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DDT INGESTION AND LIVER GLUCOSE 6-PHOSPHATE DEHYDROGENASE ACTIVITY—II*

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Abstract—A significant correlation has been observed between the dietary level of DDT and liver glucose 6-phosphate dehydrogenase activity. A dietary level of 5 ppm DDT was sufficient to induce a significant reduction in enzyme activity. The activity of liver 6-phosphogluconate dehydrogenase was not influenced by the various levels of DDT used in these experiments. It is suggested that the ratio of the activities of these two enzymes could be used as an index of DDT stress.

A CONSIDERABLE amount of information is available describing the physiological and histopathological aspects of DDT toxicity in mammals. There is little information, however, on the fundamental biochemical changes involved. Recent studies[1] in this laboratory have demonstrated that the ingestion of DDT results in a decrease in the activity of liver glucose 6-phosphate dehydrogenase. Whether this effect results from the direct or indirect action of DDT is not apparent at this stage. However, if a relation exists, one might expect that the enzyme response would be dependent on the degree of the toxic stress. This hypothesis has been evaluated by observing enzyme activities in rats raised on rations containing varying levels of DDT.

EXPERIMENTAL PROCEDURES

Ration composition and experimental design

The composition of the ration used in these experiments has been described in a previous publication.[1] Appropriate amounts of DDT were dissolved in the oil prior to incorporation in the ration. All rations were fed *ad libitum*. Gas chromatographic analysis of the p, p′-DDT† used indicated only small amounts ($< 1\%$) of the other isomeric forms of DDT.

The animals used in these experiments were from our colony of highly inbred Wistar rats. Litter-mate groups of male or female rats, 5–6 weeks of age and weighing about 100 g and 80 g, respectively, were fed the experimental rations for 4 weeks prior to sacrifice and enzyme assay. The litter-mate group was treated as a unit throughout the experiment, with enzyme preparations and assays being conducted on the same day. For statistical analysis, a litter-mate group was considered as a replicate and the variation due to these replicates could be evaluated, thus improving the sensitivity of the experiment.

* Technical Paper 1888, Oregon Agricultural Experiment Station.

† Nutritional Biochemicals analytical standard.

In experiment I, three dietary levels of DDT were fed: 0, 50 and 100 ppm. Ten (5♂ and 5♀) litter-mate groups of three animals were used. On completion of this experiment, it was evident that additional information was required at both higher and lower dietary levels of DDT. Thus in experiment II, 4 levels of DDT were fed: 0, 5, 25, and 200 ppm and 14 (7♂ and 7♀) litter-mate groups of four animals were used.

Enzyme assays

The animals were sacrificed by light ether anesthesia and exsanguination and the livers were excised. Ten per cent homogenates were prepared in 0·25 M sucrose containing 0·001 M disodium ethylenediamine tetraacetate. The homogenates were centrifuged for 10 min at 9,000 *g* to remove cell debris and the mitochondrial fraction. The soluble fraction used for enzyme assay was obtained by centrifuging for 30 min at 30,000 *g* in experiment I and 100,000 *g* in experiment II. This difference in procedure resulted in lower nitrogen levels and higher enzyme specific activity in the latter case. Enzyme activities were determined spectrophotometrically by the reaction system of Kornberg and Horecker.[2] Micro-Kjeldahl assays were run on aliquots of the soluble fraction, and enzyme activities were expressed as micromoles of nitrogen.

Statistical analysis

The data from enzyme assays were compiled and analyzed, the results from one litter-mate group considered as a replicate. Analysis of variance was run for all variables studied. In addition, regression analysis was run on some of the data obtained in experiment II. The significance of the relation between enzyme activity and log (DDT level + 1) was evaluated. This latter modification was needed so that data from control animals (0 ppm DDT) could be utilized.

RESULTS AND DISCUSSION

The levels of DDT used in these rations resulted in no differences in growth rate nor were any gross symptoms of DDT toxicity observed. DDT ingestion, however, does result in an increase in liver size (Table 1).

TABLE 1. DDT INGESTION AND LIVER SIZE*

		Liver size (g/100 g body weight*)			
		DDT level (ppm)			
Experiment I		0	50		100
	♂	4·64 (4·16–5·60)	5·44 (5·24–5·70)		5·89 (5·29–6·88)
	♀	4·79 (4·24–5·22)	5·36 (4·90–5·81)		5·70 (5·11–6·66)
		DDT level (ppm)			
Experiment II		0	5	25	200
	♂	4·49 (3·79–5·62)	4·66 (4·23–5·22)	4·98 (4·51–5·95)	5·60 (5·10–6·27)
	♀	4·74 (3·78–6·17)	4·92 (4·40–6·29)	5·42 (4·79–6·01)	5·58 (5·00–6·07)

* Mean and range.

The results from both experiments (Tables 2 and 3) indicate a significant relationship between the dietary level of DDT and glucose 6-phosphate dehydrogenase activity. Analyses of the data from experiment II indicate a highly significant linear regression of enzyme activity against log (DDT level + 1). It should be noted that a level of 5 ppm DDT was sufficient to produce a significant decrease in enzyme activity. A similar response was observed in both male and female animals, with females showing a higher level of enzyme activity.

