Effects of Acute and Chronic DDT Administration on Hepatic Microsomal Drug Metabolism in the Rat.* (28686)

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The President's Science Advisory Committee Panel on the Use of Pesticides has recently recommended that toxicity studies on pesticides should include determinations of "possible synergism and potentiation of effects of commonly used pesticides with such commonly used drugs as sedatives, tranquilizers, analgesics, antihypertensive agents, and steroid hormones, which are administered over prolonged periods"(1). Investigation of this problem has been in progress in our laboratory since 1961.

We recently reported effects of the chlorinated hydrocarbon insecticides, technical chlordane and one of its components γ-chlordane, on hepatic microsomal drug metabolism(2). These compounds caused a stimulation of enzyme activity for the metabolism of certain drugs, and these changes in enzyme activity were characterized by a latency of onset and a prolonged duration.

Other structurally related insecticides after acute administration demonstrated similar effects on drug enzyme activity as measured by barbiturate sleeping times(3). Dichlorodiphenyltrichloroethane (DDT) was the only chlorinated insecticide studied which failed to produce an alteration of enzyme activity under the conditions used (Table I).

All of these insecticides are quite lipid soluble and accumulate in body fat, after which they are slowly liberated from these tissue stores. It may be that rapid uptake of the administered DDT by the fat stores prevented it from exerting an effect on hepatic enzyme activity. With this in mind, we decided to follow the effects of DDT after chronic administration. The results of this investigation are presented here. We also studied the acute effects of certain organophosphorous anticholinesterase insecticides on drug metabolism.

Methods. DDT, as a crystalline powder, was admixed in a lab chow diet and fed to adult male Sprague-Dawley rats. The concentration of DDT in the feed was 500 ppm. Rats weighed 150-175 g at start of the experiment, while survivors after 4 months on this diet weighed 450-550 g. Prior feeding studies with ground lab chow indicated that at the concentration used, rats would ingest approximately 50 mg/kg of DDT in a 24 hour period.

Equal numbers of experimental and control animals were selected randomly for drug metabolism studies at intervals of 2 weeks, 1, 2, 3, and 4 months after beginning the diet. Hexobarbital sodium, 125 mg/kg, was injected intraperitoneally and sleeping times determined on all rats. Sleeping time was defined as the time which elapses from loss of the righting reflex until regaining this reflex. Animals were numbered so that *in vivo* results could be compared with *in vitro* enzyme activity.

Sixteen to 18 hours after sleeping time determinations, rats were sacrificed by a blow on the head and their livers immediately excised and placed in vessels immersed in ice. Samples were taken from each liver for routine histology and the remainder was homogenized with 2 volumes of cold, isotonic (1.15%) KCl in a Potter homogenizer having a plastic pestle. Homogenates were centrifuged at 9000 × *g* in a refrigerated high speed angle centrifuge to sediment nuclei, mitochondria and cell debris. The resulting supernatant fraction, composed of microsomes plus "soluble" fraction, was used for determinations of enzyme activity.

Drug metabolic pathways studied were the side chain oxidation of hexobarbital, the N-demethylation of aminopyrine, the ring hydroxylation of aniline and the reduction of the aromatic nitro group of p-nitrobenzoic acid. Disappearance of substrate was measured to follow the metabolism of hexobarbital by the method of Cooper and Brodie (4). Appearance of the metabolite was meas-

* Supported by U. S. Public Health Service grant.

TABLE I. Effects of Single Doses of Chlorinated Hydrocarbon Insecticides on Hexobarbital Sleeping Times in Mice.*

Insecticide†	Dose (mg/kg)	Time after injection of insecticide 1 hr	3 hr	1 day	3 days
DDT	25.0	64 ± 11 (9)	89 ± 15 (8) ‡	75 ± 17 (8)	68 ± 8 (8)
Trichloro 237§	25.0	63 ± 11 (7)	72 ± 14 (8)	65 ± 11 (10)	33 ± 7 (8) ‡
Control	—	65 ± 14 (9)	59 ± 13 (7)	72 ± 20 (8)	80 ±18 (10)
γ-Chlordane	25.0	—	95 ± 18 (10)	55 ± 6 (6) ‡	35 ± 7 (9) ‡
Endrin	6.25	—	48 ± 11 (9) ‡	31 ± 12 (10) ‡	37 ± 8 (9) ‡
Control	—	—	80 ± 20 (9)	79 ± 19 (7)	80 ±15 (9)

* Values in the table are time in min ± standard deviation during which righting reflex was absent. Animals received hexobarbital sodium, 125 mg/kg, intraperitoneally. Numbers in parentheses represent No. of animals per group.

