In vivo effects of Sumithion on tissue respiration and enzyme activity in the fish, *Etroplus maculatus*

S. Vijayalakshmi[1]

Department of Zoology, Sri Sathya Sai Women's College, Anantapur-515001 (India), 28 December 1979

Summary. LD_{50} exposure of a teleost fish. *Etroplus maculatus,* to Sumithion depressed the rate of oxygen consumption, concomitantly with an inhibition in succinate dehydrogenase activity in the tissues, in the order gill > brain > liver > muscle. The effect of the same pesticide on the activity of acetylcholinesterase was quite interesting, with a maximal inhibitory effect on the brain followed by liver, muscle and gill, suggesting a tissue specificity, and a differential sensitivity of the enzyme towards the pesticide.

Due to the increase in the application of organophosphate and carbamate pesticides in agricultural and public health operations, there is increased scope for disruption and havoc among the non-target organisms like fish, crabs and other aquatic ecosystems. The most tragic incidence of 'Handigod syndrome' in Karnataka (South India) has been suggested to be due to consumption of pesticide-poisoned crabs and fish by the local population[2]. According to Hiltibran[3], organophosphate insecticides such as malathion and parathion severely inhibit oxygen uptake by Blue gill (Lepomis) liver mitochondria. Several attempts have been made to evaluate their hazardous effects on many species by several workers[4-6]. The present study is directed towards a basic understanding of the effects of the organophosphorus pesticide Sumithion on tissue respiration and on succinate dehydrogenase (SDH) and acetyl cholinesterase (AChE) activity in the locally-available edible teleost fish, *Etroplus maculatus,* a proliferative breeder.

Material and methods. The fish *Etroplus maculatus* were obtained from the state fisheries department, Tirupati (A.P.) and were maintained in large laboratory aquaria. They were fed ad libitum with hydrilla plant and bits of earth-worm. Specimens within the approximate size range 10 cm long, weighing about 9 ± 1 g, were employed through out the experimentation. The organophosphate pesticide, Sumithion (dimethyl-3-methyl, 4-nitrophenyl phosphoro thionate which is most extensively used near Tirupati and is found in irrigation canals, was obtained from the Tata Fiscon Company, Bombay, and was employed in the present study. The LD_{50} value for the insecticide was determined by following the method of probit analysis[7]. 48 h after exposure to Sumithion, the tissues like brain, gills, liver and muscle were excized from the experimental and control fish and the following biochemical assays were carried out.

Oxygen consumption was measured in a Warburg Constant volume respirometer (Gallenkamp, England) by the method of Umbreit et al[8]. Fish Ringer solution with a phosphate buffer at pH 7.2 was used as the suspension medium for the tissues. The succinate dehydrogenase (EC 1.3.99.1) and acetylcholinesterase (EC3.1.1.7) were assayed by employing the methods of Nachlas et al[9] and Metcalf[10] respectively. The protein content was estimated by the technique of Lowry et al[11].

Results and discussion. The data on O_2 consumption of various tissues of normal and Sumithion-treated fish showed a significant inhibition, in the order gills > brain > liver > muscle, indicating induced respiratory distress due to pesticide stress. The decreased O_2 consumption from the surrounding medium evinces the fact that the fish may enter into an hypoxic state of 'anoxia' as a result of pesticide impact.

It is a well known fact that a depressed respiratory rate might adversely affect some of the key energy metabolising enzymes of the Krebs cycle. This type of metabolic derangement is illustrated by the decreased SDH activity in all the tissues of experimental fish (table) with a maximal expression (−61.52) registered in the gills. The in vitro studies of Hiltibran and in vivo experiments of Koundinya and Ramamurthy[12] have shown a similar diminution of O_2 uptake and cellular oxidations due to organophosphate pesticide exposure in the fish, *Tilapia mossambica.*

Concomitant with the inhibition of SDH, there is a more pronounced neuronal impairment as reflected by the significant decline in AChE activity in *Etroplus* tissues due to the toxicity of the pesticide used. However, it is interesting to note the specific trend of AChE inhibition with a maximal depression observed in the brain (−73.52% over control) > liver > muscle > gills (table). At this juncture, it could be suggested that the action of Sumithion may be analogous to that of competitive inhibitors like physostigmine[12] which act upon the active sites of AChE directly. Coppage et al.[13] also showed a dose-dependent inhibition of AChE activity in sheep brain due to malathion. The more pronounced inhibition of brain AChE and consequent disruption of nerve-impulse transmission have been suggested to be the major factors causing death in an organophosphate-poisoned organism.

