Effect of Cetain Insecticides on the Stabilization And Lysis of Human and Fish Erythocyte

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Abstract: The interaction of four insecticides with erythrocyte membrane was investigated herein. This was attempted by studying their lytic or antilytic effects on erythrocytes (human and fish blood) in critical hypotonic saline media. The insecticides used are representatives of the four major groups: organochlorines (lindane); pyrethroids (decamethrin); carbamates (lannate), and organophosphates (dursban). These insecticides were tested at concentration range % (10^{-10} - 10^{-4} M). The first two compounds exert antilytic effects on both types of erythrocytes by preventing disruption of the membrane and increased its integrity in hypotonic solution. The order of effectiveness was; decamethria > lindane. On the other hand, cell lysis was observed with the other two compounds, with the order of effectiveness; dursban > lannate. The latter effect was attributed to the disruption of cell membrane by such insecticides. Furthermore, the antilytic as well as, lytic effects depended to large extent on the molar concentration of the insecticide. The mechanisms by which such compounds exert their effects were discussed.

Key words: human blood, fish blood, insecticides

INTRODUCTION

Pesticides occupy a rather unique position among many chemicals that man encounters daily. This is because they are deliberately added to the environment for the purpose of pest control in all agricultural programs. In fact, most of such chemicals are not highly selective and generally toxic to many non-target species including man and other animals[18,9].

The most important and widely used insecticides are belonging to four classes: organochlorines, carbamates, organophosphates, and pyrethroids[8]. Being well identified, the mode of action of the carbamate (e.g. organophosphates, and pyrethroids), is the inhibition of acetylcholinesterase[6,21,1,10] and the signs of symptoms of poisoning are typically cholinergic with lacrimation, miosis, convulsions and death[14]. As a class, the organochlorine insecticides are often considered to be less acutely toxic, but of greater potentiality for chronic toxicity than organophosphate and carbamate insecticides. However, their action mechanism is not the same as that of the organophosphates and still unknown for most of them[13]. Similarly, the neurotoxocity of pyrethroids have been also reported[14,11,12]. The neurotoxicity due to organochlorines is initiated by altering specific properties of the axon membrane responsible for delayed repolarization of the action

potential[5,19,20]. Pyrethroids, however, induced similar neurotoxicity by affecting sodium channel gating kinetics[14]. These actions have been explained in terms of specific alterations of membrane permeability and conductance to the ions involved in axonic electrical events[19,20,25].

Most insecticides have low water solubility and are incorporated in lipid-rich cellular structures. Therefore, biomembranes are good candidates as targets of insecticide action. Blood may serve as carrier of insecticides. The lipid moiety of erythrocyte membrane may be also considered as a site of interaction. Moreover, erythroytes are excellent as biomembrane model for the study of the interaction of drugs and other compounds which are biologically active. In their investigation, Antunes-Madeira & Madeira[2] and Speare[25] showed that most insecticides increase the permeability of artificial lipid membranes to non-electrolytes and ion-ionophore complexes. Antunes-Madeira et al., 1980 indicated that these effects may be mediated by disordering actions on the bilayer membrane.

In the present investigation, four insecticidies (representing the four major insecticide groups) known to be neuro-toxic to both mammalian and fish species were used. The osomotic fragility of erythrocytes (for man and fish) was used as model for studying the effects of these pesticides on the barrier properties of biomembranes.

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MATERIALS AND METHODS

Preparation of biological materials: Fresh blood samples were taken from human volunteer (28 years old, healthy male) and alive adult male fish (*Tilapia niloticus*). To these samples, sodium heparin (1000 U/ml) was added so that the final concentration was 1 unit to 1 ml blood.

Aliquits of 1 ml of the heparinized blood were then centrifuged at 1500g for 5 min. The plasma and buffy coat were removed by suction. To each portion, 12.5 ml of sodium chloride solution (154 mM NaCl in 10 mM phosphate buffer, pH 7 was added). The erythrocyte was resuspended by repeated inversion and stored at 4 °C. Samples were analyzed within 24 hours.

Construction of normal hemolysis curves: To determine the concentration of sodium chloride necessary to produce 50% of hemolysis (for human and fish blood), fractional concentrations of 154 mM sodium chloride in buffer were made. The erythrocyte suspension (0.1 ml) was added to 1.5 ml of each concentration and samples were mixed several times and allowed to stand at room temperature (28 ± 1°C) for 10 min. The suspension was then centrifuged at 1500g for 1 min. The optical density of the clear supernatant was read at 540 nm. in spectronic 21 (Bousch & Lomb). The point at which 50% hemolysis occurred was read from hemolysis curve (Fig. 1). The concentrations of NaCl in phosphate buffer produced 50% hemolysis were 88 mM (human blood) and 95 mM (fish blood). Stock solutions of the two concentrations were prepared and used throughout experimentation. It was reported that within day, variation of this method was less than +3% and the colour was stable for 3 hrs[22].

