Decreased Aniline Hydroxylase Activity in Japanese Quail Due to Dietary DDT

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Activities of hepatic microsomal aniline hydroxylase (AH) and *N*-demethylase (ND) were determined in Japanese quail fed 200 ppm of DDT. AH activity in livers from newly hatched quail produced by strains fed DDT was significantly less than that of a control strain. However, AH activity of progeny from strains previously fed DDT was the same as that of the controls after a diet containing no DDT was fed for 22 days after hatch. Hepatic AH and ND activities were depressed markedly when quail were fed 200 ppm of DDT. In contrast, Cytochrome P_{450} concentration in the microsomes was increased significantly by DDT. The magnitude of the response to dietary DDT was the same in quail of the control strain and those selected for low mortality. DDT and DDE were found to inhibit AH activity when added to an *in vitro* medium at 10^{-7} *M* or more. The depressing effect of both DDT and DDE on AH activity appeared to be by way of competitive inhibition.

The influence of DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane] on liver metabolic activity has been investigated extensively during the past decade. In 1963 Hart and Fouts (1963) reported that DDT stimulated hexobarbital metabolism in the rat. Subsequently, Conney *et al.* (1965) and Hoffman *et al.* (1970), among others, reported similar findings with mammals. Peakall (1967, 1970) extended these observations to birds when he observed an increase in the rate of steroid metabolism by hepatic microsomes of pigeons and doves treated with DDT. However, Bitman *et al.* (1971) found that the ability of quail and rats to metabolize pentobarbital following the administration of DDT differed. DDT increased the rate of pentobarbital metabolism by rats, while this insecticide depressed the rate of metabolism of the same drug by quail.

Suppression in the *in vitro* activity of certain hepatic microsomal enzymes as a result of DDT administration has also been observed. Sell *et al.* (1971) found that DDT decreased the activity of hepatic aniline hydroxylase of chickens. Similarly, Gillett and Arscott (1969) reported that hepatic microsomal epoxidase activity was depressed markedly in quail fed DDT.

Considerable information has been obtained about the development of resistance to DDT among insects (Perry and Hoskins, 1950; Sternburg *et al.*, 1954; Terriere, 1968). There have also been reports that mice develop resistance to DDT after the exposure of several generations to this insecticide (Ozburn and Morrison, 1964; Barker and Morrison, 1966). Researchers at the University of Wisconsin have studied the development of tolerance to dietary DDT by Japanese quail, *Coturnix coturnix japonica* (Poonacha, 1971). The criterion used for selection was survival (low mortality) during a 30-day period of feeding 200 ppm of DDT, beginning immediately after hatch. The ninth generation of the quail populations from the Wisconsin project was made available to us to determine whether or not changes in the activities of certain hepatic mixed-function oxidases may have occurred as a consequence of this selection.

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In addition, *in vitro* experiments were performed to obtain information about the way in which DDT may exert its effect on the activity of aniline hydroxylase.

METHODS

Experiment 1. Eggs produced by three strains of quail were obtained from the Department of Poultry Science, University of Wisconsin. Two of the three strains of quail used were the culmination of eight generations of selection for resistance to the lethal effects of 200 ppm of DDT (technical grade). Strain N of the "DDT-resistant" quail was established by selecting and randomly mating, with restriction on inbreeding, survivors of the DDT challenge. Strain A differed from Strain N only in that emphasis was placed on the mating of families which demonstrated a relatively high level of survival of DDT treatment. Concurrent with these selections, a control strain of quail which was not exposed to dietary DDT was carried through the same number of generations of random mating. Further details of the selection procedures and the results may be obtained from Poonacha (1971).

The eggs produced by the eighth generation were incubated at North Dakota State University. The identities of the three strains of quail were maintained, and all quail were weighed immediately after hatching. Four groups of ten quail were selected randomly from each of the control, N, and A strains, and were killed for subsequent use in hepatic enzyme assays. The remaining quail of each strain were divided randomly into four groups and each group was assigned to a pen located in a battery brooder. Two pens of quail from each strain were fed a control diet formulated to contain the nutrients essential for growth. The remaining two pens of quail from each strain were fed the control diet to which 200 ppm of *p,p'*-DDT had been added. The quail were given feed and water *ad libitum* for 22 days. At that time all quail were weighed and eight were randomly selected from each experimental group to provide liver samples for enzyme assay.

