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EFFECT OF SOME PESTICIDES CONTAINING CHLORINE ON HEMOLYTIC RESISTANCE AND ACETYLCHOLINESTERASE ACTIVITY OF ERYTHROCYTES

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UDC 577.151.044+612.111.45

KEY WORDS: pesticides; erythrocytes; ultrasound.

Pesticides can affect the structure and biological activity of blood cells [1, 3, 7, 8, 10]. Erythrocytes are a convenient object with which to study the harmful action of pesticides on cells and their membranes, for destruction of the cell membranes leads to readily recordable hemolysis of these cells [1, 8]. Dependence of the hemolytic activity of some pesticides and herbicides on the conditions of initial activity of intracellular enzymes [8, 12] and on the ability of the substance to reduce the "flowability" of the membrane [12] has been studied. However, the kinetic characteristics of action of pesticides containing chlorine on hemolysis of erythrocytes and their resistance to mechanical action, which may characterize certain principles governing the physiological activity of these compounds, has not been studied.

The aim of this investigation was to study the kinetics of action of pesticides containing chlorine on hemolysis and mechanical resistance of erythrocytes to the action of ultrasound and on membrane-bound acetylcholinesterase (AChE) activity and also to obtain quantitative criteria with which to compare the effectiveness of action of pesticides.

EXPERIMENTAL METHOD

Suspensions of erythrocytes isolated from human blood were studied; specific AChE activity was determined by a potentiometric method with automatic recording [3]. The kinetics of erythrocyte hemolysis in isotonic medium under the influence of pesticides was studied by a photocolorimetric method based on the increase in light transmission of a suspension (10^7-10^8 cells/ml) during cell destruction [2]. The comparison cuvette contained a suspension of erythrocytes hemolyzed in distilled water. The pesticides containing chlorine which were used included herbicides: the sodium salt of trichloroacetic acid (TCA) and pentachlorophenolate (PCP-Na); the insecticide chlorophos; the fungicide pentachloronitrobenzene (PCNB), and the sulfur-containing pesticide rogor (Table 1). Resistance of erythrocytes treated with the pesticides to mechanical hemolysis through the action of ultrasound (frequency 1 MHz, intensity 0.4 W/cm^2) was investigated by automatic recording of the kinetics of erythrocyte destruction in a spectrophotometer cuvette [2]. The parameters determined from experimental kinetic curves of ultrasonic hemolysis characterize the mechanical resistance of erythrocytes and its change as a result of treatment of the erythrocytes by the chemical compounds chosen for testing.

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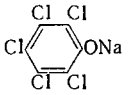
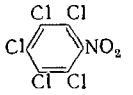
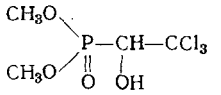
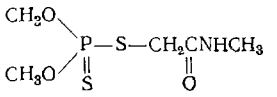
 0007-4888/83/9607-0920$07.50

TABLE 1. Effectiveness of Action of Pesticides on AChE Activity of Erythrocytes

Compound	Structural formula	$C_{A_{50}}$, mM
TCA	CCl_3COONa	120
PCP-Na		$5 \cdot 10^{-1}$
PCNB		$2 \cdot 10^{-2}$
Chlorophos		$5 \cdot 10^{-3}$
Rogor		—