Estimation of hemolytic effect of pesticides: The effects of the four pesticides on erythrocytes were studied using modified method described by Seeman & Weinstein[4] . Different pesticide concentrations (10^{-10} - 10^{-2}M) were dissolved in acetone, portions of 10-100 µl of each compound was added to 1.5 ml of the stock solution (previously described) to which 0.1 ml of erythrocyte suspension was added. The above procedure for obtaining the hemolysis curve was followed. Blanks with corresponding amounts of acetone were run concurrently and showed little effect up to 100 µl. Each concentration was run in triplicate. In some cases, especially in higher concentrations, visible turbid solutions were noticed. The centrifugation step after colour development remove this turbidity.

It is noteworthy to mention that the given concentration was tested for all compounds simultaneously. This was repeated for the new concentration. This procedure would remove, as much as possible, any variations attributable to unknown factors of curve-to-curve variation.

Pesticides used: Four pesticides, representing the main insecticide groups, were tested in the present paper. These are dursban, lannate, lindane, and decamethrin which are belonging to organophosphates; carbamates; organochlorines, and pyrethroids, respectively.

RESULTS AND DISCUSSIONS

The present investigation was attempted to study the erythrocyte hemolysis under the influence of different insecticidal representarives. Therefore, it was necessary to construct the typical hemolysis curves of both human and fish blood.

Hemolysis curve (Fig. 1) shows the variation of erythrocytic lysis with different concentrations of sodium chloride. This curve was prepared for each type of blood i.e. for human and fresh water fish (*Tilapia niloticus*). Using such curves it was easy to deduce the concentration of sodium chloride produced 50% hemolysis for each type of blood. The values obtained were 75 mM and 88 mM NaCl for both fish and human blood, respectively. These concentrations were then employed with various concentrations of the four pesticides used and the relative hemolysis was calculated. The data recoded hereafter are expressed as "relative hemolysis" from controls (NaCl produce 50% hemolysis).

The insecticides, dursban, lannate, lindane, and decamethrin were tested at concentrations ranged from 10^{-10} to 10^{-4} M. Evidently, the constructed concentration hemolysis curves (Figs. 2, 3) indicated that, at lower concentrations (10^{-10}) the relative hemolysis shows the highest values with lindane and decamethrin. The percentage values were 144%; 115% (fish blood); 156%; 164% (human blood) for the two compounds, respectively. These values were sharply declined as the concentration of the insecticide increased, indicating protection against erythrocyte fragility at higher concentration of both lindane and decamethrin. On the other hand, the other two insecticides, dursban and lannate, increased the lysis of erythrocyte in both types of blood. The percentage increases in relative hemolysis (Figs. 2, 3) depending on the concentration of the pesticides. The highest values recorded at 10^{-4} M were 95%, 98% (fish blood) and 164% and 113% (human blood) for dursban and lannate, respectively.