The procedure for procuring and processing liver samples was the same for quail killed immediately after hatch and those killed at 22 days of age. At hatch, livers from ten quail were pooled to obtain sufficient sample for analysis, while at 22 days livers from four quail provided enough sample. Sex of the quail killed at 22 days of age was determined by examination of the gonads. Representation of males and females was approximately equal in all groups. After the birds were killed by decapitation, the livers were removed, weighed, and

Table I. Average Weight of Quail at Hatch and at 22 Days of Age, and Mortality as Influenced by Dietary DDT

	Av weight per quail, g	Gain per quail to 22 days, g[a]		Mortality, %	
		No DDT	200 ppm DDT	No DDT	200 ppm DDT
Control	9.7	66.8	67.2	20	32
Strain N	10.0	66.0	66.8	0	3
Strain A	10.0	62.8	65.8	9	18
Standard error of the means		±1.8		±5.6	

[a] The number of quail per experimental group ranged from 33 to 41.

Table II. Liver Weight, and Protein and Cytochrome P_{450} Concentrations of Liver Microsomes of Progeny of Quail Fed DDT and Selected for Low Mortality

	Liver wt, mg/quail	Microsomal protein, mg/g of liver	Cytochrome P_{450}	
			Change in o.d./50 mg protein	Relative change[a]
Control	142	18.5	0.77	100
Strain N	144	18.6	0.90	117
Strain A	136	20.0	0.86	112
Standard error of the means	±3	±1.0	±0.06	

[a] Expressed as a percent of the control group.

Table III. Aniline Hydroxylase and *N*-Demethylase Activities of Hepatic Microsomes of Progeny of Quail Fed DDT and Selected for Low Mortality

	Enzyme activities[a]			
	Aniline Hydroxylase		Aminopyrine *N*-demethylase	
	μg PAP/mg protein/hr	Relative[b] activity	μg HCHO/mg protein/hr	Relative activity
Control	25.2[1c]	100	3.04	100
Strain N	18.2[2]	72	3.36	110
Strain A	18.1[2]	72	2.86	94
Standard error of the means	±1.0		±0.18	

[a] Enzyme activities expressed on the basis of micrograms of end products, *p*-aminophenol (PAP) or formaldehyde (HCHO), formed during a 1-hr incubation period. [b] Expressed as a percent of the control group. [c] Treatment means followed by different superscript numbers are significantly different at $p \leq 0.01$.

homogenized in ice-cold 0.25 *M* sucrose. The homogenate was centrifuged for 30 min at 10,000 × *g* and 4°C, and the resultant supernatant was centrifuged for 60 min at 104,000 × *g* and 4°C to obtain the microsomal fraction. The microsomes were resuspended in 5 ml of 0.1 *M* phosphate buffer (mono- and disodium phosphate mixture, pH 7.4).

The activities of aniline hydroxylase and *N*-demethylase were determined essentially as described by Schenkman *et al.* (1967). TES [*N*-tris(hydroxymethyl)methyl-2-amino ethane sulfonic acid] buffer, 50 m*M* and pH 7.4, was used instead of Tris buffer. The enzyme assay system was incubated aerobically at 40°C for 60 min before trichloroacetic acid was added to stop the reaction. Hydroxylase activity was determined by the amount of aniline hydroxylated to *p*-aminophenol, and *N*-demethylase activity was determined by the amount of formaldehyde released from the substrate aminopyrine.

Cytochrome P_{450} was determined by the method described by Kato (1966), except that 10 mg of technical sodium hydrosulfite was used to reduce the assay system, and TES buffer, 50 m*M* and pH 7.4, replaced the phosphate buffer. The difference between absorption at 450 and 500 nm was used to measure the amount of Cytochrome P_{450} present.

