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The Effects of Chlordane, DDT, and 3-Methylcholanthrene upon the Metabolism and Toxicity of Diethyl-4-Nitrophenyl Phosphorothionate (Parathion)

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The Effects of Chlordane, DDT, and 3-Methylcholanthrene upon the Metabolism and Toxicity of Diethyl-4-Nitrophenyl Phosphorothionate (Parathion). CHAPMAN, SHARON K., and LEIBMAN, KENNETH C. (1971). *Toxicol. Appl. Pharmacol.* **18**, 977–987. In rats, (1) DDT and chlordane cause equal stimulation of both pathways of parathion metabolism, similar to phenobarbital, and (2) 3-methylcholanthrene treatment results in preferential enhancement of the pathway leading to the formation of paraoxon. With mice, (1) DDT treatment results in greater enhancement of the pathway responsible for diethyl hydrogen phosphorothionate than of that producing paraoxon, (2) chlordane equally enhances both pathways of parathion metabolism, and (3) 3-methylcholanthrene treatment results in a repression of diethyl hydrogen phosphorothionate production. The differential inductive effects are discussed in terms of the hypothesis that two separate enzyme systems are involved in the metabolism of parathion. Results from toxicity studies in mice indicate that (1) no significant change in parathion toxicity results from DDT treatment, (2) toxicity is not altered significantly after treatment with 3-methylcholanthrene, and (3) chlordane treatment affords protection against the toxicity of parathion. The lack of correlation between the toxicity studies and the metabolic studies suggests that factors other than metabolism contribute to toxicity.

Parathion has been shown to be metabolized by two main pathways, both mediated by oxidative enzymes in liver microsomes. One of these involves a sulfur-oxygen exchange with the production of the toxic anticholinesterase, paraoxon (Diggle and Gage, 1951; Murphy and DuBois, 1957; O'Brien, 1959). The other results in the breakdown of parathion to diethyl hydrogen phosphorothionate and *p*-nitrophenol, and is, therefore, a "detoxication" (Nakatsugawa and Dahm, 1967; Neal, 1967a). Neal (1967b) attempted to determine whether separate mixed-function oxidase enzyme systems were involved in the reactions leading to the metabolism of parathion or whether one enzyme exists with separate binding sites. The results of experiments with various enzyme inhibitors and activators led him to postulate that the initial reactions of parathion metabolism are mediated by two separate enzyme systems.

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Protection against the toxicity of the organophosphate insecticides has been found after treatment of animals with various inducing agents, both drugs and chlorinated insecticides (Welch and Coon, 1964; DuBois and Kinoshita, 1966; Triolo and Coon, 1966b; Takabatake *et al.*, 1968). Inducers of hepatic microsomal enzymes have been classified in two groups, exemplified by phenobarbital on the one hand and polycyclic hydrocarbons on the other (Conney, 1967). It has been known for some time that chlorinated insecticides such as DDT and chlordane cause a proliferation of hepatic endoplasmic reticulum and induce microsomal mixed-function oxidase systems of mammalian liver in a manner similar to that of phenobarbital (Ortega, 1962; Hart and Fouts, 1963, 1965a; Fouts and Rogers, 1964; Conney, 1967).

The present investigation was designed to aid further in the characterization of the enzyme systems of parathion metabolism with the use of inducing agents, especially those of the chlorinated insecticide group. Particular attention was directed to the toxicologic importance of the alterations of enzyme activities that were demonstrated in vitro.

MATERIALS AND METHODS

Animals. Adult male Holtzman rats[2] weighing 100–250 g and male CF-1 mice[3] weighing 20–30 g were used. All animals were maintained on food and water ad libitum.

Chemicals. DDT,[4] chlordane,[5] and 3-methylcholanthrene[6] were dissolved in corn oil and administered ip in the amounts indicated in each study. Control animals in all cases received corresponding amounts of corn oil.

Toxicity studies. The toxicity of parathion was measured in control, DDT-treated, 3-methylcholanthrene-treated, and chlordane-treated mice. DDT and chlordane were given at a dose of 100 mg/kg/day over a period of 3 days. 3-Methylcholanthrene was administered at a dose of 20 mg/kg/day for 4 days. Control animals received the same volume of corn oil. The administration of parathion was always carried out 24 hr after the last injection. Parathion[7] was dissolved in ethanol-propylene glycol (20:80 v/v), and the amount of toxicant in solution was adjusted so that an amount of solution equivalent to 1% of the body weight was given ip. Mortalities were recorded for 24 hr after the administration of parathion. The LD50 values were calculated from the mortality data by the method of Litchfield and Wilcoxon (1949). Significant differences ($P < 0.05$) between treated and control groups were determined from their potency ratio.

