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# Enzymatic Detoxication of DDT to DDD by Rat Liver: Effects of Some Inducers and Inhibitors of Cytochrome P-450 Enzyme System

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DDT, in very first step of its detoxication is either transformed to DDD by reductive dechlorination (Walker 1969; Hassal 1973) or DDE by dehydrochlorination (Datta 1970). For further detoxication, these metabolites undergo a series of reactions which result in the elimination of DDA in urine (Peterson and Robison 1964; Datta 1970; Gold and Brunk 1982). However, enzymes involved at various steps of DDT-detoxication are not well understood. In general, these reactions are believed to be mediated by cytochrome P-450 containing enzyme system.

In earlier studies, nonenzymatic conversion of DDT to DDD was suggested (Jefferies and Walker 1966; Bunyan et al. 1966). Later, Hassal (1973) demonstrated that DDD is formed in liver in at least two ways; one protein dependent and other relatively heat resistant system. The enzyme(s) involved in the reductive dechlorination of DDT to DDD are not well known. Morello (1965) reported the induction of enzyme activity that transforms DDT to DDD in rats preadministered with multiple doses of DDT. Walker (1969) suggested the possible role of cytochrome P-450 in the conversion of DDT to DDD on the basis of inhibition by CO and the absolute requirement of NADPH for the reaction. However, studies on the *in vivo* and *in vitro* effects of some classic inducers and inhibitors of cytochrome P-450 system on the reductive dechlorination of DDT will much effectively clear the role of this enzyme system in the above conversion. Instead of the multiple administration of DDT (Morello 1965), it would be more desirable to investigate a single threshold dose of DDT for the stimulation of the enzyme involved in the reductive dechlorination of DDT. As far we know, no enzyme has been characterized for the detoxication of DDT to DDD. Therefore, DDD forming activity will be referred to as 'reductase' in this report.

This paper describes the enzymatic detoxication of DDT to DDD by rat liver preadministered with a single threshold dose of DDT. Further, effects of some inducers and inhibitors of cytochrome P-450 enzyme system on the detoxication of DDT to DDD are reported.

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## MATERIALS AND METHODS

DDT and DDD were purchased from Aldrich Chemical Co., Milwaukee WI. These compounds were recrystallized in 95% ethanol to yield a pure substance. Analysis of these compounds by gas liquid chromatography (GLC) indicated the purity greater than 99%. Chromosorb WHP 1.5% OV 17 + 1.95% OV 202 was obtained from Packard Instrument BV, Netherland. Phenobarbital (Pb), 3-methylcholanthrene (3 MC), actinomycin D (Act.D), puromycin, $\alpha$-aminitin, cyclohexamide, metyrapone, and 7, 8 - benzoflavone were obtained from Sigma Chemical Co., St. Louis, MO. Other chemicals used in the study were of analytical grade.

National Institute of Communicable Diseases colony bred male albino rats (Hissar Strain) weighing 150-160 g were used throughout the studies. Animals were maintained on laboratory diet and water ad-libitum. Analysis of six random dietary samples by GLC showed insignificant level of DDT residues. All compounds were injected intraperitoneally (ip) in normal saline (0.3ml) except DDT and 3 MC which were administered as solution in olive oil (0.3ml). The animals were killed by cervical dislocation after 72 h of the dose administration. For dose-response stimulation studies, rats were divided into seven groups of four animals each. A single dose of DDT was injected at a concentration of 0 (control), 2.5, 5, 25, 50, 100, and 200 mg/kg body wt. For the induction experiments, rats were divided into five groups of four animals each. A single dose of DDT, Pb or 3 MC (50 mg/kg body wt.) was injected into the animals of the experimental groups. The animals of control groups received oil or normal saline. For the in vivo inhibition, Act.D, 250 $\mu$g/kg; $\alpha$-aminitin, 50 $\mu$g/kg; puromycin, 10 mg/kg; and cyclohexamide, 2.5 mg/kg body wt. were administered to four groups of animals, four animals in each, 1 h before the administration of DDT (50 mg/kg body wt.). Two more doses of $\alpha$-aminitin and puromycin at the above indicated concentration were hourly injected after DDT administration. While animals of Act.D and cyclohexamide treated groups were not further injected with Act.D and cyclohexamide due to their lethal effects. In vitro inhibitors, metyrapone (8.5 m mol) and 7,8-benzoflavone (0.14 m mol) were preincubated for 30 min. with the enzyme incubation mixture under anaerobic condition prior to the addition of the substrate (DDT).