It is clear from the present result observation that two from the four insecticides studied in the present investigation protect red cells against osmotic hemolysis.
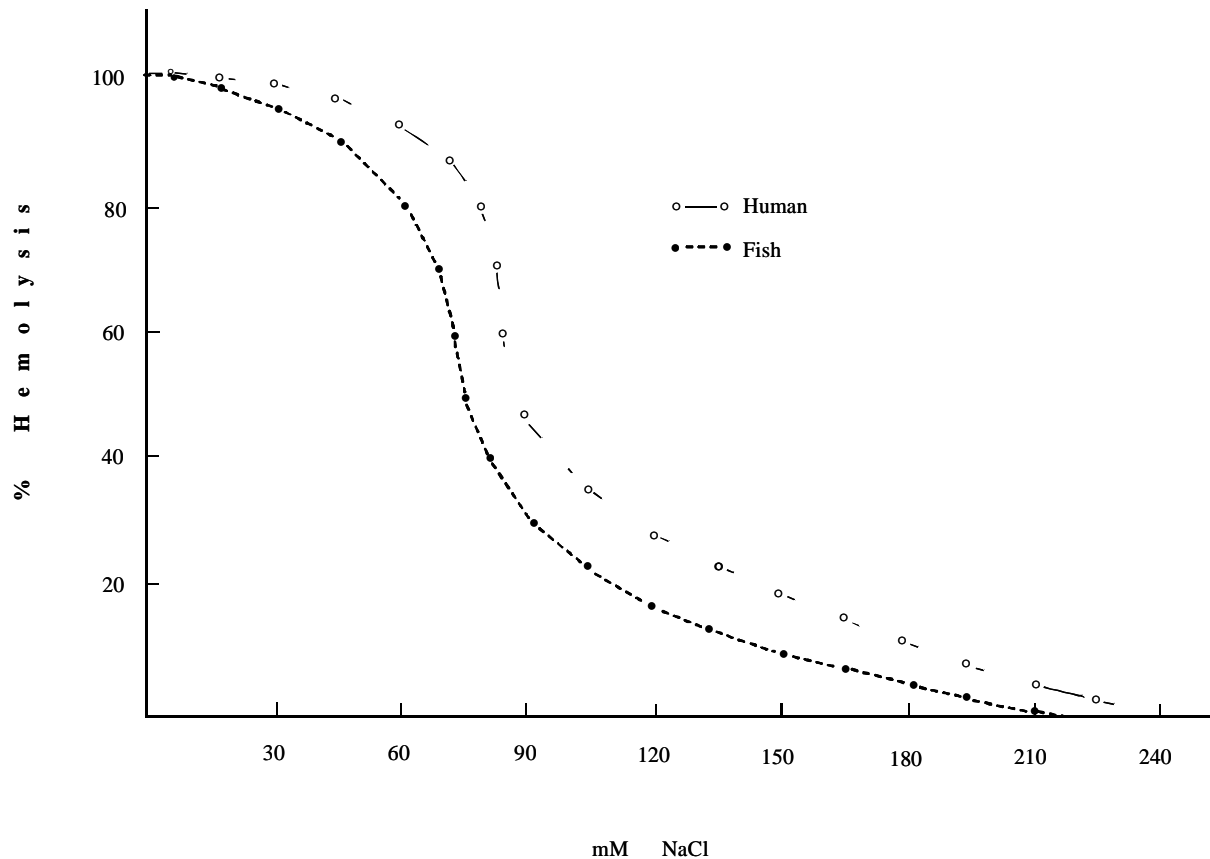
Fig. 1. Typical hermolysis curves of fish and human bloods.

The effectiveness of their protection follows the order, decamathrin > lindane. The potency of these compounds was much higher with increasing concentrations.

Essentially, these observations run in harmony with those previously described for DDT and its analogous[22], aldrin; DDT and other insecticides[4,25]. However, these investigators were not able to find any correlation between the antithemolytic effects and the degree of toxicity of these compounds.

The antihemolytic behaviour of the first two insecticides was somewhat surprising since these compounds are expected to increase the permeability of lipidionphore complexes[2,9]. These effects were also correlated with the ability of insecticides to induce disorders of lipid packing in bilayers with correspondent shifts of thermo-tropic transitions to lower temperature[3]. Therefore, the main prediction was the disaggregational effects on membrane components leading to increased hemolysis of red cells. This is true if the lytic effect of the other two insecticides (dursban and lannate) were considered. Depending on their molar concentrations, these insecticides induced their hemolytic effects on both types of red blood cells, following the order of effectiveness dursban > lannate.

Organochlorines, like DDT, appear to associate preferentially with lipid-rich biological structures[17] as a consequence of the very high partition coefficients of the compounds in a polar phases[22,17]. Therefore, it was suggested that these insecticides combine preferentially with the lipid moiety of the membrane leading to separation of protein and lipid-rich regions. Clustering of protein particles would favour their mutual interactions. Moreover, withdrawal of lipids from their interactions with protein would produce extended regions of bilayer[4]. The overall effect would decrease the number of continuity defects in contact boundaries of lipids and proteins and reinforce lipid-lipid and protein-protein interactions. These combined actions would produce strengthening of the membrane with corresponding increased resistance against osmotic lysis. The previous mechanism may be likewise contributed to action of decamethrin in performing its antihemolytic effect.

