 2 h incubation with different doses of: a, methylbromphenvinphos; b, dichlorvos; c, malathion; d, methylparathion; □, control; +, 10 μM; ○, 100 μM

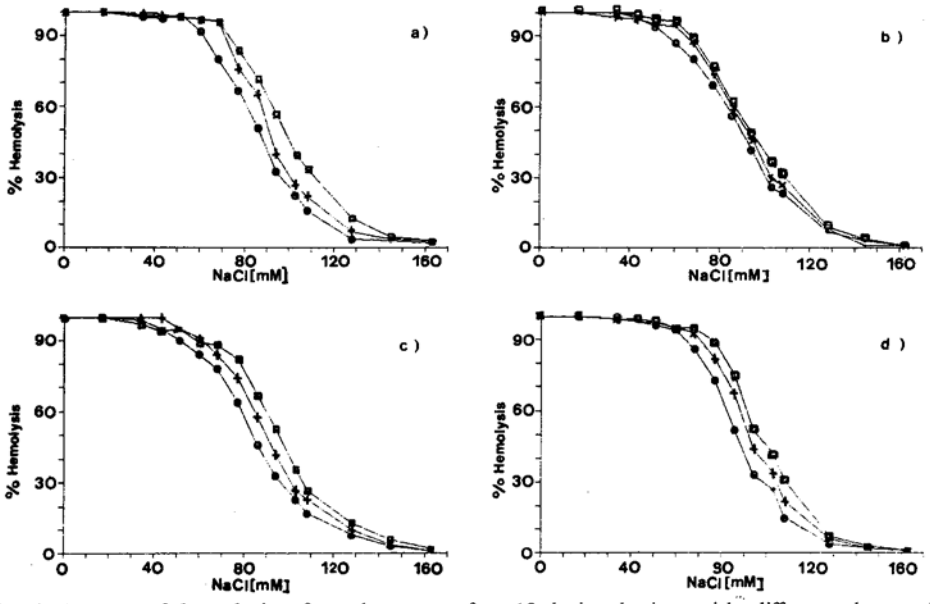

Fig. 2. Degree of hemolysis of erythrocytes after 13 h incubation with different doses of: a, methylbromphenvinphos; b, dichlorvos; c, malathion; d, methylparathion; □, control; +, 10 μM; ○, 100 μM

(EPI) which is the ratio of the hemolysis measured in absence and presence of insecticides.

$$\text{EPI} = \frac{\%\ \text{hemolysis in controls}}{\%\ \text{hemolysis in presence of insecticide}}$$

The EPI value of 1 means that OI did not affect osmotic fragility, EPI = 2 means that the degree of hemolysis in the presence of OI corresponds to half that observed for control. Generally, the higher the EPI value, the stronger the protective OI action against hemolysis. The EPI values given in Table 1 clearly show that methylbromphenvinphos (maximal EPI about 5) had the strongest protective action against hemolysis, and dichlorvos the weakest one (minimal EPI about 1). The antihemolytic effect of OI evidently depended on their concentrations, at higher concentrations higher EPI values were noted for all the insecticides applied. The protective effect of OI on erythrocytes was slightly more pronounced for the shorter (2 h) incubation time than for the longer one (13 h). The erythrocytes kept for a long time at 37°C have a stronger tendency to hemolyse and therefore the action of antihemolytic factors is weaker.

The results indicating the antilytic action of OI are in agreement with those obtained by other authors both in experiments *in vitro* [5] and *in vivo* [8]. The effectiveness of the action of OI decreases in the order: methylbromphenvinphos, methylparathion, malathion and dichlorvos. The reversed order corresponds approximately to the classification of these compounds with respect to their solubility in water, thus suggesting that the effectiveness of OI is correlated with the coefficient of their partition between lipid and water phases.

Table 1

The value of osmotic fragility index (EPI) for erythrocytes in the presence of organophosphorus insecticides

Mean ± standard error, n = 5 in each experiment. The EPI values were calculated from appropriate plots

Insecticide	Concentration [μM]	EPI Incubation time 2 h	13 h
Methylbromphenvinphos	10	2.17±0.21	1.52±0.11
	100	4.76±0.38	1.85±0.20
Methylparathion	10	1.54±0.09	1.17±0.10
	100	1.89±0.11	1.56±0.10
Malathion	10	1.36±0.09	1.27±0.09
	100	1.85±0.08	1.57±0.11
Dichlorvos	10	1.09±0.10	1.06±0.08
	100	1.21±0.09	1.16±0.07

Though these results confirm other reports, they still remain surprising since it was found that OI increase the membrane permeability to nonelectrolytes and ion-ionophore complexes [6]. Our experiments (unpublished) also showed the increase of transport kinetics of potassium ions caused by the increased activity of Na^+, K^+-ATPase induced by OI. The increased permeability for nonelectrolytes and ion-ionophore complexes seems to indicate that OI disturb the arrangement of lipid bilayer with a corresponding temperature decrease of the thermotropic transition. It could be expected that OI would affect the membrane in such a way as to lead to increased hemolysis of erythrocytes. However, the results obtained in this work as well as the ones cited above demonstrate clearly that OI increase the physical resistance of pig erythrocytes to disintegration.

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