Dieldrin Accumulation and Excretion by Rats Fed Phenobarbital and Carbon

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One of the more undesirable characteristics of the insecticide dieldrin is its tendency to accumulate in body tissues of animals. Earlier work has shown that when intake remains constant the pesticide accumulates in body tissues until it reaches a plateau, at which time further accumulation ceases and excretion of the pesticide and its metabolites approximates intake (1, 2). Metabolism of dieldrin and, hence, rate of excretion is presumably controlled by the microsomal drug-metabolizing enzymes in the liver (3-8). Activity of these enzymes can be induced by the administration of various substances including the barbiturate drugs (9-12). Activated carbon has also been cited as a method of pesticide removal because of its adsorptive properties (13-17).

The purpose of this study was to combine these two methods of detoxication to determine their effectiveness in reducing dieldrin residues.

Experimental

Female Sprague-Dawley rats were used throughout the experiment. The rats were fed 12 g of a basic diet of ground Purina Laboratory Chow. They were housed in stainless steel metabolism cages (Acme Metal Products, Inc., Chicago, Ill.) to facilitate separate collection of urine and feces.

The 12 rats of experiment I were randomly divided into six groups for a 2 X 3 factorial experiment. The factors were 0 and 1 g carbon and 0, 40, and 80 mg phenobarbital per kg body weight. The basic diet into which these additives were mixed contained 2 ppm HEOD,[2] including some dieldrin-^{14}C as a tracer. Collections of feces and urine were made three times the first week and thereafter weekly. At the end of

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[1] Conducted by the senior author as partial fulfillment of the requirements for the M. S. degree, Department of Zoology, North Dakota State University, Fargo, N. Dak.

[2] Supplied as technical dieldrin, but enough was used to supply 2 ppm of the active ingredient 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-exo-5,8-dimethano-naphthalene (HEOD).

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6 weeks, the rats were sacrificed. The livers were removed and assayed for cytochrome P-450 activity (18). Each rat carcass was ground and freeze-dried. To determine normal cytochrome P-450 levels, the livers from two nonexperimental rats were assayed for cytochrome P-450 at the time that the livers from the experimental rats were assayed. These rats were from the same colony as the experimental rats and were maintained on the same diet minus the dieldrin.

The 12 rats of experiment II were fed a diet containing 2 ppm HEOD,[2] including some tracer dieldrin-^{14}C, for 6 weeks to build dieldrin residues in their tissues. After 6 weeks, the dieldrin in the diet was discontinued. The rats were then divided randomly into six groups for a 2 X 3 factorial experiment with factors of 0 and 1 g carbon and 0, 40, and 80 mg phenobarbital per kg body weight. Collections of urine and feces were made daily the first week and three times the second week. At the end of the second week, the rats were sacrificed. The carcasses were ground and freeze-dried.

Carbon-14 in the urine was detected by liquid scintillation in a dioxane cocktail. Freeze-dried feces samples were combusted in a Schöniger flask and the resulting $^{14}CO_2$ trapped with an ethanolamine, ethylene glycol monomethyl ether solution (19). Carcass carbon-14 residues were determined by the Parr bomb oxygen combustion method (20).

While it is known that rats may metabolize dieldrin and excrete these metabolites in their urine and feces (1), no attempt was made to separate metabolites from dieldrin.

The data were analyzed statistically by analysis of variance. Phenobarbital and carbon levels were treated as discrete effects, and time was treated as a continuous variable. Statistical significance was assessed at probabilities of 5% or less.

Results and Discussion

Urinary excretion patterns of the rats in experiment I are shown in Figure 1. Rats on diets with phenobarbital alone excreted more carbon-14 than the other rats; it appears that they may have reached an excretion plateau at the end of the second week. The rats receiving diets with carbon excreted only a small percentage of the carbon-14 in their urine. Rats fed diets with both phenobarbital and carbon, however, did exhibit a slightly higher carbon-14 excretion rate than those fed carbon alone (suggesting that some of the dieldrin did reach the site of metabolism).

