should reveal if actual changes in microsomal detoxifying enzymes are brought about in *T. infestans* by DDT, as they are in the rat.

Acknowledgment

We particularly acknowledge the contributions of Kathleen Morack, Robert Schonbrod, Ian J. Tinsley, and G. H. Arscott.

Nomenclature

NADP, NADPH = oxidized and reduced forms of nicotinamide–adenine dinucleotide phosphate

DDT = 1,1,1 - trichloro-2,2 - bis(*p*-chlorophehyl)ethane

DDD = 1,1 - dichloro-2,2 - bis(*p*-chlorophenyl)ethane

DDE = 1,1 - dichloro-2,2 - bis(*p*-chlorophenyl)ethylene

Kelthane = 4,4′ - dichloro - α - (trichloromethyl)benzhydrol

Aldrin = 1,2,3,4,10,10 - hexachloro-1,4,4a,5,8,8a - hexahydro - 1,4 : 5,8 - endo-exo - dimethanonaphthalene

Isodrin = endo-endo isomer of aldrin

Dieldrin = 1,2,3,4,10,10 -hexachloro-6,7 - epoxy - 1,4,4a,5,-6,7,8,8a - octahydro-1,4 : 5,8 - endo - exo - dimethanonaphthalene

Endrin = endo-endo isomer of dieldrin

Heptachlor = 1,4,5,6,7,8,8-heptachloro-3a,4,7,7a - tetrahydro-4,7-methanoindene

Heptachlor epoxide = 1,4,5,6,7,8,8-heptachloro-2,3 - epoxy - 2,3,3a,4,-7,7a - hexahydro - 4,7-methanoindene

G-6-P = Glucose 6-phosphate

G-6-P DH = Glucose 6-phosphate dehydrogenase

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INSECTICIDE INTERACTIONS

Insecticide Interactions Affecting Residue Storage in Animal Tissues

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SURVEYS during the past few years have provided mounting evidence that food and other sectors of the environment have been contaminated with traces of several organochlorine insecticides. Correspondingly, tissues of humans residing in many parts of the world also were found to contain mixtures of such insecticides in trace amounts.

Some years ago entomologists considered the probability of notable interaction (synergism or antagonism) occurring with combinations of organochlorine insecticides. Certain combinations had greater toxicity to insects than the more toxic component alone (*6*, *13*). Whether these events should really have been described as "synergism," however, is somewhat debatable as it is not clear whether the compounds interacted directly in some biochemical system. Interaction between organochlorine and organophosphate insecticides has been reported to occur in rodents. For example, Ball found the toxicity of parathion to the rat was reduced when it was administered after either aldrin, chlordan, or lindane treatment (*1*).

The authors recently became intrigued with the possibility that the metabolic fate of one organochlorine compound in an animal conceivably might be altered by simultaneous exposure to other such insecticides. A new principle was established when it was demonstrated that DDT and dieldrin interact markedly in the rat (*9*, *10*). The major response was a reduction of dieldrin storage in the fatty tissues when DDT was administered simultaneously.

This paper presents further observations of the effect of DDT on dieldrin

Interactions among certain organochlorine insecticides influenced their storage in rat adipose tissue. DDT administration markedly depressed storage of dieldrin and heptachlor and promoted a rapid depletion of pre-existing dieldrin stores. The DDT effect on cyclodiene storage is postulated to result from stimulation of detoxifying enzymes. Supporting evidence for the postulate includes the similar effect of several enzyme-inducing drugs in depressing dieldrin storage and the effect of DDT in stimulating ascorbic acid biosynthesis. However, attempts to suppress the DDT effect with inhibitors of protein synthesis resulted in conflicting data. DDT interaction with dieldrin metabolism also was demonstrated in swine and sheep, but could not be observed in chickens. Animal variation in sensitivity to such interaction may be important in determining the ecological consequences of some pesticide usage.

metabolism and storage in rats and other vertebrates, and a consideration of the mechanism involved. An identical effect of DDT on heptachlor metabolism also is presented.

