Stimulation of Liver Detoxication Enzymes by Dietary Cadmium Acetate

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Cadmium compounds and other heavy metals usually inhibit enzymic and metabolic processes (WEBB 1966). The inhibition of detoxication activity of liver microsomal enzymes would be particularly detrimental to an organism, because the toxicity of many other substances would be increased. In fact, it has recently been reported (HADLEY and MIYA 1972) that hexobarbital sleep time was prolonged after injection of rats with cadmium acetate. In contrast, the present report contains observations on stimulation of liver microsomal enzyme activity in rats fed cadmium acetate (CdAc) and the interaction of effects of CdAc with those of phenobarbital sodium (PB).

Methods

Experimental animals were female Holtzman rats weighing 100-110 g. There were 5 individually caged rats per group. They were fed experimental diets for 15 days. Basal diet was Vitamin A test diet (USP XII) fortified with 0.69 ppm Vitamin A acetate. The uncontrolled exposure of experimental animals to cadmium was minimized. No galvanized or plated equipment was used. Cages and sipper tubes were stainless steel. Feed and water bottles were glass. Liver samples from control rats which were fed no CdAc contained less than 0.2 ppm cadmium, the approximate detection limit of the atomic absorption spectrophotometric method employed.

Liver microsomal enzyme activity for all animals in each experiment was assayed *in vivo* by 1 method and *in vitro* by 2 other methods. After 10 days *ad libitum* feeding, each rat was injected with 90 mg hexobarbital sodium per kg body weight. The period of loss of the righting reflex was recorded as hexobarbital sleep time. A decrease in sleep time was accepted as presumptive evidence of liver microsomal enzyme stimulation (Axelrod 1965). The *in vitro* procedures were performed after 15 days feeding. Each rat was killed with ether, the liver excised, and a 1 g portion ground in a glass-teflon homogenizer with 4 ml of cold 1.15% KCl. This liver homogenate was centrifuged at 5°C for 15 minutes at 9000 x g. Enzyme activity was determined in the supernatant fluid by methods of Kinoshita *et al.* (1966) for oxidative cleavage of O-ethyl-p-nitrophenylphenylphosphonothioate (EPN detoxication) and oxidative O-demethylation of p-nitroanisole (O-demethylase). Product of both assays was p-nitrophenol (PNP).

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In the first of the two studies in this paper the effects of CdAc alone were researched. Cadmium acetate was mixed into diets at the rates of 0, 100, 500, 1000, 5000, or 10,000 ppm. In the second experiment the interactions of CdAc and PB were studied in a complete randomized design with factorially arranged treatments. Six groups of rats were fed diets containing a) no CdAc and no PB, b) 500 ppm CdAc and no PB, c) 1000 ppm CdAc and no PB, d) no CdAc and 500 ppm PB, e) 500 ppm CdAc and 500 ppm PB and f) 1000 ppm CdAc and 500 ppm PB.

Statistical analyses of results from the first experiment were by Student's t test. The results of the second experiment were analyzed by analysis of variance with partitioning of the among group variance into the main effects of CdAc or PB and the interaction of CdAc and PB.

Results

Effects of Cadmium Acetate Alone

The stimulatory effects of dietary CdAc on hepatic microsomal enzymes are summarized in figure 1. Magnitude of stimulation

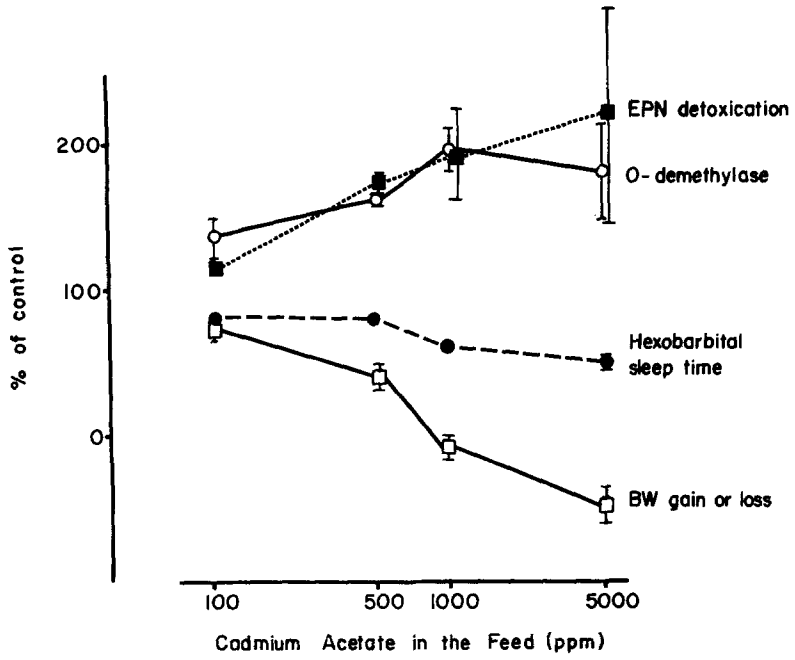

Figure 1

Effects of dietary cadmium acetate (CdAc) on selected detoxication and intoxication responses in rats fed fifteen days on the experimental diet. Standard error of mean shown by vertical bars only for values significantly (P<.05) different from control values.

increased with dosage of CdAc. Liver weight was unaffected in rats fed diets of 100 or 500 ppm CdAc but in rats fed diets of 1000 or 5000 ppm relative liver weights were significantly ($P<.05$) depressed to 86 and 88% of control respectively.