† Insecticides were dissolved in corn oil and given by intraperitoneal injection to CF#1 mice weighing 15-25 g. Mice receiving DDT and Trichloro 237 were in one group and mice receiving γ-chlordane and endrin were in another group, hence 2 separate control groups.

‡ $P \leq 0.05$. For all other values $P > 0.05$.

§ 1,2,3,4,5,6,7,8,8-nonachloro-4,7-methane-3a,4,7,7a-tetrahydroindane. It is a non-insecticidal component of technical chlordane.

ured to follow the metabolic alteration of aminopyrine to 4-aminoantipyrine by the method of Brodie and Axelrod(5), and of p-nitrobenzoic acid to p-aminobenzoic acid by the method of Fouts and Brodie(6). The metabolite p-aminophenol was measured to follow the metabolism of aniline by a method of J. R. Gillette (personal communication). Aniline HCl, 0.5 μmoles per 5 ml incubate was used as substrate. Two g of solid NaCl were added to 4 ml of the incubation mixture. Extraction was made by shaking with 30 ml ether for 20 minutes. Twenty ml of the ether phase was then extracted with 4 ml 1 N Na_2CO_3 containing 1% phenol (the phenol must be added to the Na_2CO_3 just before use). One ml of bromine-Na_2CO_3 mixture (bromine water added to 1N Na_2CO_3 until a light yellow color appears) was added to 3 ml of the Na_2CO_3 phase. This mixture is read at 620 mμ on a Coleman spectrophotometer with 3 ml Na_2CO_3-phenol solution as the blank. The conditions of incubation, cofactors used and their concentrations were the same as reported by McLuen and Fouts(7).

To assess the acute effects of DDT, the compound was dissolved in corn oil at a concentration of 25 mg/ml and injected intraperitoneally into rats at doses of 25, 50 and 100 mg/kg. Injections were made daily for 3 days with controls receiving equivalent volumes of corn oil. Sleeping times and *in vitro* metabolisms were performed in the manner described above at 1, 8 and 15 days after the last injection of DDT.

To determine whether the organophosphorous insecticides caused an alteration of hepatic drug enzyme activity, certain of these agents were screened using hexobarbital sleeping times in mice. Doses used were chosen to be well below the LD_{50}s. CF #1 white mice, weighing 15-25 g, received single intraperitoneal injections of parathion, 2.5 mg/kg; paraoxon, 0.5 mg/kg; malathion, 25 mg/kg; ethyl-p-nitrophenyl benzenethionophosphonate (EPN), 12.5 mg/kg, and octamethyl pyrophosphoramide (OMPA), 5.0 mg/kg. Sleeping times were done at 1 and 3 hours, and 1, 3 and 8 days following treatment.

Statistical evaluation of data was made using the Student's "t" distribution as described by Snedecor(8). The level of significance chosen was $P = 0.05$.

Results. Table I shows that DDT, when administered as a single dose intraperitoneally to mice, does not lead to an effect on hexobarbital sleeping times, such as can be produced in the case of γ-chlordane, endrin and trichloro 237. It was because of this apparent lack of stimulation that feeding of DDT and repeated injections of this insecticide (as described below) were studied.

Significant increases in the rate of *in vitro* drug metabolizing enzyme activity were observed 2 weeks after rats were put on the diet

TABLE II. Effects of Chronic DDT Feeding on *in vivo* and *in vitro* Hepatic Drug Metabolizing Enzyme Activity in the Rat.

Drug substrate*	Time on diet 2 wk	1 mo	2 mo	3 mo	4 mo
Hexobarbital	6.70 ±.22‡	7.55 ±.28‡	7.77 ± .08‡	8.09 ± .72‡	6.98 ± .32‡
Control	4.15 ±.77	5.60 ±.78	3.79 ±1.54	4.96 ±1.02	3.79 ± .72
Aminopyrine	1.45 ±.23‡	1.71 ±.56‡	1.14 ± .31‡	.76 ± .30‡	1.78 ± .30‡
Control	.66 ±.14	.61 ±.18	.46 ± .12	.31 ± .08	.52 ± .17
Aniline	.14 ±.04	.29 ±.09‡	.54 ± .12‡	.56 ± .20	.23 ± .12
Control	.16 ±.04	.17 ±.05	.36 ± .06	.40 ± .07	.19 ± .09
p-Nitrobenzoic acid	4.44 ±.62‡	3.18 ±.70‡	4.43 ± .64‡	3.06 ± .96‡	3.66 ±1.07‡
Control	2.20 ±.43	1.21 ±.41	1.61 ± .13	1.25 ± .39	1.49 ± .98
Sleeping times†	19 ± 4‡	13 ± 5‡	18 ± 5 ‡	25 ± 9‡	25 ± 6‡
Control	44 ± 19	43 ± 11	41 ± 12	45 ± 11	42 ± 9

