Microsomal Epoxidation: Effect of Age and Duration of Exposure to Dietary DDT on Induction

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Attempts to assess the impact of persistent environmental poisons, such as DDT [2,2-(p-chlorophenyl)-1,1,1-trichloroethane], have revealed the inductive effects of these chemicals on the microsomal mixed function oxidases active in the metabolism of drugs and pesticides (1-3). Durham (4) has noted the many factors which can operate to change the activities of the microsomal enzymes. In the rat, age, sex, nutritional status, exposure to xenobiotics, sex hormonal levels, and diurnal fluctuations of enzyme levels have all been implicated as affecting the activities of the microsomal enzyme complex.

Kato et al. (5) reported that certain drug metabolism activities reached a maximum at 30 days of age and that age was not significant in the duration of the inductive effect of phenobarbital on these activities. In preliminary studies, Gillett (3) found that adolescent male rats were most active in microsomal epoxidation, which appeared to be induced more readily in the younger rats by a given exposure to dietary DDT. More recently, a study of the effects of dietary dieldrin on microsomal epoxidation in relation to vitamin E deficiency (6) revealed a significant drop in response of the microsomal epoxidase to a given

Bulletin of Environmental Contamination & Toxicology,
Vol. 4, No. 3, 1969, published by Springer-Verlag New York Inc.

exposure of dietary dieldrin in rats 10 and 16 weeks of age as compared to six-week-old rats.

The extent of induction of microsomal epoxidation is dependent on the concentration of DDT in the diet (3,7) and has a "no effect" dietary dosage in the same range as determined by Kinoshita et al. (2) for certain drug metabolizing activities. Hart and Fouts (8) found that a single large dose of DDT was capable of maintaining elevated microsomal drug metabolism for several weeks, but little is known about the persistence of the effects of low dietary dosages of chlorinated hydrocarbon insecticides. Ortega (9) has characterized the inductive effects of DDT on liver microsomes as being adaptive, rather than pathological. Hence, to aid in the evaluation of dietary chlorinated hydrocarbon insecticides as a possible public health hazard, the effects of three parameters of dosage other than dietary concentration were needed. These parameters--age at exposure, the duration of exposure to a given dosage, and the persistence of the effect of a given exposure in the absence of dietary DDT--were therefore explored in regard to induction of epoxidase in a series of litter mate comparisons. The results of these studies, reported herein, confirm the hypothesis of Ortega by demonstrating the rapidity with which a return to normal levels of epoxidase activity occurs following withdrawal of DDT from the diet. At the same time the lesser effects dependent on the age at exposure and the duration of exposure have been examined.

Methods

Hepatic microsomes were isolated by differential centrifugation and assayed for aldrin (1,2,3,4,10,10-hexachloro-1,4,4a, 5,8,8a-hexahydro-1,4:5,8-endo-exo-dimethanonaphthalene) epoxidation to its 6,7-epoxide dieldrin by methods previously described (3). Male white rats of the Corvallis strain (a closed colony of randomly bred rats derived from Wistar rats about thirty years ago) were weaned at 28 days of age onto a semi-purified ration (10) to which analytically pure p,p'-DDT (Nutritional Biochemicals Co.) was added in the corn oil analyzed for content of chlorinated hydrocarbon pesticides but to which no DDT was added. For these experiments, all DDT diets were fortified to 25 p.p.m., which was verified by analysis (3). Rats were individually caged and allowed free access to food and water. Microsomal protein was determined by the modified Biuret method of Fincham (11). The statistical significance of the results were obtained by comparison through the Student's t test, as described by Li (12), using $P<0.05$ as the test of significance.

Results

As can be seen in FIG. 1, epoxidase activity in the microsomal fraction of all rats receiving only the control corn oil ration is progressively elevated as the rat matures. The values at 6, 8, 10, 12, 14 and 33 weeks of age are all significantly greater than the epoxidase activity at weaning; the drop at 12 weeks is not significant compared to either 10 or 14 weeks. Epoxidase activity in

