G. Tamura of Tokyo University for supplying DL-mevalonic acid, to Miss. Y. Hashimoto for her help on the feeding tests, and also to Mr. A. Matsuda for culturing *Aspergillus oryzae*.

**Summary**

The rice stem borer larva requires cholesterol as a dietary source for its growth, the optimal level of cholesterol being 0.1 to 1.3 per cent of the diet. Excessive amount of cholesterol, however, has no adverse effect on the larval growth. The sterol-precursors known for mammals were unable to be utilized for synthesis of cholesterol in the larva. These results suggest the absence of cholesterol synthesis in the rice stem borer larva.

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**Metabolic Fate of DDT in *Drosophila melanogaster*. III.** Comparative Studies.* Masuhisa Tsukamoto (Genetical Laboratory, Faculty of Science, Osaka University, Osaka, Japan). Received July 26, 1961. *Botyu-Kagaku* **26**, 74, 1961 (in English).

**14. ショウジョウバエにおける DDT の代謝. III.** 誘導体の代謝と他の昆虫での Kelthane の生成. 塚本増久（大阪大学 理学部 生物学教室） 36. 7. 26 受理

DDT から Kelthane への酸化的代謝の機構を知りまた抵抗性と代謝との関係を解明するための1つの手掛りとして，比較的毒性がすくなく，しかも DDT-Kelthane 型の代謝を示すような DDT の誘導体があるかどうかを探索した．その結果，キイロショウジョウバエでは DDT の $-CCl_3$ 部をそれぞれ $-CHCl_2$, $-CH_3$, -H などに置換してもアルコール型の代謝物に酸化されることがわかつたが，フェニール基のパラの -Cl をそれぞれ -H, -OH, $-CH_3$, $-OCH_3$ などで置換した場合にはきわめて速やかに代謝が行われるにも拘らず，アルコール型の代謝物はエーテル可溶性部分中には検出できなかつた．従つてアルキル基の脱塩酸や酸化とは全く異つた別の経路（恐らくフェニール基の）を通つて代謝されるものか，あるいはアルコール型からさらに検出不能な水溶性の形にまで代謝が進むものであろうと推測される．

また DDT-Kelthane 型の代謝はキイロショウジョウバエにのみ見られる特殊な現象ではなくて，ゴキブリやイエバエにおいてもこの酸化型の代謝経路が存在することが明らかとなつたので，むしろ一般に昆虫では DDT の代謝経路としては DDE への脱塩酸と Kelthane への酸化の両方が存在するものであろうと考えられる．

* This work was supported in part by grants from the World Health Organization, U. N., and from the Ministry of Education, Japan.
A list of all the abbreviations used is given in p. 76.

Chromatographic separations of various analogues of DDT and their possible metabolites in some insects have been performed by using resin column. Their ultraviolet absorption spectra have been compared. It has been confirmed that in *Drosophila melanogaster* DDT and some alkane-type analogues of DDT, such as TDE, K-3926 and DCPM, which have a replaceable hydrogen atom in the alkyl moiety between two *p*-chlorophenyl groups, have been metabolized to their corresponding alcohols such as Kelthane, FW-152, DMC, *iso*-DMC and DBH. On the other hand, *para*-substituted analogues of DDT, such as DØT, DOHDT, DTDT and methoxychlor, were rapidly metabolized but the corresponding alcoholic metabolites could not be detected among the recovered fractions. The ability of *D. melanogaster* to metabolize DDT to Kelthane has been found to be shared by other insect species such as *D. virilis*, *Musca domestica* and *Blattella germanica*.

The metabolic formation of Kelthane from DDT in insects first described in part I of this series[31] has recently been confirmed by radiometric techniques[15]. Such an oxidative metabolism at a tertiary carbon is rather uncommon. Comparative studies on the DDT-Kelthane type metabolism with various DDT-analogues were therefore undertaken in order to obtain a better understanding of this oxidation mechanism.

When a toxic substance such as DDT is tested against resistant and susceptible insects, it is difficult to ascribe resistance to a higer level of metabolism *in vivo* because the susceptible insects die sooner or, at least, fall into a physiologically abnormal state even at a sublethal dose of the insecticide and this in itself may account for a lower level of metabolism. If a DDT-like compound were not toxic to DDT-susceptible *Drosophila*, and if this compound were metabolized to its alcoholic derivative, as in the DDT-Kelthane conversion, it might be useful in studying the relationship between DDT-resistance and metabolism because the complicating factor of intoxication would have been diminished. The primary object of the investigation reported here was to find non-toxic analogues of DDT which underwent metabolism similar to the DDT-Kelthane conversion.