Protein concentration in the microsomal preparations was determined by the method of Lowry *et al.* (1951). Statistical analysis of the data was performed according to Snedecor (1956). Significance was assessed at probabilities of 1% or less.

Experiment 2. This experiment was conducted to obtain additional information about the effects of DDT on microsomal aniline hydroxylase activity of quail fed this insecticide and also to determine the influence of DDT on aniline hydroxylase activity when added to *in vitro* assay mixtures. Aniline hydroxylase was selected for study, since its activity had been suppressed consistently in livers of quail and chickens fed DDT.

Newly hatched quail were divided into two treatment groups and fed to 4 weeks of age either a control ration or one containing 200 ppm of *p,p'*-DDT. At 4 weeks of age female quail selected randomly from each treatment group were killed and their livers processed as described for experiment 1.

The microsomes isolated were pooled within treatment groups to comprise a large composite preparation representing each group. Prior to assay for aniline hydroxylase activity, an aliquot of each microsomal preparation was centrifuged at 104,000 × *g* and resuspended in 0.1 *M* phosphate buffer three successive times. Following this "washing," aniline hydroxylase activity was determined for the "washed" and "unwashed" microsomes representing both the control and DDT-fed quail. The procedures used for aniline hydroxylase assay were as described above.

An assay for possible competitive inhibition of aniline hydroxylase activity by DDT and DDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene] was also conducted with the "unwashed" control microsomal preparation. A series of incubation mixtures was prepared which contained the substrate aniline at concentrations of 0, 0.085, 0.142, 0.284, 0.569, and 1.138×10^{-2} *M* of incubation medium. These mixtures were incubated 1 hr at 40°C with and without DDT or DDE added to obtain a concentration of 10^{-3} *M*. DDT or DDE was added to the incubation mixture in 0.1 ml of 95% ethanol, and an ethanol-blank containing 0.1 ml of ethanol was also prepared.

Competitive inhibition of aniline hydroxylase by 10^{-3} *M* DDT and DDE was observed. Therefore, the influence of lower levels of these chlorinated hydrocarbons on activity of this enzyme was determined. Concentrations of DDT or DDE used were 0, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, and 10^{-3} *M* of incubation mixture. In one series involving both DDT and DDE, the concentration of aniline was 0.569×10^{-2} *M*, while in another utilizing only DDE, the concentration of aniline was 0.142×10^{-2} *M*.

Samples of the washed and unwashed microsomal preparations were also analyzed for DDT, DDE, and DDD [1,1-dichloro-2,3-bis(*p*-chlorophenyl)ethane]. The chlorinated hydrocarbons were extracted from aliquots of the microsomes with hexane and were quantitated by electron capture gas chromatography according to the procedure of Crosby and Archer (1966).

RESULTS

Experiment 1. The proportion of incubated eggs which hatched was relatively low (40 to 50%) but was essentially the same for the three strains of quail. The average weight of quail at hatch was nearly the same for the three strains,

and average gain in body weight from hatch to 22 days of age was not affected significantly by parental treatment or by feeding *p,p'*-DDT after hatch (Table I). Mortality was highly variable within, as well as among, treatment groups. Analysis of variance according to a complete factorial arrangement showed a significant effect of strain with regard to mortality. This was the result of the relatively high mortality in the control strain as compared with strains N and A. Although 200 ppm of dietary DDT appeared to increase mortality, particularly in the control and A strains, this main effect of DDT was not significant ($p \geq 0.10$).

The weight and microsomal protein concentration of the liver and the concentration of liver Cytochrome P_{450} did not differ significantly among the three strains at hatch (Table II). However, the *in vitro* activity of hepatic aniline hydroxylase was significantly less for quail from parents fed DDT and selected for low mortality (strains N and A) than that of control quail (Table III). The magnitude of difference from the control strain was the same for both strains N and A, as shown by the identical relative activities. *N*-Demethylase activity did not differ significantly among strains at hatch.

