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Subacute Administration of Organophosphorus Pesticides and Hepatic Drug Metabolizing Enzyme Activity in Normal and Malnourished Rats

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Organophosphate pesticides have reportedly caused numerous fatalities in man and domestic animals (Namba et al 1971). Over 100 deaths in India during the spring of 1958 resulted from eating food accidentally contaminated with parathion (Karunakaran 1958). Some reports are available on the effects of malathion on behavioural and reproductive functions (Krause 1977; Kurtz 1977). High level exposure to phosalone in rats and dogs has been reported to cause changes in body weight, liver weights, RBC etc. (FAO/WHO 1973b).

Toxic effects of these pesticides may be influenced by pre-existing nutritional conditions (Chakravarty and Ghosh 1980; Chakravarty and Sreedhar 1982). The acute toxicities of parathion (Boyd and Krupa 1970) and malathion (Boyd and Tanikella 1969) reportedly are greatly enhanced in animals fed a 3.5% casein diet compared to those fed with a 26% casein diet. Hence, it appears that the susceptibility to pesticide toxicity may be determined by the amount of protein in the diet.

The liver is the major detoxifying organ of the body (Chatterji 1975). DeCastro and Boyd (1967) have reported impairment of growth of body organs, including the liver in animals fed a protein deficient diet. Pesticides themselves may produce antihepatic effects e.g. DDT influences carbohydrate metabolism **in vivo** (Hickenbottam and Yan 1974) by promoting hepatic glycogenolysis. β and γ -isomers of BHC have been observed to cause alterations in hepatic enzyme activities **viz.** transaminases, phosphatases etc. (Srinivasan and Radhakrishnamurty 1977). Altered hepatic function in young rats exposed to malathion has been reported (Pawar and Makhija 1975). The present study was undertaken to evaluate the effect of the three most commonly used organophosphorus pesticides **viz.** parathion (O-O-diethyl-O-p-nitrophenyl-thio phosphate), malathion (O-O-dimethyl-S-(1,2-dicarboethoxy) ethyl phosphorodithioate) and phosalone (O-O-diethyl-S-(6-chlorol,3 benzoxazol-2(3H)-onyl-methyl) phosphorodithioate), on the hepatic β-glucuronidase activity in rats fed with normal and protein deprived diets.

MATERIALS AND METHODS

Adult male albino rats of Charles Foster Strain obtained from the Institute's animal house were used for the study. The animals weighed approximately 100-120gms. They were kept under controlled lighting of 12hrs dark and 12hrs light at a temperature of 26±2°C. The rats were divided into three dietary groups of 90 each. They were

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maintained on isoenergetic diets containing 16%, 6% and 3% casein protein respectively for 3 consecutive weeks. The 16% protein diet taken as the control (Ramesh 1980) was prepared by slightly modifying the composition proposed by Kalamegham et al. (1981). The 6% and 3% diets were kept isocaloric compared to the 16% diet, by replacing casein with an equivalent amount of amylum. They were fortified with minerals and vitamins as given in Table 1.

Table 1. Composition of the diets per 100 gms.

	16%	6%	3 %
Casein[a]	20.0 gms	7.5 gms	3.75 gms
Amylum	59.0 gms	71.5 gms	75.25 gms
Glucose	10.0 gms	10.0 gms	10.00 gms
Groundnut oil	6.0 gms	6.0 gms	6.00 gms
Vitamin mixture[b]	0.7 gms	0.7 gms	0.70 gms
Salt mixture[c]	4.0 gms	4.0 gms	4.00 gms
Cellulose	0.3 gms	0.3 gms	0.30 gms

a. Protein content of casein was 80%.
b. Vitamins were added as per the requirements specified by the committee of the National Academy of Sciences (1962).
c. Salt mixture was prepared according to the composition of Wesson Salt Mixture (Wesson 1932).

All animals had access to food and water **ad libitum**. Food dishes were filled and weighed, and the amount consumed was calculated from the difference of weight, 24hrs later. If there was evidence of spillage, the calculation was omitted from the estimate of weekly average. All animals were weighed weekly.

At the beginning of the treatment, each dietary group of rats were further subdivided into five individual groups for pesticide treatment, with at least 6 rats in each group. Parathion, malathion and phosalone were orally force fed to each group **per os** by using a standard feeding needle at 10.00AM for a period of 21 consecutive days. The doses used are given in Table 2. The doses were selected on the basis of 'no effect' level of these pesticides in rats (Lehman 1965; WHO 1982; FAO/WHO 1973b).

