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Profile of Drug Metabolizing Enzymes in Rats Treated with Parathion, Malathion, and Phosalone Under Various Conditions of Protein Energy Malnutrition

Saraswati Bulusu and Indira Chakravarty

Department of Biochemistry and Nutrition, All India Institute of Hygiene and Public Health, 110 Chittaranjan Avenue, Calcutta 700073, India

Pesticides particularly those of the organophosphorus group are extensively used for agricultural and various other purposes throughout the world. However, the indiscriminate use of these chemicals have often resulted in several incidences of human intoxication (Namba 1971). Recent experimental data further indicate that the toxic effects of human exposure may subsequently be influenced by their existing nutritional status (Chakravarty and Ghosh 1980). This aspect of pesticide toxicity is of great importance particularly in the developing countries where protein malnutrition is a major public health problem and use of pesticides for various purposes is also common. The toxicity of any drug or chemical, depends to a large extent on the process of detoxification of the compound in the body, which is again interdependent on the nutritional status.

Liver is the organ which takes one of the most important roles in the detoxifying functions of the body (Chatterjee 1975). The liver microsomal drug metabolising enzymes, responsible for the metabolism of a variety of endogenous and exogenous substances (Conney 1967) are also known as mixed function oxidase system (MFO). In animals, a number of agents affect the microsomal drug metabolism either by induction or inhibition of these enzymes. Exposure to environmental chemicals (Goodman and Gilman 1975) and insecticides (Wagstaff and Street 1971) induced hepatic microsomal MFO system in animals and man.

Nutritional status, starvation and stress have important effects on the activity of the drug metabolising enzymes (Kato **et al** 1962). The dietary composition of protein quality (Miranda and Webb 1973) and various micronutrients like vitamins have been reported to affect the metabolism of foreign compounds (Campbell and Hayes 1974).

Since aniline hydroxylase and aminopyrine N - demethylase are two important enzymes of the MFO system, which are essentially involved in the metabolism of many foreign compounds, activities of these two enzymes have been estimated in the present study,

Send reprint requests to Prof. Indira Chakravarty at the above address

after the administration of three most commonly used organophosphorus pesticides **viz.** parathion (O,O-diethyl-O-paranitrophenyl thiophosphate), malathion (O,O-dimethyl-S-1,2-dicarboethoxy) ethyl phosphorodithioate) and phosalone (O,O-diethyl-S-(6-chlorol, 3 benzoxazol-2(3H)-onylmethyl phosphorodithioate). The pesticides were fed to rats maintained on normal and protein deprived diets (of various grades) for a period of three weeks, subsequent to which studies were carried out.

MATERIALS AND METHODS

The experiments were performed upon adult male albino rats of Charles Foster strain weighing approximately 100-120 gms. They were kept under controlled lighting of 12 hrs dark and 12 hrs light at a temperature of $26 \pm 2^{\circ}C$. The rats were divided into three dietary groups of 90 each. They were maintained on isoenergetic diets containing 16%, 6% and 3% casein protein respectively, for 3 consecutive weeks. The 16% protein diet was taken as the control (Ramesh, 1980). The control diet was prepared by slightly modifying the composition proposed by Kalamegham **et al** (1981) and contained Casein-20%, Amylum-59%, Glucose-10%, Groundnut Oil-6%, Salt Mixture-4%, Vitamin Mixture-0.7% and Cellulose-0.3%. Protein content of casein was 80%. Vitamins were added as per the requirements specified by the Committee of the National Academy of Sciences (1962) and salt mixture was prepared according to the composition of Wesson salt mixture (Wesson 1932). The 6% and 3% protein diets were kept isocaloric compared to the 16% diet, by replacing casein with an equivalent amount of amylum. Food and water were given **ad libitum.** Food intake was calculated on the basis of difference of weight every 24 hours and all animals were weighed weekly.

At the beginning of the treatment, each major group was further sub-divided into five individual sub-groups, for each pesticide treatment, with at least 6 rats in each group. All the three pesticides under study, were orally force fed **per os** by using a standard feeding needle, daily at 10.00 hrs for a period of 21 consecutive days. The doses used are given in Table-1. The doses were selected on the basis of no-effect levels of these pesticides in rats (Lehman 1965; WHO 1982; FAO/WHO 1973b).