Treated and untreated animals were killed and each liver was perfused in situ with 75 ml of cold sucrose (0.25 M). A 12,000 g supernatant fraction was prepared as described by Hassal (1973).

The enzyme activity was measured by GLC and based on the measurement of DDD as the metabolised product of DDT. Incubation mixture was prepared as follows. In a final volume of 3.5 ml, 35.2 n mol DDT (in 25 $\mu$l ethanol) was added to an ice cold Erlenmeyer flask (25 ml) that contained 1 ml enzyme extract (20-30 mg protein), NADPH generating system (0.59 m mol NADP, 3.75 m mol glucose 6-phosphate and 3.5 Kornberg units of glucose-6-phosphate dehydrogenase), 5.62 m mol $MgCl_2$ and 50 m mol tris-buffer, pH 7.8.

Atmospheric oxygen from the incubation mixture was removed by passing a stream of $N_2$ (120 ml/min.) throughout the incubation to maintain anaerobic condition. Incubation was performed for 1 h at 37°C on water bath equipped with mechanical shaker. Reaction was terminated by 1 ml $HClO_4$ (20% v/v) and the product DDD and undegraded substrate DDT were extracted three times with 10 vol. of a mixture of hexane and acetone (4:1). The extract was purified by florisil and taken into 2 ml of hexane. An appropriate volume (2 $\mu$l) was injected into gas chromatograph. Greater than 95% of total DDT as DDT+DDD could be recovered from incubation mixture by this procedure. DDT and DDD were analysed on Packard gas chromatograph equipped with EC-detector. Conditions were:stationary phase, chromosorb WHP coated with 1.5% OV 17+1.95% OV 202, carrier gas $N_2$ (120 ml/min), temperature of detector and column 195°C each, injection port 220° and outlet 200°C.

One unit of the enzyme activity was expressed as the amount of the reductase that forms one n mol DDD/h. Specific activity was defined as unit per mg of protein.

For the determination of pH optima, 0.1M Tris-buffer of various pH ranging from pH 6 to 9 was prepared. Incubation was performed at different pH and the values reported in the text are the values determined at the end of incubation period.

## RESULTS AND DISCUSSION

Administration of a single dose of DDT with its varying concentration revealed threshold stimulation of the reductase nearly at a dose of 50 mg/kg body wt. While higher doses (100 and 200 mg/kg) exhibited lower stimulatory effect (Fig. 1). In a somewhat related study similar induction patern of DDT-dehydrochlorinase of Musca domestica was reported after DDT administration (Capdevila et al. 1973). The authors further suggested the involvement of some physiological response to microsomal detoxifying system which results in lower stimulatory effect even at higher doses. Stimulation of reductase by single threshold dose of DDT (this report), however, does not substantiate the results obtained by repeated administration of DDT (Morello 1965).

Optimum pH for the reductive dechlorination of DDT was determined to be pH 7.8. Below and above this pH the enzyme sharply looses its activity (Fig. 2A). The difference of 0.4 unit of pH resulted in appreciable loss of activity. Hassal (1973) reported optimum pH below 6.0 for avian liver. Thus a marked difference for the pH optima was observed in two species. For the better results the animals were stimulated with a single threshold dose of DDT and the reductase activity was measured at pH 7.8 in subsequent experiments. Optimum temperature for the conversion of DDT to DDD was found to be 48-50°C (Fig. 2B). In addition to the enzymatic conversion of DDT to DDD, Hassal (1973) reported the total loss of this activity at 75°C and the restoration of two fold activity from denatured system supplemented with NADPH and riboflavin. The author further suggested that DDD is formed by liver in at least