* Values in table indicate metabolism by supernatant fraction expressed as mean μmoles drug metabolized ± standard deviation per g liver (wet wt) in 2 hr (1 hr for p-nitrobenzoic acid). All groups had 7 experimental and 7 control animals.

† Time in min during which righting reflex is absent. See *Methods*.

‡ $P \leq 0.05$. For all other values $P > 0.05$.

(Table II). With the exception of the rate of metabolism of aniline, where there was no difference from control values, these increases were in the range of 2-fold. Enhancement of drug metabolism was seen for all pathways after 1 and 2 months on the diet. Again at 3 and 4 months, all pathways except aniline showed significant increases in rate of metabolism. Shortened hexobarbital sleeping times paralleled the alterations in *in vitro* enzyme activity at all times studied.

After 3 injections of DDT in rats, there were significant increases in rate of *in vitro* drug metabolizing enzyme activity at one day after the last injection for all pathways and at all 3 doses of DDT used (Table III). This is in contrast to the effects of a single dose of DDT on sleeping times in mice (Table I). This enhancement of enzyme activity was maintained at 8 days after the third injection with the exceptions of aniline at 25 mg/kg of DDT, and p-nitrobenzoic acid at 50 mg/kg of DDT where the differences were not statistically significant. Sleeping times were also depressed although differences at 8 days after the last injection were not significant due to a large variation in the control values.

Effects of the organophosphorous insecticides were characterized by a failure to shorten hexobarbital sleeping times, at least at the times studied (Table IV). Rather there was a definite trend toward lengthening of sleeping times by these agents, especially at 1 and 3 days after administration. The effects of EPN were most sustained, in that it produced significant increases in sleeping times at all times from 1 hour to 8 days after injection. OMPA was without effect at any of the times studied. Rosenberg and Coon(9) have also studied the effects of certain organophosphorous insecticides on hexobarbital sleeping times in mice. Their experimental design differed from ours in dosages used (theirs were higher) and the fact that they studied the effect of insecticides on sleeping times only at 2 hours after administration of the insecticide. They, as we, showed that only certain organophosphorous insecticides affected hexobarbital sleeping times. We also studied the effects of Parathion and paraoxon which were not studied by these authors.

Discussion. We have shown that when DDT is chronically fed to rats there is a stimulatory effect on hepatic microsomal drug metabolism, both *in vivo* and *in vitro*. This phenomenon was previously observed with several other chlorinated hydrocarbon insecticides after acute treatment(2,3).

The mechanism(s) by which these compounds exert their action has remained somewhat unclear. Ortega(10), however, has reported the effects of chronic DDT feeding on

the ultrastructure of rat liver cells. Of particular interest is his observation that the amount of smooth surfaced or agranular endoplasmic reticulum (SER) is greatly increased with conversely a partial displacement of rough surfaced or granular reticulum. Fouts (11) had previously reported that a variety of the drug metabolizing enzymes were primarily localized in the SER and in the microsomes derived from this membrane system.

These observations provide at least suggestive evidence for hypothesizing that the enhancement of enzyme activity by DDT is due to its causing an increase in the amount of SER with a consequent increase in the amount of the enzymes metabolizing the drugs studied. Further support for this line of reasoning is given by the investigations of Remmer(12) on the effects of phenobarbital on hepatic ultrastructure. Phenobarbital, like DDT, chlordane, etc., is also a non-specific "inducer" of drug enzyme activity. After phenobarbital pretreatment, there is a proliferation of SER, at least partially at the expense of rough surfaced reticulum. This mechanism of enzyme activity stimulation might be extrapolated to the other chlorinated insecticides such as chlordane, heptachlor, dieldrin, aldrin and endrin which have this stimulatory property.