Table 1. Doses of pesticide treatment

Diet for 3 weeks	Sub Groups	Parathion µg/kg b.wt	Malathion mg/kg b.wt	Phosalone mg/kg b.wt
For all rats having 16%, 6% or 3% protein in their diet	I	Nil	Nil	Nil
	II	50	5	1.25
	III	100	50	5.0
	IV	150	250	12.5
	V	200	500	60.0

At the end of the treatment, the rats were sacrificed by instant decapitation and the liver tissues were carefully dissected out. After removal of all adhering tissues and blotting on a filter paper they were weighed fresh and placed on watch glasses in crushed ice. Microsomal fractions were prepared by slightly modifying the method of Kato and Gillette (1965). A portion of liver from each rat was homogenised in ice-cold 1.15% KCL (pH 7.5). The 10% homogenate was successively centrifuged in cold at 3000 x g for 10 minutes, 20,000 x g for 1 hour and finally at 105,000 x g for 1 hour. The soluble fraction was decanted and the microsomal pellet was suspended in ice-cold 1.15% KCL and was used as the enzyme source. Aniline hydroxylase and aminopyrine N-demethylase activities were estimated by the method of Ishidate **et al** (1978), a slightly modified method of Imai **et al** (1966) and Kato and Gillette (1965). The final reaction for aminopyrine N-demethylase was completed by adding Nash reagent (Nash 1953). Microsomal protein was estimated by the method of Lowry **et al** (1951). Statistical analysis was done by taking 'n' as 4, to maintain uniformity of data. Student's 't' test and analysis of variance (ANOVA) were applied, and $P < 0.05$ was taken to be significant. Intergroup comparisons at each dose level were also done by calculating the critical difference.

RESULTS AND DISCUSSION

The rats maintained on the 6% and 3% protein diets **ad libitum** showed lower food consumption than the controls and consumed approximately 83.33% and 71.25% of the control intake, respectively.

Parathion, malathion and phosalone on daily administration for 21 consecutive days caused significant reductions in the hepatic microsomal aniline hydroxylase activity both in normal as well as protein deprived rats as seen from Table - 2. The inhibition, however, was more marked in the low protein groups where significant P values were observed even at the low doses of treatment. Protein deprivation itself caused significant reductions in the enzyme activities both at 6% and 3% levels. ANOVA analysis shows significant contribution by different doses (D) of parathion $F(4,45) = 20.63$, $P < 0.05$, malathion $F(4,45) = 20.00$ $P < 0.05$, and phosalone $F(4,45) = 18.80$, $P < 0.05$ and the dietary protein levels (DPL) for parathion $F(2,45) = 28.44$, $P < 0.05$, malathion $F(2,45) = 45.00$, $P < 0.05$ and phosalone $F(2,45) = 38.00$, $P < 0.05$ in bringing about the inhibition in the enzyme activity. The D x DPL interaction for parathion $F(8,45) = 0.47$, $P > 0.05$, malathion $F(8,45) = 1.00$, $P > 0.05$ and phosalone $F(8,45) = 0.16$, $P > 0.05$ has no synergistic effect on the enzyme activity. Intergroup comparisons at each dose level of parathion, malathion and phosalone show non-significant critical differences between 6% and 3% protein fed groups but significant differences between the means of the 16% and 6% groups, at all the doses administered.

Sub acute administration of these pesticides showed significant reductions in the hepatic microsomal aminopyrine N-demethylase activities as seen from Table - 3. Protein deficiency caused decreases

Table 2. **Effect of protein deprivation on sub-acute toxicity of pesticides in relation to hepatic microsomal aniline hydroxylase activity.**