Enzyme preparation. Animals were killed by a blow on the head followed by decapitation. The livers were quickly removed, weighed, and homogenized in 4 volumes of 0.01 M ice-cold phosphate buffer (pH 8). The whole homogenate was centrifuged at 9000 *g* for 20 min at 2°C in a Servall refrigerated centrifuge, and the supernatant fraction from this step was used for the enzyme assays.

[2] Holtzman rats were obtained from the Holtzman Co., Madison, Wisconsin.

[3] CF-1 mice were obtained from Carworth Farms, New City, New York.

[4] DDT (1,1,1-trichloro-2,2-bis[*p*-chlorophenyl]ethane) was obtained from Pesticide Repository, Pesticide Research Laboratory, Perrine, Florida.

[5] Chlordane (1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene) was obtained from Pesticide Repository, Pesticide Research Laboratory, Perrine, Florida.

[6] 3-Methylcholanthrene was purchased from Eastman Kodak Company, Eastman Organic Chemicals, Rochester, New York.

[7] Parathion (*O,O*-diethyl *O*-*p*-nitrophenyl phosphorothionate) was obtained from Pesticide Repository, Pesticide Research Laboratory, Perrine, Florida.

Incubation conditions. A solution containing 1.5 μCi of (diethyl-^{14}C) parathion[8] in benzene was added to dry incubation flasks and evaporated overnight. The following incubation system was then added to each flask: phosphate buffer (pH 8), 15 μmoles; NADP, 1.2 μmoles; glucose 6-phosphate, 5 μmoles; unlabeled parathion, 0.35 μmoles (added as a 13.6 mM solution in ethanol); 9000 *g* supernatant fraction equivalent to 150 mg liver. Distilled water was added to make a final volume of 2.0 ml. The mixtures were incubated with shaking at 37°C in 20-ml beakers open to the air. The incubation time was 60 min. Combined substrate and tissue blanks were prepared in each case as described by Neal (1967a).

Isolation and estimation of metabolites. Metabolites of (diethyl-^{14}C) parathion were isolated and identified by thin-layer chromatography, according to the procedure described by Neal (1967a). The solvent systems and scintillation fluid were modified. Incubation was terminated by the addition of 3.0 ml of cold acetone, and the reaction mixture was kept in ice for 1 hr. The precipitated proteins were removed by centrifugation, and the supernatant portion was adjusted to pH 3–4 with 1 M HCl. The volume was accurately measured and 50-μl portions of each supernatant fraction were spotted on each of two separate glass plates precoated with silica gel G.[9] After spotting, the plates were subjected to ascending chromatography in filter paper-lined glass tanks. Two different solvent systems were used, and the metabolites were identified by co-chromatography with known compounds. One plate was developed with acetonitrile-water (75:25, v/v) (Stenersen, 1968). An examination of the radioautographs revealed the presence of radioactive areas with average R_f values of 0.44, 0.61, 0.82, and 0.87. These areas were identified as diethyl hydrogen phosphate,[10] diethyl hydrogen phosphorothionate,[11] paraoxon,[12] and parathion, respectively. The second plate was developed with hexane–chloroform–methanol (8:1:1, v/v). Four areas of radioactivity appeared with average R_f values of 0.00, 0.20, 0.24, and 0.57. The area with the R_f of 0.00 was made up of more than one compound. The R_f corresponded to those of both diethyl hydrogen phosphate and diethyl hydrogen phosphorothionate. The compounds with R_f values of 0.24 and 0.57 were identified as paraoxon and parathion, respectively. The compound with R_f 0.20 was not identified, but it represented less than 10% of the total activity exclusive of that of unmetabolized parathion.

The silica gel containing the radioactive areas were scraped from the plates and placed in counting vials containing 20 ml of a mixture of 4 g of PPO (2,5-diphenyloxazole), 300 ml of methyl alcohol, and 700 ml of toluene.

The nonradioactive phosphorothionates were located on thin-layer plates by spraying with 0.5% $PdCl_2$ in 0.1 M HCl. Paraoxon was visualized with the use of an alcoholic sodium hydroxide mixture described by Beck and Sherman (1968). Diethyl hydrogen phosphate was located by molybdate sprays described by Hanes and Isherwood (1949) and Jungnickel (1967).