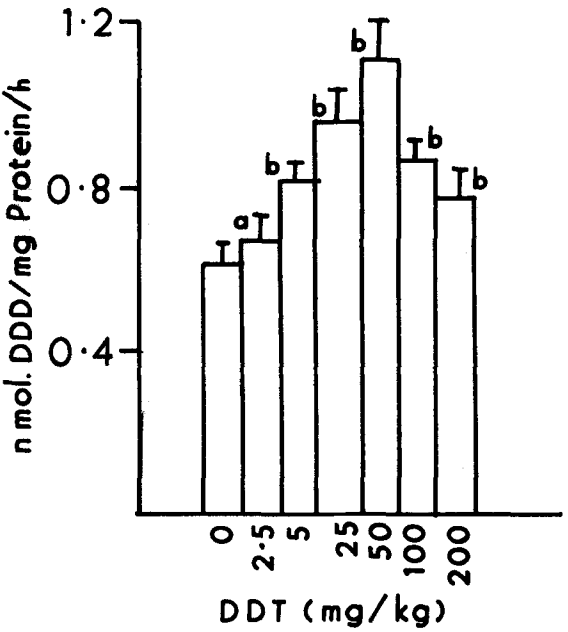


Figure 1. Effect of varying concentrations of DDT on the reductase activity. Data represents the mean ± SD from four animals. Statistical comparisons with untreated rats by students t-test: a-P < 0.05; b-P < 0.001.

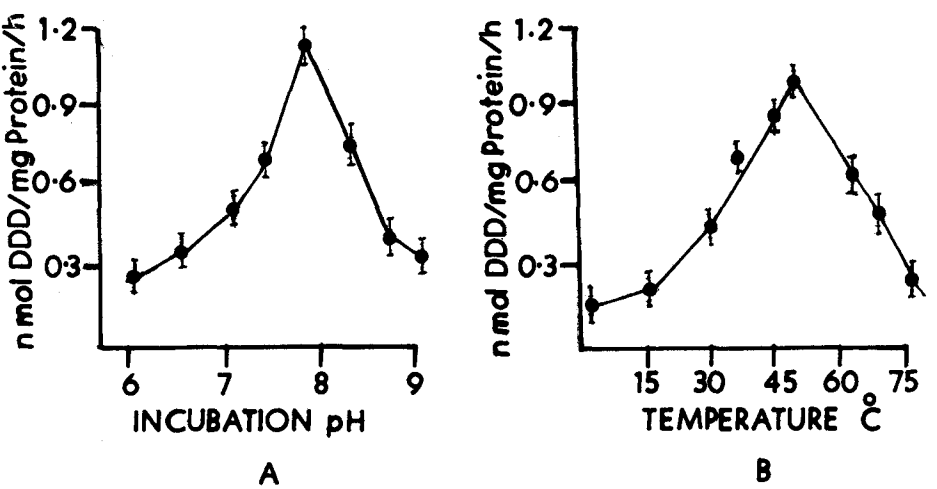


Figure 2. Effect of (A) pH, and (B) temperature on the reductase activity. Values are the mean from four experiments.

two ways: One heat liable and other relatively heat resistant system. However, the principal reaction bringing about the reductive dechlorination of DDT is almost protein dependent and relatively more rapid (Walker 1969; Hassal 1973) than the non-enzymatic process (Bunyan et al. 1966; Ecobichon et al. 1967).

The *in vivo* and *in vitro* effects of specific inducers and inhibitors of cytochrome P-450 enzyme system on the reductive dechlorination of DDT are summarized in Table 1.