Procedure

Animals. Experiments using female rats (Holtzman, Sprague-Dawley strain) were carried out following general procedures outlined elsewhere (*9*, *10*). All were caged and treated individually. Individual glass metabolism cages were employed for quantitative collection of feces and urine. Urine was frozen in the apparatus immediately upon being voided and was kept frozen until analysis. Fecal material was oven dried at 105° C. and stored dry for later analysis.

Chickens were caged individually and fed a standard layer diet. Solutions of insecticides in corn oil were administered by i.p. injection. Pigs were housed and fed in groups, and corn oil solutions of insecticides were administered by i.p. injection. The diet was a standard growing ration. In two experiments with sheep, animals were individually fed diets (alfalfa hay plus barley) treated with acetone solutions of insecticide. A small portion of the daily grain ration was treated with insecticide. The animals were required to consume that portion completely before receiving the balance of the day's ration. In other experiments, sheep were given the insecticides in corn oil by i.p. injection.

Materials. Technical dieldrin, supplied by the Shell Chemical Co., recrystallized 10 times, melting point 178–9° C.

DDT, Merck and Co. technical grade, recrystallized 10 times, melting point 107–8° C.

Heptachlor, Nutritional Biochemicals Inc., Cleveland, Ohio, recrystallized seven times, melting point 96.5–97° C.

D,L-Ethionine, Mann Research Laboratories, New York, N. Y.

Actinomycin D, a gift of Merck Institute for Experimental Research, Rahway, N. J.

Analytical Methods. Insecticides in adipose tissue were extracted with hexane and analyzed by electron capture gas chromatography after cleanup with a Florisil column; the results are expressed as the concentration in the total extractable lipid of the sample (*9*). DDT and DDE were, of course, present in the fat of all treated animals in amounts proportional to the duration of DDT treatment and the time lapse between the end of treatment and sacrifice. Those values are not presented. Dried, ground fecal material was extracted repeatedly with hexane under reflux. That extract was passed over a Florisil column before gas chromatography. Ascorbic acid in urine was determined colorimetrically (*8*).

Statistics. Standard analysis of variance techniques was used to aid interpretation in all experiments. The level of significance chosen was $P < 0.05$.

Results and Discussion

Duration and Timing of the DDT Effect. The rate in which the interaction effect develops and its subsequent duration are of major importance. They are particularly relevant to problems associated with agricultural insecticide residues and the possible development of effective therapeutic agents for organochlorine toxicity by capitalizing on the interaction mechanism. The discovery of the DDT-dieldrin interaction was made in an experiment with 10-week treatment of rats (*9*, *10*). Later research demonstrated that DDT was highly effective by the tenth day of continuous oral treatment and after only 3 days of successive daily i.p. injections (*12*).

The effectiveness of DDT administered either early or late during a period of dieldrin exposure, of pretreatment with DDT before initiating dieldrin treatments, and of DDT treatment following dieldrin exposure have been studied.

Groups of female rats (five per group) were fed for 6 weeks a commercial laboratory diet which had been fortified with 1 p.p.m. of dieldrin. Individual groups were given DDT (50 p.p.m. added to the feed) either prior to, following, or simultaneously with the dieldrin feeding period. All DDT treatments caused highly significant reductions in dieldrin storage. The results are summarized in Figure 1.

The DDT effect on dieldrin retention persisted for at least 3 weeks after ceasing treatment, but was definitely weakened. DDT treatment for the final 3 weeks of the 6-week dieldrin period was as effective as continuous DDT treatment. Pretreatment with DDT resulted in reduced storage of dieldrin during the following 6 weeks. Post-dieldrin administration of DDT was highly effective in reducing tissue storage of dieldrin. The results indicate the potential value of a DDT-like effect in preventive or therapeutic treatment of individuals for insecticide exposure. Such an effect has been found in the use of certain drugs (*12*).

