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Inhibition of Gill Na^+ K^+-ATPase Activity in Dragonfly Larva, *Pantala flavescens*, by Endosulfan

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Evidence from a variety of studies suggests that odonates might prove practical agents of mosquito control (Luh 1981; Brown 1983). Thus, larval odonates, like larvivorous fish, can be used to control mosquito larvae. Pesticides, however, are commonly used to control larval mosquitoes. But in recent years the increase in the use of pesticides caused general deterioration of the environment and such widespread use of pesticides adversely affects the natural population of the aquatic fauna. Jacob *et al* (1982) have reported the toxicity of certain mosquito larvicides to the larvivorous fish. Aquatic organisms, particularly fish, are highly sensitive to endosulfan (NRC Report 1975) and its toxicity to fish has been studied (Rao *et al.* 1982). Earlier studies have demonstrated that aerial spraying of endosulfan for insect pest control cause acute toxicity to fish (Fox and Matthiessen 1982). Endosulfan has been most successfully used against variety of insects (NRC Report 1975; Holihosur 1985). Inhibition of ATPases and changes in the flux of carbohydrate and lipid metabolism have been proposed as mechanisms that contribute to the insecticidal action of endosulfan in insects (Holihosur 1985). The increase in the use of endosulfan in India to control insect pests including mosquitoes is reported to affect the non-target organisms (Yadwad 1989).

Adenosine triphosphatases (ATPases) are a group of enzymes which play an important role in intracellular functions and considered to be a senstive indicators of toxicity. Na^+ K^+- ATPase has a role in osmoregulation, in that provides energy for the active transport of Na^+ and K^+ accross the cell membrane and also effect the transepithelial movements of cations in gills (spencer *et al.* 1979). Inhibition of Na^+ K^+-ATPase by xenobiotics may produce adverse effects to the organism. Interference of xenobiotics with ionic homeostasis may be reflected as altered Na^+ K^+-ATPase activity (Haya and Waiwood 1983). The xenobiotic can alter Na^+ K^+-ATPase activity due to disruption of energy producing metabolic pathways or interact directly with the enzyme (Watson and Beamish 1980; Verma *et al.*1978). Earliear studies have demonstrated that several xeno biotics including organochlorine pesticides inhibit gill Na^+ K^+-ATPase in

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fishes and invertebrate species (Davis and Wedemeyer 1971; Leadem *et al.* 1974; Neufled and Pritchard 1979; Dhavale *et al.*1988)

Aquatic insects are known to possess specialized cells which are involved in the ionic regulation. In larval odonates they are present among the rectal gill basket and play central role in osmoregulation and respiratory mechanism. The Na^+ K^+-ATPase activity has been described in larval odonate (Komnick and Achenbach 1979). In the present study Na^+ K^+ - ATPase activity in the rectal gills of dragonfly larvae *Pantala flavesens* has been investigated as a potential indicator of endosulfan intoxication.

MATERIALS AND METHODS

Larvae of the dragonfly *Pantala flavesens* were collected from local paddy fields and ponds and maintained in our laboratory. Technical grade endosulfan dissolved in acetone was used. Lethal concentration was determined (LC_{50} for 48 hr was 15 ppm). The naiads were exposed to different concentration of endosulfan for specified time. Acetone alone in the medium served as control. After an appropriate time, the rectal gills were quickly separated and homogenized immediately in 1mL of homogenzing medium (0.5 mM sucrose, 1mM EDTA and 50 mM Tris HCl buffer pH 7.4). The homogenate was centrifuged at 1000 *g* for 10 min and the supernatant was further centrifuged at 28,000 *g* for 10 min. The pellet was washed twice and resuspended in Tris HCl buffer pH 7.4 and used as the source of enzyme. All operations were carried out at 2-4^0 C. Na^+ K^+-ATPase activity was determined based on the method described by Bonting (1970). Incubation medium contained 10 mM KCl, 8 mM $MgCl_2$, 80 mM NaCl, 25 mM ATP, enzyme source and with and without 10^{-4} M oubain in a final volume of 0.5 mL. Incubation was carried out for 30 min at 37^0 C The reaction after 30 min was terminated by the addition of 0.1 mL of 30% cold trichloroacetic acid. The phosphate released was measured by the method of Chen *et al* (1956). Protein content was determined by the method of Lowry *et al* (1951).