Table 1. Effects of some inducers and inhibitors of cytochrome P-450 enzyme system on the reductive dechlorination of DDT.

| Treatment | Reductase activity[a] |
|---|---|
| Control (Oil) | 0.322 ± 0.016 |
| Control (Saline) | 0.315 ± 0.021 |
| *Inducers (in vivo)* | |
| DDT | 0.352 ± 0.031[b] |
| DDT | 0.453 ± 0.103[c] |
| DDT | 0.739 ± 0.148 |
| Pb | 0.375 ± 0.026 |
| 3MC | 0.319 ± 0.010 |
| *Inhibitors (in vivo)* | |
| DDT + Act.D | 0.726 ± 0.165[d] |
| DDT + ∝Aminitin | 0.776 ± 0.186[d] |
| DDT + Cyclohexamide | 0.203 ± 0.043[d] |
| DDT + Puromycin | 0.327 ± 0.078 |
| *Inhibitors (in vitro)* | |
| DDT + Metyrapone | 0.331 ± 0.078 |
| DDT + 7,8-Benzoflavone | 0.368 ± 0.123 |

a. Activity was expressed as n mol DDD/mg of protein/h. More than 55% conversion of the substrate was observed. All values are the mean ± SD from four animals.
b. Incubation was performed aerobically.
c. Incubation in absence of NADPH.
d. Values are from single administered dose of inhibitor.

Treatment of animals with DDT, Pb or 3 MC (50 mg/kg body wt.) showed that rats fed with DDT were capable of carrying out reductive dechlorination of DDT at a rate of 2.25 while Pb registered a 1.12 increase in activity over the control. No significant induction was observed in 3 MC treated rats. Thus, DDT itself proved to be the most potent inducer, tested in this study, of its own metabolism. The relative differences in the metabolism of DDT after the administration of these inducers seems due to their differential effects on each forms of cytochrome P-450 participating in the reaction. (Hildebrandt et al. 1968, Comai and Gaylor 1973). The increase in reductase activity attendant to the administration of DDT was completely abolished by cyclohexamide (2.5 mg/kg) while puromycin (10 mg/kg) appeared to inhibit 45%

reductase activity. Inhibitory effects of cyclohexamide on the reductive dechlorination of DDT has not been reported earlier. Inhibition of reductase activity by puromycin was in close agreement with the activity reported to prevent DDT stimulatory effect (Morello 1965). In a related study inhibition by puromycin and cyclohexamide of β-naphthoflavone and Pb-mediated induction of N-demethylase and aryl hydrocarbon hydroxylase were reported (Cutroneo and Bresnick 1973). Specific inhibitors of RNA synthesis, Act.D (250 μg/kg) and α-aminitin (2.5 mg/kg) were found to have no affect on the transformation of DDT to DDD. Act.D has been reported to effectively inhibit polycyclic hydrocarbon mediated induction (Gelboin and Blackburn 1963). Hence, results of our study indicate that the reductase activity is governed at translational level by the de novo synthesis of the enzyme protein rather than the activation of the enzyme. In vitro inhibitors, metyrapone (8.5 m mol) and 7,8 - benzoflavone (0.14 m mol) when added to the assay system reduced the reductase activity by 52% and 56%, respectively (Table-1). These in vitro inhibitors can affect cytochrome P-450 system either by specific interaction with heme or protein moiety or by serving as competing substrate. Binding of metyrapone to the reduced cytochrome P-450 to inhibit the metabolism was reported by Luu-The et al. (1980). While suggesting the involvement of cytochrome P-450 on the basis of inhibition of metabolism observed by metyrapone or 7,8-benzoflavone, a lack of effect must be interperated with caution due to the presence of different forms of cytochrome P-450. For instance, oxidative metabolism of aminopyrene, hexobarbital and morphine were inhibited by metyrapone while acetanilide hydroxylation was enhanced in presence of metyrapone (Leibnman 1969), showing thereby its differential effect in the metabolism.

Thus an increase or decrease metabolism of DDT following in vivo treatment of animals with inducers and inhibitors or the absolute requirement of NADPH strongly suggest a role of cytochrome P-450 in the reductive dechlorination of DDT. The mechanism of formation of DDD from DDT is not fully known. Owing to the structural similarity of DDT to $CCl_4$ and halothane, Baker and Van-Dyke (1984) suggested that DDT is reductively dechlorinated to DDD via free radical intermediate in a way similar to the formation of $CF_3CHCl$ from halothane (Ahr et al. 1982). This free radical intermediate abstracts hydrogen atom to form DDD. Further purification of the enzyme involved in the reductive dechlorination of DDT is in progress.

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