[8] [Diethyl-^{14}C] parathion was prepared by Mallinckrodt/Nuclear, Orlando, Florida.

[9] Silica gel G (Merck No. F-254) was purchased from Brinkman Instruments, Inc., Westbury, New York.

[10] Diethyl hydrogen phosphate was purchased from Eastman Organic Chemicals, Rochester, New York.

[11] Diethyl hydrogen phosphorothionate was obtained as the potassium salt from American Cyanamid Co., Princeton, New Jersey.

[12] Paraoxon (*O,O*-diethyl-*O*-*p*-nitrophenyl phosphate) was obtained from Pesticide Repository Pesticide Research Laboratory, Perrine, Florida.

Recovery of (diethyl-^{14}C) parathion. The activity of the stock solution of labeled parathion was 150 μCi per ml of benzene. Pilot experiments were performed to determine whether radioactivity was lost on evaporation of the stock solution overnight or during the incubation, precipitation, or centrifugation steps. In all cases, 85–95% recovery was obtained.

Statistical evaluation of results. Statistical significance of the results was calculated by Student *t* test. The values for the ratios of paraoxon: diethyl hydrogen phosphorothionate represent the mean ±SE of the ratios calculated for each individual animal, not a ratio of the mean values.

RESULTS

The demonstration that the two oxidative reactions of parathion were equally enhanced by treatment with phenobarbital (Neal, 1967a) was verified early in this work with the use of both isolated microsomes and 9000*g* supernatant fractions.

Pilot experiments were performed to establish optimum conditions for induction in rats with either DDT or chlordane. Both single and multiple dosage schedules were studied. It appeared that for either DDT or chlordane, a dosage schedule of 100 mg/kg/day × 3 days afforded optimum induction of activity for parathion metabolism.

The data in Table 1 show that diethyl hydrogen phosphorothionate production from parathion in DDT-treated rats was stimulated 210% as compared with untreated animals, and the production of paraoxon was stimulated 206%. The ratio of paraoxon: diethyl hydrogen phosphorothionate was not significantly different from that of control rats. Therefore, DDT treatment caused equal enhancement of both oxidase path-

TABLE 1

THE EFFECTS OF CHLORDANE, DDT, AND 3-METHYLCHOLANTHRENE UPON THE METABOLISM OF PARATHION BY RAT LIVER 9000 *g* SUPERNATANT FRACTIONS[a]

Treatment	Diethyl hydrogen phosphorothionate (nmoles/hr/g liver)	Paraoxon[b] (nmoles/hr/g liver)	Ratio paraoxon: diethyl hydrogen phosphorothionate
Corn oil control (7)[c]	120 ± 21	151 ± 28	1.24 ± 0.03
DDT (7)	373 ± 14	462 ± 13	1.24 ± 0.03
100 mg/kg/day × 3	(<0.01)[d]	(<0.01)	(NS)
Corn oil control (6)	93 ± 10	115 ± 13	1.24 ± 0.06
Chlordane (6)	229 ± 20	307 ± 28	1.34 ± 0.03
100 mg/kg/day × 3	(<0.01)	(<0.01)	(NS)
Corn oil control (6)	41 ± 8	50 ± 9	1.25 ± 0.05
3-Methylcholanthrene (6)	75 ± 9	141 ± 15	1.90 ± 0.06
20 mg/kg/day × 4	(<0.02)	(<0.01)	(<0.01)

[a] Procedures for incubation and for isolation and measurement of metabolites are given in the materials and methods section. The concentration of (diethyl-^{14}C) parathion in each incubation flask was 0.175 mM.

[b] The values for paraoxon represent the sum of the paraoxon and the diethyl-hydrogen phosphate formed during the incubation. The values are the means ± SE.

[c] Numbers in parentheses in the treatment column represent the number of animals.

[d] The *P* values, in parentheses, indicate the significance of differences between mean results from groups of treated and untreated animals. NS, not significant.

ways. Results also indicated that chlordane treatment resulted in equal enhancement of the metabolism of parathion to diethyl hydrogen phosphorothionate and paraoxon (146 and 166%, respectively).

After administration of 3-methylcholanthrene in an optimum induction schedule (20 mg/kg/day × 4) (Sladek and Mannering, 1969), a differential stimulation was observed (Table 1). Diethyl hydrogen phosphorothionate production from parathion in treated rats was stimulated 83%, as compared with untreated animals, whereas the production of paraoxon was stimulated 182%. A significant difference in the ratio paraoxon:diethyl hydrogen phosphorothionate between preparations from 3-methylcholanthrene-treated and control animals, was observed. This effect was seen at 20-, 60-, and 80-min incubation intervals.

