Aniline Hydroxylase, *N*-Demethylase, and Cytochrome P_{450} in Liver Microsomes of Hens Fed DDT and Dieldrin

Jerry L. Sell,* Kenneth L. Davison,[1] and Robert L. Puyear

The insecticides *p,p'*-DDT and dieldrin were fed to White Leghorn laying hens for 12 weeks and the effects on liver microsomal enzymes were determined. Liver aniline hydroxylase activity was reduced significantly ($P \leq 0.01$) by feeding diets containing 100 or 200 ppm *p,p'*-DDT. *p,p'*-DDT did not significantly affect *N*-demethylase activity or cytochrome P_{450} concentration. Dieldrin fed at 20 ppm significantly ($P \leq 0.01$) increased liver microsomal demethylase activity and cytochrome P_{450} but had no effect on hydroxylase. Feeding 10 ppm dieldrin had no noticeable effect on the activities of the liver microsomal enzymes studied. There were highly significant ($P \leq 0.01$) negative correlation coefficients between hydroxylase and *N*-demethylase activities and cytochrome P_{450} concentration, and average length of clutch of individual hens. Since length of clutch indicates intensity of reproductive activity (egg laying), these results suggest a relationship between endocrine secretions associated with reproduction and activities of enzymes involved in the metabolism of these secretions and other compounds.

Induction of mixed-function oxidase enzymes of liver microsomes following the administration of certain drugs and insecticides has been reported in mammals (Conney *et al.*, 1965; Hart and Fouts, 1963; Kuntzman, 1969) and birds (Peakall, 1967, 1970; Puyear *et al.*, 1970). Additional research has shown that liver enzyme systems which metabolize the insecticides DDT and dieldrin appear to closely resemble those which metabolize steroid hormones (Kuntzman, 1969). The possibility that some chlorinated hydrocarbons and steroid hormones may be alternate substrates for common enzyme systems has led to much concern recently with regard to the health of man and animals. It has been suggested that the decline in population of some species of birds has been due to a derangement in hormone metabolism as a consequence of chronic ingestion of chlorinated hydrocarbons such as DDT or dieldrin (Heath *et al.*, 1969; Peakall, 1970).

The data reported here show that the activities of certain mixed-function oxidases of liver microsomes are changed by feeding DDT and dieldrin to female chickens. Also, an inverse relationship was observed between the *in vitro* activity of liver mixed-function oxidases and length of clutch of the hens.

EXPERIMENTAL

Sixty White Leghorn hens, 28 weeks of age, were housed in individual cages. Following a 2-week adjustment period, the hens were divided randomly into five groups of 12 hens each. Individual hens served as the experimental units.

A nutritionally balanced laying hen diet obtained commercially was used for all hens. The chlorinated hydrocarbons, dieldrin or DDT, were added to the diet to obtain the following treatments: Control, 10 ppm dieldrin [1,2,3,4,10,10,-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo*:5,8-*exo*-dimethanonaphthalene (HEOD)], 20 ppm dieldrin, 100 ppm *p,p'*-DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)-ethane], 200 ppm *p,p'*-DDT. To equalize feed intake among all hens, 90 g of diet was fed to each hen daily throughout the experiment. This amount was found previously to be completely consumed within each 24-hr period. The hens were fed the experimental rations up to the time they were killed.

Individual egg production records were kept daily during the 12-week experiment. At the end of 12 weeks the hens were killed by exsanguination, and the livers removed and weighed. Ten gram samples of liver were homogenized in ice-cold 0.15 *M* KCl and the homogenate was centrifuged for 30 min at 10,000 × *g* at 4° C. Eleven milliliters of 10,000 × *g* supernatant was centrifuged for 60 min at 104,000 × *g* and 4° C to obtain the microsomal fraction, and the microsomes were resuspended in 5 ml of 0.15 *M* KCl.

The activities of aniline hydroxylase and *N*-demethylase were determined essentially as described by Schenkman *et al.* (1967). TES (*N*-tris[hydroxymethyl]methyl-2-aminoethane sulfonic acid) buffer, 50 mM and pH 7.4, was used instead of the Tris buffer. The enzyme assay system was incubated aerobically at 37° 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 4 mM NADPH was used to reduce the assay system and TES buffer at 50 mM 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.

