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Acute Toxicity of Endosulfan to Crab: Effect on Transport Property of Haemocyanin

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It is well known that organochlorine insecticides interfere with axonic transmission causing nervous dysfunction (O'Brien 1967). The toxic effects of endosulfan [Thiodan (R) 6,7,8,9,10-hexachloro 1,5,5a,6,9a-hexahydro-6, 9-methano-2,4,3-benzodioxathiepine-3 oxide] -an organochlorine insecticide used extensively for crop protection in this part of India - to various fish species have been documented (Schoettger 1970; Arora et al. 1971, 1972; Moulton 1973; Toor et al. 1973; Reddy and Gomathy 1977; Amminikutty and Rege 1977; Gopal et al. 1980; Rao and Murthy 1980; Gopal et al. 1982). However the toxicity of endosulfan to a freshwater field crab, *Oziotelphusa senex senex* - another nontarget organism of aquatic ecosystem - has not been fully evaluated. In view of this it would be interesting to examine the toxic effects of endosulfan on the transport property of crab haemocyanin since the respiratory pigments provide a prime example of a link between molecular and whole organ biology.

MATERIALS AND METHODS

Freshwater field crabs *O. senex senex* in the weight range of 28±1 gm were collected from the local paddy fields under ground (well) water irrigation prior to spraying operations thus making sure that crabs are not being collected from previously contaminated areas. Crabs thus collected were acclimated to the laboratory conditions for about a week during which time they were fed with *ad libitum* minced meat. The animals were not fed for one day before commencing the experiment to overcome differences, if any, due to differential feeding. Technical grade endosulfan (99%) dissolved in acetone was used. Sublethal (3.8 mg/L) and lethal (15.14 mg/L =96h LC_{50}) concentrations were chosen to study acute toxic effects.

Uninjured, intermoult (Stage 4) crabs were chosen and divided into three batches of six each. First two batches were exposed for 96h to sublethal and lethal concentration of endosulfan, respectively. The third batch served as controls. Acetone was added to the control batch in an amount equal to the largest aliquot of stock used in the experimental batches. After completion of specified time interval the haemolymph was drawn through the arthrodial membrane present at the base of the 4th walking leg by means of a hypodermic syringe. The haemolymph was allowed to clot and centrifuged at 3000 rpm for 15 min. Clear serum was collected and used for biochemical estimations.

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The copper content was estimated by the method of Barnes and Rothschild (1950). A known volume of the sample was wet ashed with concentrated sulphuric acid in microkjeldahl flask and the colour was cleared with hydrogen peroxide. After adjusting the final volume to 10 ml with distilled water a known quantity of 20% citric acid and liquid ammonia were added followed by 0.5 ml of 0.5% sodium diethyldithiocarbamate. The resultant yellow colour was extracted into a known quantity of chloroform and measured at 420 nm using a spectrophotometer. Total protein content was estimated by the method of Lowry et al. (1951).

The oxygen equilibrium curves (OECs) were determined at 580 nm - the wavelength of maximum absorption in the visible range of the spectrum-following the spectrophotometric method of Redmond (1955) modified by Padmanabha Naidu (1966). The absorption of oxyhaemocyanin was determined by comparing an oxygenated sample with that of a deoxygenated sample obtained by the addition of a pinch of sodium hydrosulphite (Redmond 1955). The absorption of oxyhaemocyanin was measured at various pressures and OECs were plotted. Haemolymph pH was measured with the help of a digital grip pH meter.

The carbondioxide content was determined by mixing the haemolymph sample with acid phosphate buffer in an SR analyser (Scholander-Roughton analyser) and the gases were extracted in vacuum as described by Scholander and Roughton (1943). The volume of the gas was measured before and after absorption with sodium hydroxide after equilibration to laboratory temperature. Statistical treatment of the data was done by the method of Pillai and Sinha (1968).

RESULTS AND DISCUSSION

Haemocyanin, a copper containing respiratory pigment, is found dissolved in the haemolymph of arthropods and molluscs. Oxygen combines reversibly with copper in the ratio of 1 oxygen: 2 copper and as such copper

Table 1. Variations in haemolymph(HL) and hepatopancreatic (HP) copper and haemolymph protein in the freshwater field crab, *O. senex senex* exposed for 96h to sublethal and lethal concentration of endosulfan. Values are mean±SD of six individual observations.