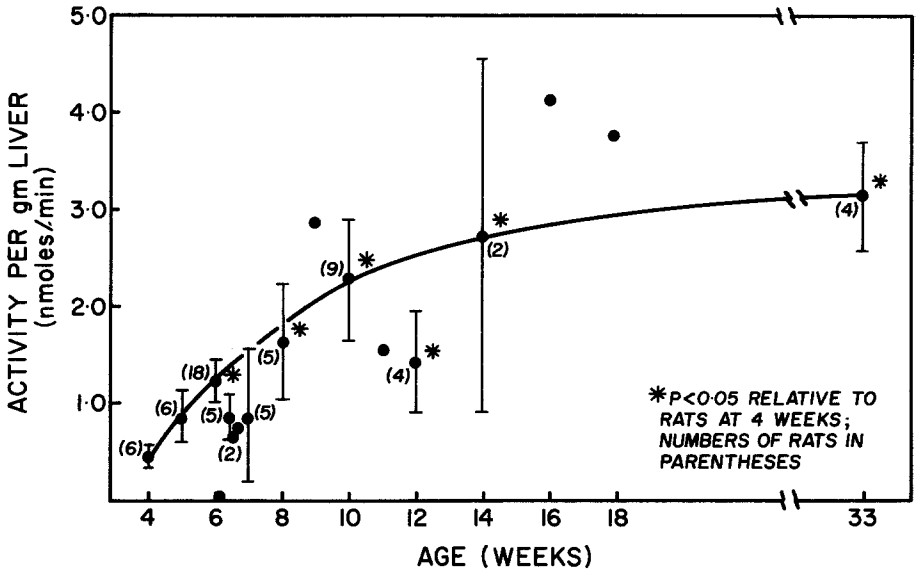

FIG. 1: Increase in Hepatic Microsomal Epoxidation with Age.

33 week-old rats is also higher than the rats at six and 10 weeks. Individuals from the same litter sacrificed at different ages show similar trends, although too small a sample was taken to provide a complete age range. Wide variations at some ages are particularly evident, even though the variation in replicate analyses of pooled liver samples is less than 1% (13) and averages 4% between litter mates. Although the techniques used were not changed over the course of these experiments (about 18 months), during some periods most litters were much lower in epoxidase activity than others determined previously or subsequently. No statistical correlation could be found for this

distribution, however.

Although the basic level of microsomal epoxidase increases with age, the absolute amount of DDT-induced increase per gm of liver or per mg of microsomal protein remains about the same. In Table 1 are shown the changes in epoxidase observed in rats fed 25 p.p.m. of DDT for two weeks prior to sacrifice at 6 and 33 weeks of age. The final level of activity and the amount of increase over unexposed litter mates are not significantly different, but the percentage increase of the younger rats is markedly higher, due to the lower basal level. These same trends were observed in experiments comparing pairs of the same litter fed for two weeks prior to sacrifice at different ages. Since food consumption for these rats is at 15 to 20 gm per 100 gm of body weight per day, intake, and therefore dosage, remains fairly constant.

TABLE 1

Effect on Aldrin Epoxidase of 25 p.p.m. of Dietary DDT for Two Weeks

Age of rats (weeks)	No. of litters	Microsomal Epoxidase Specific Activity[a]			Percentage increase[b]
		Control	DDT-treated	Increase over litter mate controls	
6	6	32.7±8.5	269±80	235±74	852±249
33	4	222±44	414±98	192±65	87±10

a Nano moles of dieldrin formed per min per mg of microsomal protein ± S.E.

b Mean percentage increase ± S.E., calculated by $\frac{(\text{treat-control})}{(\text{control})} \times 100$, for each litter mate pair.

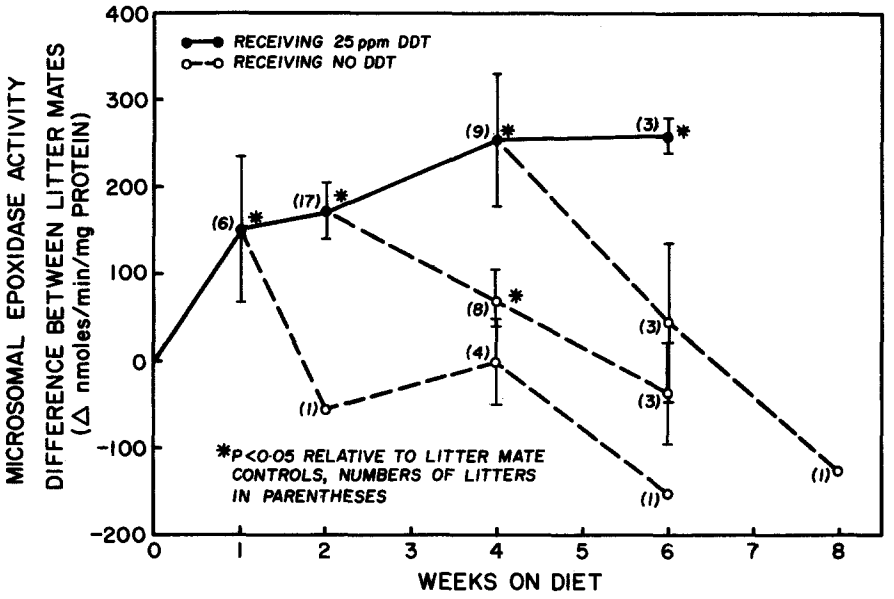

FIG. 2. Effect of Withdrawl of DDT from the Diet on Hepatic Microsomal Epoxidase Activity.