Fecal excretion patterns for rats of experiment I are illustrated in Figure 2. Rats receiving phenobarbital or carbon, or both, in their diets exhibited higher excretion rates of carbon-14 than the control rats. Excretion rates of rats receiving carbon and/or phenobarbital approached a maximum level by the end of the first week, whereas control rats show a much slower increase in excretion rates. The fecal excretion rates were similar whether the rats were fed carbon or phenobarbital. A significant ($P<.01$) phenobarbital X carbon interaction suggests that the effects of the two were not additive.

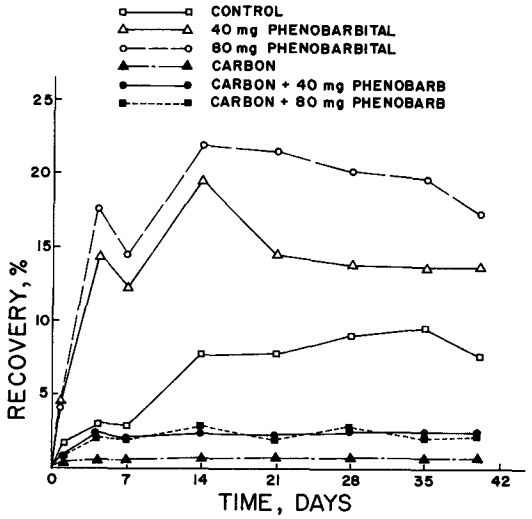

Figure 1. Urinary excretion of ^{14}C (% of daily dose) by rats fed dieldrin-^{14}C and combinations of phenobarbital and carbon in experiment I. Phenobarbital X carbon X time interaction significant, P<.01.

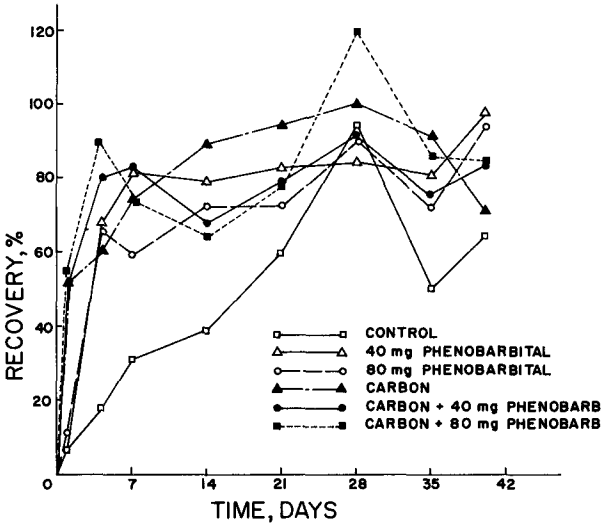

Figure 2. Fecal excretion of ^{14}C (% of daily dose) by rats fed dieldrin-^{14}C and combinations of phenobarbital and carbon in experiment I. Phenobarbital X carbon interaction significant, P<.01; Phenobarbital X time and carbon X time interactions significant, P<.01 and P<.05, respectively.

TABLE I

Body weights, recovery of carbon-14, and cytochrome P-450 in liver microsomes of rats fed carbon and phenobarbital simultaneously with dieldrin-^{14}C

Treatment	Body Weight, g		Recovery, %				Liver Cytochrome P-450 OD units/mg protein
	Initial	Final	Urine	Feces	Carcass	Total	
Control	237	223	7.4[a]	53.4[a]	38.4[b]	99.2	9.0[b]
Phenobarbital, 40 mg/kg	231	230	14.6	79.2	4.5	98.3	21.4
Phenobarbital, 80 mg/kg	231	224	19.2	73.6	4.4	97.2	28.8
Carbon	230	217	0.7	84.6	7.8	93.1	4.9
Carbon + Phenobarb, 40 mg	234	212	2.4	77.2	2.2	81.8	28.6
Carbon + Phenobarb, 80 mg	225	214	2.3	83.9	2.0	88.2	12.3
Nonexperimental							4.6

[a] Interaction of phenobarbital level X carbon significant, $P<.01$
[b] Interaction of phenobarbital level X carbon significant, $P<.05$

TABLE II

Body weights and recovery of carbon-14 from rats fed carbon and phenobarbital after accumulation of dieldrin-^{14}C had occurred