Dietary groups	Groups	Parathion		Malathion		Phosalone	
		Doses µg/kg b.wt.	Aniline Hydroxylase activity MEAN±SEM	Doses mg/kg b.wt.	Aniline Hydroxylase activity MEAN±SEM	Doses mg/kg b.wt.	Aniline Hydroxylase activity MEAN±SEM
	I	Nil	1.61±0.15	Nil	1.88±0.06	Nil	1.82±0.13
	II	50	1.21±0.11	5	1.71±0.12	1.25	1.63±0.12
16%	III	100	1.20±0.10	50	1.53±0.13	5.0	1.33±0.14
protein	IV	150	1.09±0.08[a]	250	1.13±0.09[a]	12.5	1.20±0.10[a]
	V	200	1.04±0.11[a]	500	1.12±0.14[a]	60.0	1.17±0.18[a]
	VI	Nil	1.23±0.04[b]	Nil	1.43±0.09[b]	Nil	1.36±0.05[b]
	VII	50	1.03±0.09	5	1.04±0.15	1.25	1.26±0.08
6%	VIII	100	0.89±0.11[a]	50	0.97±0.10[a]	5.0	0.85±0.08[a]
protein	IX	150	0.73±0.14[a]	250	0.95±0.08[a]	12.5	0.76±0.06[a]
	X	200	0.67±0.03[a]	500	0.75±0.06[a]	60.0	0.66±0.07[a]
	XI	Nil	1.20±0.01[b]	Nil	1.18±0.07[b]	Nil	1.14±0.13[b]
	XII	50	0.97±0.05[a]	5	1.01±0.08	1.25	1.06±0.14
3%	XIII	100	0.73±0.09[a]	50	0.91±0.06[a]	5.0	0.74±0.04[a]
protein	XIV	150	0.71±0.06[a]	250	0.80±0.11[a]	12.5	0.68±0.06[a]
	XV	200	0.52±0.06[a]	500	0.63±0.11[a]	60.0	0.59±0.11[a]

Nil dose signifies vehicle control group.

Activities expressed as nmol. PAP liberated/mg MP/min.

a = In comparison with vehicle control group within the same dietary group $P < 0.05$.

b = In comparison with vehicle control group in the 16% protein group $P < 0.05$.

in the enzyme activities, the change being significant both at the 6% and 3% protein levels. The enzymatic inhibition induced on pesticides administration was more marked in the protein deprived rats than in the normal protein fed rats. ANOVA shows that the different doses (D) of parathion $F_{(4,45)} = 12.11$, $P < 0.05$, malathion $F_{(4,45)} = 7.10$, $P < 0.05$ and phosalone $F_{(4,45)} = 14.58$, $P < 0.05$ and the dietary protein levels (DPL) for parathion $F_{(2,45)} = 54.53$, $P < 0.05$, malathion $F_{(2,45)} = 39.44$, $P < 0.05$ and phosalone $F_{(2,45)} = 25.76$, $P < 0.05$ all contribute significantly to bring about the change in the demethylase activity. The D x DPL interaction however, has no synergistic effect for parathion $F_{(8,45)} = 0.67$, $P > 0.05$, malathion $F_{(8,45)} = 0.18$, $P > 0.05$ and phosalone $F_{(8,45)} = 0.67$, $P > 0.05$. Intergroup comparisons at each dose level of pesticides show significant critical differences at all the doses between 16% and 6% protein groups but not between 6% and 3% dietary groups.

From the foregoing results it is seen that protein deprivation for a period of 3 weeks caused a decrease in the microsomal aniline hydroxylase and aminopyrine N-demethylase activities which were significant both at the groups receiving 6% as well as 3% protein in their diet. Previous reports (Campbell and Hayes 1974) have also clearly demonstrated decreased microsomal MFO activity in conditions of malnutrition (Kato **et al** 1962). The role of protein in regulating the microsomal enzyme status and determining the toxicity of foreign compounds has also been shown by Miranda and Webb (1973).

Sub-acute administration of parathion, malathion and phosalone caused inhibition of microsomal aniline hydroxylase and aminopyrine demethylase activities both in the groups receiving normal as well as low protein levels in their diets. Exposure to pesticides have been reported to alter MFO system as well. Halogenated hydrocarbon insecticides on the other hand are reported to induce microsomal enzyme activity (Hoffman and Anderson 1970). The work of Welch **et al** (1967) indicated that these hydrocarbons can either stimulate or inhibit microsomal testosterone hydroxylation depending upon whether the agents are given chronically or acutely.

