Evidence for Conversion of DDT to TDE in Rat Liver: II. Conversion of p,p'DDT to p,p'TDE in Axenic Rats

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Several studies have been conducted to elucidate the site of origin of TDE found in the livers of animals (1, 2, 3, 4). There is ample evidence that some microorganisms, including a few types of bacteria isolated from intestinal microflora, are capable of metabolic conversion of DDT to TDE (3, 5, 6). These data support the hypothesis that TDE found in intact animals is the product, at least in part, of metabolic activity of intestinal microorganisms. Evidence for hepatic metabolism of DDT to TDE is less clear. *In vitro* studies of hepatic conversion have either failed to demonstrate conversion (7) or have not excluded the possibility that TDE produced in the *in vitro* system was the result of putrefaction (4) or nonenzymic conversion (8). *In vitro* studies will fail to

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demonstrate conversion if the animal donors of liver tissues to be used in the in vitro system are not preconditioned to DDT, a condition essential for success in view of the evidence that the hepatic enzymes responsible for production of TDE from DDT are inductive enzymes (9).

In vivo assessment of the role of the liver in the conversion is difficult; oral administration of DDT is unsuitable in conventional animals and administration by routes other than oral does not obviate bacterial contact with DDT because of biliary excretion of DDT (10). The problem of separating the intact animal from its intestinal microflora can be overcome by use of axenic animals. In the present study, germ-free animals were employed to evaluate the requirement of intestinal organisms for the production of TDE found in animal tissues.

Materials and Methods

Two male weanling axenic rats and two male weanling conventional rats, both of the Fisher strain, were obtained from the Charles River Breeding Laboratories. The axenic animals were transferred upon receipt to a sterile Bioquest ISOLAB portable isolator. The conventional rats were housed in standard caging next to the isolator so that conditions of temperature, light, and humidity would be as like as possible between the two groups. Weighed, sterilized packets of commercial rat feed prepared to contain 200 ppm p,p'DDT plus sufficient thiamine, niacin, riboflavin, and β-carotene to

make up for loss during autoclaving were fed ad libitum to both groups. Each group consumed an average of 0.1 g feed/g of rat/per day, thus their oral exposures to DDT were comparable. Feed samples were analyzed after autoclaving to verify DDT compound content.

Microbial monitoring of the oral and anal orifices of the axenic animals was performed upon receipt and on days 5, 10 and 14 of the experiment. It was planned to feed the DDT diet to both groups for as long as microbial monitoring confirmed the sterile state of the germ-free animals, up to a period of 28 days. It was felt that a feeding period of 5 days or longer would be sufficient for enzyme induction; Morello showed that DDT-metabolizing enzyme activity becomes appreciable 72 hours after initial exposure to DDT (9).

It was discovered that a spore-forming contaminant was present in the thioglycollate media employed in the microbial monitoring on day 10. Introduction of this contaminated media into the isolator provided a source for contamination of the axenic animals. On day 14 the oral cavities of both axenic animals were still sterile, but the anal orifices of both animals produced growth of the same type of organism as present in the contaminated media used on day 10. The animals were sacrificed on day 16 when the first trace of growth appeared in the media inoculated on day 14. The animals were sacrificed by exsanguination, and livers, epididymal fat and perirenal fat were excised and stored frozen

until chemical analyses were performed.

The question of whether the spore-forming contaminant could effect the conversion of DDT to TDE in otherwide germ-free animals was investigated by the method of Barker, et al. (6). A subculture from each thioglycollate monitoring tube that showed growth was incubated aerobically for 6 days in brain-heart infusion broth in a tube coated with 6 mg p,p'DDT. A duplicate set was incubated under anaerobic conditions. Uninoculated tubes containing brain-heart infusion media plus DDT served as controls. Analyses of all tubes showed that the contaminant organisms did not convert DDT to detectable quantities of any other metabolite.

Tissues were analyzed by the method of Stanley and LeFavoure (11). Samples were digested in a mixture of perchloric and acetic acids and the pesticide-containing fat was extracted with cyclohexane. After quantitation of the fat content of the tissues, clean-up was effected on a sulfuric-fuming sulfuric acid-celite column. Feed samples were extracted with n-hexane for 16 hours in a Soxhlet apparatus, prior to acid-celite clean-up. The contents of all media tubes were analyzed by dissolving all DDT in n-hexane. After dilution, the solution was injected in the gas chromatograph without clean-up.

Pesticides were determined by electron capture gas chromatography using an Aerograph Pestilyzer with a 6-foot x 1/8" glass column packed with a mixture of equal parts of 5% QF-1 and 5% DC-200 on

Chromosorb G 70/80 mesh. Column temperature was 190°C and the nitrogen gas flow was 60 ml per minute. No detectable breakdown of p,p'DDT to p,p'TDE occurred under these conditions. Precision of the method was estimated to be ± 10%.

Results and Discussion

The data in Table 1 show that the axenic rats employed in this study cannot be distinguished from their conventional counterparts with respect to concentration and distribution of p,p'DDT and metabolites in their fatty tissues or livers.

Analyses of feed (Table 1) showed that loss of total DDT content, in part due to reduction to TDE, occurred during the autoclaving procedure. The breakdown of DDT during autoclaving resulted in inadvertent dietary exposure of TDE to both sets of animals. Presumably this dietary TDE contributed to the TDE found in the animal tissues. The change in TDE/DDT ratio from that in the feed to that in the liver (1:3 to 3:1) indicates that conversion from DDT to TDE also occurred in the animals.

The fact that the axenic animals became contaminated during the course of the experiment makes it necessary to consider the possibility that TDE found in the tissues was the product of microbial metabolism of DDT. If intestinal microorganisms were solely responsible for production of TDE it could not be expected that the axenic animals would receive and store the same quantities of TDE as their conventional counterparts. The latter had the full complement of their normal intestinal microflora for the entire duration

TABLE 1

DDT and Metabolites in Tissues of Individual Animals and in Feed

	p,p'DDT	p,p'TDE	p,p'DDE
Tissues (ug/g lipid)			
Epididymal Fat			
Axenic #1	360	13	32
Axenic #2	300	16	31
Conventional #1	360	9	33
Conventional #2	320	14	31
Perirenal Fat			
Axenic #1	370	6	37
Axenic #2	310	4	26
Conventional #1	420	8	29
Conventional #2	300	11	32
Liver			
Axenic #1	100	420	36
Axenic #2	130	370	37
Conventional #1	140	410	28
Conventional #2	110	330	30
Feed (ug/g feed)			
Average of all feed samples analyzed	93	34	1

of DDT feeding whereas the axenic animals were known to be in a sterile state for at least 10 of the 16 days of the experiment. On the 16th day their contamination, by what appeared to be a monocontaminant, can be considered minimal as evidenced by the fact that no organisms were cultured from oral swabbings taken on day 14. Further, the contaminant organism incubated in the presence of p,p'DDT gave no evidence of ability to convert DDT to TDE. This excluded the contaminant organism as a significant source of TDE found in the axenic animals.

The data presented in this paper support the hypothesis that metabolism of DDT to TDE occurs in the intact animal independent of microbial activity. The fact that the liver lipids of axenic and conventional animals contained the same levels of TDE indicates that contribution by intestinal microflora to tissue TDE is negligible compared to that produced by the animal organism itself.

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