Parameter	Control	Experimental	
		Sublethal	lethal
HL copper *(µg/mL)*	40.45±2.86	53.98±2.18	28.82±2.17
% change	-	33.45[a]	-28.75[a]
HP copper *(µg/gm dry weight)*	139.19±3.99	108.48±3.23	149.86±3.66
% change	-	-22.06[a]	7.67[a]
HL protein *(mg/mL)*	29.56±2.65	41.76±2.15	27.35±2.08
% change	-	41.27[a]	-7.48[a]

[a]Differences are significant at 0.05 level.

concentration gives a measure of haemocyanin concentration (Prosser 1973). It is obvious from the results (Table 1) that while there was a significant increase in haemolymph copper concentration upon sublethal exposure, there was a significant decrease upon lethal exposure indicating an increase in haemocyanin concentration upon sublethal exposure and a decrease upon lethal exposure. It has been reported that there was a decrease in the oxygen consumption of crabs exposed to sublethal and lethal concentration of endosulfan resulting in greater oxygen demand (Subhadra Devi 1985). Hence upon sublethal exposure the animal might have stepped up haemocyanin synthesis (as reflected by increased copper concentration) to meet the increased demand for oxygen. This view gains further support by the fact that haemocyanin synthesis increases with decrease in oxygen consumption (Prosser 1973). On the contrary there was a drastic decrease in haemolymph copper-and as such in haemocyanin-concentration upon exposure to lethal concentration of endosulfan. Probably all synthetic activities of the animal might have been disrupted upon exposure to lethal concentration and this could well be the reason for a drastic fall in the pigment concentration upon exposure to lethal concentration. That there may be an increase in haemolymph copper (haemocyanin) concentration upon sublethal exposure and a decrease upon lethal exposure is further supported by a corresponding decrease in hepatopancretic copper concentration upon sublethal exposure and an increase upon lethal exposure since, in decapod crustaceans, the hepatopancreas acts as a copper donor. The copper necessary for synthesis of the haemocyanin might be derived from the hepatopancreas (Ogura 1959; Ghiretti-Magaldi et al. 1973). In accordance with the variations in haemolymph copper, haemolymph protein also varied in both sublethal and lethal exposures since the protein fraction of crustacean haemolymph predominantly consists of the respiratory pigment haemocyanin (Florkin 1960).

Table 2. Variations in P_{50} (partial pressure of oxygen at which 50% of the haemocyanin is in oxygenated state) and 'n' (degree of interaction among oxygen combining sites of the haemocyanin molecule) values of the haemocyanin in haemolymph of the freshwater field crab, *O. senex senex* exposed for 96h to sublethal and lethal concentration of endosulfan.

Parameter	Control	Experimental	
		Sublethal	lethal
'P_{50}' (mm Hg)	12.5	15.0	21.0
'n'	2.750	2.658	2.248

The relationship that exists between per cent saturation of the respiratory pigment and oxygen tension (PO_2) of the haemolymph is represented by the OEC. It is obvious that the maximum absorption band of oxyhaemocyanin did not show any variation between control and experimental groups (Fig 1) and as such OECs for control and experimental animals have been determined at this wavelength. The OECs of crabs exposed to both sublethal and lethal concentration shifted to the right relative to that of control (Fig 2). Correspondingly there was an increase in 'P_{50}' and a decrease in 'n' at sublethal and lethal exposures respectively (Table 2) indicating a decrease in the affinity of oxygen for the pigment.

