Influence of Dietary Methionine on Metabolism of DDT in the Rat

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Weanling rats have been raised for a period of 12 weeks on a ration containing 150 ppm. of DDT [ethane 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)] and 22% alpha-protein as the sole source of nitrogen. In addition to the DDT, significant levels of DDE [ethylene 1,1-dichloro-2,2-bis(*p*-chlorophenyl)] and DDD [ethane 1,1-dichloro-2,2-bis(*p*-chlorophenyl)] were detected in liver tissue with DDD being present in the highest concentration. When a methionine supplement is added to the ration, the concentrations of DDE and DDT in liver tissue increase with an increase in level of the supplement, while the concentration of DDD remains constant. These observations are interpreted in the light of present knowledge of the metabolism of DDT and a possible effect of methionine on its transport.

In a recent publication we reported that the effect of DDT on the amount of vitamin A stored in the liver was influenced by the level of dietary methionine (Tinsley, 1969). This observation was made in experiments designed to evaluate the potential interaction between nutritional and toxic stresses using a protein low in sulfur-containing amino acids as the nutritional stress and DDT as the toxic stress. This result illustrates the effect of a chemical on vitamin A nutrition. In extending this study we have determined the levels of DDT and its metabolites accumulated in the liver as an index of the effect of the nutritional variable on the response to the toxicant.

EXPERIMENTAL

Ration Composition. With the exception that α-protein was substituted for casein, the composition of the semisynthetic ration used was the same as that given in a previous publication (Tinsley, 1966). The ration contained 22% protein, 5% fat, 4% salts, and 67% carbohydrate, with adequate amounts of micronutrients being incorporated as the pure compounds. Supplements of DL-methionine were added at the expense of the protein. *p,p'*-DDT (ESA Pesticide Reference Standard, City Chemical Corporation, New York) was dissolved in the oil and incorporated into the ration to give a level of 150 ppm. With the feeding period used, this level did not produce any gross symptoms of toxicity.

Experimental Design. Rations were prepared with 0, 0.5, 1.0, and 4.0 g/kilo of added DL-methionine with highest level of supplement approximating that found in a lab chow. Litter mate groups (4 ♂ or 4 ♀) of 28-day weanling rats from our closed colony of Wistar rats were distributed on the 4 rations which were fed *ad libitum*. A total of 8 rats (4 ♂ and 4 ♀) were used for each ration. The animals were housed in individual cages, weighed weekly, and food intakes recorded. After a 12-week feeding period the animals were sacrificed and samples of liver tissue taken for analysis.

Analytical Procedures. The liver tissue was macerated with acetone in an Omni-mixer, filtered, and washed twice in the filtering flask. The DDT was watered out into hexane by diluting the acetone (one part) with two parts of water and extracting twice with 0.5 part of hexane. An aliquot of the hexane solution was diluted to a known volume for analysis by gas chromatography using an electron capture detector and a microcoulometric halide detector. *p,p'*-DDT, *p,p'*-DDD, and *p,p'*-DDE were readily separated on a 183 cm (6 ft.) × 2 mm i.d. glass column packed with 1.8 parts of 5% QF-1 and 1.0 parts of 5% DC-11 on 60/80 mesh Gas Chrom Q. The QF-1 support was packed in series in front of the DC-11 support.

To test the method, additional extractions were made with other solvents and additional water was added in the watering out step. Less than 1% additional DDT was found with these steps. Recoveries were within the error of measurement.

RESULTS

With a level of 150 ppm in the diet, significant amounts of DDT, DDE, and DDD were detected in liver tissue. The effect of the experimental variables on the concentration of these compounds in liver tissue is illustrated in Figure 1. Table I summarizes the results of the analysis of this 2 × 4 factorial experiment using procedures given by Li (1957).

Considering the overall response of both males and females, increasing levels of dietary methionine produced a significant increase in the concentrations of DDE and DDT in liver tissue. Female rats showed a more pronounced response to the dietary variable and at the two highest supplement levels showed higher concentrations of DDE and DDT than did male rats. The concentration of DDD was not influenced by the dietary variable. The data in Figure 1 would indicate a higher concentration of DDD in the livers of female rats raised on rations containing 4.0 g of methionine per kilo.