The effects of chlordane, DDT, and 3-methylcholanthrene upon the metabolism of parathion by mouse liver 9000 *g* supernatant fractions were investigated. In this species, DDT treatment resulted in greater enhancement of the pathway responsible for diethyl hydrogen phosphorothionate production (161%) than of that which produces paraoxon (113%) (Table 2). This was reflected in the significant decrease in the ratio paraoxon:diethyl hydrogen phosphorothionate.

TABLE 2

THE EFFECTS OF CHLORDANE, DDT, AND 3-METHYLCHOLANTHRENE UPON THE METABOLISM OF PARATHION BY MOUSE LIVER 9000 *g* SUPERNATANT FRACTIONS[a]

Treatment	Diethyl hydrogen phosphorothionate (nmoles/hr/g liver)	Paraoxon[b] (nmoles/hr/g liver)	Ratio paraoxon: diethyl hydrogen phosphorothionate
Corn oil control (6)[c]	119 ± 10	142 ± 11	1.19 ± 0.02
DDT (6)	310 ± 38	302 ± 33	0.98 ± 0.02
100 mg/kg/day × 3	(<0.01)	(<0.01)	(<0.01)
Corn oil control (9)	69 ± 11	89 ± 15	1.31 ± 0.07
Chlordane (9)	172 ± 29	203 ± 29	1.21 ± 0.05
100 mg/kg/day × 3	(<0.01)	(<0.01)	(NS)
Corn oil control (6)	90 ± 14	89 ± 14	0.98 ± 0.08
3-Methylcholanthrene (6)	48 ± 6	63 ± 8	1.32 ± 0.28
20 mg/kg/day × 4	(<0.02)	(NS)	(<0.02)

[a] The experimental conditions and statistical analyses were as in Table 1.

[b] The values for paraoxon represent the sum of the paraoxon and the diethyl hydrogen phosphate formed during the incubation. The values are the means ± SE.

[c] Numbers in parentheses in the treatment column represent the number of animals.

The pattern of equal stimulation of both pathways observed in chlordane-treated rats was also observed with mice. No significant difference was noted in the ratios between control and chlordane-treated mice.

The effects of 3-methylcholanthrene administration on the metabolism of parathion in mice were quite unusual. A statistical analysis revealed a significant depression of diethyl hydrogen phosphorothionate production (49%). The apparent depression of paraoxon formation (29%) was not significant when compared to control values. The ratio paraoxon:diethyl hydrogen phosphorothionate was increased.

In view of the metabolic alterations observed in vitro after pretreatment with DDT, chlordane, and 3-methylcholanthrene, experiments were conducted to see whether such alterations had toxicologic importance. The LD50 values of parathion for control and treated mice were compared (Table 3). No significant alteration in parathion toxicity occurred after pretreatment with either 3-methylcholanthrene or DDT. In contrast, chlordane treatment resulted in a significant decrease in parathion toxicity.

TABLE 3

EFFECT OF TREATMENT WITH DDT, 3-METHYLCHOLANTHRENE AND CHLORDANE ON THE TOXICITY OF PARATHION IN MICE

Treatment	No. of mice	LD50 (mg/kg)	19/20 Confidence limits (mg/kg)	Ratio of LD50 treated/control
Control	50	10.0	(7.4–13.6)	—
DDT				
100 mg/kg/day × 3	45	7.2	(4.9–10.0)	0.72
3-Methylcholanthrene				
20 mg/kg/day × 4	45	15.0	(9.7–23.1)	1.50
Chlordane				
100 mg/kg/day × 3	50	35.0	(17.5–70.0)	3.50[a]

[a] Treated group is significantly different from control group ($P < 0.05$).

DISCUSSION

It has been proposed (Conney, 1967) that inducers can be divided into two groups: (a) compounds such as phenobarbital, which induce the metabolism of a large number of substrates and (b) those which exert specificity as enzyme inducers and stimulate a limited number of reactions. In rats and rabbits, the chlorinated hydrocarbon insecticides have been shown to possess the induction properties characteristic of the first group (Hart and Fouts, 1965a).

