STUDIES ON THE ROLE OF *FOLSOMIA FIMETARIA* IN METABOLISM OF SELECTED CHLORINATED HYDROCARBON INSECTICIDES

Thesis for the Degree of M. S.
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Erik Kirk Kirknel

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ABSTRACT

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By

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Folsomia fimetaria has been exposed to pp' DDT, pp' TDE and op' TDE. The experiments have been analyzed for pp' DDE, op' DDE and the methyl ester of pp' DDA. Previously described metabolism of pp' DDT to pp' DDE was confirmed. No metabolism of pp' DDT to the methyl ester of pp' DDA could be detected. There was no metabolism of pp' TDE or pp' TDE to pp' DDE or to the methyl ester of pp' DDA. An unidentified compound in extracts of Collembola showed up in the GLC analysis but did not appear on TLC, as the expected compound op' DDE.

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Erik Kirk Kirknel

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INTRODUCTION

Since it became available for insecticide use, metabolism of DDT and related compounds has been a subject of investigation for several reasons. First, it has been an insecticide used in relatively large amounts against a wide variety of insects, and second, its half life is relatively long.

It has been shown in numerous papers (1, 6, 9, 10 and 11), that different systems are capable of decomposing DDT to different compounds and in different degree. Five different routes of DDT metabolism have been described including, 1) DDA, 2) Kelthane, 3) dichlorobenzophenone, 4) DDE, and 5) TDE. In insects the most common described route is probably metabolism of DDT to DDE. To the authors knowledge, metabolism of TDE has not been reported in insects, although it is widely used as an insecticide, and is frequently reported as a contaminant. It is known that lake water is capable of degrading DDT to TDE (11) in the presence of reduced porphyrins and it is reported (2) that porphyrins in many milieus have a half life of billions of years. TDE was found to be common in samples of water, soil plants and animal tissue, in areas where only DDT had been sprayed and was shown to be able to degrade DDT to TDE (10). Metabolism of DDT to DDA has been reported for the body louse Pediculus humanus humanus, which was able to degrade DDT to three metabolites, DDE, DBP and DDA in the ratio 1:2:2 (12).

It has recently been shown by Butcher, Kirknel and Zabik 1969,[1] that in the presence of Folsomia fimetaria Linn., pp' DDT is metabolized to pp' DDE, in significant quantities; but the mechanism of metabolism is not defined.

Folsomia fimetaria is a commonly distributed Collembolan in soils, particularly those rich in organic matter. It could be potentially important in soil decontamination since it has been shown that Collembola can survive heavy application of DDT. Folsomia fimetaria itself appears to be highly resistant to DDT (13). Although large quantities of pp' DDE were produced by Collembolans in culture in previous experiments (Butcher, Kirknel and Zabik 1969), large quantities of TDE were also observed in these same cultures. This raised the question: is TDE also metabolized by Folsomia fimetaria? In trying to answer this question it seemed logical to investigate two metabolic routes for both pp' TDE and op' TDE; especially metabolism to pp' DDE, op' DDE and pp' DDA; and op' DDA; and pp' DDT to pp' DDA.

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[1] Manuscript (in press).

METHODS

The Folsomia fimetaria used in these experiments had been kept at 21 C° and saturated atmosphere in glass jars half filled with a mixture of plaster of paris and charcoal substrate to which powdered Brewers yeast was added as a food supply. The Folsomia strain had been cultured in the Michigan State University Laboratory under these conditions for about 3 years.

The metabolism experiments reported here were carried out in pyrex culture tubes 150 mm long, with an inside diameter of 14 mm. The substrate for the insecticide to be tested for metabolism was a 1-1/2% Difco bacto-agar, control No. 511791 from Difco Laboratories, Detroit, Michigan. The agar was poured into cold water and heated up to 90 C°. After being cooled down to 60 C°, a 400 ppm concentration of DDT in glass distilled hexane was slowly poured into the agar, while stirring with a magnetic stirrer. The ratio between the water agar and the solvent in which the pesticide was dissolved was 19:1. The hexane had a boiling point of 68-69°F. The final concentration of the insecticide in the agar was 20 ppm. The insecticides used were obtained from Pesticides Repository, Pesticides Research Laboratory, Perrine, Florida. The insecticide purity was represented as follows: pp' DDT, 99%; pp' TDE, 99+% and op' TDE, 98%. Gas-liquid chromatography analyses of the insecticides showed that pp' DDT contained about 11.1% pp' TDE. No other compounds could be detected with GLC in the samples of pp' TDE and op'TDE.