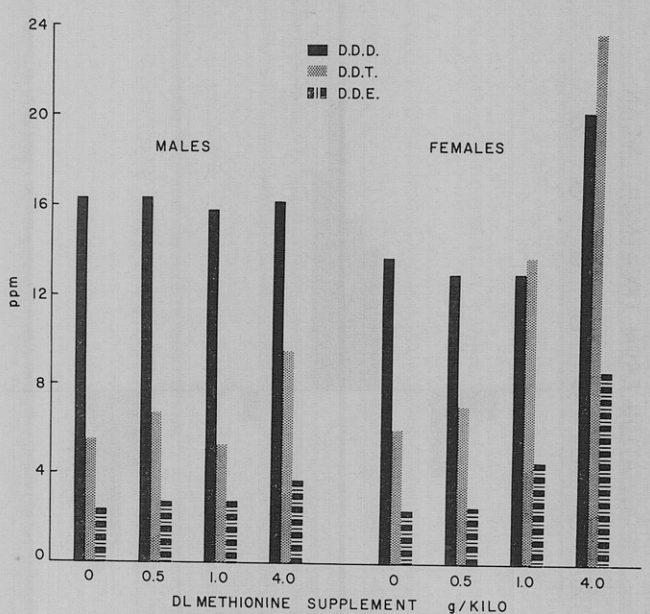

Figure 1. Influence of dietary methionine on concentration of DDT and its metabolites in liver tissue

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Table I. Effect of Sex and Dietary Methionine on Concentration of DDT and Its Metabolites in Rat Liver—Summary of Statistical Analysis

Component Analyzed	F Values and Level of Significance			Error Mean Square (23 degrees of freedom)
	Sex	Methionine	Sex × methionine	
DDT	15.8 ($p < 0.005$)	9.23 ($p < 0.005$)	4.90 ($p < 0.01$)	20.3
DDE	6.13 ($p < 0.025$)	6.33 ($p < 0.005$)	3.00 n.s.	3.92
DDD	1.03 n.s.[a]	2.32 n.s.	2.38 n.s.	10.7

[a] Not significant.

Table II. Influence of Experimental Variables on DDE/DDT Ratio

Sex	Methionine Level, g/kilo			
	0	0.5	1.0	4.0
Male	0.424	0.408	0.459	0.412
Female	0.395	0.374	0.365	0.365

However, the values observed in this group were particularly variable and, hence, this difference was not statistically significant.

Although the increasing level of methionine supplement improves growth rate (Tinsley, 1969), the data reported in this paper cannot be explained on the basis of associated changes in liver size since similar relationships are obtained when the data are expressed as micrograms liver/100 g body weight. The methionine variable did not result in a statistically significant change in food intakes (Tinsley, 1969) although there was a tendency toward lower intake with animals raised on the unsupplemented ration. The dietary variable did not produce any significant change in the amount of fat in the liver, another factor which could influence the amount of DDT stored in the tissue.

It is of interest to note that the level of methionine supplement does not influence the DDE/DDT ratio (Table II). Statistical analysis, however, does indicate a highly significant litter effect. This is illustrated in Figure 2 by plotting for each individual in a litter mate group its deviation from the appropriate treatment mean. Each litter shows a most consistent response. This observation is consistent with that of Rothe *et al.* (1957) and could result from variations in the level of the enzymes involved. Such changes could be due to genetic differences or possibly variations in the pre-experimental environment. In any case the expedient of using litter mate comparisons to reduce experimental error is clearly illustrated.

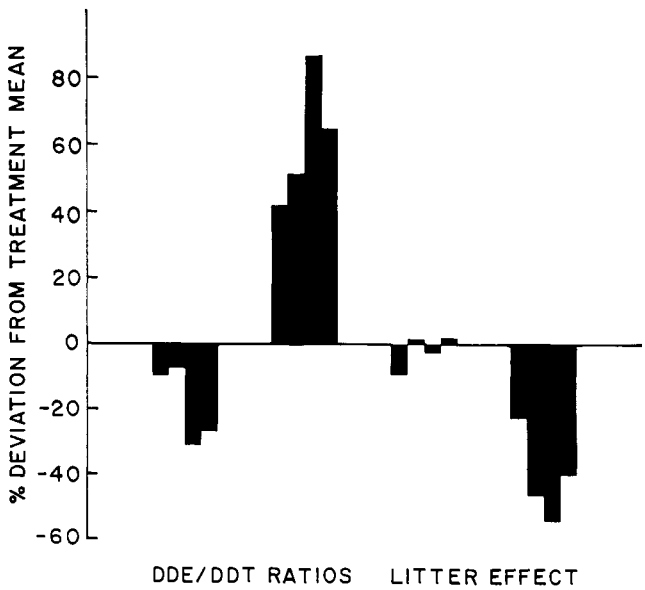

Figure 2. Litter mate effect on DDE/DDT ratios in male rats

Thus, an increase in the level of dietary methionine increases the total concentration of chlorinated compounds in liver tissue of rats fed DDT. A change in the relative proportions of the chlorinated compounds is observed also.