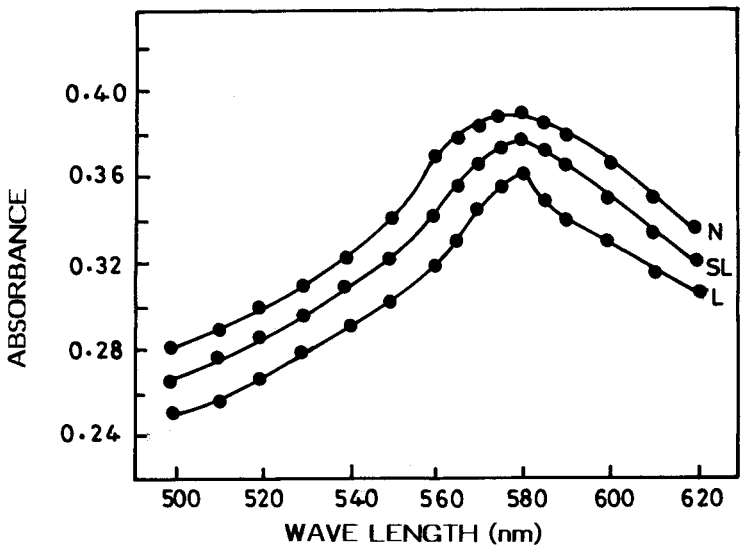

Figure 1. Absorption spectra of the Oxyhaemocyanin of normal (N) crabs and crabs exposed to sublethal (SL) and lethal (L) concentration of endosulfan

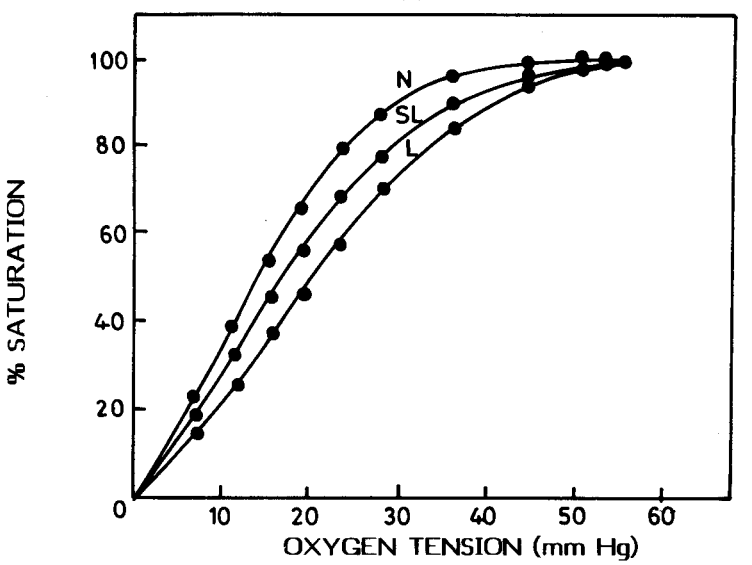

Figure 2. Oxygen equilibrium curves (OECs) of normal (N) crabs and crabs exposed to sublethal (SL) and lethal (L) concentration of endosulfan

Further haemolymph CO_2 increased and pH decreased (Table 3) in both sublethal and lethal concentrations the increase/decrease being concentration dependent. The increased CO_2 and decreased pH might be responsible for the shift right of OEC since it has been shown that

Table 3. Variations in haemolymph CO_2 and pH of the freshwater field crab, *O. senex senex* exposed for 96h to sublethal and lethal concentration of endosulfan. For CO_2 values are mean ± SD of six individual observations.

Parameter	Control	Experimental	
		Sublethal	lethal
Carbondioxide content	9.03±1.05	10.75±0.429	14.22±1.52
% change	-	19.05[a]	57.48[a]
pH	6.96	6.60	6.10

[a]Differences are significant at 0.05 level.

increased haemolymph CO_2 and decreased pH play a major role in the shift right of OEC (Prosser 1973). Moreover shifting of OEC to the right indicates that the rate of oxygen delivery is more pronounced at sublethal and lethal exposures than that at normal state. Probably through this mechanism the animal might have had more oxygen available at tissue level for sustenance of the required metabolic rate. Based on the foregoing observations it appears that there exists, in crab, a double edged mechanism of enhancing haemocyanin synthesis (as reflected by increased haemolymph copper) and decreasing the affinity of oxygen for the pigment (as reflected by shift right of OEC)- for its continued survival after being exposed to sublethal concentration of endosulfan. On the contrary, in the crab exposed to lethal concentration of endosulfan the amount of O_2 being carried by the pigment (as reflected by decreased haemolymph copper and hence haemocyanin) and as such the amount of O_2 being available for tissues might have been reduced (though the OEC is shifted to the farthest right) thus contributing to the onset of pathologic condition in the animal.

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