

sumption balance in the ecosystem. Further it is suggested that *S. costatum* may be taken as an indicator species in pollution monitoring studies.

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DDT IMPACT ON ASPARTATE AND ALANINE AMINO TRANSFERASE ACTIVITY LEVELS IN THE LIVER OF FROG, *RANA HEXADACTYLA*

Introduction

THE organochlorine insecticide (DDT) was known to be a neurotoxicant affecting peripheral sensory organs and nervous system¹, by inhibiting the acetylcholinesterase activity². It also inhibits the microsomal ATP phosphorylases³, and Mg⁺⁺, Na⁺, and K⁺ dependent ATPases as well as actomyosin ATPases of nerves and muscles⁴⁻⁶. DDT also causes the depletion of carbohydrate reserves, the loss of body weight and a general increase in blood amino nitrogen content^{7,8}. The depletion of carbohydrate reserves and increase in blood amino nitrogen content indicate the possible involvement of transcrases. In the present investigation an attempt has been made to study the *in vitro* effects of DDT on the enzymatic systems involved in the amino acid metabolism of liver of frog, so as to assess the specific role of this insecticide on transamination reaction systems.

Materials and Methods

The liver of frog, *Rana hexadactyla* was excised after pithing the animal and 10% (W/V) homogenate was prepared in 0.25 M sucrose solution at 5°C. The extract was centrifuged at 2,500 rpm for 15 minutes to remove cell debris and the supernatant was used for the assays of aspartate and alanine aminotransferase activities. The activities were estimated by the method of Reitman and Frankel⁹ as given by Bergmeyer¹⁰.

The activities were expressed as μ moles of sodium pyruvate formed/mg protein/hr.

The free amino acid levels were estimated by the method of Moore and Stein as given by Colowick and Kaplan¹¹. Sucrose soluble and insoluble proteins were estimated by the method of Lowry *et al.*¹² with folin phenol reagent.

Experimental tubes received 0.01 to 0.1 ml containing 2 to 20 μ M of DDT in addition to the contents of reaction mixture, whereas control tubes received distilled water in the place of DDT (as per the method described by Desai *et al.*¹³).

Results and Discussion

The results obtained with control and experimental (preincubated with DDT) homogenate preparations from frog liver indicated that the protein content showed a continuous increment as the concentration of DDT increased from 2 to 20 μ M (Table I). Similar increase in protein levels was observed by Cappan and Nicholls¹⁴, and it is also suggested that DDT and polychlorinated biphenyls cause the induction of drug-metabolizing enzymes in liver and cause marked changes in the protein synthetic machinery^{15,16}. The preincubation of liver homogenates with DDT resulted in changes not only in the total protein level but also in the soluble and structural fractions. The variation in the levels of sucrose soluble protein is presumed to indicate the variation in the general solubility of denaturation of protein which could correspondingly reflect in the structural protein level. To verify this aspect the ratios of soluble/structural proteins were determined and a decrease in the ratio showed a lesser solubility, greater denaturation while an increase in the ratio showed an elevation in the solubility and lesser denaturation. When compared to the control, the experimental homogenates, in general showed increased soluble/structural ratio with increase in DDT concentration in the incubation mixture showing an elevatory tendency of proteins to solubilize. Thus the DDT preincubation alters the denaturation patterns in addition to proteolysis.

The preincubation with DDT showed a general drop in the level of FAA with the increase in the concentration of DDT (Table I). Hence it is presumed that the free amino acids might have been mobilized for the synthesis of proteins. Earlier *in vivo* and *in vitro* studies have shown that the increased synthesis of microsomal protein after the addition of DDT depends on an increased incorporation of amino acids into microsomal proteins¹⁷⁻²⁰ and the present investigation adds credence to the above statement. Since FAA content was found to decrease in the experimental tissue homogenates, it was felt desirable to study the possible involvement of aminotransferase reacting systems in the mobilization of free amino acids.