Since finding that DDE was a principal metabolite of DDT in the housefly[10,27,35], many workers have employed the Schechter-Hallar method[25] to determine DDT-metabolism in various insects, and a metabolite giving a red color reaction in this test was assumed to be DDE[7,8,9,18]. Various compounds structurally related to DDT including Kelthane also react positively in this test, and recent investigations using $C^{14}$-labeled DDT have proved that DDT is converted not only to DDE but also several unidentified metabolites in various insect species[11,12,14,18,21,30]. The second object of these comparative studies was, therefore, to determine if the DDT-Kelthane conversion was a property peculiar to *D. melanogaster* or possessed by other insect species.

## Materials

Both resistant and susceptible strains of *D. melanogaster* were used. Descriptions for the strains have been given in the part II of this series[32]. A DDT-resistant strain of *D. virilis* was also used, in which DDT-resistance has been analysed genetically by Oshima and Hiroyoshi[17]. For studying metabolism in the *Drosophila* species, DDT or its chemical relatives were added at doses of 1000-2000 μg per ml of larval breeding medium.

For comparing the metabolism by highly DDT-resistant and susceptible strains of the housefly, *Musca domestica*, the insecticide or its analogues were topically applied to adult flies or added to the larval media as in the case of *Drosophila*. Resistance levels of these strains to DDT and to Kelthane are given later.

Adults of the German cockroach, *Blattella germanica*, were supplied by Mr. M. Sakai, Research Laboratory, Takeda Chemical Industry Ltd., Kyoto, and by Yamamoto Agricultral Chemicals Ltd., Osaka. Adults of the American cockroach, *Periplaneta americana*, were also supplied by Mr. T. Kasai, Japan Agricultural Chemicals and Insecticides Co., Osaka.

The names of the reference compounds and the abbreviations used in this paper are given

below*:

1. DDT: 1,1-bis (*p*-chlorophenyl)-2,2,2-trichloroethane.
2. Kelthane: 1,1-bis (*p*-chlorophenyl)-2,2,2-trichloroethanol.
3. TDE: 1,1-bis (*p*-chlorophenyl)-2,2-dichloroethane.
4. FW-152: 1,1-bis (*p*-chlorophenyl)-2,2-dichloroethanol.
5. K-3926: 1,1-bis (*p*-chlorophenyl)-ethane.
6. DMC: 1,1-bis (*p*-chlorophenyl)-ethanol.
7. *iso*-DMC: 2,2-bis (*p*-chlorophenyl)-ethanol.
8. DCPM: bis (*p*-chlorophenyl)-methane.
9. DBH: bis (*p*-chlorophenyl)-methanol.
10. *iso*-DDT: 1,1-bis (*p*-chlorophenyl)-1,2,2-trichloroethane.
11. Cl-DDT: 1,1-bis (*p*-chlorophenyl)-1,2,2,2-tetrachloroethane.
12. DØT: 1,1-bis (phenyl)-2,2,2-trichloroethane.
13. DØTOH: 1,1-bis (phenyl)-2,2,2-trichloroethanol.
14. DOHDT: 1,1-bis (*p*-hydroxyphenyl)-2,2,2-trichloroethane.
15. DTDT: 1,1-bis (*p*-tolyl)-2,2,2-trichloroethane.
16. Methoxychlor: 1,1-bis (*p*-methoxyphenyl)-2,2,2-trichloroethane.
17. DANP: 1,1-bis (*p*-methoxyphenyl)-2,2,2-trimethylethane.
18. DANPOH: 1,1-bis (*p*-methoxyphenyl)-2,2,2-trimethylethanol.
19. DOHNP: 1,1-bis (*p*-hydroxyphenyl)-2,2,2-trimethylethane.
20. DDA: bis (*p*-chlorophenyl)-acetic acid.
21. PCBA: *p*-chlorobenzoic acid.
22. DDE: 1,1-bis (*p*-chlorophenyl)-2,2-dichloroethylene.
23. TDEE: 1,1-bis (*p*-chlorophenyl)-2-chloroethylene.
24. DME: 1,1-bis (*p*-chlorophenyl)-ethylene.
25. DBP: 4,4'-dichlorobenzophenone.
26. BP: benzophenone.
27. Bz: Benzil.