Mechanism. Because of the similarities between the DDT effect on dieldrin storage and effects of DDT in inducing drug-metabolizing enzymes of liver microsomes (*5*), it was postulated that the DDT effect might also result from enzyme induction. The reduced storage of dieldrin would follow from enhancement of its metabolic degradation. Circumstantial confirmation of the postulated mechanism was obtained by demonstrat-

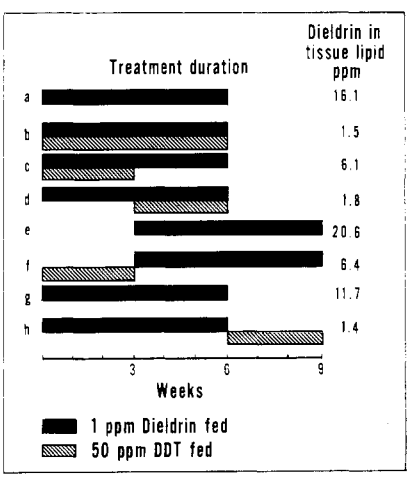

Figure 1. Effects of time and duration of DDT administration on the storage dieldrin in rat adipose tissue

Values listed are means for groups of five rats. All DDT treatments caused highly significant reductions in dieldrin storage. The DDT effect persisted for at least 3 weeks after ceasing DDT treatment, but was definitely weakened (cf. *a*, *b*, and *c*). DDT treatment for the final 3 weeks of a 6-week dieldrin exposure was as effective as DDT given continuously (*b* and *d*). Pretreatment with DDT for 3 weeks was effective throughout the following 6 weeks of dieldrin exposure (*e* and *f*). Administering DDT after the dieldrin treatment was highly effective in reducing residual dieldrin in fat (cf. *g* and *h*). The rats in groups *a*, *b*, *c*, and *d* were sacrificed at the end of the sixth week and those in groups *e*, *f*, *g*, and *h* at the end of the ninth week

ing significant reductions in dieldrin storage while treating rats with drugs known to induce liver microsomal enzymes (*12*).

Additional indirect evidence has been obtained to support the postulate. Ascorbic acid is synthesized in the rat liver in a reaction sequence involving microsomal enzymes. Consequently, agents that induce hepatic microsomal enzymes generally stimulate ascorbic acid production (*2*). In dieldrin treated rats, DDT has been shown to stimulate ascorbic acid production significantly (as measured by its excretion in urine) while dieldrin excretion in feces was reduced simultaneously (Figure 2). Dieldrin excretion later increased and ascorbic acid excretion decreased after discontinuing DDT treatment. The symmetry between the two responses, increased ascorbic acid excretion and reduced dieldrin excretion, suggests they may be related to one mechanism, that of increased liver microsomal enzyme activity. With increased dieldrin metabolism by such an enzyme(s), less intact dieldrin may remain for storage in body fat and be excreted via the intestine. Thus, both responses support the postulated induction of enzyme by DDT. Such a theory requires that increased quantities of dieldrin metabolite(s) should be recoverable in urine and/or feces owing to the action of DDT. Recent preliminary evidence indicates that this is so (*11*).

However, other evidence has been obtained which is seriously contradictive to the postulated enzyme induction mechanism. In theory, inhibitors of protein synthesis would counteract the induction effect of DDT. Experiments have been completed using D,L-ethionine, a methionine antagonist, and actinomycin D which inhibits the formation of *m*RNA. Ethionine has been reported to block the action of DDT in stimulating drug detoxification rates (*1*), and actinomycin D blocks the enzyme induction effect of polycyclic hydrocarbons such as 3-methylcholanthrene (*3*).

Ethionine was administered by diet to female rats receiving either dieldrin or dieldrin + DDT. The resulting dieldrin storage data are shown in Table I. The effect of DDT on dieldrin storage was not significantly different when ethionine was administered. In contrast, Hart, using a similar ethionine dosage (*1*), largely blocked the DDT stimulation of drug metabolism.

