The Effect of Exogenous Administration of Thyroxine on the Accumulation of o,p'-DDT in Rat Abdominal Fat[1]

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Discovery that many pesticides, herbicides and other noxious chemicals induce microsomal enzyme activity and thus stimulate their own metabolism or elimination has encouraged studies to find therapeutic agents that may be used to lessen their potential harmful effects, or enhance their rapid elimination. SELL and DAVIDSON (1973) observed that simultaneous administration of DDT and dieldrin enhances deildrin metabolism and loss from the body; both DDT (CONNEY, 1967) and diphenylhydantoin (KUNTZMAN, 1969) have been used to stimulate enhanced steroid hormone metabolism in diseases of steroid overproduction. Numberous examples can be found, many of these reviewed by CONNEY (1967), that employ one noxious compound to enhance the loss of another.

Most of the compounds that induce increased microsomal enzyme activity are fat soluable at body pH (CONNEY, 1967) and tend to accumulate in body fat stores. Recently, LILLARD and NOLES (1973) found that injection of thyroxine, slightly in excess of normal secretion rate, into laying hens with previously accumulated DDT in body fat stores, significantly increased the rate of loss of DDT from those fat stores. This work was undertaken to determine if thyroxine pretreatment of rats, at a level slightly above normal secretion rate (50% excess), would reduce the rate of incorporation of DDT into body fat.

MATERIALS AND METHODS

Sprague-Dawley male rats weighing 280 to 340 grams were maintained three to a cage at 22° C, and fed Purina Lab Chow and water ad libidum. At the beginning of the experiment they were randomly divided into three groups: controls; DDT treated; and T_4-DDT treated.

The control rats were injected i.p. with 1.0 ml/kgbw corn oil and 48 hours later were sacrificed under ether anesthesia. All visible abdominal fat was dissected free, weighed, finely minced with scissors and frozen until analyzed.

The DDT treated rats were injected i.p. with 20 mg o,p'-DDT/ml/kgbw corn oil and sacrificed 48 hours later. Fat samples were treated as outlined for controls.

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The T_4-DDT rats were treated as follows: 1. normal thyroid secretion rate (TSR) was determined by the method of GREGERMAN and CROWDER (1963), 2. the rats then received the equivalent of their normal secretion rate of thyroxine plus 50% in daily injections administered for a total of 28 days, 3. on day 26 of T_4 injections, the rats received a single i.p. injection of 20 mg o.p'-DDT/ml/kgbw in corn oil. The rats were sacrificed on day 28; fat samples were treated as outlined for controls.

Minced fat from all three groups was ground with clean sand and Na_2SO_4; extracted with 50 ml pet ether; the extract filtered and reduced to approximately 2-3 ml in a 40° C water bath, under an airstream, as outlined by THOMPSON, 1971. The extract was placed on top of a florisil column containing 1.6 g florisil overlayed with 1.6 g anhydrous Na_2SO_4 (CAHILL and ESTESEN, 1970) that had been previously conditioned by successive 40 ml washes of hexane and methanol. The extract was washed onto the forisil with a 25 ml hexane wash, followed by three successive 5 ml washes. The pesticide was then eluted with 36 ml of 1% methanol in hexane. The elutant was reduced to 3 to 4 ml volume under an airstream and stored refrigerated in the dark until analysis.

GLC analysis of the fat-accumulated and possible metabolites of o,p'-DDT was performed on a Tracor MT-220 instrument fitted with a three-foot U-tube column containing chromosorb WHP 80/100 mesh support coated with 3% OV-1 (Varian Aerograph). The detection system was a ^{63}Ni electron capture detector. The column oven was maintained at 140° C; injection ports at 200° C; detector temperature at 250° C; and nitrogen flow rate at 70 ml/min. The system was able to detect quantities of DDT as low as 2 ng.

O,p'-DDT (1-(o-chlorophenyl)-1-(p-chlorophenyl)-2,2,2-(trichloroethane) was obtained from Aldrich Chemical Company. All reagents used for GLC analysis were chromatography grade.

The levels of T_4 administered to the rats, 50% above normal secretion rate, in this study amounted to a total mean of approximately 1.30 ug/100 gbw T_4/day/rat. There were no deleterious effects noted, and we judged from weight gains that the rats were not severely hyperthyroid.

RESULTS

In order to determine the extent to which o,p'-DDT or any metabolites were incorporated into fat stores, the chromatographic behaviors of the injected compound vs. that extracted from fat stores were compared. GLC analysis of the injected compound indicated 100% o,p'-DDT with no detectable contaminants. All pesticide extracted from fat stores, 48 hours after its injection, behaved as o,p'-DDT with no detectable contaminants or metabolites. Fat from the control rats (corn oil injected) had

no detectable levels of o,p'-DDT or metabolites.

The DDT-treated group accumulated DDT in abdominal fat varying from 0.74 µg to 12.40 µg/g fat, with a mean of 5.57 µg/g fat (Table 1). The group receiving T_4 for 26 days prior to, and for 2 days after the DDT injection, accumulated DDT to levels varying from 0.96 µg to 6.20 µg/g fat, with a mean of 3.43 µg/g fat (Table 1). Rats pretreated with thyroxine exhibited significantly lower ($P < .05$) mean uptake of DDT per gram fat than those not pretreated with thyroxine.

TABLE 1.

Concentration of o,p'-DDT[a] in Abdominal Fat of Rat

Treatment	Control	DDT	T_4-DDT
Number of Animals	11	12	11
BW at time of Sacrifice $\bar{X} \pm SE$	307 ± 5	310 ± 7	470 ± 24
Abdominal fat g/100 gbw $\bar{X} \pm SE$	1.2 ± .05	1.4 ± .05	3.1 ± .28
Range of DDT uptake µg/rat	0	3.6 - 48.3	8.4 - 118.8
µg DDT/g fat $\bar{X} \pm SE$	0	5.57 ± 0.8	3.44 ± 0.6[b]

[a] All DDT determinations were corrected for loss of pesticide in florisil column, and analysis of each sample by GLC was performed in triplicate.

[b] Significantly less than DDT group, $P < 0.05$.

DISCUSSION

The data presented indicates that pretreatment of rats with a slight excess of thyroxine reduces DDT incorporation into body fat stores. The thyroxine treated rats, though larger and possessing more fat than controls, incorporated significantly less DDT per gram of body fat than controls. The thyroxine treated group received approximately 35% more DDT (in mgs) than the controls because of greater body weight, but incorporated only about 25% more per rat.

It is speculated that the reduction of DDT incorporation into fat may be due to increased lipolysis of fat, thyroxine's most notable effect on that tissue (ARMSTRONG et al., 1974; KRISHNA et al., 1968). It has been found that thyroxine injections increase production of NADPH (COHEN and ESTABROOK, 1971); NADPH Cytochrome C reductase (FAIRHURST et al., 1959; PHILLIPS and LANGDON, 1956); and NADH Cytochrome C reductase (FAAS et al., 1974). Since the incorporation of DDT into fat is a phenomenon of concentration gradient between blood and fat stores (HAYES, 1965), lowering of blood levels by stimulating hepatic metabolism would result in reduced fat incorporation.

Regardless of the possible mechanism responsible for the reduced DDT incorporation, it seems that using one of the body's naturally produced substances to reduce DDT incorporation into fat or hasten its loss, is much more desirable than using another noxious drug. The possibility of antagonism between two stimulatory compounds might be much less if thyroxine is one of them, making the treatment less hazardous to the animal.

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