The catalytic efficiency of enzymes is usually represented in terms of activation energy and in the present study the activation energy barrier may be more pronounced in various tissues of fish after treatment with Sumithion. Thus,

Sumithion effects on tissue respiration and enzyme activity of *Etroplus maculatus*

Tissue	O_2 consumption*			Succinate dehydrogenase**			Acetylcholinesterase***		
	Control	Experimental	% change	Control	Experimental	% change	Control	Experimental	% change
Brain	596±20.18	308±27.4	−48.32 p>0.002	0.536±0.017	0.528±0.024	−51.8 p>0.001	68.85±5.34	18.24±3.41	−73.52 p>0.001
Gill	1438±40.15	692±22.7	−51.88 p>0.001	0.148±0.009	0.057±0.012	−61.52 p>0.001	37.42±2.8	26.03±1.15	−30.43 p>0.005
Liver	607±18.6	328±28.5	−45.96 p>0.005	0.287±0.021	0.166±0.007	−42.17 p>0.002	44.79±3.18	21.52±2.64	−51.9 p>0.001
Muscle	216±15.32	188±11.4	−12.96 p>0.02	0.264±0.058	0.187±0.026	−29.1 p>0.005	29.16±1.83	17.18±2.36	−41.09 p>0.001

* µl of O_2 consumption/g/h. ** µmoles of formazon/mg protein/h. *** µmoles of acetylcholine hydrolysed/mg protein/h. p = probability value.

the toxic effects of Sumithion as evinced by the impaired energy metabolism and brain acetylcholinesterase measurement are a good general index of organophosphate poisoning of fish in the environment[14].

1 I wish to express may gratitude to Dr K. Sasira Babu and Dr J. Hemalatha for their valuable discussions and encouragement.

2 National Institute of Nutrition: Annual report. Indian Council of Medical Research publication, 184 (1977).
3 R.C. Hiltibran, Trans-III Stat. Acad. Sci. *67*, 228 (1974).
4 R.R. Bozena, Rocz, Panstw. Zakl. Hig. *26*, 293 (1975).
5 G.P.K. Paul and S. Balbir, Arch. envir. Health *29*, 167 (1974).
6 P.T.L. Kaminskaya, Gig. Tr. *1*, 91 (1969).
7 D.J. Finney, probit analysis. Cambridge University Press, 1964.
8 W.W. Umbreit, R.H. Burris and J.F. Stanffer, Monometric techniques. Burgess Minneapolis 1959.
9 M.M. Nachlas, L.M. Stanley and A.M. Seligman, J. biol. Chem. *253*, 499 (1960).
10 R.L. Metcalf, Methods in Biochemical Analysis, vol. 5. Interscience Publishers, New York 1951.
11 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. *193*, 265 (1951).
12 P.R. Koundinya and R. Ramamurthy, Indian J. envir. Health *20*, 426 (1978).
13 D.L. Coppage, E. Mathews, G.H. Cook and J. Knight, Pest. Biochem. Physiol. *5*, 536 (1975).
14 S. Vijayalakshmi, P. Murati Mohan and K. Sasira Babu, J. Insect Physiol. *23*, 195 (1977).

Zinc-deficiency and activities of urea cycle-related enzymes in rats[1]

M. Rahmatullah, Louise Y.Y. Fong, J.S.K. Lee and T.R.C. Boyde

Department of Biochemistry, University of Hong Kong, Hong Kong (Hong Kong), 12 February 1980

Summary. In contrast to previous reports, an increase in glutamate dehydrogenase activity and no change in arginase activity were observed in rats fed a zinc-deficient diet for 15 weeks. The discrepancies could be due to a difference in degree and duration of zinc-deficiency.