TABLE I
In vitro effect of DDT on soluble, structural protein levels and free amino acid content in liver of frog

Sl. No	DDT in μ M	Protein type (mg/gm wet wt. of tissue)				Soluble/ structural ratio	Free amino acid content mg/gm wet wt.	% Change
		Soluble proteins	% Change	Structural proteins	% Change			
1.	Control	22.01 \pm 0.8704	..	22.15 \pm 0.1779	..	0.9936	0.900 \pm 0.032	..
2.	2	46.01 \pm 0.9802*	+109.04	28.13 \pm 0.2533*	+ 26.99	1.6356	0.866 \pm 0.01154**	- 3.78
3.	4	51.00 \pm 0.9901*	+131.71	30.02 \pm 5.665*	+ 35.53	1.6988	0.840 \pm 0.006*	- 6.67
4.	6	54.00 \pm 0.0031*	+145.34	32.50 \pm 0.448*	+ 46.73	1.6615	0.746 \pm 0.0871*	-17.11
5.	8	63.33 \pm 0.5718*	+187.73	38.31 \pm 0.6002*	+ 72.96	1.6531	0.610 \pm 0.01*	-32.22
6.	10	76.00 \pm 0.000*	+245.29	39.34 \pm 0.5917*	+ 77.61	1.9318	0.543 \pm 0.0057*	-39.67
7.	12	62.61 \pm 1.149*	+184.46	41.01 \pm 0.0908*	+ 85.15	1.5267	0.436 \pm 0.0023*	-51.56
8.	14	63.66 \pm 0.927*	+189.23	44.31 \pm 0.5854*	+100.04	1.4366	0.420 \pm 0.01*	-53.3
9.	16	61.33 \pm 1.34*	+178.65	46.03 \pm 0.1681*	+107.81	1.3323	0.383 \pm 0.0003*	-57.44
10.	18	97.01 \pm 1.02*	+340.75	47.00 \pm 0.03108*	+112.19	2.0640	0.350 \pm 0.01*	-61.11
11.	20	97.31 \pm 1.34*	+342.12	47.89 \pm 0.6134*	+116.21	2.0319	0.323 \pm 0.221*	-64.11

* P < 0.01; ** P < 0.05; All the values are mean of six observations.

TABLE II
In vitro effect of DDT on Aspartate (AAT) and Alanine (AlAT) amino transferase activity levels of liver homogenates of frog

(Activity expressed in μ M of pyruvate formed/mg protein/hr.)

Sl. No.	DDT in μ M	AAT	% Change	AlAT	% Change
1.	Control	1.746	..	1.848	..
		\pm 0.326		\pm 0.472	
2.	2	1.749	+ 2.75	1.858	+ 0.54
		\pm 0.243*		\pm 0.462*	
3.	4	1.964	+ 12.48	2.066	+ 11.79
		\pm 0.565*		\pm 0.855*	
4.	6	2.914	+ 66.89	2.514	+ 36.09
		\pm 0.428**		\pm 0.775*	
5.	8	2.944	+ 68.61	3.029	+ 63.91
		\pm 0.452**		\pm 0.456**	
6.	10	3.222	+ 84.54	3.228	+ 74.67
		\pm 0.384**		\pm 0.681**	
7.	12	3.658	+109.51	3.659	+ 97.99
		\pm 0.639**		\pm 0.472**	
8.	14	3.829	+119.30	4.033	+118.23
		\pm 0.30**		\pm 0.606**	
9.	16	3.594	+105.74	2.550	+ 37.99
		\pm 0.706**		\pm 0.438†	
10.	18	2.558	+ 46.51	1.889	+ 2.22
		\pm 0.447**		\pm 0.424*	
11.	20	1.227	- 29.72	1.639	- 11.31
		\pm 0.177***		\pm 0.404*	

* Not significant; ** P < 0.001; *** P < 0.01; † P < 0.02.
All the values are mean of six observations.

In general the AAT and ALAT activity levels show a continuous elevation with increase in DDT concentrations. The activity levels of both AAT and ALAT showed a continuous increment upto 14 μ M and decreased at higher concentrations of DDT (Table II). The decrease in amino acid level with increase in DDT concentration in incubation mixture corresponds to the increase in amino-transferase activity showing a possibility of mobilization of amino acids into pathways mediated by transaminases. At higher concentrations of DDT, the aminotransferase activity showed a decline but FAA content showed a continuous decrease indicating that the amino acid depletion at higher concentration of DDT need not be essentially by aminotransferase reactions but probably due to general degradation of amino acids or their incorporation into proteins¹⁷⁻²⁰.

Hence it can be presumed that both protein and amino acid metabolism seem to alter with insecticide concentration.

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ERRATA

In the article "A NEW RECORD OF SCLEROTINIA ROT OF CABBAGE IN INDIA" by M. N. Alagianagalingam, R. Moman, C. L. Subramanian and S. Sampanthamoorthy" published in CURRENT SCIENCE, 1978, 47, 967, the claim that it was the first

record of India is incorrect. In place of INDIA it should be SOUTH INDIA. The error was noticed by Dr. A. K. Roy, ICAR Research Complex, Assam Agricultural University, Diphu, Assam 782 460.