* The sources of the compounds listed above were as follows: Asahi Glass Co., Japan, compounds 1 and 8; Dr. E. D. Bergmann, Ministry of Defence, Israel, compounds 10, 11, 13, 15 and 27; Dr. H. D. Brown, Merck & Co., Inc., U. S. A., compounds 17, 18 and 19; J. R. Geigy S. A., Switzerland, compounds 12, 14, 20, 21 and 25; Dr. C. W. Kearns, University of Illinois, U. S. A., compounds 5 and 9; Dr. H. H. Moorefield, Union Carbide Chemicals Co., U. S. A., compound 7; Dr. A. G. Rogers, E. I. du Pont de Nemours & Co., U. S. A., compound 16; Rohm & Haas Co., U. S. A., compounds 2, 3 and 4; Sherwin-Williams Co., U. S. A., compound 6.

## Results

### A. Chromatographic Separation and Ultraviolet Absorption Spectra of DDT-like Compounds

Non-ionic adsorption chromatography for the separation of various DDT-analogues was performed on ion-exchange resins, Amberlite CG-50 and Duolite CS-101, with the isopropanol-water (3:2 by volume) system described in the part I of this series[31]. Column sizes were 0.8 cm in inside diameter and 69 cm (A-column) or 73 cm (B-column) in length for the Duolite resin, and 0.8 cm and 73 cm for the Amberlite resin, respectively. Elutions were performed at 20–23° C. Each one-ml fraction of effluent was collected automatically into a small test tube with a graduation mark at 3.0 ml, and was diluted with the isopropanol-water solution (3:2) to give 3.0 ml. The elution profile was determined by recording the optical density reading at 230 mμ. After the elution profile was obtained at this wavelength, the absorption spectrum for each DDT-analogue or its possible metabolite was continuously recorded from 220 mμ to 340 mμ.

Reference elution profiles were obtained by chromatography of mixtures of known compounds. The DDT-analogues used gave rise to 4 groups of elution profiles. The first group contained non-adsorptive substances and polar compounds which were rapidly eluted near the solvent front. This group included the acidic analogues of DDT, such as DOHDT, DOHNP, DDA and PCBA. The second group contained the alcohols such as DBH, DMC, *iso*-DMC, DØTOH, FW-152, Kelthane and DANPOH. The third group included the methane-, ethane- and ethylene-type derivatives such as DCPM, K-3926, TDE, DDT, Cl-DDT, *iso*-DDT, DDE, TDEE, DME, methoxychlor, DTDT, DØT,

etc. Finally, DBP represented the fourth group. Non-chlorinated benzophenone(BP)and benzil(Bz) eluted from the column into a fraction between the second and the third groups. While the separation of compounds belonging to different groups was satisfactory, the separation of members within a group was difficult in spite of differences in the number of chlorine atoms and in the substituents in the *para*-position of the aromatic moiety. These four groups are called hereafter the DDA-, Kelthane-, DDT-, and DBP-regions, respectively. The upper parts of Figs. 1-7 illustrate the elution profile obtained with known reference compounds.

The ultraviolet absorption spectra of the DDT-analogues in the 3:2 isopropanol-water solvent are shown in Figs. 8-13. These spectra suggest the following relations between chemical structure and absorption maxima:

1) Absorption maximum shifts progressively to shorter wavelengths as chlorine is subtracted from the 2-carbon of DDT-analogues; for example,

| | $-CH_3$ | | $-CHCl_2$ | | $-CCl_3$ |
|---|---|---|---|---|---|
| *Alkanes*: | K-3926 (228 mμ) | < | TDE (232 mμ) | < | DDT (238 mμ) |
| *Alcohols*: | DMC (227.5 mμ) | < | FW-152 (230 mμ) | < | Kelthane (231 mμ) |