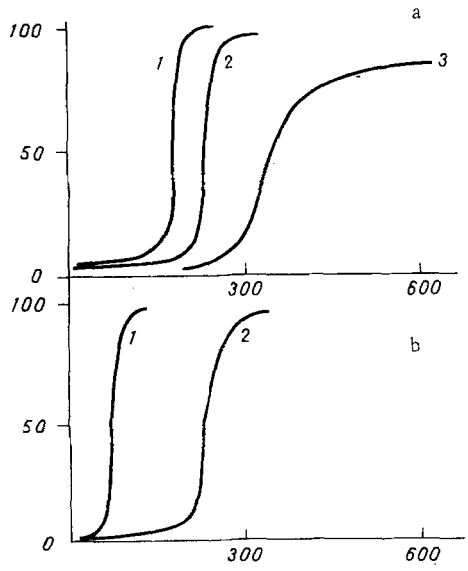


Fig. 1

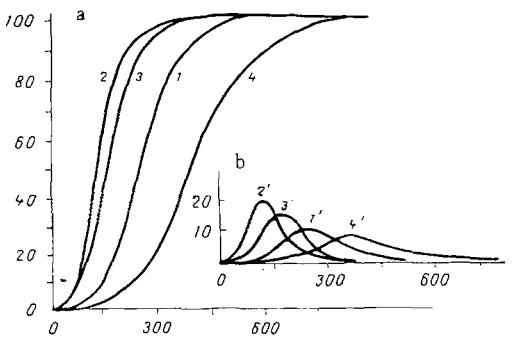


Fig. 2

Fig. 1. Typical kinetic curves of hemolysis of erythrocytes treated with various concentrations of chlorphos (a) and PCP-Na (b). Concentrations of chlorophos: 1) 10^{-2} M; 2) 9×10^{-3} M; 3) 5×10^{-3} M. Concentration of PCP-Na: 1) 5×10^{-3} M; 2) 10^{-2} M. Abscissa, hemolysis time (in sec); ordinate, light transmittance of suspension (in %).

Fig. 2. Dependence of parameters of hemolysis: hemolysis time (t), half-destruction time (t_{50}), and velocity of hemolysis (v) of erythrocytes under the influence of chlorophos (a) and PCP-Na (b), on pesticide concentration in sample. 1) t, 2) t_{50}, 3) v. Abscissa, pesticide concentration (in $M \times 10^3$); ordinate: on left, t and t_{50} (in sec); on right, v (in optical density units/sec).

EXPERIMENTAL RESULTS

As criterion of the action of pesticides on erythrocyte AChE the parameter of 50% inactivation of the enzyme (CA_{50}) was chosen. These pesticides were found to have definite anti-AChE activity. The compounds chosen are not specific AChE inhibitors (Table 1), for they depress activity of this enzyme in much higher concentrations (CA_{50} 120 and 10^{-1}-10^{-3} mM) than the known anti-AChE agents phosphine, amiton, etc., for which CA_{50} is 10^{-6}-10^{-8} mM [5]. Inactivation of the external enzyme AChE found in these experiments indicates that

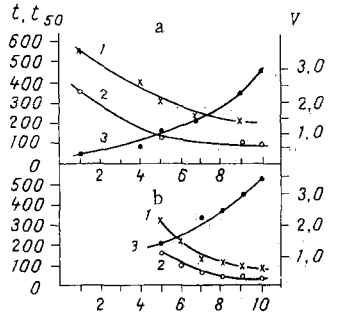


Fig. 3. Kinetic curves of ultrasonic hemolysis of erythrocytes in control and in presence of pesticides (a); corresponding curves for distribution of erythrocytes by resistance, in percent, as a function of hemolysis time (b). 1, 1') Control; 2, 2') 1.8×10^{-3} M PCNB; 3, 3') 1×10^{-4} M PCP-Na; 4, 4') 1×10^{-3} M PCNB. Abscissa: time (in sec); ordinate: a) light transmittance (in percent); b) number of erythrocytes (in percent).

the inhibitory action of organochlorine pesticides is due to injury to the erythrocyte membranes with which AChE is bound [11]. An extreme manifestation of the modifying action of chemical compounds on the erythrocyte membrane, leading to a disturbance of integrity of the cells, is hemolysis. It was shown that PCP-Na, chlorophos, and rogor have natural hemolytic activity in isotonic medium, but treatment of erythrocytes with TCA and PCMB does not cause their hemolysis. Kinetic curves of erythrocyte hemolysis induced by different concentrations of PCP-Na and chlorophos in isotonic medium are illustrated in Fig. 1. The curves are S-shaped with a definite induction period (latent period), and the velocity of hemolysis is concentration-dependent. Dependence of measured and calculated parameters of the structural and functional state of erythrocytes (time and rate of hemolysis, half-destruction time) treated with pesticides on the concentration of the compound is shown in Fig. 2. It will be clear from Fig. 2 that with an increase in concentration of the compound in the incubation medium the hemolysis time and half-destruction time are reduced whereas the velocity of hemolysis increases correspondingly. The method used to study quantitative characteristics of erythrocyte hemolysis under the influence of pesticides enabled the region of concentrations of the compound with a definite degree of hemolytic activity to be established in each case.