Average weight of liver and microsomal protein concentration in liver at the end of the 22-day growing period were not affected significantly by feeding 200 ppm of DDT after hatch, nor did they differ significantly among strains (Table IV). Changes in hepatic microsomal enzyme activities and Cytochrome P_{450} concentration were related only to the feeding of DDT after hatch (Tables IV and V) and not to previous history of the strains. Hydroxylase activity was depressed markedly by feeding 200 ppm of dietary DDT from hatch to 22 days of age, regardless of the strain of quail involved. Concurrently, DDT caused a significant increase in Cytochrome P_{450} concentration of liver microsomes, again irrespective of parental history.

Data obtained after 22 days of feeding a diet containing no DDT to progeny from strains N and A showed that the relative activities of the adaptative enzymes studied and the concentration of Cytochrome P_{450} of hepatic microsomes were nearly the same as the control group. The relative enzyme activities of the three strains fed 200 ppm of DDT were similar to one another, but were less than those activities observed in their counterparts not fed DDT.

Experiment 2. Dietary DDT decreased aniline hydroxylase activity and increased Cytochrome P_{450} of hepatic microsomes but did not affect the protein concentration in the microsomal preparations (Table VI). Washing the microsomes three times with 0.1 *M* phosphate buffer decreased the protein concentration of the microsomes, but the decreases were of relatively the same magnitude for control and DDT-treated quail. The aniline hydroxylase activity and Cytochrome P_{450} concentration per mg of microsomal protein were increased significantly by washing.

A small concentration of DDT, DDE, and DDD was found in the microsomal preparation of the control quail (Table VI), probably reflecting the widespread occurrence of these chlorinated hydrocarbons in the environment. Feeding quail 200 ppm of DDT markedly increased the amounts of DDT, DDE, and DDD in microsomes. In addition, the relative concentrations of these substances were changed when DDT was fed. Of the total amount present, the microsomes of control quail

Table IV. Liver Weight, and Protein and Cytochrome P_{450} Concentrations in Liver Microsomes of 22-Day-Old Quail

	Liver wt, g/quail	Microsomal protein, mg/g liver	Cytochrome P_{450}: Change in o.d./50 mg protein	Cytochrome P_{450}: Relative change[a]
Control	1.99	26.5	0.68[1b]	100
Control + 200 ppm DDT	2.14	24.2	1.10[2]	162
Strain N	2.15	24.2	0.71[1]	104
Strain N + 200 ppm DDT	2.17	26.3	1.11[2]	163
Strain A	1.99	26.3	0.68[1]	100
Strain A + 200 ppm DDT	2.24	25.6	1.20[2]	176
Standard error of the means	±0.40	±1.1	±0.07	

[a] Expressed as a percent of the controls. [b] Treatment means followed by different superscript numbers are significantly different at $p \leq 0.01$.

Table V. Activities of Hepatic, Microsomal Aniline Hydroxylase, and *N*-Demethylase of 22-Day-Old Quail

	Enzyme activities[a]			
	Aniline hydroxylase		Aminopyrine *N*-demethylase	
	μg PAP/mg protein/hr	Relative[b] activity	μg HCHO/mg protein/hr	Relative activity
Control	16.0[1c]	100	2.14[1]	100
Control + 200 ppm DDT	8.8[2]	55	1.54[2]	72
Strain N	15.8[1]	99	2.06[1]	96
Strain N + 200 ppm DDT	7.9[2]	49	1.34[2]	63
Strain A	17.0[1]	106	2.23[1]	104
Strain A + 200 ppm DDT	7.8[2]	49	1.42[2]	66
Standard error of the means	±0.97		±0.11	

[a] Enzyme activities expressed on basis of micrograms of end products, *p*-aminophenol (PAP) or formaldehyde (HCHO), formed during a 1-hr incubation period. [b] Expressed as a percent of the controls. [c] Treatment means followed by different superscript numbers are significantly different at $p \leq 0.01$.