Actinomycin D also failed to inhibit the action of DDT. The insecticides were administered in the diet and actinomycin D by injection, once daily for 10 days. The resulting insecticide residues in adipose tissue are summarized in Table II. Actinomycin D blocked completely the glucocorticoid-induced synthesis of glucose-6-phosphatase in rat liver at dosages of 5 and 10 μg. (*11*). Therefore, it was assumed that the 10- and 25-μg. dosages in this experiment would likewise block enzyme synthesis. Although identical negative results were obtained with both inhibitors, the mounting nonspecific toxic effects of those compounds during 10 days' treatment may have influenced the results. Weight gain and vigor, for example, were reduced notably by the inhibitor treatments. However, the postulated induction, by DDT, of a dieldrin-metabolizing enzyme system is compromised by the results with these protein synthesis inhibitors. To be acceptable, a mechanism is required which will reconcile these differing results. Since it is highly probable that enhancement of dieldrin biotransformation is the effective result of the DDT action, the alternative to the enzyme induction postulate is that DDT stimulates the enzyme system, either by direct activation or indirectly by influencing the supply of substrates, energy, or required cofactors. Work is in progress in an attempt to clarify the mechanism.

DDT Effect with Heptachlor. One study has been completed of DDT interaction with another chlorinated cyclodiene, heptachlor. The basal rat diet was fortified with either 1 or 10 p.p.m. of recrystallized heptachlor and 0, 5, or 50 p.p.m. of DDT. Female rats were fed the treated diets for 10 days and sacrificed. Adipose tissue was analyzed for insecticide residues. The results are presented in Table III. Interaction was as marked as in results previously obtained with dieldrin (*9*, *10*).

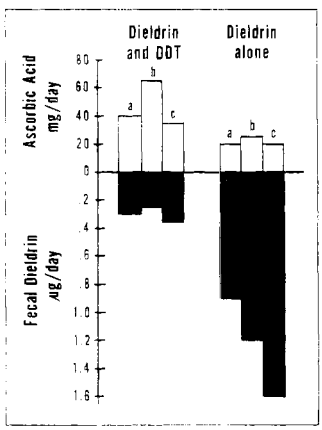

Figure 2. Ascorbic acid excretion in urine (upper bars) and fecal dieldrin excretion (lower bars) during continuous oral dieldrin treatment, with and without concomitant oral DDT

(a) after 1 week of treatment; (b) after 4 weeks, at peak ascorbic acid excretion; (c) 3 weeks after DDT treatment was discontinued (i.e., 7 weeks total dieldrin treatment). Dieldrin was fed at the rate of 1 p.p.m. and DDT at 50 p.p.m. DDT treatment was discontinued beyond the fourth week

Table I. Effects of Ethionine on DDT-Stimulated Processes in the Rat

Treatment	Dieldrin Storage,[a] μG./G. Lipid in Adipose Tissue	Treatment	Aniline[b] Metabolism, μM./G. Liver	Aminopyrine[b] Metabolism, μM./G. Liver
1 p.p.m. dieldrin	7.12	Control	1.06	0.50
1 p.p.m. dieldrin + 4 mg./kg. DDT	1.30	Control + 50 mg./kg. DDT	2.55	1.73
1 p.p.m. dieldrin + 4 mg./kg. DDT + 250 mg./kg. ethionine	1.90	Control + 50 mg./kg. DDT + 200 mg./kg. ethionine	1.40	0.48
1 p.p.m. dieldrin + 250 mg./kg. ethionine	7.52	Control + 200 mg./kg. ethionine	1.10	0.18

[a] Mean storage after 10-day treatment of five rats. The DDT effect was significant but ethionine was ineffective ($P < 0.05$).

[b] Data taken from Hart (*1*). Both the DDT and ethionine effects were significant ($P < 0.05$).