The agar with the insecticide (presumably now in a microcrystalline phase), was pipetted into culture tubes. Each culture tube received 7 ml and the culture tubes were plugged with cotton. In experiments without insecticides, only hexane was poured into the agar.

Culture tubes with DDT were cooled at room temperature with the tube tilted to give the agar a 40 mm long surface. Culture tubes containing TDE were autoclaved for 20 min. at 121 C°, 1 atmosphere pressure and cooled as described above for DDT.

DDT was not autoclaved, because, for unknown reasons, conversion of DDT to TDE in the agar proceeded more rapidly in the autoclaved experiments.

Twenty-four hours after the agar was poured, an antibiotic was added to the culture tubes in a Microvoid inoculation chamber with a syringe attached to a Swinnex millipore filter holder equipped with a 0.45 micron 13 mm millipore filter. Antibiotics were used in these experiments to see what metabolism, if any, was suppressed or eliminated. Dihydrostreptomycin was used because of its reputation as a wide spectrum antibiotic. The particular antibiotic was a crystalline sulfate from Pfizer Laboratory (Control No. 70944; exp. date January, 1973). One gram, containing approximately 10^6 units was dissolved in 50 ml distilled water, and 0.08 ml was injected (1600 units/culture tube or 400 units/cm^2 agar surface) into the culture tubes. After 24 hours, Folsomia were added to the culture tubes. Approximately 30 mg (live weight) were added to each culture tube. The age of the Collembola was 1 week at this time. They were not supplied with any food except for the agar in the culture tubes. The culture tubes were placed in a constant temperature cabinet at 21 C° without light.

After exposing the Collembola for predetermined periods of time, the culture tubes were removed from the cabinet and analyzed. Three culture components were analyzed for metabolites: 1) macerated Collembola, 2) surface agar, and 3) agar in the bottom of the culture tube. The GLC detected compounds were quantitatively determined in the Collembola, and qualitatively determined in both of the agar samples.

For purposes of extraction the Collembola were all removed from the culture tubes and while still alive were transferred to a 125 mm long, 14 mm inside diameter test tube, and closed with a Teflon lined screw cap. In order to prepare Collembola for analysis, a cottom plug saturated with ethylacetate was inserted into each test tube opening. After a one minute exposure, the Collembola were weighed on a Cahn electro balance, returned to the test tube and macerated with a glass rod. One milliliter of diethyl ether was added immediately and the test tube was capped. After 24 hours the Collembola extract was injected directly into the GLC without any clean-up. Concentration was calculated on a weight by weight basis.

In addition to the Collembola, a 2 mm thick layer of the surface agar was removed from the culture tubes with a spatula and transferred to a test tube, to which 1.5 ml diethyl ether was added. After 24 hours in the test tube, the extract was injected in the GLC without any clean-up. Concentration was determined in the extract and relationships between the observed metabolites were calculated.

The GLC used was a Beckmann GC-4 with an electron capture detector. The column was a 1/8", 3' copper column vibrator packed with 7.3% OV-17 + 3% QF-1 on a Gas-Chrom Q. column: temperature was 220 C°. The helium flow through the column was 40 ml/min. Concentrations were calculated using

peak height. The recorder was running at 1/2"/min. and the retention times for the insecticides were:

op' DDE	1.77 min.
pp' DDE	2.20 min.
Methyl ester of pp' DDA	2.20 min.
op' TDE	2.60 min.
pp' TDE	3.39 min.
op' DDT	3.23 min.
pp' DDT	4.09 min.

