Effects of Diphenylhydantoin upon Estrogen Metabolism by Liver Microsomes of DDT-treated Japanese Quail

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Introduction

Estradiol-17β metabolism has been implicated in such aspects of calcium metabolism as eggshell formation and the deposition of medullary bone (OESTREICHER 1971). The negative relationship between p,p'-DDT and eggshell thickness has been well documented in various avian species including the Japanese quail, Coturnix coturnix japonica (RATCLIFFE 1967, JEFFERIES 1971, BITMAN et al. 1969, KENNEY et al. 1972). One mechanism that may be operative is induction of liver microsomal enzyme activity by the pesticide. Among the pertinent observations is the stimulation of the rate of hydroxylation of estradiol-17β by liver microsomes from White Leghorn cockerels and pullets pretreated with p,p'-DDT reported by NORWICKI and NORMAN (1972).

One way of alleviating the resulting reproductive distress is to reduce the organism's pesticide burden. It has been reported that 5,5-diphenylhydantoin (DPH) fed to human males at a level of 300 mg per day for up to nine months caused a reduction of adipose levels of DDT, DDE and dieldrin to about 30% of pretreatment levels (DAVIES et al. 1971).

The present study was undertaken to determine whether DPH affects estradiol metabolism in p,p'-DDT-treated birds.

Experimental

Male and female Japanese quail, 42 days old, were placed for three weeks on a standard breeder ration to which was added: 1) control - no additions, 2) 100 ppm p,p'-DDT, 3) 100 ppm p,p'-DDT and 130 ppm DPH, 4) 100 ppm p,p'-DDT and 520 ppm DPH and 5) 100 ppm p,p'-DDT and 1040 ppm DPH. The birds were maintained under a light-dark regime of 16 hr and 8 hr respectively.

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Liver microsomes were prepared according to the procedure described by LAZIER and JELLINCK (1965). The liver was homogenized in a Potter-Elvehjem homogenizer with Teflon pestle, centrifuged at 8,000 x g for 10 min to remove nuclei and mitochondria, and centrifuged at 100,000 x g for 1 hr to obtain the microsomal pellet. The pellet was washed in 0.25 M sucrose, resuspended, and recentrifuged at 100,000 x g for 1 hr. The resulting pellet was suspended in 0.1 M potassium phosphate buffer, pH 7.4, for incubation.

An NADPH-generating system was added consisting of $NADP^+$ (the monosodium salt from yeast, Sigma Chemical Co., St. Louis, Mo.), glucose-6-phosphate (monosodium salt, Sigma), glucose-6-phosphate dehydrogenase (from Torula yeast, lyophilized, Sigma), and $MgCl_2$ reagent grade.

Into a chilled incubation vessel were placed in order:

1) 0.5 mg glutathione (crystalline, reduced, Sigma).
2) 200 µl glucose-6-phosphate dehydrogenase solution (0.1 mg/ml in H_2O).
3) 500 µl of glucose-6-phosphate solution (9.34 mg/ml in 0.05 M tris).
4) 85 µl of 0.05 M $MgCl_2$.
5) 0.023 µCi of estradiol-17β-6,7-^3H (New England Nuclear, Boston, Mass.) in 10 µl absolute ethanol.
6) Microsome suspension from 0.5 g liver, 1 ml.
7) 250 µl of $NADP^+$ (8 mg/ml in 0.05 M tris).

Incubation was carried out in the Parafilm-covered vessel at 37° with moderate shaking for 30 min. The reaction was stopped by placing the reaction vessel in a boiling water bath for 5 min. The incubation mixture was then extracted 3 times with equal volumes of anhydrous ether. A 0.2 ml aliquot of the residual aqueous phase (centrifuged free of precipitate) was placed into a scintillation vial containing 20 ml of a 3:2 toluene: ethanol solution containing 0.4% PPO and 0.01% POPOP. Counts per minute were recorded in a Packard Tri-Carb liquid scintillation counter and averaged for a 20-min period. Counts per minute were corrected to disintegrations per minute (DPM) using the channels-ratio method. Quenching correction was made.