Table 2. Doses of pesticide treatment

Diet for 3 weeks	Group	Parathion µg/kg body weight	Malathion mg/kg body weight	Phosalone mg/kg body weight
16%, 6% or 3% dietary protein	I	Nil	Nil	Nil
	II	50	5	1.25
	III	100	50	5.00
	IV	150	250	12.50
	V	200	500	60.00

The rats were sacrificed by decapitation, exactly 24hrs after the last day of treatment. The intact liver was carefully dissected out after removal of all adhering tissues, blotted on a filter paper, weighed and placed in crushed ice on watch glasses. A

part of the liver from each rat was homogenised in ice-cold 0.32 (M) Sucrose solution and the 10% homogenate was centrifuged at 4°C at 3000 x g. The supernatent was used as the enzyme source. β-glucuronidase activity was estimated by the method of Fishman et al. (1948), using P-nitrophenyl- β-D-glucuronide (PNP- β-D-glucuronide) as the substrate. Protein was estimated by the method of Gornall et al. (1949). At the completion of the experiment, four healthiest rats were chosen from each group for carrying out the studies to have compatible results and uniformity in data.

Statistical analysis of the data was done by using students 't' test and the analysis of variance, and $P < 0.05$ was taken to be significant. Intergroup comparisons at each dose level were also done by calculating the critical difference and 't'-statistic was also used, to find out whether there existed a significant difference between the two sample means.

RESULTS AND DISCUSSION

It is evident from Table 3 that all the three pesticides studied caused a dose dependent increase in the activity of hepatic β-glucuronidase. The change in the enzyme activity in the 16% group was significant only at the higher doses of pesticide treatment. In the groups containing low protein however, pesticide administration even at the lower doses caused significant increase in the enzyme activity. Protein deficiency itself also caused a significant decrease on the enzyme activity as is evident from Groups VI and XI when compared to Group I.

Table 4. Anova table for β-glucuronidase after subacute administration of parathion, malathion and phosalone.

Source	df	Mean Squares		
		Parathion	Malathion	Phosalone
D	4	28.23*	28.23*	44.23*
DPL	2	97.96*	57.84*	78.48*
DxDPL	8	1.36**	1.77*	1.72*
Error	45	0.65	0.32	0.42

'n' per dose = 4 *P < 0.05 **P > 0.05

On applying analysis of variance individually for parathion, malathion and phosalone (Table 4) it is seen that the various doses (D) and the dietary protein levels (DPL) contribute significantly to bring about the change in β-glucuronidase activity for each of the three pesticides studied. The DxDPL interaction also, however, caused significant change except in the case of parathion.

The intergroup comparisons at each dose level of parathion (Table 5), malathion (Table 6) and phosalone (Table 7) showed significant reductions in the β-glucuronidase activity when compared between the 16% and 6% protein groups. Comparisons between 6% and 3% protein groups showed significant changes for malathion and phosalone. In case of parathion, at the 100 and 150 µg doses, though the reduction is apparent, the change was not statistically significant.

The present study indicates that β-glucuronidase activity increases in a dose-dependent manner with pesticide administration. Low protein diets however appeared to reduce the enzyme activity in the liver. In protein deprived rats, there was initially a decrease in the activity of the enzyme which is greater after pesticide treatment.

Injuries to the lysosomal membrane produced by toxic agents often lead to the release of this enzyme into the cytoplasm, subsequently resulting in cell death and necrosis

Table 3. Effect of sub-acute administration of parathion, malathion and phosalone on hepatic β-glucuronidase activity

Dietary groups	Groups	Doses of parathion µg/kg b.wt.	β-glucuronidase activity Mean±SEM*	Doses of malathion mg/kg b.wt.	β-glucuronidase activity Mean±SEM*	Doses of phosalone mg/kg b.wt.	β-glucuronidase activity Mean±SEM*
	I	Nil	10.68±0.48	Nil	10.08±0.34	Nil	11.47±0.31
16%	II	50	10.63±0.43^{ns}	5	10.11±0.17^{ns}	1.25	11.59±0.21^{ns}
casein	III	100	10.02±0.49^{ns}	50	10.67±0.42^{ns}	5.0	12.29±0.36^{ns}
protein	IV	150	12.57±0.45^{s}	250	10.97±0.15^{ns}	12.5	13.19±0.20^{s}
	V	200	13.32±0.52^{s}	500	12.46±0.44^{s}	60.0	15.20±0.27^{s}
	VI	Nil	7.18±0.43^{s}	Nil	7.71±0.17^{s}	Nil	7.74±0.25^{s}
6%	VII	50	7.64±0.31^{ns}	5	7.78±0.39^{ns}	1.25	8.40±0.34^{ns}
casein	VIII	100	7.77±0.39^{ns}	50	8.52±0.22^{s}	5.0	9.98±0.47^{s}
protein	IX	150	9.17±0.26^{s}	250	10.03±0.32^{s}	12.5	11.81±0.53^{s}
	X	200	10.99±0.40^{s}	500	11.21±0.12^{s}	60.0	13.47±0.34^{s}
	XI	Nil	4.63±0.52^{s}	Nil	5.20±0.34^{s}	Nil	6.50±0.24^{s}
3%	XII	50	6.15±0.24^{s}	5	5.82±0.23^{ns}	1.25	7.89±0.35^{s}
casein	XIII	100	6.92±0.39^{s}	50	6.87±0.60^{s}	5.0	8.21±0.44^{s}
protein	XIV	150	8.49±0.22^{s}	250	9.15±0.19^{s}	12.5	10.60±0.30^{s}
	XV	200	9.28±0.30^{s}	500	10.23±0.31^{s}	60.0	10.90±0.16^{s}

