Effect of dietary protein, dichlorodiphenyltrichloroethane (DDT) and hexachlorocyclohexane (HCH) on hepatic microsomal enzyme activity in rats

BY GIRIJA BALAKRISHNAN, M. RAMACHANDRAN*, B. D. BANERJEE AND Q. Z. HUSSAIN

Biochemistry Division, National Institute of Communicable Diseases, 22 Sham Nath Marg, Delhi-110 054, India

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1. Albino rats were fed on diets containing 30, 120 or 200 g protein/kg with or without the incorporation of dichlorodiphenyltrichloroethane (DDT) or hexachlorocyclohexane (HCH) at 100 mg/kg diet for 4 weeks.

2. The activities of the liver microsomal enzymes NADPH-cytochrome reductase (*EC* 1.6.2.4), flavoprotein-linked monooxygenase (*EC* 1.14.14.1) and *O*-demethylase were significantly greater in animals fed on 120 and 200 g protein/kg diet compared with those fed on 30 g protein/kg diet.

3. The inclusion of DDT or HCH at all protein intakes led to further significant rises in microsomal enzyme activities but the increases were much greater for animals receiving the 120 and 200 g protein/kg diets than for those receiving the 30 g protein/kg diet.

4. The results imply that detoxification of DDT or HCH was carried out more effectively at the higher protein intakes.

The widespread use of dichlorodiphenyltrichloroethane (DDT) and hexachlorocyclohexane (HCH) over a number of years, coupled with their extreme stability and slow metabolism, have led to environmental contamination and the ultimate carry-over from the food chain into the mammalian system (Ramachandran *et al.* 1973; Kalra & Chawla, 1981). A serious outbreak of poisoning due to consumption of HCH-admixed foodstuffs occurred recently in Uttar Pradesh, India (Ramachandran *et al.* 1982).

It is now known that the metabolism of these xenobiotics is mediated by hepatic microsomal enzymes. It was also observed that the level of protein in the diet influenced the activities of these enzymes and thus the toxicity of DDT and HCH (McLean & McLean, 1966). Since the level of dietary protein varies in the Indian population depending on socio-economic status, the capacity of different levels of protein to stimulate microsomal enzymes was studied in albino rats given diets containing one level of pesticide.

MATERIALS AND METHODS

Technical HCH was obtained from Hindustan Insecticides Ltd, India and was recrystallized to yield a product containing (g/kg): α-isomer 107, β-isomer 110, γ-isomer 783. DDT was obtained from Aldrich Chemical Co., Gillingham, Dorset, UK. NADPH, NADP, glucose-6-phosphate, glucose-6-phosphate dehydrogenase (*EC* 1.1.1.49) and cytochrome *c* were the products of Sigma Chemical Co., St. Louis, Missouri, USA. Vitamin-free casein was obtained from ICN Pharmaceuticals, Ohio, USA. Other chemicals were of analytical reagent grade.

Male albino rats (Hissar strain) of average weight 100 g were used for the experiment. They were divided into three sets of three groups each. The first, second and third sets received diets containing 30, 120 and 200 g protein/kg respectively. Casein was used as the

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Table 1. *Changes in body-weight and liver weight in rats fed on diets containing different levels of protein with or without dichlorodiphenyltrichloroethane (DDT; 100 mg/kg) or hexachlorocyclohexane (HCH; 100 mg/kg)*

(Mean values with their standard errors for ten animals)

Diet		Initial body-wt (g)		Final body-wt (after 4 weeks)		Liver:body-wt	
Protein (g/kg)	Pesticide (mg/kg)	Mean	SEM	Mean	SEM	Mean	SEM
30	0	110·0	4·47	108·3	1·68	0·039	0·002
	100 HCH	110·8	0·41	106·7	2·37	0·078***	0·007
	100 DDT	111·4	2·64	110·6	3·94	0·062***†††	0·002
120	0	100·9	2·12	124.4‡‡‡	5·44	0·039	0·002
	100 HCH	100·0	3·54	142·2‡‡‡	5·10	0·106***†††	0·004
	100 DDT	102·4	1·53	128·7‡‡‡	3·36	0·081***	0·002
200	0	105·3	1·25	137·7‡‡‡	2·09	0·040	0·002
	100 HCH	107·9	1·04	141·3‡‡‡	2·18	0·074***†††	0·009
	100 DDT	105·0	2·42	133·5‡‡‡	2·16	0·054***	0·003

Mean values were significantly different from their respective control values: ***$P < 0·001$.