It has been shown for certain pesticides [10] and bactericidal preparations [9] causing hemolysis of erythrocytes in isotonic medium that they also induce stabilization of erythrocyte membranes to hemolysis in hypotonic medium. It might be expected that the modifying action of pesticides on erythrocyte membranes would also lead to a change in their mechanical resistance to the action of ultrasound. Typical kinetic curves of ultrasonic hemolysis of erythrocytes treated with various concentrations of pesticides are given in Fig. 3a. PCP-Na, which possesses natural hemolytic activity, also accelerates mechanical ultrasonic hemolysis in concentrations not inducing natural hemolysis (10^{-4}-10^{-5} M, curve 3). A similar effect was found for chlorophos in a concentration of 10^{-3} M. TCA and PCNB, which do not themselves induce erythrocyte hemolysis, differed in their effect on ultrasonic hemolytic resistance of the erythrocytes. TCA accelerated (curve 2) mechanical destruction due to ultrasound, but PCNB stabilized the cells to it (curve 4). Differential curves showing the percentage distribution of erythrocytes, for resistance to ultrasonic hemolysis for instance (Fig. 3b), were obtained from experimental kinetic curves of ultrasonic hemolysis of erythrocytes in the absence and in the presence of pesticides. The curves were extremal in character with an approximately uniform distribution of erythrocytes around the maximum. Under the influence of PCP-Na and TCA (curves 2' and 3') the position of the maximum was changed with a shift to the left, indicating a decrease in mechanical stability of the erythrocytes. In the case of PCNB, however, the maximum of the distribution curve (curve 4') was shifted to the right compared with the control (curve 1'), confirming an increase in erythrocyte resistance to ultrasonic hemolysis in the presence of this compound. Table 2 gives quantitative parameters characterizing changes in erythrocyte resistance to ultrasound under the influence of different concentrations of pesticides. The changes in mechanical stability of erythrocytes under the influence of pesticides thus revealed may reflect disturbances of micro-

TABLE 2. Effect of Pesticides on Parameters of Ultrasonic Hemolysis of Erythrocytes (M ± m)

Parameter	Control	PCNB, m		TCA, M		PCP-Na, m			
		10^{-4}	10^{-3}	$3 \cdot 10^{-3} - 6 \cdot 10^{-3}$	$2 \cdot 10^{-2} - 3 \cdot 10^{-2}$	10^{-6}	10^{-5}	$10^{-4} - 10^{-3}$	10^{-2}
t	500±10	700±22	1200±150	420±17	360±19	484±37	450±27	415±46	250±52
v	1,09±0,03	0,60±0,02	0,30±0,09	1,12±0,04	1,30±0,05	1,02±0,07	1,22±0,09	1,30±0,05	3,00±0,08

Legend. Frequency of ultrasound 1 MHz, intensity 0.4 W/cm^2. t) Hemolysis time (in sec); v) velocity of hemolysis (in optical density units/sec).

viscosity of the protein–lipid system [6, 7]. Under these circumstances pesticides may either be incorporated into the lipoprotein structure of the erythrocyte membrane [3] or interact with erythrocytes by distribution on the hydrophobic surface sites of the membrane where the AChE which they inactivate is located.

Quantitative characteristics of the effect of organochlorine pesticides on structural and functional changes in erythrocytes were thus obtained on the basis of parameters reflecting hemolytic resistance and AChE activity. Since disturbances of erythrocyte membranes may also reflect the characteristics of changes in the plasma membranes of other tissues [4], there is reason to suppose that determination of these characteristics can also be used to judge responses of other biological cells to these substances.

The authors are grateful to Dr. Chem. Sci. Professor K. E. Kruglyakova for help in the discussion of the results.

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