A single dose of phenobarbital, 15 mg/kg, in rabbits will produce a stimulatory effect in 12 hours (unpublished observation) with this effect disappearing quite rapidly unless more drug is administered. The discrepancy between onset and duration of action of phenobarbital as compared to DDT and other chlorinated hydrocarbons would seem to be due in part to the physico-chemical properties of the insecticides. DDT is highly lipid sol-

TABLE III. Effects of 3 Daily Injections of DDT on *in vivo* and *in vitro* Hepatic Drug Metabolizing Enzyme Activity in the Rat.

Drug substrate*	Daily DDT dose (mg/kg)	Time after last injection: 1 day	8 days
Hexobarbital	25	7.15 ±.17 (4)‡	7.22 ± .12 (3)‡
	50	7.03 ±.44 (4)‡	7.58 ± .12 (3)‡
	100	6.98 ±.11 (4)‡	7.38 ± .40 (4)‡
	—	4.39 ±.41 (4)	3.33 ± .25 (3)‡
Aminopyrine	25	1.52 ±.24 (4)‡	2.48 ± .37 (3)‡
	50	1.57 ±.27 (4)‡	2.12 ± .33 (3)‡
	100	1.80 ±.28 (4)‡	2.41 ±1.32 (4)‡
	—	.58 ±.08 (4)	.61 ± .17 (3)
Aniline§	25	2.16 ±.34 (4)‡	.27 ± .11 (3)
	50	2.27 ±.17 (4)‡	.37 ± .05 (3)‡
	100	2.10 ±.20 (4)‡	.35 ± .06 (4)‡
	—	1.49 ±.22 (4)	.21 ± .07 (3)
p-Nitrobenzoic acid	25	3.66 ±.66 (4)‡	3.21 ± .45 (3)‡
	50	3.73 ±.69 (4)‡	4.15 ±2.14 (3)
	100	4.20 ±.53 (4)‡	4.66 ±1.72 (4)‡
	—	2.24 ±.33 (4)	1.65 ± .54 (3)
Sleeping times†	25	17 ± 5 (4)‡	23 ± 1 (3)
	50	16 ± 6 (4)‡	24 ± 7 (3)
	100	12 ± 3 (3)‡	19 ±10 (4)
	—	30 ± 4 (4)	43 ±22 (3)

* Values in table indicate metabolism by supernatant fraction expressed as avg μmoles drug metabolized ± standard deviation per g liver (wet wt) in 2 hr (1 hr for p-nitrobenzoic acid). Numbers in parentheses represent No. of animals per group.

† Time in min during which righting reflex is absent. See *Methods*.

‡ $P \leq 0.05$. For all other values $P > 0.05$.

§ At 1 day, concentration of the substrate, aniline HCl, was increased to 5.0 μmoles per 5 ml incubate. This larger substrate concentration caused a higher rate of metabolism. Qualitatively, the results with 5.0 μmoles are the same as with 0.5 μmoles substrate.

TABLE IV. Effects of Single Doses of Organophosphorus Insecticides on Hexobarbital Sleeping Times in Mice.*

Insecticide†	Dose (mg/kg)	Time after injection of insecticide: 1 hr	3 hr	1 day	3 days	8 days
Parathion	2.5	70 ± 23 (7)	68 ± 9 (7)	121 ± 20 (9)‡	118 ± 23(10)‡	57 ± 9 (8)
Paraoxon	1.0	74 ± 10(10)‡	62 ± 8 (9)	91 ± 16 (9)‡	105 ± 17(10)‡	71 ± 16(10)
OMPA	5.0	64 ± 7 (9)	68 ± 20(10)	83 ± 12 (9)	73 ± 9 (7)	71 ± 12 (7)
EPN	12.5	110 ± 4 (7)‡	104 ± 9 (8)‡	106 ± 19 (9)‡	108 ± 25 (7)‡	95 ± 22 (9)‡
Malathion	25.0	61 ± 13 (8)	64 ± 24 (8)	102 ± 13 (8)‡	78 ± 10 (9)	—
Control	—	58 ± 10 (7)	59 ± 10 (9)	72 ± 20 (8)	80 ± 18(10)	68 ± 14 (9)

* Values in table are the times in min ± standard deviation during which righting reflex was absent. Animals received hexobarbital sodium, 125 mg/kg, intraperitoneally. Numbers in parentheses represent No. of animals per group.

† Insecticides were dissolved in corn oil and given by intraperitoneal injection.