Mean values were significantly different compared with DDT-fed animals given the same level of dietary protein: †††$P < 0·001$.

Mean values were significantly different compared with initial body-weight in the respective groups receiving 30 g protein/kg diet: ‡‡‡$P < 0·001$.

protein source. The composition of the diet used was (g/kg): vitamins 1; minerals 11; protein 30, 120 or 200; groundnut oil 40; the diet was made up to 1 kg with maize starch. In each set, one group served as the control, the second received DDT at 100 mg/kg and the third group HCH at 100 mg/kg; the insecticide was dissolved in groundnut oil and incorporated in the diet. The animals were fed for 4 weeks and were weighed every week. At the end of the experiment they were killed by cervical dislocation. Livers were perfused with ice-cold saline (9 g sodium chloride/l), rapidly excised, weighed and liver microsomes prepared by the calcium aggregation method of Schenkman & Cinti (1972). The microsomal pellet was homogenized gently and suspended in 0·2 M-Tris hydrochloric acid buffer, pH 7·5. This suspension was used for enzyme studies. NADPH-cytochrome reductase (*EC* 1.6.2.4) was measured spectrophotometrically by the method of Gigon *et al.* (1969). Flavoprotein-linked monooxygenase (*EC* 1.14.14.1) and *O*-demethylase were assayed by determining *p*-aminophenol and *p*-nitrophenol formed respectively (Kinoshita *et al.* 1966). Protein concentration was determined by the method of Lowry *et al.* (1951).

All the analyses were carried out in duplicate. The average was used in the calculation of means and standard errors. Significance of the differences was assessed by Student's *t* test. A *P* value of 0·05 or less was considered to be significantly different.

RESULTS AND DISCUSSION

Results of the experiment are summarized in Tables 1 and 2. The animals maintained on a 30 g protein/kg diet showed a slight decrease in weight. There was a significant increase in weight in animals fed on the 120 and 200 g protein/kg diet and the HCH-fed groups showed a much higher increase compared with the DDT-fed groups. A significant increase in liver weight ($P < 0·001$) was noted in all the three groups which were given DDT and

Table 2. *Changes in the activities of hepatic microsomal enzymes in rats fed on diets containing different levels of protein with or without dichlorodiphenyltrichloroethane (DDT; 100 mg/kg) or hexachlorocyclohexane (HCH; 100 mg/kg)*

(Mean values with their standard errors for ten animals)

Diet		NADPH cytochrome reductase (*EC* 1.6.2.4) (nmol cytochrome *c* reduced/g liver per min)		Flavoprotein-linked monooxygenase (*EC* 1.14.14.1) (μg *p*-aminophenol formed/min per mg protein)		*O*-Demethylase (μg *p*-nitrophenol formed/50 mg liver per 60 min)	
Protein (g/kg)	Pesticide (mg/kg)	Mean	SEM	Mean	SEM	Mean	SEM
30	0	0·074	0·003	0·186	0·009	3·99	0·025
	100 HCH	0·091**	0·007	0·263**	0·027	4·23**	0·018
	100 DDT	0·088**	0·012	0·247**	0·048	4·18**	0·050
120	0	0·192†††	0·025	0.397†††	0·007	5·34†††	0·019
	100 HCH	2·069***	0·050	0·749***	0·012	6·58***	0·063
	100 DDT	1·690***	0·023	0·586***	0·083	6·43***	0·025
200	0	0·232	0·010	0·428	0·026	5·45	0·036
	100 HCH	2·440***	0·017	0·744***	0·020	6·95***	0·019
	100 DDT	2·073***	0·076	0·615***	0·010	6·56***	0·025

Mean values were significantly different from their respective controls: $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}001$.
Mean values were significantly different from 30 g protein/kg diet group: †††$P < 0{\cdot}001$.

