CHANGES IN THE ACTIVITY OF SOME HEPATIC ENZYMES DURING ORGANOPHOSPHORUS INSECTICIDE-ACEPHATE (ORTHENE) TREATMENT IN ALBINO RATS

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Summary : Acephate, an organophosphorus insecticide (60 *mg/day/rat)* disturbed the carbohydrate metabolism in albino rats (wt. between 100-150 *gms*). Changes in the enzyme activities in the liver were estimated in the rats after oral administration of Acephate. The specific activities of succinic dehydrogenase was decreased in experimental rats than control. A gradual decrease in blood and liver glutathione was also observed. Increase in total lactate dehydrogenase was also noted. It has been observed that in the liver homogenate of treated rats, the isoenzymes LDH_{4+5} were increased. LDH_{1+2} were decreased while LDH_3 remain unchanged with respect to control value.

Key words : acephate (orthene) lactic dehydrogenase
reduced glutathione succinic dehydrogenase

INTRODUCTION

Liver plays very important role in the metabolism of carbohydrate. Due to toxic effect of organophosphorus insecticides, carbohydrate metabolism is affected to some extent. The activity of succinic dehydrogenase was significantly depressed by Di-(-2 ethyl hexyl) phthalate (11).

Maneb, Cineh and Kelthane decreased the activity of SDH in liver (16). When rats were treated with DDT showed decrease in hepatic SDH and LDH activities (15). Decrease in activity of hepatic SDH was noted histochemically by toxic and lethal Gurza Venom doses in rats (18). Increase in hepatic LDH while decrease in SDH activities were found in rats treated with Chloropromazine (14). Hepatic LDH activity has been found to be increased in rats after Dieldrin and Thioacetamide treatment (2, 22). Rats

treated with Lindane and DDT showed changes in activities of LDH isoenzymes (1). The lethal exposure of sumithione (Fenitriothion) decreased Glutathione, SDH while increased LDH activity in fish (19). Significant increase in LDH level was observed in dogs after injection of Scorpion Venom (7). Penicillic acid, 1, 1, Dicholoroethlene, hydrazine hydrate, Sumithion have been found to deplete hepatic glutathion (3, 23, 24).

We have recently observed an increase in Pyruvic and Lactic acid contents in Acephate fed albino rats (17). In light of these findings we thought worthwhile to study the changes in hepatic enzymes such as lactic dehydrogenase, succinic dehydrogenase, and reduced glutathione in liver tissue and blood with Accephate treatment.

The effect of 75 sp. Acephate (Orthene), a water soluble organophosphorus insecticide on some hepatic enzymes related to carbohydrate metabolism is observed in the present investigation. Water soluble Acephate has been field tested in major growing regions for rice, vegetables, tobacco, cotton, oil palm and citrus.

MATERIALS AND METHODS

Adult albino rats of either sex weighing between 100–150 *gms* were distributed into two groups. The animals of group I were given standard basal diet and animals of group II were given standard diet with Acephate orally (60 *mg/day/rat*). (Oral LD_{50} for rat for 75 sp. Orthene is 1,494 *mg/kg* body wt.) Animals were given diet and water *ad libitum*. Animals were periodically killed by stunning and decapitation. Livers were immediately removed and chilled in ice. Different estimations were carried out with the chilled tissues. All the enzyme assays were performed in duplicate. Statistical analysis has been done by students 't' test (12).

The activity of SDH was determined by the method of Kuhn and Abood (10). The coloured compound triphenyl tetrazolium chloride (TTC) after reduction by SDH present in liver homogenate in presence of succinate was extracted with acetone and its O.D. read at 470 *nm*. The reaction mixture was incubated for ½ hr at 37°C. The unit is expressed as *µg* TTC reduced/10 *min/mg* fresh liver. The enzyme source was 10% liver homogenate in ice cold sucrose (0.25 M) solution.