The results obtained from GLC analysis were checked on thin layer using glass plates covered with aluminum oxide, F(254) type T. These plates show flourescence when illuminated with a light source with a 2537 Angstrom wavelength. The solvent system used was 85% glass distilled hexane with a boiling point of 68-69 C°, and 15% Diethyl ether with a boiling point of 34.6 C°.

R_f values for	pp' DDT	0.463
	pp' TDE	0.362
	pp' DDE	0.558
	Methyl ester of pp' DDA	0.290

The pp' DDA was methylated so that it could be detected in an electron capture detector. The samples were first injected in GLC, and then methylated with diazomethane. A new injection was then done to determine if pp' DDA had been in the sample. Diazomethane was prepared in the following way: Diazald in ether was slowly dropped down into a 100 ml reaction bottle containing an alcholic solution of potassium hydroxide (25 ml ethyl alchol, plus 5 gram potassium hydroxide, plus 8 ml distilled water). The

reaction bottle was in a water bath heated up to 65 C°. The diazomethane and ether vapor were condensed in 2 receiving flasks and cooled down to 0 C°. The second receiving flask was filled to 1/4" with ether. This was just enough to reach the glass tube coming from the first receiving flask. Care had to be taken when preparing because of the explosion hazard.

RESULTS

Analyses for pp' DDT metabolites.

In this experiment the earlier observations made by Butcher, Kirknel and Zabik 1969, that pp' DDT was metabolized to pp' DDE, were confirmed (Table 2). Analysis was made 1, 6, 12, and 22 days after the Collembola were introduced into the agar. After 22 days the Collembola extract contained between 23% and 33% pp' DDE compared to pp' DDT. The metabolism did not seem to be affected by the antibiotic added to the culture tube, Tables 1, 2, 3 and 4, and pp' DDA could not be found in any of the extracts. A compound with the same retention time as the methylated acid did show up in all 3 extracts but could not be identified on thin layer plates as the methyl ester of pp' DDA. As Table 3 shows, the concentration of pp' DDT in the Collembola in some cases reached 1000 ppm. After 22 days the concentration of pp' DDE was higher. The cultures after 22 days did not have the same viability as they had in the beginning. An explanation for the decreased concentration of pp' DDT in the Collembola might be because they stopped feeding. Support for this argument may be derived from the following observations. After 12 days the Collembola in the culture tube were split into two groups. One group remained in the culture tube with 20 ppm pp' DDT and the other group was transferred to a culture tube with 1-1/2% agar but no pp' DDT. Table 5 shows these Collembola have almost the same relationship between pp' DDE and pp' DDT as the first group. Concentrations

of DDT in the Collembola are shown in the Tables 1, 2, 3, 4 and 5 and the Figures 5, 6, 7, 8, 9 and 10.

Analysis for pp' TDE metabolites

Neither the pp' DDE nor pp' DDA methyl ester metabolites of pp' TDE were found. However, an unidentified compound appeared in the Collembola extract in the GLC analysis, with the same retention time as op' DDE (1.77 min.), and the concentration increased slightly with time. It could not be identified on thin layer plates as op' DDE. Measured in GLC as op' DDE the relationship between pp' DDT and the compound in Collembola extracts after 3 days was 100: 3.6 and 100: 1.8 in experiments with antibiotic. In experiments without the antibiotic the relationship was 100: 1.5 and 100: 1.1. After 16 days, in experiments with the antibiotic, the relationship was 100: 6.5 and 100: 3.1. Without the antibiotic it was 100: 4.5 and 100: 4.5. The antibiotic did not seem to affect the presence of this compound. In the experiment designed to observe metabolism of pp' DDT to pp' DDE, pp' TDE was present. The experiment described here with pp' TDE shows that metabolism to pp' DDE started out with pp' DDT. Evidently pp' TDE was not metabolized to pp' DDE in the Collembola. As in the previously described experiment with pp' DDT, a compound with the same retention time as the methyl ester of pp' DDA showed up in the GLC analysis after methylation of the extracts. It was found in all three extracts, but it was not possible to confirm the finding on TLC. Concentrations of pp' TDE in the Collembola are shown in Table 6 and the Figures 1 and 2.