Table II. Effect of Actinomycin D on the DDT-Dieldrin Interaction

Dieldrin in Diet, P.P.M.	DDT in Diet, P.P.M.	Actinomycin D, Daily[a] 0 μg.	10 μg.	25 μg.
1	0	10.2	12.0	11.4
1	50	1.4	1.5	0.5

[a] Values are the mean dieldrin residue (p.p.m.) in rat adipose tissue lipids after 10 days' treatment. There were five female rats in each group. The DDT effect was significant but Actinomycin D was ineffective ($P < 0.05$).

Table III. Effect of DDT on the Storage of Heptachlor Epoxide in Rat Tissue upon Oral Treatment with Heptachlor

DDT Added to Diet, P.P.M.	Heptachlor Epoxide in Tissue, P.P.M.[a] 1 P.p.m. heptachlor fed	10 P.p.m. heptachlor fed
0	4.4 ± 0.55	40.7 ± 1.2
5	3.5 ± 0.22	31.0 ± 2.9
50	0.4 ± 0.03	8.4 ± 1.1

[a] Values are the mean dieldrin residues ± S.E. after 10 days' treatment. There were five female rats in each group. The DDT effect was significant ($P < 0.05$).

Therefore, other chlorinated cyclodienes also should be subject to the DDT interaction effect. Koransky *et al.* (*7*) have shown that phenobarbital stimulates the metabolism of BHC. Therefore, BHC and lindane should interact also with DDT.

DDT-Dieldrin Interaction in Other Species. Exploratory studies have been carried out with sheep, swine, and chickens. The interaction effect was obtained with sheep, although DDT produced only a marginally effective response. In a 12-week experiment, lambs receiving diets fortified with 0.7 mg. per kg. of body weight of dieldrin and either 0.7 or 3.6 mg. per kg. of body weight of DDT showed no evidence of a DDT effect on dieldrin storage. However, a significant interaction was obtained when wether sheep were dosed by i.p. injection (0.5 mg. per kg. of body weight of dieldrin and 1.7 mg. per kg. of body weight of DDT); dieldrin storage was reduced 45% during the 2-week trial (Table IV). In another study, storage of injected dieldrin was significantly lower in fat samples of DDT-treated ewes and wethers at the end of 20 weeks of daily treatment (Table V). Since both sexes and various DDT dosages were used in that experiment, the statistical analysis was planned to test for possible dose level or sex effects and a dose level–sex interaction. Of these, only the DDT dosage significantly influenced dieldrin storage (in the twentieth week sample). Biopsied fat samples taken after 3 and 9 weeks showed no significant effect of DDT on dieldrin storage.

The digestive tract of the ruminant appeared to be involved in the differing results obtained between insecticide injection and feeding. However, a small but statistically significant depression of the dieldrin level in sheep milk was obtained by feeding DDT (Figure 3). A trend toward lowered dieldrin was apparent after the third week of treatment. Significant differences were observed only in milk samples taken at 49, 77, and 91 days.

Table IV. Storage of Dieldrin in Adipose Tissue of Wether Sheep[a]

Treatment	Tissue Dieldrin Level,[b] P.P.M.
30 mg. of dieldrin	17.1
30 mg. of dieldrin + 110 mg. of DDT	9.4

Analysis of Variance

Source	Degrees of Freedom	Mean Sq.
Treatment	1	87.09 ($P < 0.01$)
Replication	2	15.75 ($P < 0.01$)
Error	2	0.81

[a] Three sheep received each treatment by daily i.p. injection for 15 days.
[b] Table values are mean dieldrin levels based on extractable lipid.

Dieldrin storage in pork fat was reduced 60 to 65% by DDT (0.4 mg. per kg. of body weight of dieldrin daily and either 0.4 mg. per kg. or 3.1 mg. per kg. of body weight of DDT daily for 21 days). The insecticides were administered by i.p. injection to young male and female pigs. The results were highly significant (Table VI).