The DPM for the aqueous phase was taken as a measure of estradiol-17β metabolism to polar metabolites. When the microsome suspension was placed in a boiling water

bath for 5 min prior to incubation, the DPM in the aqueous aliquot fell sharply.

25 µl aliquots of the microsome suspension in 0.1 M potassium phosphate buffer, pH 7.4, were taken for protein determination by the method of LOWRY et al. (1951) for difficult-to-dissolve protein. Bovine serum albumin (fraction V, Nutritional Biochemicals Corp., Cleveland, Ohio) standards were similarly prepared.

Results and Discussion

Both DDT and DPH plus DDT caused increases in liver weight in both males and females, as shown in Table 1.

TABLE 1

Liver Wet Weight[1]

DDT,ppm	DPH,ppm	Female	Male
0	0	2.98 ± 0.13 (6)	1.62 ± 0.17 (4)
100	0	3.44 ± 0.18 (6)	1.98 ± 0.06 (5)
100	130	3.76 ± 0.29 (6)*	2.09 ± 0.12 (6)*
100	520	3.64 ± 0.10 (5)**	2.09 ± 0.15 (3)
100	1040	4.04 ± 0.18 (5)**	2.08 ± 0.10 (6)*

[1]Weight in grams, expressed as mean ± S.E.M., number of specimens in parentheses.
*P<0.05 **P<0.01

The content of microsomal protein in the entire liver of each bird was calculated. This was also found to be elevated in both males and females as shown in Table 2.

TABLE 2

Total Liver Microsomal Protein[1]

DDT,ppm	DPH,ppm	Female	Male
0	0	33.8 ± 1.0 (5)	21.7 ± 2.3 (4)
100	0	42.7 ± 4.3 (5)	24.0 ± 1.2 (5)
100	130	42.4 ± 4.3 (5)	32.5 ± 2.3 (6)*
100	520	54.0 ± 1.2 (4)**	37.8 ± 2.6 (3)**
100	1040	52.3 ± 3.6 (5)**	39.0 ± 1.9 (6)**

[1]Given as mg equivalent bovine serum albumin, expressed as mean ± S.E.M., number of individuals in parentheses.
*P<0.05 **P<0.01

Although DPH fed with DDT induced an increase in the weight and microsomal protein content of the liver, it caused a decrease in estradiol-17β metabolism in females at the lowest level fed, as shown in Figure 1.