Analysis for op' TDE

No op' TDE metabolites were detectable with the above described analytical tools, not even after methylation with diazomethane. Concentrations of

op' TDE in the Collembola are shown in Table 7 and in Figures 3 and 4.

DISCUSSION AND CONCLUSION

This study confirms that in the presence of Folsomia fimetaria, pp' DDT is metabolized to pp' DDE rapidly and in relatively large quantities. This substantiates earlier work by Butcher, Kirknel and Zabik 1969. However, it has not been possible to show metabolism of pp' DDT to pp' DDA. Neither was it possible to show any metabolism of either pp' TDE or op' TDE to pp' DDE, op' DDE, methyl ester of pp' DDA or the methylester of op' DDA.

The data presented here appear sufficient to justify these conclusions. On the other hand had the technique been different, further information could have been obtained. A ring labelled isotope would have given better information about the real rate of metabolism. Furthermore, variations from culture tube to culture tube were in some cases quite large. It would have been better for example if the Collembola which were extracted at different times had been taken from the same culture. Although these and earlier studies do not deal with the mechanism of pp' DDT conversion to pp' DDE, it is felt that Collembola gut flora or those associated with the media substrate may be involved. Collembola reared from surface sterilized eggs in sterile culture, with sufficient nutrition, may provide answers to this question. Some preliminary work has been done on this problem, but results are inconclusive. Also, consideration has been given to the possibility that detoxifying Collembolan enzymes are involved. This work is continuing.

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Table 1. Experiment extracted after 1 day.

Culture Tubes	Replic.	Culture Compound	ppm in the Collembola x 100 pp'DDT	pp'TDE	pp'DDE	100 x pp'TDE / pp'DDT	100 x pp'DDE / pp'DDT	100 x pp'DDE / pp'TDE
With Collembola	1)	Collembola	2.60	0.36	0.08	13	3	20
		surface				16	2	10
		agar				18	1	6
With antibiotic	2)	Collembola	3.00	0.56	0.08	14	3	19
		surface				20	0	0
		agar				14	1	6
With Collembola	1)	Collembola	1.54	0.22	0.04	14	2	17
		surface				12	1	7
		agar				14	1	7
Without antibiotic	2)	Collembola	2.34	0.54	0.06	18	3	15
		surface				14	1	7
		agar				12	1	8
Without Collembola	1)	surface				16	2	12
		agar				15	1	7
With antibiotic	2)	surface				16	2	10
		agar				15	0.5	3
Without Collembola	1)	surface				12	1	7
		agar				12	1	7
Without antibiotic	2)	surface				Analysis not made.		
		agar				17	0	0

Table 2. Experiment extracted after 6 days.

Culture Tubes	Replic.	Culture Compound	ppm in the Collembola x 100 pp'DDT	pp'TDE	pp'DDE	100 x pp'TDE / pp'DDT	100 x pp'DDE / pp'DDT	100 x pp'DDE / pp'TDE
With Collembola	1)	Collembola	1.62	0.22	0.08	13	5	38
		surface				14	1	8
		agar				11	0.4	4
With antibiotic	2)	Collembola	1.27	0.16	0.07	13	15	43
		surface				12	0.3	3
		agar				12	0.4	3
With Collembola	1)	Collembola	2.22	0.30	0.13	14	6	42
		surface				16	1	4
		agar				16	0	0
Without antibiotic	2)	Collembola	1.76	0.23	0.09	13	5	38
		surface				14	0	0
		agar				10	0.3	3
Without Collembola	1)	surface				18	1	3
		agar				15	0.4	3
With antibiotic	2)	surface				12	1	5
		agar				12	1	4
Without Collembola	1)	surface				13	0.2	2
		agar				12	0.4	3
Without antibiotic	2)	surface				12	1	5
		agar				12	0.4	4

Table 3. Experiment extracted after 12 days.