TABLE 2. EFFECT OF DIETARY LEVEL OF DDT ON GLUCOSE 6-PHOSPHATE DEHYDROGENASE ACTIVITY

Experiment I	(μmoles NADP reduced/min/mg N)	
	♂	♀
DDT level, ppm		
0	0·188	0·231
50	0·133	0·154
100	0·119	0·130
Analysis of variance		
Treatment F value	9·81*	9·84*
Degrees of freedom	2 and 8	2 and 7
l.s.d.† P = 0·05	0·00345	0·00501

* Significant at the 1 per cent level.
† Least significant difference.

TABLE 3. EFFECT OF DIETARY LEVEL OF DDT ON GLUCOSE 6-PHOSPHATE AND 6-PHOSPHOGLUCONATE DEHYDROGENASES

Experiment II DDT Level (ppm)	Glucose 6-phosphate dehydrogenase		6-Phosphogluconate dehydrogenase		Glucose 6-phosphate/6-phosphogluconate dehydrogenase	
	(μmoles NADP reduced/min /mgN)				(ratio)	
	♂	♀	♂	♀	♂	♀
0	0·239	0·327	0·385	0·423	0·618	0·764
5	0·198	0·240	0·356	0·401	0·573	0·598
25	0·170	0·235	0·374	0·419	0·465	0·621
200	0·152	0·210	0·362	0·391	0·422	0·531
Analysis of variance Treatment F value	7·60*	16·70*	1·95†	1·72†	6·36*	6·65*
Degrees of freedom	3, 18	3, 16	3, 18	3, 18	3, 18	3, 17
l.s.d. P = 0·05	0·056	0·038	0·028	0·034	0·108	0·113
Regression analysis F value for linear regression	21·78†	40·86‡			17·99*	16·26*
Deviation from linearity	0·50†	4·62§			0·54†	1·85†

* Significant at the 0·5 per cent level.
† Not significant.
‡ Significant at the 0·1 per cent level.
§ Significant at the 5·0 per cent level.

In experiment II, studies were made of the effect of DDT ingestion on the activity of another NADP-dependent enzyme of the soluble fraction, 6-phosphogluconate dehydrogenase. The activity of this enzyme was not influenced by the level of DDT in the ration (Table 3). If the activity of glucose 6-phosphate dehydrogenase were used as a measure of DDT stress, it is possible that the activity of 6-phosphogluconate dehydrogenase could be used as an internal standard, and the ratio of the activities of these two enzymes would be the quantity of significance. Statistical analysis indicated a significant effect of DDT-intake level on this ratio and a significant linear regression of the enzyme activity ratio on log (DDT level + 1).

Analyses of these data have established a significant relation between a DDT stress and the specific activity of liver glucose 6-phosphate dehydrogenase. Since this stress also increases liver size, it is possible that the decrease in activity of glucose 6-phosphate dehydrogenase may be due to a dilution effect. If this were so, the total liver glucose 6-phosphate dehydrogenase activity per unit of body weight should not be influenced materially by the level of DDT intake. It is evident that this is not the case, since DDT ingestion produces a significant depression in the total level of this enzyme in liver (Table 4). By contrast, the higher dietary levels of DDT tend to increase the total level of 6-phosphogluconate dehydrogenase in the liver.

TABLE 4. MEAN LIVER ENZYME ACTIVITY PER UNIT BODY WEIGHT

	(μmoles NADP reduced/min/weight of liver equivalent to 100 g body weight)			
	DDT level (ppm)			
Experiment I	0	50	100	
Glucose 6-phosphate dehydrogenase				
♂	13·0	10·0	11·4	
♀	16·0	12·9	13·4	
	DDT level (ppm)			
Experiment II	0	5	25	200
Glucose 6-phosphate dehydrogenase				
♂	14·3	10·9	9·8	10·2
♀	17·7	12·8	12·3	13·5
6-Phosphogluconate dehydrogenase				
♂	20·9	19·0	21·9	24·3
♀	22·2	21·4	25·6	25·4

Various organic chemicals, *in vivo*, are able to produce an increase in the level of certain microsomal enzymes.[3, 4] Some interaction in protein metabolism is suggested, and if this response is due to an increased rate of synthesis of specific enzymes, one might anticipate a parallel decrease in the rate of synthesis of other enzymes. It has been suggested that the primary biochemical response to carbon tetrachloride intoxication is a decrease in the rate of protein synthesis.[5] Incidental studies in this laboratory have suggested that DDT likewise influences microsomal function and, in a recent report,[6] we have described an effect of DDT ingestion on the composition of the soluble fraction of liver tissue. In the light of this evidence, it would seem more

probable that the response of glucose 6-phosphate dehydrogenase to a DDT stress is associated with a change in protein metabolism rather than being the result of a direct interaction of DDT or its metabolites with the enzyme. One might thus explain the lower activity of liver glucose 6-phosphate dehydrogenase by either a decrease in the rate of synthesis of this enzyme or by the synthesis of a modified enzyme which is less effective. Further studies are necessary to evaluate this hypothesis.

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