Another hypothesis is that antihemolytic insecticides may potentiate the binding capacity of divalent cations which was reported to have condensing effects on membranes and reducing physical disorder[7,26,25]. This
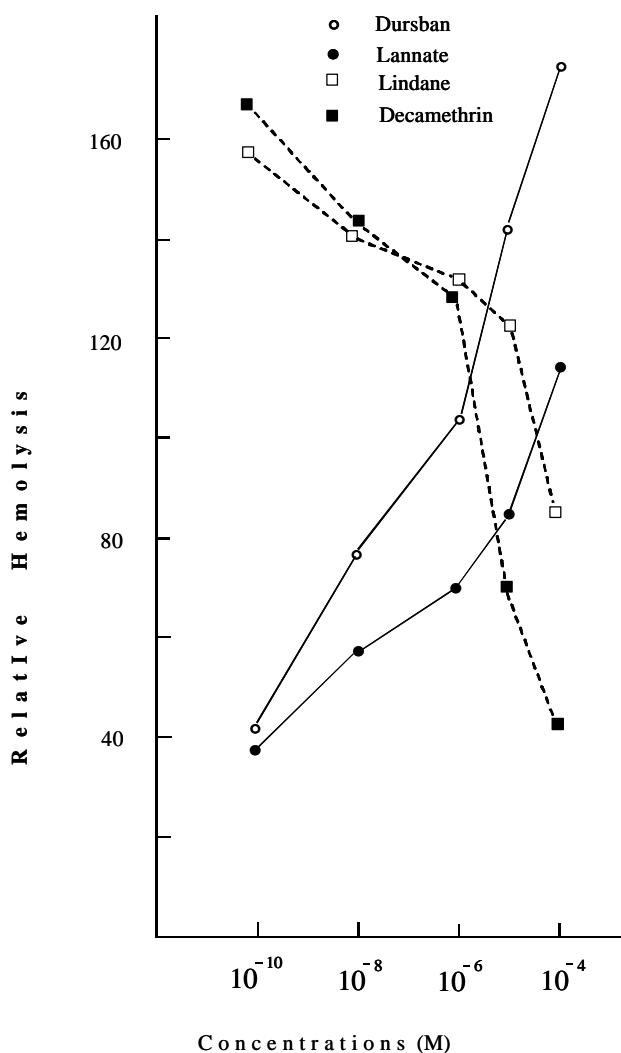
Fig. 2: Hemolysis of hman erythrocyte (in vitro) in the presence of insecticides; dursban; lannate; lindane and decemathyrin (relative to controls which gave 50% hemolysis).

strengthening may offer a more rigid and stable conformation which would provide the membrane with resistance to stretch produced by hypoosmotic effects. The forementioned hypothesis is further supported by the findings of other investigators who have shown that Ca^{++} and other divalent cations acting in the intracellular spaces induce shape-alterations and shrinkage of red cells and ghosts[23,16]. Moreover, addition of divalent cations during hypotonic hemolysis prevents extensive disruption of erythrocyte membrane[15]. These effects have been also explained in terms of the ability of divalent cations to bridge adjacent membrane components, thereby stiffening the membrane[23,15]. However, the ability
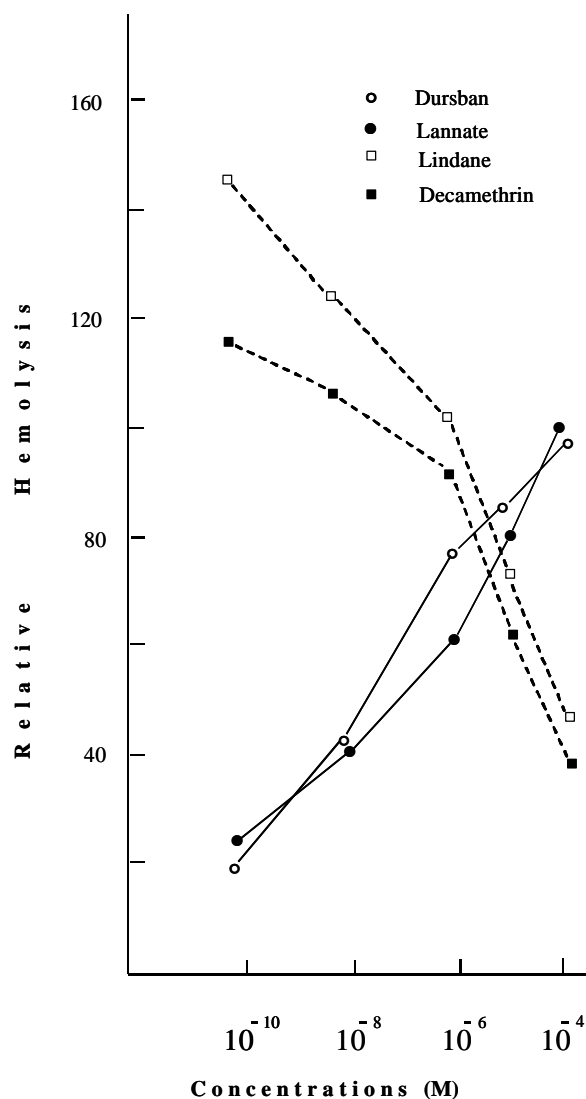

Fig. 3: Hemolysis of fish erythrocyte (in vitro) in the presence of insecticides; dursban; lannate; lindane and decemathyrin (relative to controls which gave 50% hemolysis).

of insecticides to potentiate the binding capacity to cell membrane of divalent cations remained to be elucidated and needs more investigations.

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