Table VI. The Effects of Dietary DDT and Washing of Microsomal Preparations on Protein, Cytochrome P_{450}, and DDT Concentrations, and on Aniline Hydroxylase Activity of Quail Microsomes

Treatment group	Microsomal protein, mg/g liver	Aniline hydroxylase[a]: μg PAP/mg protein/hr	Aniline hydroxylase[a]: Relative activity[b]	Cytochrome P_{450}: Change in o.d./25 mg protein	Cytochrome P_{450}: Relative change	DDT,[c] ng/mg protein
Control	20.1	23.6[2d]	100	0.53[1]	100	12
Control, washed	11.4	35.2[1]	149	0.82[2]	155	22
200 ppm DDT	20.9	9.1[4]	39	0.84[2]	158	175
200 ppm DDT, washed	13.5	13.9[3]	59	1.05[3]	198	310

[a] Aniline hydroxylase activity expressed on basis of micrograms of *p*-aminophenol (PAP) formed during a 1-hr incubation period. [b] Expressed as percent of controls. [c] Value includes total of DDT, DDE and DDD. [d] Treatment means followed by different superscript numbers are significantly different at $p \leq 0.01$.

Table VII. Effect of Including DDT or DDE in Incubation Mixtures on the *in vitro* Activity of Hepatic Aniline Hydroxylase from Quail

Concentration of DDT or DDE	Relative activity of aniline hydroxylase[a] DDT[b]	DDE[b]	DDE[c]
	(Expressed as % of blank)		
O-blank	100	100	100
Ethanol-blank	97	98	100
10^{-7} M	82[1d]	84[1]	46[1]
10^{-6} M	68[1]	73[1]	44[1]
10^{-5} M	70[1]	67[1]	39[1]
10^{-4} M	66[1]	61[1]	34[1]
10^{-3} M	57[1]	55[1]	30[1]

[a] Expressed as percent of O-blank controls. [b] The concentration of aniline in the incubation mixture was 5.69×10^{-3} M. [c] The concentration of aniline in the incubation mixture was 1.42×10^{-3} M. [d] Means followed by the superscript number differ significantly from the O-blank at $p \leq 0.01$.

contained 43% DDT, 44% DDE, and 13% DDD, while the distribution in microsomes of DDT-fed quail was 61% DDT, 22% DDE, and 17% DDD. The increased proportion of DDT in the latter case probably reflects an influx of DDT into the liver at a rate greater than that which could be metabolized to DDE. Washing the microsomes three times with phosphate buffer did not reduce the concentration of DDT or its metabolites. Rather, the concentrations of DDT, DDE, and DDD per mg of protein were increased by washing. The magnitude of increase was relatively the same for microsomes from control and DDT-fed quail.

The addition of 10^{-3} M DDT or DDE to incubation mixtures containing varying levels of aniline decreased hydroxylase activity. Graphically plotting the data as in Figure 1 showed that DDT and DDE were acting as competitive inhibitors of aniline hydroxylase. The lines were drawn on the basis of least squares analysis. Extrapolation of each line was used to estimate the Km's. The Km for the reaction with aniline alone was 0.196×10^{-2} M, while those for mediums containing DDT or DDE were 1.0×10^{-2} and 0.91×10^{-2} M, respectively.

Since the concentrations of DDT and DDE used in the competitive inhibition assays were relatively high (10^{-3} M), it seemed advisable to determine the effects of lower concentrations of DDT and DDE on aniline hydroxylase activity. The addition of DDT or DDE at a concentration of 10^{-7} M significantly decreased aniline hydroxylase activity when the incubation mixture contained 0.569×10^{-2} M of aniline per liter (Table VII). Relative hydroxylase activity was further depressed with each succeeding increment increase in DDT or DDE level. When aniline was used at 0.142×10^{-2} M, the addition of 10^{-7} M of DDE caused a more severe depression in hydroxylase activity than observed previously.