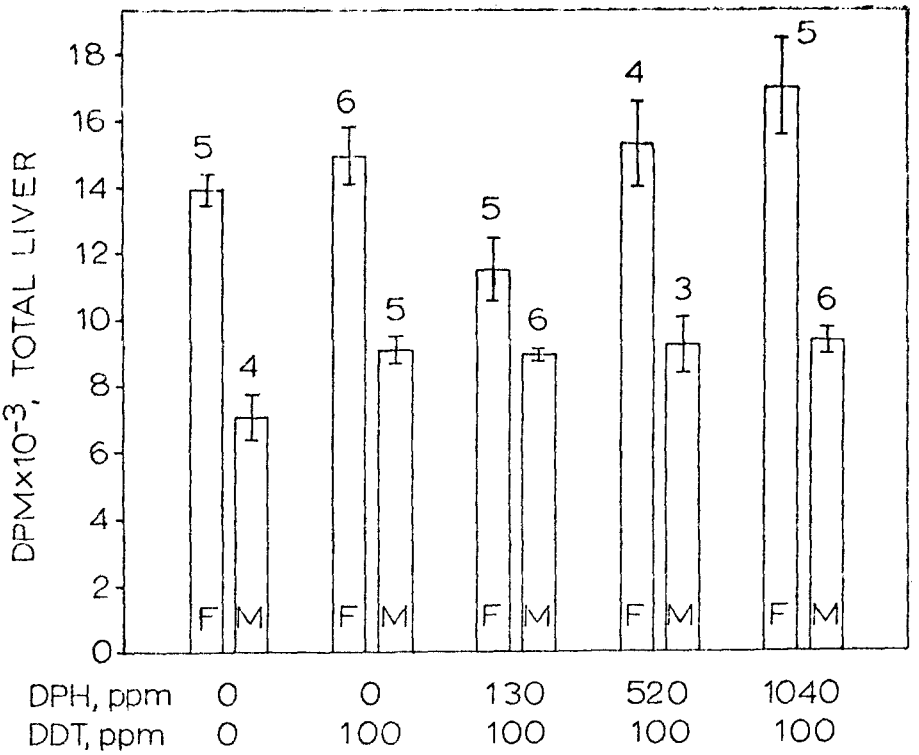

FIGURE 1. Effect of DDT and DPH plus DDT on estradiol-17β metabolism of total liver microsomes. The disintegrations per minute (DPM) used in the calculation was that obtained using the aliquot specified in Materials and Methods. The error bars represent the standard error of the mean, the numbers above them the number of specimens. F denotes females, M denotes males.

At the higher levels in females and at all levels fed in males, DPH fed with DDT increased estradiol-17β metabolism. The effect of still lower dietary levels has not been tested.

The reduction of metabolism at 130 ppm DPH can be attributed to the reduction in metabolism per mg microsomal protein seen in Figure 2. Reduced activity of micro-

somal protein at high DPH levels in males was outweighed by increased total liver content of microsomal protein.

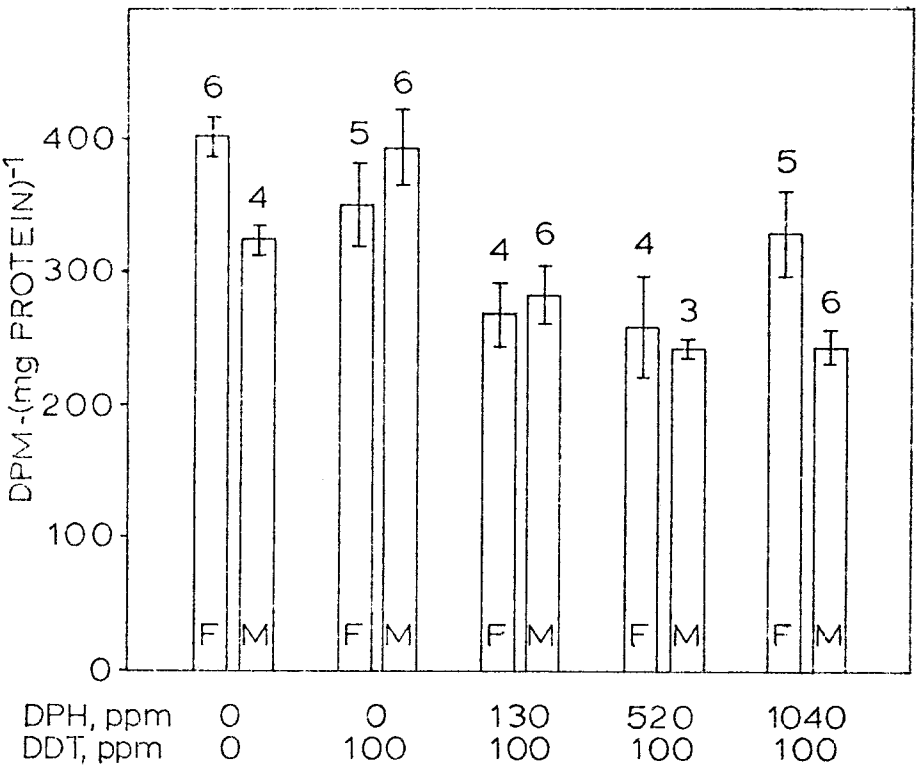

FIGURE 2. Aliquot disintegrations per minute per mg microsomal protein. The error bars represent the standard error of the mean, the numbers above them the number of specimens. F denotes females, M denotes males.

No adverse effects of DPH on the general health of the quail were noted. DPH had no evident narcotic effect, but did reduce excitability and aggressive behavior, particularly at the 130 ppm level.

DPH thus appears suitable for further study as a means of mitigating the effects of DDT on avian reproduction. Studies on the effect of DPH on DDT residue plasma levels in Coturnix have been initiated.

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