Treatment	Body weight, g		Recovery, %				
	Initial	Final	1st 6 weeks[a]	Decontamination Period[b] Urine	Decontamination Period[b] Feces	Carcass	Total
Control	148	199	64.5	1.1[c]	10.2[c]	16.2	92.0
Phenobarbital, 40 mg/kg	146	198	64.8	4.6	23.3	1.2	93.9
Phenobarbital, 80 mg/kg	134	178	66.1	2.9	13.8	0.7	83.5
Carbon	157	188	64.4	0.4	9.7	14.2	88.7
Carbon + Phenobarb, 40 mg	139	186	69.6	2.7	15.4	0.9	88.6
Carbon + Phenobarb, 80 mg	156	188	66.6	3.2	17.6	1.2	88.6
Rats killed at end of 6 wk	152	197	66.0	---	---	28.8	94.8

[a] Recovery of carbon-14 in feces and urine while dieldrin-^{14}C was being fed
[b] Recovery of carbon-14 during the 2 wk period when carbon and phenobarbital were fed. Dieldrin-^{14}C was not fed during this time
[c] Interaction of phenobarbital level X carbon significant, $P<.01$

Carcass residues for experiment I are listed in Table I. Carbon and phenobarbital together were the most effective in reducing carcass residues; carbon alone was the least effective additive.

Cytochrome P-450 data are also summarized in Table I. Cytochrome P-450 levels of rats fed dieldrin alone (control) were twice those of the nonexperimental rats, while cytochrome P-450 levels of rats fed only carbon and dieldrin were identical to those of the nonexperimental rats. These results indicate that dieldrin in the experimental diet did induce cytochrome P-450. Phenobarbital greatly increased cytochrome P-450 levels.

Recovery of carbon-14 in urine and feces of the rats from experiment II are illustrated in Figures 3 and 4. After an initial 6-week contamination period, these rats were fed "dieldrin-free" feed with carbon and phenobarbital beginning the 43rd day, day "0." Following a one-day lag, rats fed phenobarbital exhibited a marked increase in urinary and fecal carbon-14 excretion. This increase in excretion peaked between the 2nd and 4th days and then declined to control values. Carbon had little effect on carbon-14 excretion in either urine or feces.

Carcass residues for experiment II are listed in Table II. Rats receiving phenobarbital in their feed retained the least carbon-14 in their tissues. There was no difference in the residues in the carcasses of control rats and those receiving only carbon in their feed. Some decontamination did occur in these rats during the 2-week period, however, as can be seen by comparing their residue levels with those of rats killed immediately following the 6-week contamination period.

The effectiveness of carbon as an antidote for dieldrin poisoning probably lies in its ability to adsorb the pesticide present in the gastrointestinal tract. The adsorbed pesticide is then excreted in the feces. Three observations here support this conclusion. When carbon was fed with the dieldrin, the amount of cytochrome P-450 and the amount of carbon-14 excreted in the urine were reduced, while the amount of carbon-14 excreted in the feces was increased, suggesting that little dieldrin was absorbed for metabolism. Carbon was of little value in removing residues from the body when administered after contamination had occurred, probably removing only the pesticide present in the gastrointenstinal tract and not affecting the absorbed dieldrin. Thus, as stated in earlier work (16), carbon is an effective antidote for dieldrin poisoning when administered while the pesticide is still in the gastrointestinal tract.

In a recent publication (17), carbon was effective in reducing storage of DDT in rats when fed along with DDT, but carbon was ineffective in reducing residues when fed after storage had occurred. In this same publication, carbon did not affect excretion of dieldrin, DDT, DDD, or DDE in milk by contaminated dairy cows. Their observations substantiate the results reported here.