Toxicity of CdAc increased with dietary dosage. Out of 5 rats in each group there was 1 death among rats fed the diet of 1000 ppm CdAc, 3 deaths among those fed 5000 ppm and 5 deaths at 10,000 ppm. Depression of body growth is illustrated in figure 1. Feed consumption in rats fed 500, 1000, or 5000 ppm CdAc was significantly ($P<.05$) depressed to 75, 55 and 65% of control respectively.

Interactions of Cadmium Acetate and Phenobarbital Sodium

The results from the study of interactions of CdAc and PB are summarized in table 1. The stimulatory effects of 500 and 1000 ppm CdAc on the microsomal enzyme responses and the depressing effect of these levels of CdAc on feed consumption and body growth were similar to the results in the first study. Phenobarbital increased liver weight and increased activity in each of the 3 measures of microsomal enzyme activity. The stimulatory effects on the *in vitro* enzyme assays of feeding the combinations of 500 or 1000 ppm CdAc and 500 ppm PB were synergistic, i.e., the effects of the combinations of CdAc and PB were greater than the summation of their effects when fed singly. Hexobarbital sleep time in rats fed phenobarbital was of such short duration that analysis of variance was not computed for this parameter. There were no deaths in this experiment.

Discussion

The concentrations of cadmium employed in these studies were much above present levels in the general environment. However, there are local concentrations of the metal or its compounds (Cannon 1969). Some vegetation concentrates cadmium from the soil and adds further to the risk of high exposure (Bowen 1969). If the microsomal enzyme stimulatory effects of cadmium compounds are synergistic with other compounds in the environment, it may be that the threshold for stimulation of microsomal activity by cadmium is much lower than the levels used in the present studies.

The observations reported here are not sufficient to support strong speculation on mechanism of the microsomal enzyme stimulation by CdAc. The site of action may or may not be in the hepatocyte. It can be suspected that the stress of cadmium intoxication, as observable by decreased body growth, may cause an endocrine response the result of which is altered microsomal activity. However, stress response would not account for the synergism of CdAc and PB. There was no evidence that PB added to diets containing CdAc caused any increase in stress.

The reason is unknown for the difference between the observation reported here of decreased hexobarbital sleep time following feeding

TABLE I

Effects of Dietary Cadmium Acetate (CdAc), Phenobarbital Sodium (PB) and the Combination of the two on Body Weight Gain, Feed Consumption, Liver Weight, and Liver Microsomal Enzyme Activities

Dietary Additive and Conc. (ppm)		Body Weight Gain	Feed Consumption	Liver Weight	Liver Microsomal Enzyme Activities		
					Hexobarbital Sleep time	EPN Detoxication	O-demethylase
CdAc	PB	(% of initial BW)	(% of initial BW)	(% of BW)	(min)	(μmoles PNP/g liver/hr)	(μmoles PNP/g liver/hr)
0	0	67 ± 15[a]	187 ± 15	4.9 ± 0.3	81 ± 5	.15 ± .01	.60 ± .10
500	0	33 ± 5	141 ± 15	5.4 ± 0.1	46 ± 7[b]	.26 ± .01	1.27 ± .06
1000	0	9 ± 5	119 ± 10	5.0 ± 0.1	53 ± 3[b]	.27 ± .02	1.27 ± .11
0	0	66 ± 5	196 ± 9	6.5 ± 0.3	<1[c]	1.47 ± .12	2.37 ± .20
500	500	33 ± 4	150 ± 7	6.2 ± 0.3	<1[c]	2.06 ± .05	3.51 ± .16
1000	500	1 ± 6	127 ± 11	5.9 ± 0.5	2 ± 0.7[b]	2.33 ± .09	3.84 ± .17

Analyses of variance

SV	*df*	Mean Squares					
CdAc	2	9347**	12205**	.02	-	.641**	3.33**
PB	1	69	579	12.62**	-	22.390**	36.05**
PBxCdAc	2	43	1	.49	-	.355**	.41*
Within groups	24	166	667	.28	-	.022	.10

a mean + SEM

b statistically (P<.05) different from values for rats fed no CdAc or PB by Student's t test

c exact magnitude of variance unknown but mean obviously different from values for rats fed no CdAc or PB

* significant at P<.05

** significant at P<.01

of cadmium acetate and the report by other researchers (HADLEY and MIYA 1972) that injection of this compound produces prolongation of hexobarbital sleep time. Perhaps in feeding experiments the rate of absorption is slow enough to permit processes supporting enzyme stimulation to occur but after injection some of these processes are overwhelmed.

Questions also have been raised regarding mechanism of cadmium detoxication and ability of animals to adapt to toxic dosages of this metal (GABBIANAI *et al.* 1967, TERHAAR *et al.* 1965). It can be speculated that there may be a relationship of liver microsomal activity and detoxication of and adaptation to cadmium. Despite uncertainties about mechanisms, it is concluded that in rats fed a diet containing a cadmium compound the liver microsomal enzyme system continues to function and to adapt to changes in exposure levels of foreign compounds.

Acknowledgements

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