The unique feature of parathion metabolism is that in both oxidative reactions the site of attack by oxygen appears to be the phosphorus atom (Wolcott and Neal, 1969). Neal (1967b) postulated that two separate mixed-function oxidase systems were involved. With the use of various inhibitors, electron acceptors, and sulfur-containing compounds, he demonstrated that differential inhibition and stimulation existed. This was taken as evidence against the possibility that a common metabolic pathway exists for both the metabolism of parathion to paraoxon and the conversion of parathion to diethyl hydrogen phosphorothionate.

The results of the present experiments indicated that, with rats, DDT and chlordane acted to stimulate both pathways of parathion metabolism, analogous to the action of phenobarbital (Table 1). In the absence of differential enhancement, little could be said concerning the possibility that more than one enzyme system was involved. However, when 3-methylcholanthrene was used as the inducing agent, preferential enhancement of the pathway leading to the formation of paraoxon was observed. This was demonstrated by a significant rise in the ratio paraoxon: diethyl hydrogen phosphorothionate

after 3-methylcholanthrene treatment. Neal (1967a) also observed a preferential enhancement of paraoxon production after 3,4-benzopyrene with microsomal preparations.

The differences between inducing agents have allowed differentiation among various hydroxylating systems in liver microsomes (Conney *et al.*, 1959, 1960; Takemori and Mannering, 1958). Creaven and Parke (1966) demonstrated that 3,4-benzopyrene stimulated the 2-hydroxylation of biphenyl but not its 4-hydroxylation, whereas phenobarbital caused a large increase in the 4-hydroxylation relative to the change in activity for 2-hydroxylation. These results were interpreted to mean that separate enzyme systems catalyzed the hydroxylation of biphenyl in the 2- and 4-positions and that the syntheses of the two enzyme systems were under separate control. The results in the present study, with 3-methylcholanthrene-treated rats, suggest that the two pathways of parathion metabolism are also under separate control. If the reactions were catalyzed by the same enzyme system, an equal enhancement of the production of the two metabolites would be expected after induction.

Further support of this hypothesis was obtained from the in vitro study of parathion metabolism in mice. This species was chosen in view of the apparently limited ability of DDT to alter drug metabolism in the mouse (Conney, 1967). Among the pathways unaffected by pretreatment with DDT were hexobarbital metabolism in vitro and in vivo (Hart and Fouts, 1963, 1965b), *p*-nitroanisole *O*-demethylation (Netter, 1969), and naphthalene hydroxylation (Netter, 1969). Recently, however, DDT was shown to stimulate the metabolism of at least one drug (zoxazolamine) in mice (Cram and Fouts, 1967). This latter finding indicated that DDT exerted specificity for at least one metabolic pathway. Therefore, its ability to differentiate the two pathways of parathion metabolism was investigated.

Results from the present study with mice showed that DDT treatment resulted in greater enhancement of the pathway responsible for diethyl hydrogen phosphorothionate production than of that producing paraoxon (Table 2). With the exception of zoxazolamine metabolism, this is apparently the only reported case of enzyme induction by DDT in mice.

Differential effects on parathion metabolism were also noted with 3-methylcholanthrene-treated mice. Unlike the results with rats, in which 3-methylcholanthrene treatment caused an enhancement of paraoxon production (Table 1), those with mice indicated that such treatment depressed diethyl hydrogen phosphorothionate production (Table 2). This effect was not caused by stimulation of the conversion of diethyl hydrogen phosphorothionate to diethyl hydrogen phosphate, as no increase in diethyl hydrogen phosphate production in 3-methylcholanthrene-treated mice occurred. Therefore, the depression of diethyl hydrogen phosphorothionate production could not be explained on the basis of an increase in desulfuration.

When mouse liver was used as the enzyme source in the metabolic studies, three different effects were observed on the ratio of toxic metabolite (paraoxon): breakdown product (diethyl hydrogen phosphorothionate). The ratio was reduced in DDT-treated mice, increased in 3-methylcholanthrene-treated animals, and unaltered in mice pretreated with chlordane (Table 2). Therefore, experiments were conducted to study the effect of such metabolic alterations on the toxicity of parathion, in order to see whether the metabolic alterations that were demonstrated in vitro had toxicologic importance.

It was expected that the toxicity of parathion would depend upon a balance between the two oxidase reactions. If this were so, a notable stimulation of the production of diethyl hydrogen phosphorothionate at the expense of that of paraoxon would result in protection against the toxic effects of parathion, whereas the opposite situation should prevail in vivo if paraoxon production were preferentially stimulated.