Culture Tubes	Replic.	Culture Compound	ppm in the Collembola x 100 pp'DDT	pp'TDE	pp'DDE	100 x pp'TDE / pp'DDT	100 x pp'DDE / pp'DDT	100 x pp'DDE / pp'TDE
With Collembola	1)	Collembola	7.25	0.97	1.09	13	15	112
		surface				12	1	12
		agar				15	0	0
With antibiotic	2)	Collembola	11.4	1.37	0.93	12	8	68
		surface				10	1	12
		agar				11	0	0
With Collembola	1)	Collembola	4.35	0.72	0.45	17	10	63
		surface				15	2	13
		agar				14	0	0
Without antibiotic	2)	Collembola	8.32	1.08	1.32	13	16	122
		surface				12	1	6
		agar				16	0	0
Without Collembola	1)	surface				10	0.3	3
		agar				13	0	0
With antibiotic	2)	surface				11	0	0
		agar				12	0	0
Without Collembola	1)	surface				11	0	0
		agar				13	0.3	3
Without antibiotic	2)	surface				14	0	0
		agar				13	0	0

Table 4. Experiment extracted after 22 days.

Culture Tubes	Replic.	Culture Compound	ppm in the Collembola x 100 pp'DDT	pp'TDE	pp'DDE	100 x pp'TDE / pp'DDT	100 x pp'DDE / pp'DDT	100 x pp'DDE / pp'TDE
With Collembola	1)	Collembola	5.40	0.69	0.97	13	19	141
		surface				15	2	12
		agar				10	1	8
With antibiotic	2)	Collembola	5.35	0.90	1.50	17	28	167
		surface				16	3	19
		agar				12	1	11
With Collembola	1)	Collembola	3.21	0.72	1.18	22	37	165
		surface				11	2	22
		agar				9	1	9
Without antibiotic	2)	Collembola	4.76	0.80	1.36	17	28	145
		surface				10	3	30
		agar				12	1	8
Without Collembola	1)	surface				11	1	9
		agar				9	1	6
With antibiotic	2)	surface				12	2	17
		agar				10	1	7
Without Collembola	1)	surface				11	0	0
		agar				13	0.3	3
Without Antibiotic	2)	surface				22	0	0
		agar				21	0	0

Table 5. Collembola exposed to pp' DDT for 12 days and then transferred to non-pp' DDT agar.

Culture Tubes	Replic.	Culture Compound	ppm in the Collembola x 100 pp'DDT	pp'TDE	pp'DDE	100 x pp'TDE / pp'DDT	100 x pp'DDE / pp'DDT	100 x pp'DDE / pp'TDE
With antibiotic	1)	Collembola	4.60	0.39	0.74	8	16	190
	2)	Collembola	2.87	0.32	0.84	11	29	266
Without antibiotic	1)	Collembola	2.51	0.26	0.80	12	32	257
	2)	Collembola	2.91	0.38	1.00	13	34	264

Table 6. ppm pp' TDE x 100 in Collembola after exposure.

Culture tubes	Replic.	3 days	7 days	12 days	16 days
With antibiotic	1)	2.19	2.70	1.43	3.26
	2)	2.90	1.80	15.00	5.36
Without antibiotic	1)	1.90	6.20	3.72	12.00
	2)	4.70	7.40	1.80	1.90

Table 7. ppm op' TDE x 100 in Collembola after exposure.

Culture tubes	Replic.	3 days	7 days	12 days	16 days
With antibiotic	1)	7.80	18.00	4.20	6.24
	2)	6.60	10.00	8.15	57.00
Without antibiotic	1)	no analysis made	12.60	10.80	4.44
	2)	4.80	8.10	10.00	41.2