HCH as compared with the controls (Table 1). A significant increase was seen in liver weights in the HCH-fed animals when compared with the controls (Table 1). A significant increase was seen in liver weights in the HCH-fed animals when compared with DDT-fed animals in all the three groups ($P < 0{\cdot}001$).

A previous study reported that albino rats fed on a diet containing HCH (50 mg/kg) for 12 weeks had significantly higher liver *O*-demethylase activity compared with control rats (Bikram Chand & Ramachandran, 1980). In the present study the activities of the microsomal enzymes, NADPH-cytochrome reductase, flavoprotein-linked monooxygenase and *O*-demethylase were significantly increased in all groups treated with DDT or HCH (Table 2). Animals given the 200 g protein/kg diet showed a slightly higher *O*-demethylase activity than those given 120 g protein/kg diet, but this was not significant.

Animals fed on a 30 g protein/kg diet had significantly lower microsomal enzyme activities when compared with the groups fed on diets containing 120 and 200 g protein/kg. A 30 g protein/kg diet supplemented with DDT and HCH did not induce microsomal enzyme activity ($P < 0{\cdot}01$) to as significant a level as in the 120 and 200 g protein/kg fed animals ($P < 0{\cdot}001$). This is in agreement with the work of McLean & McLean (1966) who have shown that protein deprivation (critical protein concentration is between 30 and 60 g/kg) leads to an 80% fall in activity of microsomal enzymes. In their study the addition of a single dose of DDT increased microsomal enzyme activities by a much smaller value in animals fed on a protein-deficient diet when compared with animals fed on a stock diet. Boyd & Chen (1968) have also reported that a low-protein diet increased lindane toxicity, decreasing LD_{50} values in experimental animals. The results show that the hepatic microsomal enzyme activities are highly dependent on the dietary protein level, being very low at 30 g protein/kg diet and significantly higher at the 120 and 200 g/kg levels. Addition of DDT or HCH led to a further significant elevation of the microsomal enzymes but this

effect was minimal in the low dietary protein group. Hence the level of dietary protein appears to be important in the detoxification of DDT and HCH.

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REFERENCES

Bikram Chand & Ramachandran, M. (1980). *Indian Journal of Experimental Biology* **18**, 735–736.
Boyd, E. M. & Chen, C. P. (1968). *Archives of Environmental Health* **17**, 156–163.
Gigon, P. L., Gran, T. & Grillete, A. R. (1969). *Molecular Pharmacology* **5**, 109–122.
Kalra, R. L. & Chawla, R. P. (1981). *Journal of Bombay Natural History Society* **78**, 1–15.
Kinoshita, F. K., Frawley, J. P. & Dubois, K. P. (1966). *Toxicology and Applied Pharmacology* **9**, 505–513.
Lowry, O. H., Rosebrough, N. T., Fars, A. L. & Randall, R. J. (1951). *Journal of Biological Chemistry* **193**, 265–275.
McLean, A. E. M. & McLean, E. K. (1966). *Biochemical Journal* **100**, 564–571.
Ramachandran, M., Bikram Chand, Aravindakshan, A., Pyare Lal & Hussain, Q. Z. (1982). *Nutrition Reports International* **26**, 377–380.
Ramachandran, M., Sharma, M. I. D., Sharma, S. C., Mathur, P. S., Aravindakshan, A. & Edward, G. J. (1973). *Bulletin of the World Health Organization* **49**, 637–638.
Schenkman, B. J. & Cinti, D. L. (1972). *Life Sciences* **11**, 247–257.

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