Several investigations have indicated that defects in urea cycle enzyme systems might be present in rats fed a zinc-deficient diet. Excessive amounts of nitrogen, urea and uric acid were found in urine of rats fed a zinc-deficient diet for 2–3 weeks accompanied by an increase in arginase activity[2]. On the other hand, no change in arginase or glutamate dehydrogenase activity was observed in pigs after 6 weeks of a zinc-deficient diet[3]. Rabbani et al.[4] found that blood urea nitrogen in zinc-deficient rat declined sharply and significantly during the 4th week of the dietary regimen. It appeared that duration of zinc-deficiency might affect activities of these enzymes. This led us to measure the levels of ornithine carbamoyltransferase (OCT), arginase, glutamate dehydrogenase (GDH) and aspartate aminotransferase (AAT) in rats fed a deficient diet for a prolonged period.

Materials and methods. Animals. Male, weanling rats (50–55 g) of Sprague-Dawley strain obtained from the Animal Laboratory Unit, University of Hong Kong were divided randomly into 2 groups. They were housed in plastic cages[5]. Experimental animals were fed ad libitum a zinc-deficient diet[5] using EDTA-washed soybean protein as the protein source. The zinc-deficient diet contained 5–6 ppm of zinc as determined by atomic absorption spectroscopy. Controls were pair-fed the same diet except that supplementary zinc sulphate was given to provide a dietary zinc level of 100 ppm. Deionized water was supplied to both groups. These animals were maintained for 15 weeks and were then killed by cervical dislocation. Livers, kidneys and small intestines (a 10-cm segment distal to the stomach) were removed quickly. Activities of enzymes were determined in tissues where they have maximum activities.

Determination of enzyme activities. OCT; Triethanolamine buffer (0.27 M, pH 7.7) containing 2.5 mM ornithine and 5 mM carbamoylphosphate was used for assay of OCT activity[6]. Arginase was assayed in arginine-glycine buffer (pH 9.5) at 37 °C following activation of tissue homogenates for 10 min at 52 °C[7]. Assays for AAT[8] and GDH[9] were followed by measuring decrease of absorbance at 340 nm in a LKB 2086 Reaction Rate Analyzer (LKB Instruments Ltd, Bromma, Sweden).

Results and discussion. After being fed a deficient diet for 2 weeks, the rats began to develop deficiency symptoms characterized by growth retardation, hair loss, dermal lesions and fissures at the mouth corners. Pair-fed controls showed none of these signs. The 5–6 ppm of zinc in the deficient diet permitted a chronic rather than acute (probably early) lethal deficiency. Before the animals were killed, serum zinc levels, determined by atomic absorption spectroscopy, were 0.27 ± 0.07 and 0.95 ± 0.06 µg/ml respectively for zinc-deficient and control rats. This difference was significant at the $p < 0.001$ level.

Results presented in the table showed that zinc-deficiency had no effect on tissue weight and tissue protein content. The level of intestinal OCT in zinc-deficient rats is not significantly lower than that in the controls ($p \leqslant 0.05$). The reason might be that the contribution of intestinal mucosa to ammonia utilization in the animal as a whole is not significant[10,11]. On the other hand, zinc-deficient rats showed significantly lower liver OCT and higher liver GDH activities as compared to those in controls ($p < 0.01$ in both cases). These data lent support to the observation of Rabbani et al.[4] that ammonia utilization was defective in zinc-deficient rats and resulted in its elevation in the plasma. These authors also noted a diminished hepatic OCT activity but did not measure GDH. Though bovine GDH has earlier been considered a zinc metalloenzyme[12], Colman et al.[13] showed that zinc was not an essential constituent of the enzyme but functioned as an inhibitory allosteric modifier. Therefore, it might be possible that under prolonged and severe deficiency there would be less free zinc available in the tissue for interaction with the enzyme, and as a result, GDH activity would be increased. The fact that other investigators observed no change in hepatic GDH levels in their deficient animals[2,14] might be due to a shorter and less severe deficiency. The same argument could apply to our observation of no change in hepatic arginase activity, while Hsu et al.[2] reported an