LDH was determined by the method of Roman (21). The assay was carried out at three different temperatures i.e. 60°C, 50°C and room temperature. The assay was started by the addition of 1 *ml* Sorenson–glycine buffer (pH 4.5) in four tubes. Then 0.02 *ml* of the appropriate enzyme i.e. liver homogenate 10% (w/v) in 0.25 M sucrose

solution was added to the four tubes. The 1st tube was kept at 60°C, the 2nd tube at 50°C in water bath and the third and fourth tubes kept at room temperature. The fourth tube served as a blank. After 14 *min* incubation period the first two tubes were removed and cooled under tap water. Then all the four tubes were incubated for 15 minutes in a water bath at 37°C. After incubation period added 0·2 *ml* of 0·5% NAD with mixing and kept at same temperature for 15 min. After 15 min. 1 *ml* of 2 : 4 dinitrophenyl hydrazine was added and with mixing and read at 440 *nm*. For standard sodium pyruvate was used. The total protein was estimated by Lowry *et al.*, method (13).

Liver and blood reduced glutathione (GSH) was determined by the method of Woodward and Fry (25). Results are as shown in tables.

RESULTS AND DISCUSSION

The activity of SDH in liver, level of glutathione in liver and blood has been found to be decreased in rats after oral administration with Acephate (Orthene). It is evident from the Table I that the animals receiving Acephate for 60 days showed gradual decrease in the activity of SDH in liver. The decline in the activity of SDH was found to be more pronounced in the animals after 60 days oral administration. The decreased SDH activity will affect cellular oxidation. A diminished O_2 uptake by liver mitochondria in the presence of organophosphorus insecticides has already been observed by Ranganatha *et al.* (19).

The liver and blood GSH levels have been found to be depleted after 60 days in the treated rats than controls.

The enzymes which require –SH group for their activity may also be affected by the depletion of glutathione. This depletion may affect the metabolism of carbohydrate, lipid and protein. GSH plays an important role for detoxication of organophosphorus compounds (4, 5, 6, 8). Hepatic GSH depletion or even extra hepatic GSH depletidn (20) can provide a useful indication of the protective role of GSH against potentially toxic foreign compounds. The toxic effects of some substance e.g. vinylidene chloride could be greater if administered when GSH levels are at their lowest (9).

Thus GSH may be regarded as an endogenous protective agent for drugs, pesticides

and other compounds. The depletion of GSH will affects –SH containing enzymes and metabolism.

The activity of total LDH and its isoenzymes have been also changed by Acephate treatment. Significant in total LDH and its isoenzymes LDH_{4+5} have been observed in liver after 60 days treatment. This result indicate that pyruvate is not properly routed to TCA cycle. Even though the pyruvate dehydrogenase complex which catalyses the reaction of conversion of pyruvate to acetyl COA, has not been examined, the unequivocal depression of SDH and elevation of LDH indicate favouring of anaerobic demands.

TABLE I : Effect of Acephate (Orthene) on succinate dehydrogenase, reduced liver Glutathione and blood Glutathione.

Sr. No.	*Experimental period*	*No. of animals*	*SDH activity*	*Liver GSH (mg/g wet tissue)*	*Blood GSH (mg/100 ml)*
1.	Control	6	1.60±0.85	1.25±0.41	29.8±2.9
2.	30 days	6	1.22±0.71	0.88±0.36	28.4±2.3
3.	45 days	6	0.90±0.72	0.65±0.47*	27.5±2.7
4.	60 days	6	0.85±0.68*	0.41±0.49**	26.0±2.4*

SDH Activity : μg T.T.C. reduced 10 *min/mg* of wet weight.

* $P < 0.05$.

** $P < 0.02$.

TABLE II : Effect of Acephate (Orthene) on total lactic dehydrogenase and its Isoenzymes.

Sr. No.	*Days of expts.*	*No. of animals*	*Total LDH*	LDH_{4+5}	LDH_3	LDH_{1+2}
1.	Control	6	37.3±1.5	18.3±2.2	9.7±1.0	8.4±0.89
2.	30 days	6	38.1±1.6	19.4±1.9	10.1±0.85	7.9±0.62
3.	45 days	6	39.4±1.8	20.9±2.3	10.4±1.1	7.3±0.98
4.	60 days	6	40.4±2.4*	22.01±2.8*	10.8±0.98	6.6±1.10**

Specific activity is expressed as μg of pyruvate formed/15 *min/mg* protein of enzyme extracted.

* $P < 0.05$

** $P < 0.02$

P values represent significance of difference between normal and experimental based on student's 't' test.

Values given in the tables are mean±SD.

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