When mice were pretreated with DDT, no change in the toxicity of parathion was observed (Table 3). This result was in contrast to the in vitro data that showed an enhancement of the direct detoxification of parathion. Discrepancies between in vivo and in vitro observations were also noted in the case of 3-methylcholanthrene administration. Metabolic studies revealed an increase in the ratio paraoxon:diethyl hydrogen phosphorothionate, but this was not reflected in increased toxicity; no significant alteration in parathion toxicity was noted after 3-methylcholanthrene treatment. Although chlordane was shown in vitro to stimulate both the activation and detoxication of parathion, the in vivo study demonstrated that pretreatment with this agent decreased the toxicity of parathion to mice.

The present experiments suggest that alterations in toxicity do not parallel the changes in the microsomal metabolism of parathion. Alary and Brodeur (1969) found the same discrepancy in their study of the mechanism of the phenobarbital-induced protection against parathion in adult female rats. They showed that the stimulating effect of phenobarbital on the activation of parathion to paraoxon, as observed under in vivo conditions, was not reflected in increased toxicity. After measuring the urinary metabolites of parathion, they suggested that, in the intact animal, phenobarbital stimulates only the direct degradation of parathion to diethyl hydrogen phosphorothionate and *p*-nitrophenol. However, their failure to measure the formation of paraoxon did not eliminate the possibility that phenobarbital enhanced the production of paraoxon, which was in turn bound by tissue or plasma proteins.

Several investigators have studied the characteristics and mechanisms of the protective action of various inducing agents against poisoning by the organophosphate insecticides (Crevier *et al.*, 1954; Main, 1956; Brodeur, 1967; Alary and Brodeur, 1969; Triolo and Coon, 1966a, 1969; Lauwerys and Murphy, 1969; Ball *et al.*, 1954). The concept that protection was attributed to a rise in liver and serum esterases did not prove to be a satisfactory explanation (Brodeur, 1967). The low paraoxonase activities of tissues other than liver (Alary and Brodeur, 1969) tends to minimize the possibility that extrahepatic tissues protect significantly from paraoxon poisoning by catalyzing its hydrolysis. Brodeur (1967) suggested that phenobarbital protection against *O*-ethyl *O*-(4-nitrophenyl) phenylphosphonothioate (EPN), a compound structurally related to parathion, was the result of a direct effect on hepatic biotransformation.

If the results from the present study in vitro can be extrapolated to the intact animals, parathion toxicity cannot be completely dependent upon the amount of paraoxon produced in the liver. Both DDT and chlordane treatment enhanced paraoxon production in liver preparations but neither agent increased the toxicity of parathion. On the contrary, chlordane treatment afforded protection against parathion toxicity. Therefore, if paraoxon production is enhanced in vivo after pretreatment with DDT and chlordane, the protective effect can be explained only if the increased amounts of paraoxon are detoxified before reaching the cholinesterases.

The influences of DDT, 3-methylcholanthrene, and chlordane upon tissue and

plasma binding of parathion and its metabolites were not investigated in this study. The possibility exists that one or both of these processes may be responsible for the lack of correlation between the in vitro and in vivo observations. Lauwerys and Murphy (1969) have suggested that liver or plasma, when incubated with low concentrations of paraoxon, detoxifies this organophosphate by a nonenzymatic tissue-binding process. The opposing results in this study between metabolic and toxicologic observations could be explained if such a process is affected by DDT, 3-methylcholanthrene, and chlordane.

Triolo and Coon (1969) investigated plasma protein-binding as a mechanism for the protection against parathion in aldrin-treated mice. They found that the protein-binding of paraoxon was increased in the plasma of animals treated with aldrin, resulting in a decrease in the amount of free paraoxon. They further demonstrated that in mice pretreated with several of the organochlorine insecticides, there was a significant correlation between the percent mortality after the administration of paraoxon and the "free paraoxon" levels in the plasma when it was incubated with paraoxon (Triolo *et al.*, 1970). It may be postulated that a similar mechanism is involved in the present study with DDT and chlordane. If both agents act to stimulate the conversion of parathion to paraoxon, as demonstrated in vitro, their failure to produce increased toxicity may be due to a mechanism similar to that mentioned above.

In conclusion, the in vitro studies on the effects of 3-methylcholanthrene, DDT, and chlordane upon parathion metabolism support the hypothesis that parathion is metabolized by two separate mixed-function oxidase systems. The lack of correlation between the toxicity studies and the metabolic studies suggests that alterations in toxicity are not mediated predominantly by changes in the biotransformation of the insecticide.

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