No DDT effect was obtained in either of two experiments with chickens. In one, aged producing hens were treated

Table V. Storage of Dieldrin in Sheep Adipose Tissue[a]

Daily Treatment[b]	3-Week Treatment,[c] P.P.M.	9-Week Treatment,[d] P.P.M.	20-Week Treatment,[e] P.P.M.
0.44 mg. of dieldrin/kg.			
Males	8.52	53.75	124.18
Females	4.53	61.20	96.94
0.44 mg. of dieldrin + 1.60 mg. of DDT/kg.			
Males	7.45	49.24	63.37
Females	5.24	53.26	69.75
0.44 mg. of dieldrin + 6.40 mg. of DDT/kg.			
Males	6.49	76.62	83.65
Females	5.59	46.26	77.41

Analysis of Variance

Source	Degrees of Freedom	Mean Sq.	Mean Sq.	Mean Sq.
Treatment	5	7.67	601.79	2433.50 ($P < 0.01$)
DDT dose	2	0.27	267.76	5055.85 ($P < 0.01$)
Sex	1	32.16	296.86	612.19
Sex × dose	2	3.59	1092.29	721.32
Error	24	8.76	504.69	384.06

[a] Values are expressed as mean concentrations in extractable lipid of adipose tissue.
[b] Administered by i.p. injection of corn oil solution. There were five males and five females in each treatment group.
[c] Subcutaneous biopsy.
[d] Abdominal biopsy (mesenteric).
[e] Abdominal sample (perirenal) at sacrifice.

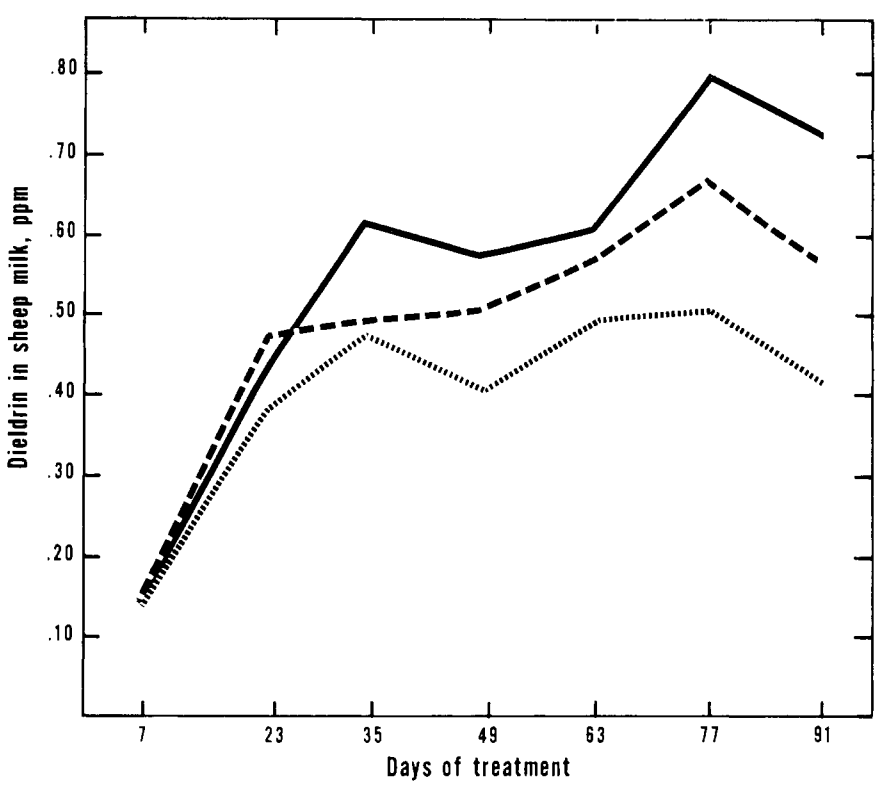

Figure 3. Effect of DDT administration on the level of dieldrin in sheep milk

The insecticides were administered orally using fortified feed. Each point represents the mean dieldrin level in milk of six sheep
—— 5 mg. of dieldrin daily
– – – 5 mg. of dieldrin + 50 mg. of DDT daily
..... 5 mg. of dieldrin + 200 mg. of DDT daily