DISCUSSION

With the development of appropriate gas chromatographic procedures, DDD has been identified along with DDE as a major metabolite of DDT. Klein *et al.* (1964) fed adult rats rations containing 50 ppm of *p,p'*-DDT and observed a DDT:DDD:DDE ratio in liver tissue of 1.00:1.41:0.18. In our study, considering data from both male and female rats, these three components were present in the ratio of 1.00:1.11:0.38 in rats receiving the highest level of methionine supplement. The proportion of DDD increased as the level of dietary methionine decreased. DDD is not accumulated in fat to the same extent as DDT and DDE (Klein *et al.*, 1964). Intestinal flora have been implicated in the conversion of DDT to DDD (Mendel and Walton, 1966); however, it would not appear to be the only site involved since DDD has also been detected in the livers of rats when DDT was administered intramuscularly or interperitoneally (McCully *et al.*, 1968).

DDT is absorbed slowly from the small intestine with the larger proportion of the absorbed material being transported through the lymphatic system (Rothe *et al.*, 1957). For a given rat, a constant proportion of the absorbed DDT is converted to DDE in the intestine with a constant ratio of these two components being observed in the chyle. No observations have been made of the level of DDD in chyle.

Liver preparations from birds and rats can convert appreciable proportions of added DDT to DDD under anerobic conditions and it has been suggested that the transformation is nonenzymatic (Bunyan *et al.*, 1966). The same preparations convert DDT to DDE at a much slower rate with the reaction being stimulated by glutathione. Peterson and Robison (1964) have fed various metabolites of DDT to rats and tissues were analyzed for the presence of metabolites. DDD was converted to DDMU [ethylene 1-chloro-2,2-bis-(*p*-chlorophenyl)] which was subsequently transformed to DDA [acetic acid, bis(*p*-chlorophenyl)] in which form it is excreted in the bile (Burns *et al.*, 1957). The sequence of reactions proposed by Peterson and Robison for the transformation of DDT to DDA has recently been confirmed by Abou-Donia and Menzel (1968) in similar studies with the chicken. DDE is not involved in this process and little is known about its metabolic breakdown and excretion.

One must conclude that the system responsible for the

formation of DDD in liver tissue is saturated under the conditions of these experiments. The concentration of this metabolite in the liver remains constant despite an increase in the concentration of its precursor, DDT. Either the enzyme system or some reactant must be limiting, depending on whether the process is enzymatic or nonenzymatic. This constant concentration of DDD must reflect a balance between the rates with which it is formed and broken down, neither of which is influenced by the dietary variable. Since the metabolites of DDD are detected in only small amounts (Abou-Donia and Menzel, 1968), the breakdown of this compound appears to be very slow compared with the rate at which its metabolites are metabolized and subsequently excreted.

The experiments of Rothe *et al.* (1957) suggest that in the intestine the transformation of DDT to DDE is quite rapid and this could be the preferred site for this reaction in view of the fact that it occurs to only a limited extent in liver (Bunyan *et al.*, 1966). The constant DDE/DDT ratio observed in the liver would then merely reflect that condition existing in the chyle.

The most significant observation of this experiment is the buildup of DDT in liver tissue with increasing levels of dietary methionine. This response might well be associated with an effect of methionine on lipid transport. Possibly, limiting the availability of methionine restricts the formation of chylomicrons and thus reduces the rate of lipid ransport (Hyams *et al.*, 1966; Sabesin and Isselbacher, 1965). Since DDT is probably associated with chylomicrons in the chyle, the transport of this compound would be likewise affected. With an increase in dietary methionine, fat transport would be increased and as a consequence more DDT would be absorbed and carried to the liver. Since the DDT to DDD system is saturated under the conditions of these experiments, it is the DDT which accumulates. The proportionate increase in DDE in liver tissue could also develop from such a mechanism.

These experiments would indicate that feeding proteins low in methionine would not accentuate the chronic response to DDT. Low levels of methionine result in lower levels of DDT and its metabolites accumulating in the tissues.

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