Nitrogen (*Ass. Offic. Agr. Chem.*, 1960) and glycogen (Siefter *et al.*, 1950) contents of the livers were also determined. Statistical analysis of the data was performed according to Snedecor (1956). Significance was assessed at probabilities of 5% or less.

RESULTS AND DISCUSSION

Liver weights were not affected significantly ($P \leq 0.10$) by feeding dieldrin or DDT for 12 weeks (Table I). This was true whether liver weight was expressed as g of total liver per hen or as a percent of body weight. Similarly, the concentrations of nitrogen and glycogen in liver were not changed noticeably by dieldrin or DDT (Table I).

Animal Science and Zoology Departments, North Dakota State University, Fargo, N.D. 58102

[1] U.S. Department of Agriculture, Metabolism and Radiation Research Laboratory, Animal Husbandry Research Division, ARS, Fargo, N.D. 58102

Table I. The Effect of Dietary Dieldrin and DDT on Liver Weight, Liver Nitrogen, and Liver Glycogen

Ration	Number of Observations	Average Liver Weight (g/hen)	Liver Nitrogen[a] (%)	Liver Glycogen[a] (mg/g)
Control	12	42 ± 2.5[b]	2.39 ± 0.10	28.2 ± 3.1
10 ppm dieldrin	11	38 ± 1.2	2.54 ± 0.10	28.3 ± 4.3
20 ppm dieldrin	11	37 ± 1.4	2.66 ± 0.08	30.8 ± 3.9
100 ppm *p,p'*-DDT	12	42 ± 1.8	2.44 ± 0.08	28.7 ± 3.3
200 ppm *p,p'*-DDT	12	40 ± 1.4	2.51 ± 0.07	30.8 ± 4.2

[a] Expressed on a wet weight basis. [b] Treatment means ± standard error.

Hydroxylase and *N*-demethylase activities and cytochrome P_{450} concentration of liver microsomes were altered by feeding the chlorinated hydrocarbons, but in a variable manner (Table II). Hydroxylase activity of livers from hens fed 100 or 200 ppm DDT was significantly ($P \leq 0.01$) lower than that of livers from hens fed the control diet or dieldrin. Dieldrin fed at 20 ppm significantly ($P \leq 0.01$) increased liver *N*-demethylase and P_{450} activity as compared with all other treatments.

These observations support previous findings that dieldrin and DDT affected the activity of certain liver enzymes (Kuntzman 1969; Peakall, 1967, 1970; Welch *et al.*, 1967). In addition, our results demonstrate that the effects of each insecticide may be fairly specific in the laying hen. For example, dieldrin increased the activity of *N*-demethylase and amount of P_{450}, but did not affect hydroxylase activity. In contrast, DDT reduced hydroxylase activity considerably.

Peakall (1967) reported that the *in vitro* metabolism of testosterone and progesterone by liver enzymes of male and female pigeons was increased markedly when DDT or dieldrin was fed. But there appeared to be a difference in the metabolites of these hormones formed as a result of DDT administration in comparison with dieldrin, so Peakall suggested that different microsomal enzyme systems were affected by the two compounds.

Our results also show that DDT and dieldrin affect liver enzyme systems differently. However, our observations with DDT are opposite to those of Peakall (1967, 1970) in that DDT reduced hydroxylase activity in hens, while Peakall reported an apparent induction of hydroxylase activity in pigeons and ring doves. These contradictory findings may be related to major differences between our approach to the problem and that of Peakall. Peakall fed 10 ppm DDT to pigeons for 1 week (Peakall, 1967), and 10 ppm DDT to ring doves for about 5 weeks (Peakall, 1970), while we fed 100 and 200 ppm DDT to chickens for 12 weeks. Little information is available concerning the effects that differences among species, levels of dietary DDT, or duration of treatment may have on liver microsomal enzyme activities. Thus, correlative interpretation of the above data is difficult. Nevertheless, it is evident that species used as well as levels of DDT fed and length of treatment are important factors to consider in assessing the effects of DDT on liver mixed-function oxidases.