* = Mean values expressed as µg para-nitrophenol liberated per mg protein per hour (4 rats per group)
ns = denotes not significant P value > 0.05
s = denotes significant P value < 0.05
Groups II to VI and XI compared to Gr-I
Groups VII to X compared to Gr-VI
Groups XII to XV compared to Gr-XI

Table 5. Significance of difference table for β-glucuronidase activity after subacute administration of parathion

Doses µg/kg b.wt.	0		50		100		150		200	
Dietary groups	Mean[a]	Difference	Mean[a]	Difference	Mean[a]	Difference	Mean[a]	Difference	Mean[a]	Difference
16% protein	10.68	3.50[b]	10.63	2.99[b]	10.02	2.25[b]	12.57	3.40[b]	13.32	2.33[b]
6% protein	7.18	2.55[b]	7.64	1.49[b]	7.77	0.85[c]	9.17	0.68[c]	10.99	1.71[b]
3% protein	4.63		6.15		6.92		8.49		9.28	

a = Mean values expressed as µg PNP liberated per mg protein per hr (4 rats per group) b = Significant $P < 0.05$ c = Nonsignificant $P > 0.05$

Table 6. Significance of difference table for β-glucuronidase activity after subacute administration of malathion

Doses mg/kg b.wt.	0		5		50		250		500	
Dietary groups	Mean[a]	Difference	Mean[a]	Difference	Mean[a]	Difference	Mean[a]	Difference	Mean[a]	Difference
16% protein	10.08	2.37[c]	10.11	2.33[c]	10.67	2.15[c]	10.97	0.94[b]	12.46	1.25[c]
6% protein	7.71	2.51[c]	7.78	1.96[c]	8.52	1.65[c]	10.03	0.88[b]	11.21	0.98[b]
3% protein	5.20		5.82		6.87		9.15		10.23	

a = Mean values expressed as µg PNP liberated per mg protein per hr (4 rats per group) b = Significant $P \leq 0.05$ c = Significant $P < 0.01$

Table 7. Significance of difference table for β-glucuronidase activity after subacute administration of phosalone

Doses mg/kg b.wt.	0		1.25		5.0		12.5		60.0	
Dietary groups	Mean[a]	Difference	Mean[a]	Difference	Mean[a]	Difference	Mean[a]	Difference	Mean[a]	Difference
16% protein	11.47	3.73[b]	11.59	3.19[b]	12.29	2.31[b]	13.19	1.38[b]	15.20	1.73[b]
6% protein	7.74	1.24[b]	8.40	0.51[c]	9.98	1.77[b]	11.81	1.21[b]	13.47	2.57[b]
3% protein	6.50		7.89		8.21		10.60		10.90	

a = Mean values expressed as µg P-nitrophenol liberated per mg protein per hour (4 rats per group)

b = Significant $P < 0.01$

c = Nonsignificant $P > 0.05$

(Deckers-Passau 1957). Hence, the increase in this lysosomal enzyme activity induced by the pesticides as observed in the present study, may also be due to the possible damage to the lysosomal membrane. All the organophosphorus pesticides studied appear to have a similar action in undernourished condition, but of the three pesticides studied, malathion seems to have the least significant effect while parathion appears to be most toxic. Enhanced **in vivo** toxic effect of parathion and its metabolite paraoxan under conditions of water and food restriction have also been reported (Baetjer 1983; Chakravarty and Sreedhar 1982). In case of malathion, increased susceptibility under the protein deprived conditions have been previously reported from our laboratory (Chakravarty and Sreedhar 1981; Bulusu and Chakravarty 1983; Bulusu and Chakravarty 1984). Hence, protein deprivation may lead to increased **in vivo** toxicity of the three organophosphorus pesticides studied. Hence, it may be advisable to determine the nutritional status of a community prior to its exposure to any new pesticide.

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