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Effect of Endosulfan on Glutathione S-transferase and Glutathione Content of the Premoult Field Crab, *Paratelphusa hydrodromus*

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The widespread use of insecticides causes concern about the effects of persistent exposure to toxicants on non-target organisms. The field crab *Paratelphusa hydrodromus* is an important human food source in parts of southern India, but is exposed constantly to the insecticide, endosulfan, a chlorinated hydrocarbon which is used extensively to control agricultural pests in India. Detectable quantities of insecticides are present in the native crabs although no external behavioral abnormalities are apparent.

Conjugation of xenobiotics with reduced glutathione (GSH), catalyzed by glutathione S-transferases (GSH S-transferase), is an important physiological process in the elimination of toxic substances from the body. In insects, several insecticides induce the GSH S-transferase activity (Hayaoka and Dauterman, 1982; Yadwad and Kallapur, 1988). Alteration of glutathione content is of toxicological importance in this process since it is used as a substrate for GSH S-transferase. Several chemicals affect glutathione levels in fishes and insects (Dalich and Larson,1980; Thomas *et al,* 1982; Yadwad and Kallapur, 1988). In the present study, GSH S-transferase activity and the GSH content of the crab has been investigated as a potential indicator of sublethal dose endosulfan intoxication.

MATERIALS AND METHODS

Batches of 40 healthy crabs weighing 55-60 g were collected from local paddy fields. Procedure for the maintainance and identification of the moult phase of crabs under laboratory conditions and for the preparation and administration of insecticide have been described (Kallapur and Yadwad, 1986). Repetitive sublethal doses of endosulfan (4 µg/crab every 24 hr) were injected directly into the hemocoel through the arthrodial membrane of the walking leg using a Hamilton microsyringe. Following treatment, crabs were individually maintained in rectangular glass jars containing moist sand.

After an appropriate time, the hepatopancreas was exposed by cutting open carapace. The tissue was removed and homogenized immediately

in ice cold Tris HCl buffer (pH 8.5) using a Potter-Elvehjem glass homogenizer with teflon pestle. The homogenate was centrifuged at 8000 *g* to separate mitochondria, nuclei and cell debris. The supernatant was used immediately as the source of the enzyme. All operations were carried out at 2-4°C .

GSH S-transferase activity was determined spectrophotometrically based on the method described by Motoyama and Dauterman, (1975). Incubation medium contained, in a final volume of 3 mL, 16 mM GSH, 0.6 mM 3,4-dichloronitrobenzene (DCNB) and 25 µL of enzyme source. The incubation was carried out at 26°C. The enzyme activity expressed was the maximum obtainable under the conditions specified in the present study. The glutathione content of the tissue was determined colorimetrically according to the method of Moron *et al,* (1979). Protein content was determined by the method of Lowry *et al,* (1951). Incorporation of labelled leucine studies were carried out by injecting five µL of a solution containing 0.05 mCi of [U-14C] leucine (Sp. activity 335 mCi/m mol) into the hemocoel. Incorporation of labelled leucine into proteins was determined by a filter paper disc method (Mans and Novelli, 1961). The radioactivity was measured by using Beckman liquid scintillation counter model No. LS-1701.

RESULTS AND DISCUSSION

Repetitive sublethal doses of endosulfan caused significant induction of GSH S-transferase activity in the hepatopancreas of the crab (Table. 1). Induction of enzyme activity was time dependent with a statistically significant increase evident within 48 hr of initial exposure to the toxicant and the maximal induction expressed between 96 and 192 hr after the first exposure.

The GSH level also increased during the treatment with the maximal level achieved within 96 hr of initial exposure. The maximal level in GSH was not sustained and some decrease was apparent after 96 hours. However, the GSH level remains significantly higher than in the control animals during the entire course of treatment (Table. 2). The observations indicated that GSH S-transferase took more time for the maximum induction (144 hr) than the GSH (96 hr) by endosulfan treatment.

Incorporation of labelled leucine by the hepatopancreas following a single dose of endosulfan intoxication was higher than in untreated crabs (Table. 3).