Kuntzman *et al.* (1964) postulated that the same hydroxylases in liver microsomes metabolized steroids and many foreign compounds. This postulation might be used to further the contradiction between our data and those of Peakall (1967, 1970). We used the *in vitro* hydroxylation of aniline to *p*-aminophenol to estimate liver microsomal hydroxylase activity. Peakall used a similar system for studying the metabolism of testosterone, progesterone, and estradiol to more polar compounds by liver microsomes—the metabolic mechanism presumably being hydroxylation. Since Kuntzman's postulation has not been clearly established in the case of aniline and steroids, it is possible that DDT administration could stimulate steroid metabolism through induction of certain liver enzymes which might not be involved in the hydroxylation of aniline.

Table II. Activities of Aniline Hydroxylase and *N*-Demethylase and Concentration of Cytochrome P_{450} of Liver Microsomes as Affected by Dieldrin and DDT

Diet	Enzyme activities as % of controls		
	Hydroxylase[a]	*N*-demethylase	Cytochrome P_{450}
Control	100 ± 12[a,b]	100 ± 12[b]	100 ± 20[B]
10 ppm dieldrin	81 ± 8[a]	102 ± 13[b]	83 ± 11[B]
20 ppm dieldrin	87 ± 12[a]	171 ± 12[a]	162 ± 15[A]
100 ppm *p,p'*-DDT	52 ± 11[b]	74 ± 28[b]	83 ± 11[B]
200 ppm *p,p'*-DDT	45 ± 12[b]	70 ± 21[b]	92 ± 15[B]

[a] The average amounts of *p*-aminophenol and formaldehyde produced per mg liver protein were 264 and 125 ng, respectively, for the control group. The average change in optical density associated with cytochrome P_{450} was 0.384 per 100 mg liver protein for the controls. [b] Treatment means ± standard error which are followed by different superscript letters are significantly different at $P \leq 0.01$ (a,b) or $P \leq 0.05$ (A,B)

Table III. Relationship Between Length of Clutch and Activity of Aniline Hydroxylase and *N*-Demethylase and Concentration of Cytochrome P_{450}

	Correlation Coefficient
Length of clutch and hydroxylase activity	−0.44[a]
Length of clutch and *N*-demethylase activity	−0.44[a]
Length of clutch and P_{450} concentration	−0.42[a]

[a] Statistically significant at $P \leq 0.01$, $n = 56$.

The possibility also exists that the reproductive status of the bird modified the liver enzyme response to DDT. The hens of the current study were in a state of relatively intensive reproductive activity (45 to 50 eggs per 60 hens per day) throughout the 12-week experiment, and it would be expected that steroid hormone secretion and metabolism would be in an exceptionally dynamic state. The possible effect that this type of physiological status may have on the enzyme induction response to DDT or dieldrin is not known but merits consideration.

Examination of the egg production data from our research shows that the intensity of reproductive activity, as indicated by length of clutch (number of eggs produced on successive days), did affect the activities of liver microsomal enzymes studied. The average length of clutch determined for each individual hen varied from 2 to 12. Statistical analysis re-

vealed no significant differences in lengths of clutch related to dietary treatments. These data were then pooled across treatments and correlation coefficients between the activities of microsomal enzymes and length of clutch were calculated. Activities of liver hydroxylase and *N*-demethylase and concentration of cytochrome P_{450} were negatively correlated with length of clutch, and the correlation coefficients were highly significant ($P \leq 0.01$) (Table III).

The implications of these observations are not known. However, it is conceivable that the level of circulating steroids in the hen during intensive reproductive activity could influence the ability of some liver enzymes to metabolize foreign compounds. Juchau and Fouts (1966) found that progesterone administered to rats changed the activities of those microsomal drug-metabolizing enzymes which formed hydroxylated or oxidized products. Kuntzman (1969) reported that treatment of animals with steroids altered the metabolism of certain drugs. Variations in natural secretions of steroids have also been shown to affect drug metabolism; Radzialowski and Bousquet (1967) observed a circadian rhythm in drug metabolism by rats and stated that this rhythm was related to diurnal variation in circulating levels of corticosteroids.

Our observations of negative correlations between the activities of certain liver enzymes and length of clutch indicate that an important relationship may exist between endocrine secretions essential for specific physiological needs, and the activities of enzymes associated with the metabolism of these secretions and other compounds. This facet of metabolism deserves further investigation.

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