Table VI. Effect of DDT on Dieldrin Storage in Swine

Daily Treatment[b]	Tissue Dieldrin,[a] P.P.M.
5.45 mg. of dieldrin	
Males	25.5
Females	13.0
5.45 mg. of dieldrin + 22.55 mg. of DDT	
Males	5.4
Females	7.8
5.45 mg. of dieldrin + 45.10 mg. of DDT	
Males	6.7
Females	8.3

ANALYSIS OF VARIANCE

Source	Degrees of Freedom	Mean Sq.
Treatment	5	116.33 ($P < 0.01$)
DDT	2	192.89 ($P < 0.01$)
Sex	1	28.63
DDT × sex	2	83.61 ($P < 0.01$)
Error	9	8.85

[a] Values are the mean dieldrin levels in adipose tissue after 15 days of treatment. There were three males and two females in each group. [b] The insecticides were administered daily by injection of corn oil solutions.

daily with 0.15 mg. per kg. of body weight of dieldrin and either 0.60 or 2.40 mg. per kg. of body weight of injected DDT. Neither eggs, collected during the 15-day experiment, nor abdominal adipose tissue showed lowered dieldrin values resulting from DDT. A similar experiment was conducted for 21 days with young hens just beginning to lay. Dieldrin dosages were fixed at 0.075 and 0.375 mg. per kg. daily and DDT dosages were increased to 2.0 and 10.0 mg. per kg. Again, no influence of DDT on dieldrin storage was observed.

Animal variation in sensitivity to such an interaction effect may be important in determining the ecological consequences of some agricultural chemical usage. In particular, it leaves the question of possible interaction in humans quite problematical.

The findings in this report point out an important new aspect of the toxicology of a major class of environmental contaminants, the chlorinated hydrocarbon insecticides. Much more study is needed to determine the ultimate significance of the findings with those insecticides, and the principles learned may well apply to the toxicology of many other chemical pollutants being placed in our environment. An intriguing possibility is that effective agents might be developed which would safely reduce insecticide storage in animals and man. Such agents also might be used for the treatment of individuals who may become overexposed to insecticides and other foreign chemicals.

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INTERACTIONS

Toxicologic Interactions of Chlorinated Hydrocarbon and Organophosphate Insecticides

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THE first study of the influence of the chlorinated hydrocarbon insecticides on the toxicity of a subsequently administered organophosphate insecticide was that of Ball *et al.* in 1954 (*3*). They reported that rats given a single oral dose of aldrin, chlordan, or lindane were protected 4 days later against the oral toxicity of parathion, an effect which they attributed to the increase in serum aliesterase activity induced by the insecticides. Main (*18*) noted in 1956 that similar aldrin pretreatment reduced the mortality of rats challenged with parathion, paraoxon, and TEPP. He attributed this protective effect of aldrin to an increase in liver A-esterase activity. In 1958 Neubert and Schaefer (*20*) reported that pretreatment with α-hexachlorocyclohexane protected mice against the toxicity of paraoxon and OMPA, but not DFP.

As knowledge developed of the role of the liver microsomal enzymes in the metabolism of drugs and other chemicals, several groups of investigators reported findings indicating that the chlorinated hydrocarbon insecticides stimulate microsomal enzyme activity. The present authors found that a single dose of aldrin in mice markedly decreased the sleeping time due to hexobarbital, a drug which is metabolized mainly by the microsomal fraction of the liver (*27*). Hart *et al.* (*16*) and Hart and Fouts (*15*) pretreated rats with chlordan or DDT and noted an increased rate of hexobarbital metabolism by the liver microsomes. Isomers of hexachlorocyclohexane, DDT, and dieldrin have been reported to accelerate several detoxication processes of rat liver microsomes—including hydrolysis of paraoxon, oxidation of hexobarbital, *O*-dealkylation of phenacetin, and *N*-dealkylation of nikethamide—and to lower