IN VIVO SUB-ACUTE PHYSIOLOGICAL STRESS INDUCED BY SUMITHION ON SOME ASPECTS OF OXIDATIVE METABOLISM IN THE FRESH WATER CRAB

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Abstract. Specimens of fresh water crab, Oziotelphusa senex senex were exposed to sub-lethal concentration (25%, of LC_{50}/48 hr) of Sumithion for 7 days. Sumithion was found to inhibit the activity levels of acetylcholinesterase, succinate dehydrogenase, isocitrate dehydrogenase and pyruvate dehydrogenase, activate lactate dehydrogenase and cause accumulation of acetylcholine in the hepatopancreas and muscle. The increase in lactate dehydrogenase and the decrease in succinate, isocitrate and pyruvate dehydrogenases in these tissues indicate the development of anaerobic conditions and is interpreted as a functional adaptation to pesticide induced metabolic stress.

1. Introduction

The organophosphate insecticides are highly effective for the control of mosquitos and agricultural pests in many parts of the world (Muirhead-Thompson, 1971; Duke, 1977). Since these insecticides are non-persistent, they are used repeatedly, and the danger to non-target organisms such as fish, prawns and crabs, the natural enemies of the insect larvae and important members of the food chain, are adversely affected. The tragic incidence of ‘Handigodu syndrome’ in Karnataka has been attributed to long term consumption of pesticide poisoned crabs and fish by the local population (NIN, 1977). In view of this an elaborate program to evaluate the impact of pesticides on the physiology and biochemistry of several non-target species of aquatic ecosystem has been undertaken. The present paper explains the toxic effects of sublethal concentrations of Sumithion (0-0-dimethyl(0-3 methyl-4-nitrophenyl) phosphorothioate), which is extensively used locally on certain biologically important enzymes, i.e., acetylcholinesterase, succinate dehydrogenase, isocitrate dehydrogenase, pyruvate dehydrogenase and lactate dehydrogenase, and acetylcholine levels in hepatopancreas and muscle of the rice field edible crab, Oziotelphusa senex senex. The purpose of this study is to obtain information about the biochemical disturbances in the crab on exposure to Sumithion and to increase our understanding of its mode of action.

2. Materials and Methods

Animals: Healthy, adult, intermolt (Stage C4 according to Sreenivasula Reddy, 1981) male specimens of Oziotelphusa senex senex (weight, 30 ± 2 g; carapace width, 30 ± 4 mm) were collected from local rice fields. They were acclimated to laboratory...
conditions for 10 days before experimentation. They were fed frog muscle daily, but food was withheld during the experimental period. The properties of tap water were as follows: Temperature 25 ± 1 °C; pH 7.3; dissolved oxygen content, 6.2 ppm; hardness 38 ppm of CaCO₃.

*Pesticide:* Technical grade (96% W/V) Sumithion (Fenitrothion; 0-0-dimethyl-0-(3 methyl-4-nitrophenyl) phosphorothioate) obtained from Rallis India Ltd (Bangalore) was used. Sumithion was dissolved in acetone and diluted with tap water so that the final concentration was 0.1 mg L⁻¹ in 0.001% acetone. Control crabs were also kept in tap water containing the same acetone concentration (0.001%).

*Experimental design:* Sixty crabs were exposed for 7 days at 0.1 mg L⁻¹, 25% of the 48 hr LC₅₀ of Sumithion (Bhagyalakshmi and Ramamurthi, 1981). Controls were also set up for comparison. The experimental medium was changed every 24 hr with freshly prepared medium of the same concentration so as to compensate for any change in the concentration of chemical due to degradation or uptake. After 1, 3, and 7 days hepatopancreas and muscle tissues were dissected over ice, from both control and exposed crabs, placed in crab ringer (Van Harreveld, 1936) and stored in prechilled glass tubes until analyzed.

*Enzyme assays:* The isolated tissues were minced with scissors and homogenized (2% weight/volume) in cold sucrose solution (0.25 M) at 4 °C in an ice-Jacketed-glass homogenizer with a motor-driven Teflon-coated pestle. The homogenate was centrifuged at 1000 × g for 10 min at 4 °C to remove unbroken cells and particulate debris. The obtained cell-free supernatants was utilized as the enzyme source.

Measurements of enzyme activities were performed at 37 °C with appropriate enzyme and reagent blank using a spectrophotometer. Preliminary experiments established the optimal conditions for each enzyme with respect to pH, and substrate and cofactor concentration (Bhagyalakshmi, 1981). Optimal assay conditions for individual enzymes (nomenclature, IUB, 1978) were as follows:

- **Acetylcholinesterase, (AChE)** acetylcholin acethylhydrolase EC 3.1.1.8: AChE was assayed (Metcalf, 1957) in a medium of 2 mL which contained: 8 μmoles of acetylcholine chloride, 100 μmol of phosphate buffer (pH 7.4) and 1 μL of enzyme source.

- **Succinate dehydrogenase, (SDH)** Succinate oxidoreductase, EC 1.3.99.1: SDH was assayed according to Nachlas *et al.* (1960) using 100 μmol of phosphate buffer (pH 7.4), 40 μmol of sodium succinate, 2 μmol of INT (2-4 Iodophenyl-3(4 nitrophenyl)-5 phenyl tetrazolium chloride) and 0.5 mL of enzyme source in 2 mL of volume.

- **Pyruvate dehydrogenase, (PDH)** Pyruvate oxidoreductase, EC 1.2.3.3: PDH was assayed (Srikanthan and Krishnamurthy, 1955) in a medium of 2 mL which contained: 100 μmol of phosphate buffer (pH 7.4), 40 μmol of sodium pyruvate, 2 μmol of INT, 0.1 μmol of NAD (Nicotinamide adenine dinucleotide) and 0.4 mL of enzyme source.

- **Isocitrate dehydrogenase, (ICDH)** Isocitrate oxidoreductase, EC 1.1.1.41: ICDH was assayed by the method of Kornberg and Pricer (1951). The reaction mixture in a final volume of 2 mL contained: 20 μmol of DL-isocitrate, 100 μmol of phosphate buffer (pH 7.4), 4 μmol of INT, 10 μmol of magnesium chloride, 0.2 μmol of ADP, 0.2 μmol of NAD and 0.4 mL of enzyme source.