DISCUSSION

At time of hatch, hepatic aniline hydroxylase activity of progeny from strains of quail fed 200 ppm of DDT was markedly lower than that of progeny from a control strain. Apparently, this difference between strains at hatch was caused by a carryover of DDT in the eggs produced by quail fed this insecticide. The data of Smith *et al.* (1969) and Wesley *et al.* (1969) showed that considerable DDT would be expected in the egg yolk of reproducing quail even though they had not received dietary DDT for 5 to 6 weeks. It appears that sufficient DDT was present in the eggs from strains N and A to depress aniline hydroxylase activity in hatchlings but not

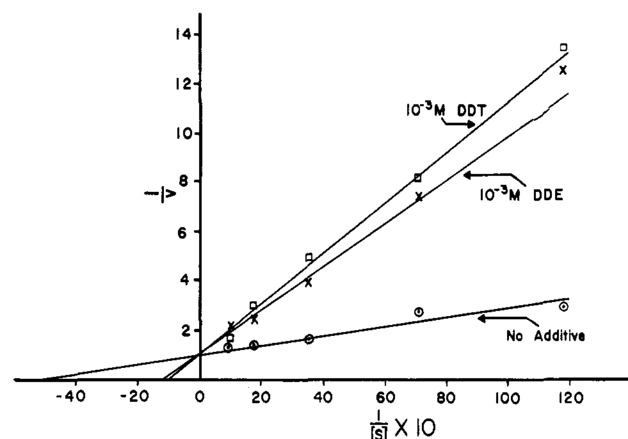

Figure 1. The influence of 10^{-3} M DDT or 10^{-3} M DDE on the *in vitro* activity of aniline hydroxylase. The reciprocals of aniline concentration ([S]) and of reaction velocity (v) were plotted to illustrate competitive inhibition of hydroxylase activity by DDT and DDE

enough was present to affect N-demethylase activity or Cytochrome P_{450} concentration detectably.

By the end of the 22-day growing period of experiment 1, the hepatic aniline hydroxylase activity of quail from strains N and A had returned to a level equal to that of their respective controls when these groups were not fed DDT. Gillett (1969) has shown that dietary DDT increased microsomal epoxidase activity of rat livers and that the activity of this mixed-function oxidase rapidly returned to the control level following withdrawal of DDT from the diet. It appears that aniline hydroxylase activity follows the pattern of returning to a "normal" level subsequent to termination of exposure of quail to DDT even though the effect of DDT on hydroxylase activity of the liver was opposite to that on epoxidase in rat liver.

It is of interest that in the current study the activities of aniline hydroxylase and N-demethylase of microsomes from control quail were much lower at 22 days of age than at time of hatching (Tables III and V). Similarly, Gillett and Arscott (1969) reported that microsomal epoxidase activity of quail liver decreased with age. These observations differ from those made with rats where the activities of some microsomal enzymes and the rate of drug metabolism were found to increase with age (Conney, 1967; Gillett, 1969).

These data also indicate that repeated, periodic exposure of quail to DDT over a number of generations did not cause a "permanent" change in the activity of the mixed-function oxidases studied. Similar results were obtained by Guthrie *et al.* (1971), who found that mice surviving an intraperitoneal injection of an LD_{50} dose of DDT once each generation for 14 successive generations had essentially the same relative activity of hepatic aniline hydroxylase as the controls.

The results obtained in both experiments with quail agree with those of Sell *et al.* (1971) in that dietary DDT decreased the activity of hepatic aniline hydroxylase. Dietary DDT also decreased N-demethylase activity of quail in the current study. Gillett and Arscott (1969) reported that the activity of hepatic epoxidase was depressed in mature quail fed 100 ppm of DDT. Stephens *et al.* (1970) also observed that DDT decreased the rate of phenobarbital metabolism by chickens but were unable to detect any consistent effect of this insecticide on hepatic O- or N-demethylase activity.

The means by which the effects of dietary DDT on aniline hydroxylase or N-demethylase activity are mediated is not

known. It was found in experiment 2 that washing hepatic microsomes with a phosphate buffer did not change the relative activity of aniline hydroxylase in microsomes of quail fed DDT as compared with the controls, indicating that the substance(s) responsible for depressed hydroxylase activity was not removed by washing with a polar solvent. Washing also failed to decrease the concentration of DDT or its metabolites in the microsomes. This might be expected, though, since DDT, DDE, and DDD are lipophilic and would not be removed easily by washing with a polar solvent.