‡ $P \leq 0.05$. For all other values $P > 0.05$.

uble and as such is taken up rapidly by body fat. This "site of loss" especially after a low dose could account for the latency in onset, and at higher doses or after repeated administration, for the prolonged duration of action. Phenobarbital is not stored to as great an extent as the insecticides in fat and is more rapidly handled by the liver and routes of excretion, with subsequently more rapid inactivation and/or excretion. There may be of course other explanations for these differences.

In general, the organophosphorous agents that we tested had either no detectable effects on enzyme activity or produced slight inhibitory effects. It is possible that the lengthening of sleeping times seen with several of the organophosphorous insecticides may be due to other than effects of these compounds on hepatic drug metabolism.

It should be emphasized that not all drugs are inactivated or "detoxified" by metabolism, but rather some are converted from an inactive parent compound to an active or toxic metabolite. The organothiophosphates and phosphonates for example usually require metabolic conversion to give the active cholinesterase inhibitor(13). An increase in rate of metabolism of drugs made more toxic by metabolism could lead to increased effects or duration of action of these drugs. On the other hand, if metabolism does result in inactivation, the increases in rate of metabolism which we have observed after animals are treated with halogenated hydrocarbon insecticides would lead to decreased effects or duration of action as we have shown with hexobarbital and other drugs. In either event, animals treated with chemicals altering drug metabolism will often be pharmacologically "abnormal" and the usual drug dosages employed will not give the "usual" effects.

We are investigating the herbicides for possible effects on hepatic drug metabolizing enzyme activity.

Summary. DDT, 500 ppm, was fed in a lab chow diet to rats. Hexobarbital sleeping times and *in vitro* assays were measured after 2 weeks, 1, 2, 3 and 4 months on the diet to observe the effects of DDT on hepatic microsomal drug metabolizing enzyme activity. With the exception of aniline, which was not different from control at 2 weeks, 3 and 4 months, significant increases in rate of drug enzyme activity were seen at all times on the diet for the drugs studied. Shortened sleeping times paralleled these increases. Acute treatment of rats with DDT at 3 dose levels, each for 3 days, produced significant stimulatory effects on drug metabolism *in vitro* at both 1 and 8 days. Sleeping times were depressed at both times, although this depression was significant only at 1 day. In mice, acute injection of a single dose of DDT did not shorten hexobarbital sleeping times even at 3 days after treatment. Several of the organophosphorous insecticides were screened acutely with sleeping times in mice and produced either no effects or in several cases a prolongation of sleeping times. The proposal was made that the stimulatory effects of DDT on enzyme activity were due to its causing a proliferation of smooth surfaced endoplasmic reticulum in the liver cell, with a consequent increase in the amount of the drug metabolizing enzymes.

The authors wish to express their appreciation to Mr. John Mullen and Mrs. Roberta Pohl for technical assistance. We also wish to thank Professor W. L. Kerr, Drug Service, Univ. of Iowa, for DDT.

1. Report of The President's Science Advisory Committee, *Use of Pesticides,* May 15, 1963, U. S. Gov't Printing Office.

2. Hart, L. G., Shultice, R. W., Fouts, J. R., *Toxicol. Appl. Pharmacol.,* 1963, v5, 371.

3. Fouts, J. R., *Ann. N. Y. Acad. Sci.,* 1963, v104. 875.

4. Cooper, J. R., Brodie, B. B., *J. Pharmacol. Exp. Therap.,* 1955, v114, 409.

5. Brodie, B. B., Axelrod, J., *ibid.,* 1950, v99, 171.

6. Fouts, J. R., Brodie, B. B., *ibid.,* 1957, v119, 197.

7. McLuen, E. F., Fouts, J. R., *ibid.,* 1961, v131, 7.

8. Snedecor, G. W., *Statistical Methods,* 5th ed., Iowa State Univ. Press, Ames, 1956.

9. Rosenberg, P., Coon, J. M., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 650.

10. Ortega, P., *Fed. Proc.,* 1962, v21, 306.

11. Fouts, J. R., *Biochem. Biophys. Res. Commun.,* 1961, v6, 373.

12. Remmer, H., Merker, H. J., *Klin. Wochensch.,* 1963, v41, 276.

13. Diggle, W. M., Gage, J. C., *Nature,* 1951, v168, 998.

Received July 25, 1963. P.S.E.B.M., 1963, v114.