The effect of continued feeding of DDT or of withdrawing it after a certain period of time can be seen in FIG. 2. By disregarding the various ages of the animals employed, a comparison of the effects of dietary DDT on the increase or decrease in the activity of microsomal epoxidase in the livers of litter mates shows that (a) the effectiveness of a 25 p.p.m. diet plateaus after a one week exposure and epoxidase continues to be significantly elevated as long as the rat remains exposed, and (b) that the withdrawal of DDT from the diet results in a relatively prompt return of the activity levels to that of unexposed animals, re-

gardless of the length of exposure. Previously, when rats were fed a diet containing 2.5 p.p.m. of DDT for several weeks, the values of the treated rats fell down to or below those of the unexposed animals (3).

Discussion

Inducibility versus age. Whereas epoxidation continues to rise within the liver microsomes of rats not exposed to DDT, the response to DDT seems fixed to a level determined by the concentration of DDT in the diet (7). Given a specific basal ration, which will be reproducible in terms of low residue (less than 0.05 p.p.m. of total chlorinated hydrocarbon insecticides) and nutritional composition, the addition of DDT elicits a response proportional to the concentration in the diet. However, once the cell has responded, the activity continues essentially at the level of response. In part, the effect of a given dosage of DDT at any one age would depend on the absorption from the diet, distribution within the body and within the target hepatcytes, rates of metabolism to products more or less active as inducers, and rates of excretion of both DDT and any active metabolites. If inducible metabolism of DDT (14) is similarly regulated by the dietary level, and if the products are either approximately as active as DDT (3) or can be considered inactive (as for p,p'-DDD), the feeding of any particular level of dietary DDT would establish a set of rates of metabolism and excretion which would maintain residue levels at a nearly fixed value. This condition

is observed in the case of dieldrin, a compound analogous in effect but not in structure. In rats receiving dietary dieldrin, the microsomal residues have been found to be altered either by the concentration in the diet or by the age at which exposure began, but not be the duration of the exposure (6). Similarly, the dietary concentration of dieldrin evokes a corresponding response in microsomal epoxidation that may be correlated with the residues of dieldrin found in the microsomes after the treatment (15).

Induced epoxidase activity following withdrawal of dietary DDT. In the earlier experiments of Hart and Fouts (8) on the effects of chlorinated hydrocarbon insecticides on microsomal drug metabolism, significantly induced activities were obtained one day after a single large (500 mg/kg) injected dose of DDT. Repeated large doses were capable of maintaining the activity at significantly elevated levels for several weeks. When previously DDT-treated animals were starved, induction again occurred, probably due to the mobilization of fat reserves which in turn released "bound" or stored DDT to the liver. The response to the lower dosages used in the experiments reported herein probably is not due to any difference in mechanism of induction, but rather to the quantitative compartmentalization of the DDT. Rats receiving 30 mg per kg per week apparently develop mechanisms to regulate the concentration of DDT available as inducer and these mechanisms continue to operate after exposure has ceased. Storage of DDT must play a smaller role in this regulation than in

the case of the higher dosages (>1000 mg per kg per week), where sustained release of stored DDT (or its metabolites active as inducers) maintains induced activity for a longer period. Without adequate residue data, speculation as to the maximal achievable residues and the probable effect of such large doses may be fruitless. Nevertheless, the experiments of Hart and Fouts point the way to the significance of tissue residues of chlorinated hydrocarbon insecticides as determinants of microsomal function.

Concern regarding the real and potential hazards accompanying the existence of residues of the persistent chlorinated hydrocarbon insecticides in the environment has resulted in several evaluations of possible interaction of these compounds with microsomal metabolism. Kinoshita et al. (2) found various drug metabolizing activities in the rat elevated by DDT and toxaphene to have a dietary "no effect" level of between 1.0 and 5.0 p.p.m.; microsomal epoxidation has a similar "no effect" range in response to chlorinated hydrocarbon insecticides (7,15). If cyclodiene epoxidation is considered a typical activity of the microsomal mixed function complex (16), transient exposures to dietary DDT concentrations even 100 times those found in normal human diets would not be detectable in regard to effects on rat microsomal activities a short time after the exposure was withdrawn.

The technical assistance of Mrs. K. M. Morack and the advice of I. J. Tinsley, who supplied the rats and diets, are hereby acknowledged. This work was supported by a grant (ES 00040-03) from the United States Public Health Service. Technical Paper No. 2522 of the Oregon Agricultural Experiment Station.

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