That DDT or its metabolites remaining in the microsomes may be inhibited hydroxylase activity was shown by the results of experiment 2. When added to the assay medium at a concentration of 10^{-3} *M*, both DDT and DDE inhibited aniline hydroxylase activity in a manner corresponding to the classical description of competitive inhibition (West *et al.*, 1966). Hart *et al.* (1963) found that chlordane at concentrations up to 1.7×10^{-3} *M* in the incubation mixture did not affect hexobarbital or aminopyrine metabolism by rat microsomal preparations. However, Welch *et al.* (1967) reported that DDT inhibited the metabolism of testosterone to more polar metabolites by liver microsomes of rats when it was included in an *in vitro* medium at a concentration of 10^{-4} *M;* however, they did not determine the nature of the inhibition.

Further investigation in experiment 2 showed that concentrations of DDT or DDE as low as 10^{-7} *M* in the assay medium markedly reduced aniline hydroxylase activity. It was also found that hepatic microsomes of quail fed 200 ppm of DDT for 4 weeks contained sufficient DDT (plus DDE and DDD) to result in a final concentration of 0.5×10^{-6} *M* in the hydroxylase assay mixture, a level approaching that shown to inhibit hydroxylase activity when added *in vitro*. Thus, it seems that the DDT concentration in microsomes of quail fed 200 ppm of DDT may be sufficient to depress hydroxylase activity by way of competitive inhibition.

The observations reported herein that dietary DDT depressed the activities of enzymes representing the hepatic mixed-function oxidases are contrary to those made with rats (Hart and Fouts, 1963; Hoffman *et al.*, 1970; Gillett, 1969) but coincide with those of Bitman *et al.* (1971). Bitman *et al.* (1971) found that dietary DDT increased pentobarbital-induced sleeping time in quail, suggesting a suppression in activity of some mixed-function oxidases. In the same study they observed that DDT markedly decreased pentobarbital-induced sleeping time in rats. Quail were observed to consume more feed per unit body weight and had a lower concentration of body fat than rats. As a consequence, the level of *p,p'*-DDT and *p,p'*-DDE which accumulated in body fat of quail was ten times that of rats.

The relationship that the different concentrations of DDT in body fat of quail, as compared with rats, may have with changes in microsomal enzyme activities of the liver is not known. However, on the basis of relative accumulations of *p,p'*-DDT and *o,p'*-DDT in body fat and the relative effects of these two forms of DDT on pentobarbital metabolism, Bitman *et al.* (1971) stated that the changes in metabolic activity of the liver caused by DDT were more closely related to intake than to body burden of the insecticide. Gillett (1969) also found that changes in the activity of liver epoxidase of rats in response to DDT seemed to be determined by the concentration of DDT in the diet and was not related to body burden of DDT. However, it seems unlikely that differences in rates of DDT consumption alone would cause completely opposite effects on the activities of microsomal enzymes of quail, as compared with those of rats.

It is possible that differences in the relative effects of dietary DDT on specific metabolic activities of livers from birds and rats may be a reflection of species differences in the rate and/or route of DDT metabolism. Quail apparently metabolize *o,p'* isomers of DDT more readily than rats (Bitman *et al.*, 1971) and relatively high concentrations of DDE have been found in tissues and eggs of birds (Bitman *et al.*, 1971; Risebrough, 1969).

Cytochrome P_{450} is considered to be an important component of the electron transfer system associated with the microsomal mixed-function oxidases, and changes in enzyme activities and Cytochrome P_{450} concentration might be expected to parallel one another. The results of the experiments reported here show that DDT affected Cytochrome P_{450} in a manner opposite to its effect on the activity of aniline hydroxylase or *N*-demethylase and illustrate that specific mixed-function oxidases and Cytochrome P_{450} do not necessarily respond similarly to DDT treatment.

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