FIGURE 1

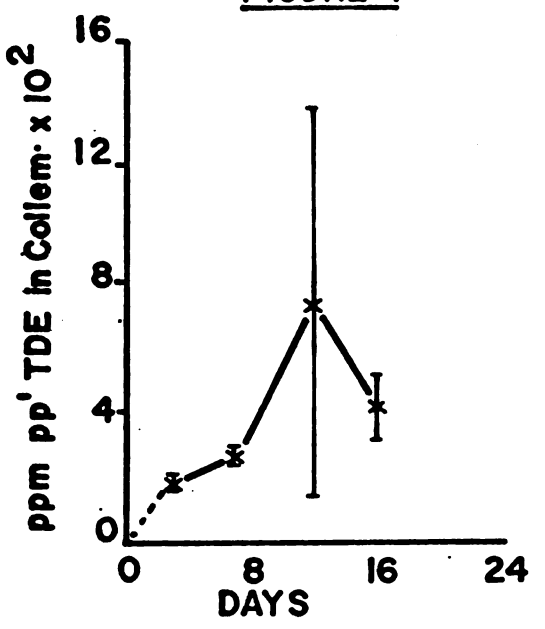


FIGURE 2

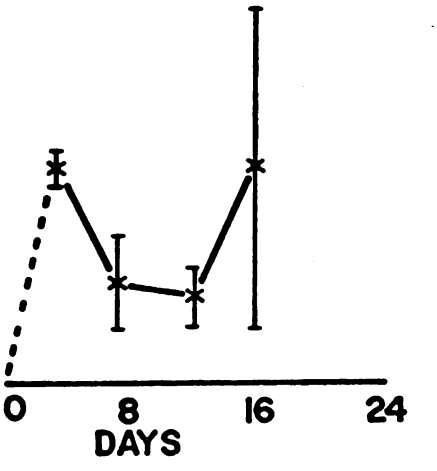
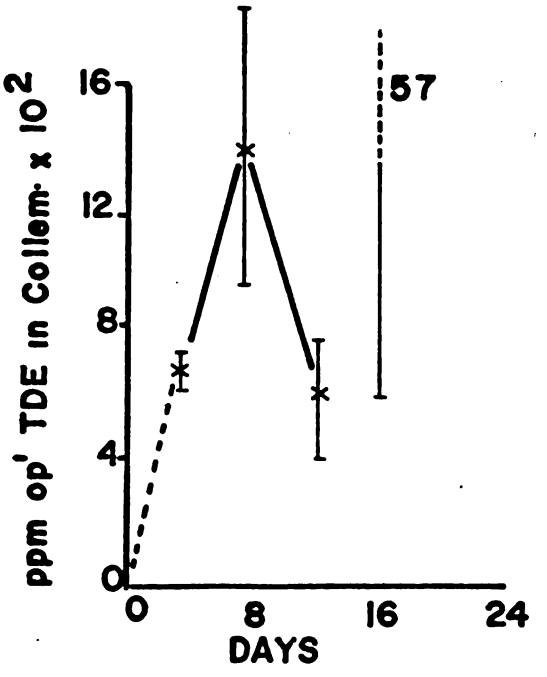
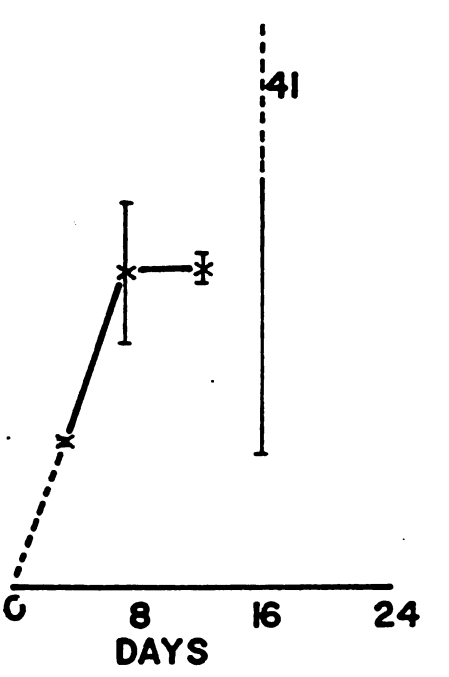