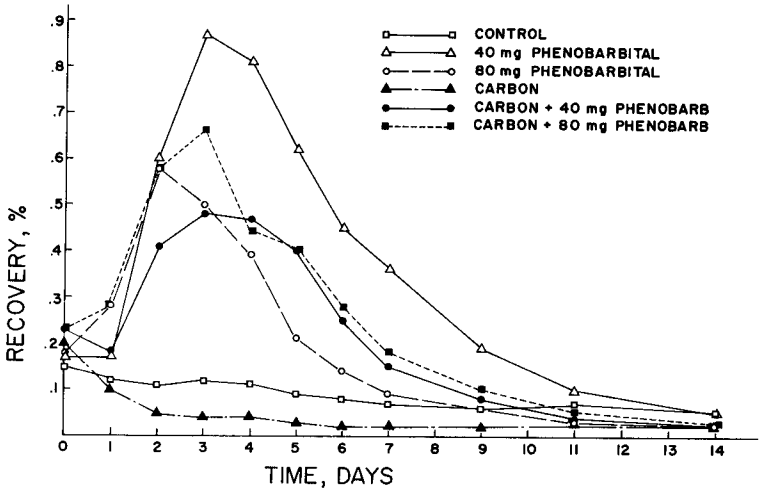

Figure 3. Urinary excretion of dieldrin-^{14}C residues (% of total dose) from rats fed combinations of phenobarbital and carbon in experiment II. Phenobarbital X carbon and phenobarbital X time interactions significant, P<.01.

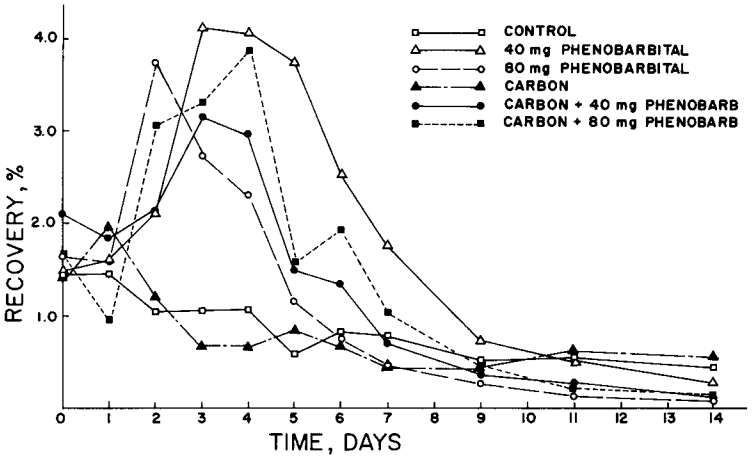

Figure 4. Fecal excretion of dieldrin-^{14}C residues (% of total dose) from rats fed combinations of phenobarbital and carbon in experiment II. Phenobarbital X carbon interaction significant, P<.01; Phenobarbital X carbon X time interaction significant, P<.05.

Phenobarbital has been used as a treatment for symptoms of severe dieldrin poisoning (21); its anesthetic properties effectively reduced tremors and tetany. Phenobarbital now appears to be an effective treatment not only because of its anesthetic effect but also because of its stimulation of drug-metabolizing enzymes in the liver, which speed the metabolism and excretion of dieldrin. In these studies, phenobarbital was effective in reducing tissue residues of dieldrin whether administered simultaneously with the pesticide or after storage in tissues had occurred. The one-day lag observed in the phenobarbital-treated rats in Figures 3 and 4 suggests that approximately 24 hours are needed for induction to occur.

Summary

Activated charcoal and phenobarbital have both been proposed as agents for reducing accumulations of dieldrin in the body. Rats maintained on diets containing 2 ppm dieldrin spiked with dieldrin-^{14}C were fed 0 and 1 g charcoal and 0, 40, and 80 mg phenobarbital per kg body weight in 2 x 3 factorial experiments. Measurements were made of the carbon-14 accumulated in the carcasses and excreted in the feces and urine. In one experiment, charcoal effectively reduced dieldrin storage in tissues when fed simultaneously with the pesticide; but, in another experiment, it was ineffective in reducing dieldrin residues when fed after storage of the pesticide had occurred and dieldrin feeding was stopped. Phenobarbital, a potent inducer of drug-metabolizing enzymes in the liver, dramatically reduced dieldrin storage whether fed simultaneously with dieldrin or following dieldrin accumulation.

Acknowledgements

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Reference to a company or product name does not imply approval or recommendation of the U. S. Department of Agriculture to the exclusion of others that may be suitable.

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