RESULTS AND DISCUSSION

The dragonfly naiads were exposed to different concentrations of endosulfan for 24 hr. The data summerized in Table 1 shows the inhibition of Na^+ K^+-ATPase by endosulfan. Significant inhibition of enzyme activity was observed when naiads were exposed to 5, 10 and 15 ppm of endosulfan for 24 hr. The maximum inhibition (59.66%) was observed at the concentration of 15 ppm. Endosulfan at 2 ppm concentration did not show any significant effect on the enzyme activity for 24 hr. When naiads were exposed to sublethal concentration of endosulfan (5 ppm) a time dependent inhibition of Na^+ K^+-ATPase was observed (Fig. 1). The maximal inhibition (62.5%) was expressed between 24 and 48 hr, but the rate of inhibition was decreased at 48 hr. However, the inhibition of ATPase remained significant during the entire course of treatment.

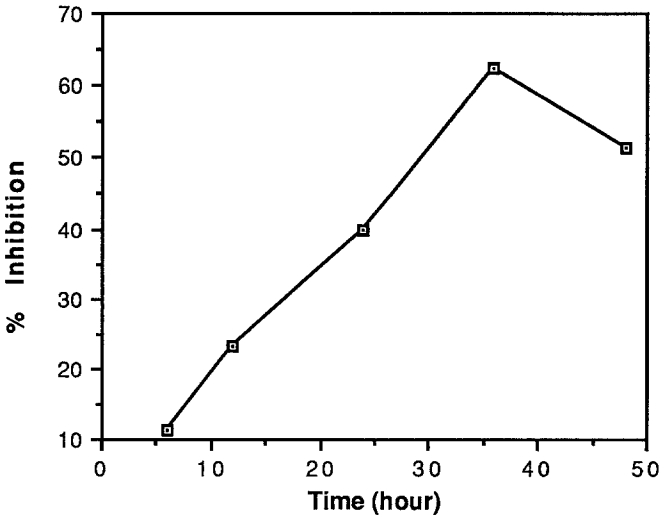

Figure 1. Inhibition of Na K-ATPase activity in gills of dragonfly larva by sublethal dose of endosulfan.

The present investigation has demonstrated the inhibition of gill Na^+ K^+ ATPase by endosulfan. This effect of xenobiotics has been shown previously in invertebrate species (Neufled and Pritchard 1979; Dhavale *et al.* 1988). Studies were conducted to evaluate the impact of pesticides on odonate larvae (Wilkes and Weiss 1971; Muirhead-Thomson 1973). However, no attempt has been made to investigate the biochemical mechanism involved in the toxicity of xenobiotics in these important predators of mosquito larvae.

In aquatic insects gills serve as a major organ for osmoregulation and respiration. Since Na^+ K^+-ATPase plays a key role in osmoregulation and maintaining Na^+, K^+ transmembrane gradients, its inhibition may alter the ionic concentration of hemolymph and damage gill tissue. Organochlorine insecticides including endosulfan are known to inhibit Na^+K^+ ATPase in insects and fishes (Koch *et al.*1969; Davis and Wedemeyer 1971; Leadem *et al.*1974; Holihosur 1985). It is believed that xenobiotics interact with membrane proteins thereby destabilizing membrane bound enzymes. Indeed such disruptive effect on plasma liver membrane of rats treated with DDT and Toxaphene has been suggested (Mourelle *et al* .1985). The results of the present study suggest that endosulfan may interact with membrane bound enzymes. However, the exact mechanism involved in the inhibition of ATPases by endosulfan is not clear. More detailed studies are required to elucidate the biochemical mechanism involved in the action of endosulfan on ATPases.

Table. 1. Effect of endosulfan on Na^+ K^+-ATPase activity in gills of dragonfly larva *Panatala flavesens.*

Dose (ppm)	Na^+ K^+-ATPase activity μM P_i / mg protein / 30 min Control	Treated	P value
2	7.75 + 0.16	7.09 + 0.37	NS
5	7.32 + 0.24	4.39 + 0.23 (38.08)	P <0.01
10	7.40 + 0.35	3.82 + 0.48 (46.26)	P <0.01
15	8.02 + 0.41	2.86 + 0.39 (59.66)	P <0.01

All values are mean + SEM of 5 experiments.
Values in parenthesis indicate % inhibition.

The present study has shown that endosulfan inhibits rectal gill Na^+ K^+ ATPase activity in the larvae of *Pantala flavesens.* Thus, the inhibition of Na^+ K^+-ATPase may affect the cellular functions including ion transport and osmoregulation and contribute to the lethal effects of endosulfan.

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