FIGURE 3



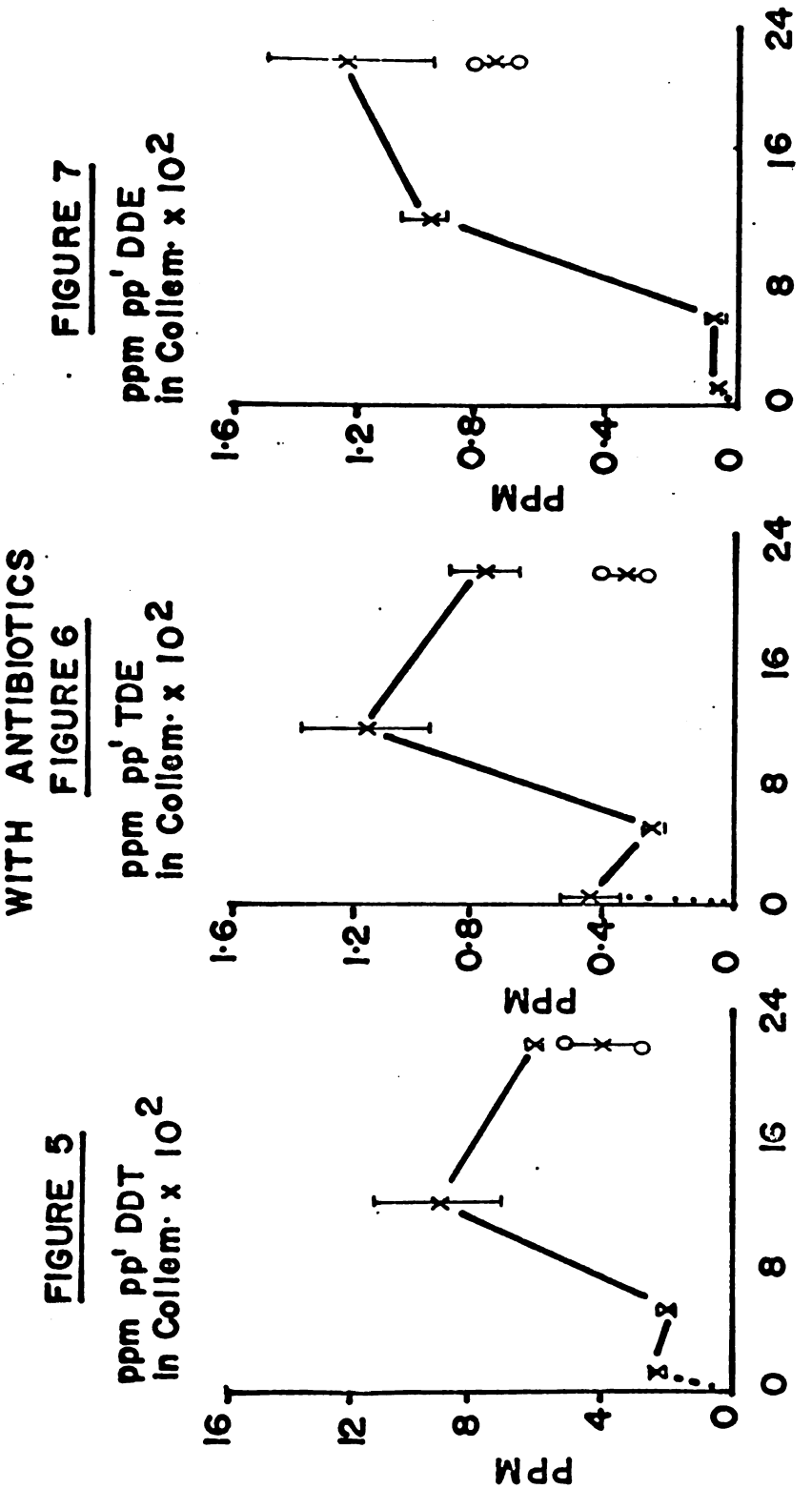
WITH ANTIBIOTIC

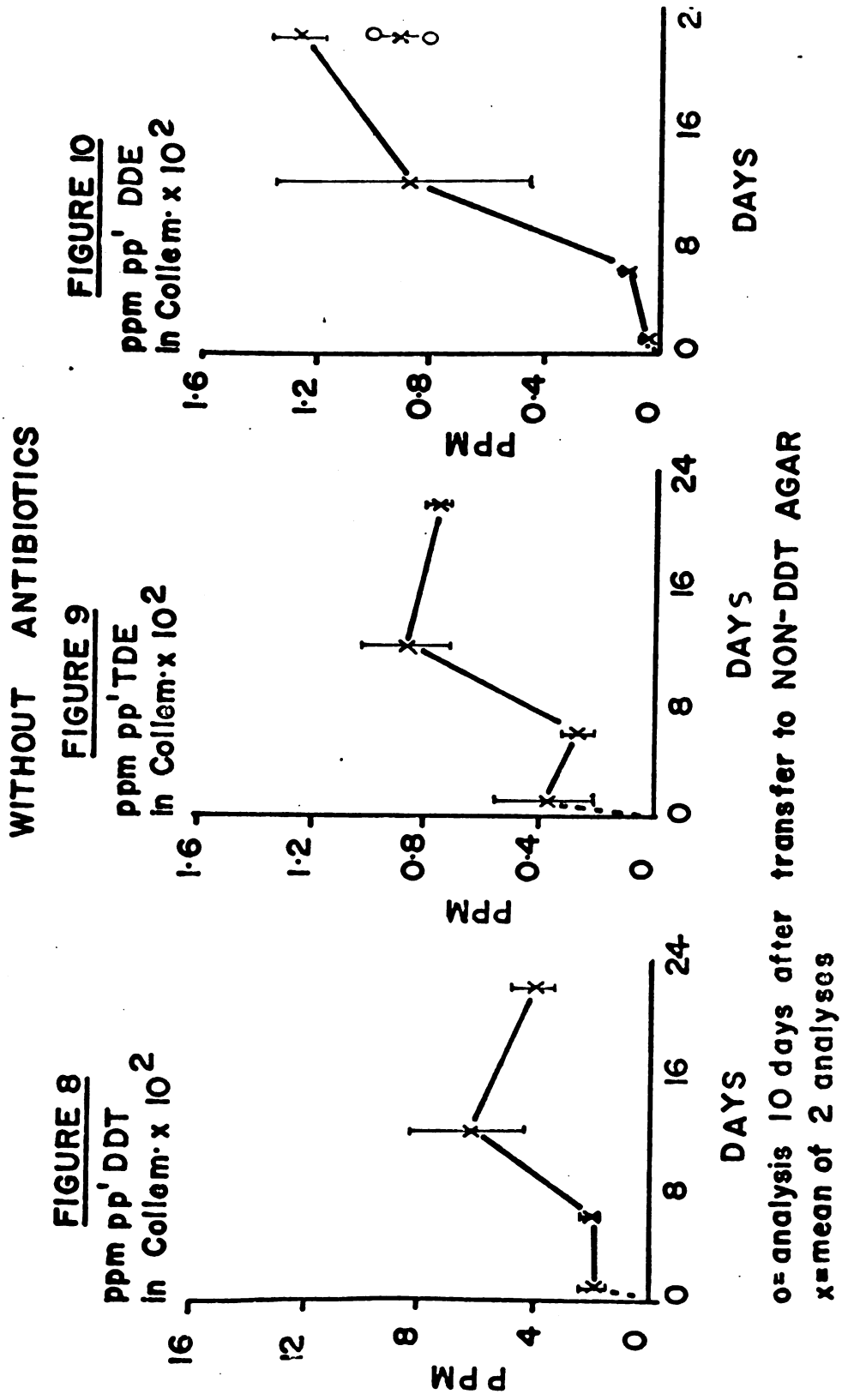
FIGURE 4



NO ANTIBIOTIC

x= mean of 2 analyses


WITH ANTIBIOTICS
FIGURE 5
ppm pp' DDT
in Collem· x 10²
FIGURE 6
ppm pp' TDE
in Collem· x 10²
FIGURE 7
ppm pp' DDE
in Collem· x 10²
PPM
DAYS
o= analysis 10 days after transfer to NON-DDT AGAR
x= mean of 2 analyses


WITHOUT ANTIBIOTICS
FIGURE 8
ppm pp' DDT
in Collem· x 10²
FIGURE 9
ppm pp'TDE
in Collem·x 10²
FIGURE 10
ppm pp' DDE
in Collem· x 10²
PPM
DAYS
o= analysis 10 days after transfer to NON-DDT AGAR
x=mean of 2 analyses