Table. 1. Effect of repetitive sublethal doses of endosulfan on GSH S-transferase activity of the field crab *Paratelphusa hydrodromus.*

Time after treatment (hr)	nm DCNB conjugated / mg protein / min		P value
	Control	Treated	
0	1329.30 ± 176.1	1301.70 ± 105.8	NS
48	1291.18 ± 199.6	1604.13 ± 141.8	P < 0.01
96	1315.50 ± 121.4	1970.25 ± 110.5	P < 0.01
144	1300.89 ± 115.7	2353.28 ± 158.0	P < 0.01
192	1321.45 ± 099.8	2003.10 ± 136.0	P < 0.01

All values are mean ± SE of mean of 5 experiments.

Table. 2. Effect of sublethal doses of endosulfan on the glutathione level of the field crab *Paratelphusa hydrodromus.*

Time after treatment (hr)	µg glutathione / 100 mg wet wt		P value
	Control	Treated	
0	18.86 ± 0.53	17.45 ± 0.91	NS
48	17.98 ± 0.67	39.51 ± 1.30	P < 0.01
96	18.01 ± 0.96	49.06 ± 2.16	P < 0.01
144	18.41 ± 0.44	46.95 ± 0.57	P < 0.01
192	17.65 ± 1.01	40.43 ± 1.38	P < 0.01

All values are mean ± SE of 5 experiments

Table. 3. Effect of sublethal endosulfan treatment on the [U-14C]-Leucine uptake in the field crab *Paratelphusa hydrodromus.*

	Labelled leucine uptake	
	dpm / mg wet wt	dpm / mg protein
Control	896.01 ± 94.11	13601.53 ± 301.38
Treated	2177.45 ± 168.12	29512.31 ± 477.69

All values are mean ± SE of mean of 4 experiments.

The present investigation has demonstrated the induction of GSH S-transferase activity and increased glutathione content by sublethal dose of endosulfan. This effect of pesticides and other xenobiotics has been shown previously in insects and vertebrate species (Motoyama and Dauterman, 1980; Dalich and Larson, 1980; Ottea and Plapp, 1981; Hayaoka and Dauterman, 1982; Thomas *et al,* 1982; Chatterjee and Bhattacharya, 1984; Yadwad and Kallapur, 1988). Insects with induced GSH S-transferase are tolerant to several insecticides (Motoyama and Dauterman, 1980; Yadwad and Kallapur, 1988) probably as a result of the enzymes enhancing the conjugation of glutathione to xenobiotics. In the present study the GSH content increased in response to endosulfan treatment and this could contribute to the induction of increased enzyme activity. The exact mechanism involved in the increase of GSH content is not clear. Xenobiotics could prevent glutathione from exerting negative feedback on gamma-glutamyl cysteine synthetase activity by forming a glutathione conjugate so that the total tissue concentration of the glutathione increases (Thomas *et al,* 1982). Although, the level of GSH remained significantly higher throughout the experiment over the control animals, slow depletion was observed when GSH S-transferase activity was maximal. This slow depletion of GSH may have toxicological significance since GSH is a substrate for the GSH S-transferase system. However, it has been suggested that GSH S-transferase itself acts as a binding protein and the enzyme is known to bind diverse group of chemicals including carcinogens (Motoyama and Dauterman, 1980). Hence, the increased GSH S-transferase activity regardless of GSH depletion may play an idependent role in reducing the toxicity. The observed induction of GSH S-transferase and GSH undoubtedly facilitates the detoxification and elimination of endosulfan. Increased GSH S-transferase activity

and polysubstrate monooxygenases (PSMO) activity is usually associated with an increase in protein synthesis (Agosin *et al,* 1966; Kato *et al,* 1966; Yadwad and Kallapur, 1988). In the present study increased protein synthesis was evident from the increased incorporation of labelled leucine into tissue protein following insecticide treatment (Table. 3).

In conclusion, the study has shown that endosulfan induces GSH S-transferase activity and GSH content in the field crab *Paratelphusa hydrodromus.* The present observation also indicate that field crabs have an effective detoxification system with which to overcome exposure to various toxicants. More detailed studies are required to examine whether glutathione conjugation catalyzed by GSH S-transferase is a major detoxification pathway in this group of animals.

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