Very little information is available relating to the effects of the cholinesterase-inhibiting class of insecticides on the MFO system and that too in malnutrition. However, Pawar and Makhija (1975) reported an increase in demethylase and hydroxylase activity on malathion treatment in rats. This finding is however, not in support of the present observations, where a decrease in enzyme activities were noted. These results are in accordance with the findings of MacDonald **et al** (1970) who found O - and N-demethylase activity to be diminished by parathion. Stevens **et al** (1972) also reported parathion, malathion, disulfoton and carbaryl to be potent inhibitors of the **in vitro** metabolism of ethylmorphine and aniline in the mouse.

It may be suggested that probably a competition exists between the insecticides and microsomal substrates used in the study. The

Table 3. **Effect of protein deprivation on sub-acute toxicity of pesticides in relation to hepatic microsomal aminopyrine N-demethylase activity.**

Dietary groups	Groups	Parathion Doses μg/kg b.wt.	Parathion Aminopyrine N-Demethylase activity MEAN±SEM	Malathion Doses mg/kg b.wt.	Malathion Aminopyrine N-Demethylase activity MEAN±SEM	Phosalone Doses mg/kg b.wt.	Phosalone Aminopyrine N-Demethylase activity MEAN±SEM
	I	Nil	5.91±0.17	Nil	6.58±0.17	Nil	6.35±0.25
	II	50	5.17±0.27	5	6.36±0.17	1.25	6.17±0.37
16%	III	100	4.29±1.06	50	6.07±1.29	5.0	5.24±0.43
protein	IV	150	4.24±0.37[a]	250	5.55±0.26[a]	12.5	5.14±0.21[a]
	V	200	4.14±0.61[a]	500	5.07±0.15[a]	60.0	4.26±0.61[a]
	VI	Nil	4.14±0.15[b]	Nil	5.33±0.26[b]	Nil	5.33±0.32[b]
6%	VII	50	3.95±0.12	5	5.04±0.33	1.25	5.00±0.21
protein	VIII	100	2.80±0.24[a]	50	4.02±0.44[a]	5.0	4.53±0.26
	IX	150	2.70±0.16[a]	250	3.86±0.17[a]	12.5	4.32±0.19[a]
	X	200	2.37±0.21[a]	500	3.79±0.45[a]	60.0	3.96±0.10[a]
	XI	Nil	3.27±0.18[b]	Nil	4.45±0.17[b]	Nil	4.86±0.12[b]
3%	XII	50	2.67±0.16[a]	5	4.08±0.17	1.25	4.24±0.18[a]
protein	XIII	100	2.29±0.33[a]	50	3.36±0.34[a]	5.0	4.23±0.16[a]
	XIV	150	2.28±0.13[a]	250	3.03±0.30[a]	12.5	3.99±0.13[a]
	XV	200	2.18±0.17[a]	500	2.95±0.06[a]	60.0	3.38±0.28[a]

Nil dose signifies vehicle control group.

Activities expressed as nmoles HCHO liberated/mg MP/min.

a = in comparison with vehicle control group within the same dietary group $P < 0.05$.

b = in comparison with vehicle control group in the 16% protein group $P < 0.05$.

existence of a competition is supported by the finding that increases in pesticide doses lead to greater inhibition of the substrate metabolism. Stevens **et al** (1972) even stated that the insecticides may be functioning as alternative substrates since Lykken and Casida (1969) have shown that many of them are metabolized by microsomal enzymes. Since, increased lipid peroxidation results in diminished enzyme activities (Wills 1971) it may also be assumed that these pesticides may be enhancing lipid peroxidation, more so in protein deprived conditions thereby resulting in increased inhibition of the enzyme activities.

Hence, from these studies it may be indicated that the cholinesterase inhibiting insecticides parathion, malathion and phosalone can alter **in vitro** rates of microsomal drug metabolism, more so in protein deprived rats than in normal ones and is probably responsible for their increased toxicity in protein malnourished conditions. Augmented hepatic susceptibility to pesticide toxicity under protein deprived conditions has been previously reported from this laboratory (Bulusu and Chakravarty 1984a; Bulusu and Chakravarty 1984b). Altered drug metabolism with respect to glucuronidase activity on repeated pesticide exposure has also been reported from this laboratory (Bulusu and Chakravarty 1986). Therefore, it may be concluded that people sustaining on nutritionally inadequate diets may be more prone to the toxic effects of these pesticides as compared to those having nutritional adequacy.

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