2) Change of the chlorinated alkanes to the corresponding alcohols results in decrease of the absorption maximum, but the non-chlorinated alkyl analogues tested have approximately similar absorption maxima; for example,

| | *Alcohols* | | *Alkanes* |
|---|---|---|---|
| *Chlorinated alkyl group* | Kelthane (231 mμ) | < | DDT (238 mμ) |
| | FW-152 (230 mμ) | < | TDE (232 mμ) |
| | DØTOH (220 mμ) | < | DØT (228 mμ) |
| *Non-chlorinated alkyl group* | DMC (227.5mμ) | ≒ | K-3926 (228 mμ) |
| | DBH (228 mμ) | ≒ | DCPM (228 mμ) |
| | DANPOH (233 mμ) | ≒ | DANP (233 mμ) |

3) Replacement of the chlorine atom at the *para*-position of the benzene ring of DDT decreases the absorption maximum; for example,

*p*-H (228 mμ), *p*-OH (228 mμ), *p*-$OCH_3$ (228.7 mμ), *p*-$CH_3$ (235 mμ) < *p*-Cl (238 mμ).

These relations might be useful in the identification of unknown metabolite of DDT-analogues.

B. Metabolism of DDT-Analogues in *Drosophila melanogaster*

(1) Untreated control : Larval, pupal and adult tissue samples of *D. melanogaster* were prepared from untreated insects as controls. When ether extracts of these samples were partly purified by the partitioning method between acetonitrile and petroleum ether[32] and eluted through a resin column, several unknown substances were observed, which interfered with the spectrometric analysis in the ultraviolet region. The chromatographic profile of these interfering substances from untreated insects is illustrated in the upper part of Fig. 1 as a black histogram superimposed upon the separation profile of the reference compounds. Most of these interfering substances are eluted near the solvent front or the DDA-region (see above) and the other into the Kelthane-region. Ultraviolet absorption curves of these peaks are also given as broken lines in Fig. 8. The first black peak in Fig. 1 seems to contain several unknown substances with rather non-characteristic spectra within the 220–340 mμ range (curves 4 and 5 in Fig. 8), but the second peak at the Kelthane-region shows a characteristic absorption spectrum from 260 mμ to 300 mμ (curve 6 in Fig. 8). No attempt has been made to identify these interfering metabolites. They were almost completely removed by the conc. $H_2SO_4$ treatment[31], but as already mentioned some of the DDT-analogues and/or their metabolites are also destroyed by this somewhat drastic treatment. Therefore, the partitioning method between acetonitrile and petroleum ether was employed for the present work.

(2) Variations in the alkyl moiety : When *Drosophila* larvae were bred on media containing bis (*p*-chlorophenyl)-alkanes such as DDT, TDE, K-3926 and DCPM, their corresponding alcohols such as Kelthane, FW-152, DMC and DBH were detected respectively as the major metabolites (Figs. 1-4, lower parts). Identication of these metabolites was based upon coincidence of the chromatographic fraction and ultraviolet absorption spectrum with those of the reference compounds. In addition to the principal metabolite DMC,

Fig. 1

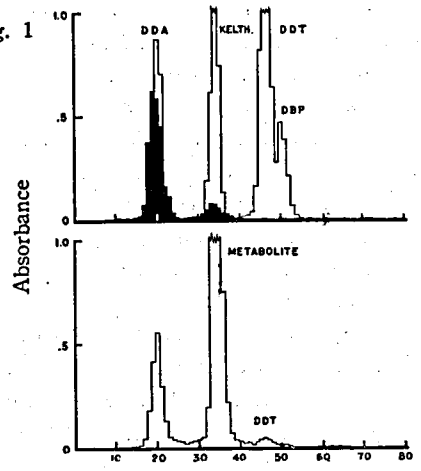



Fig. 2

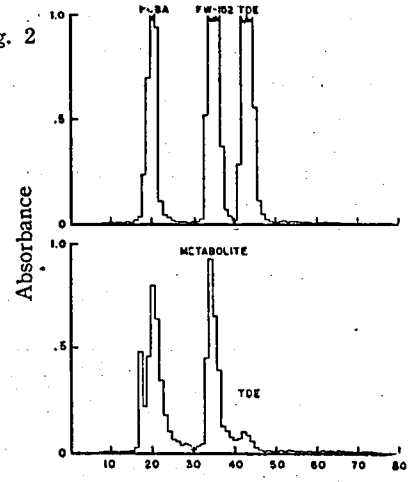



Fig. 3

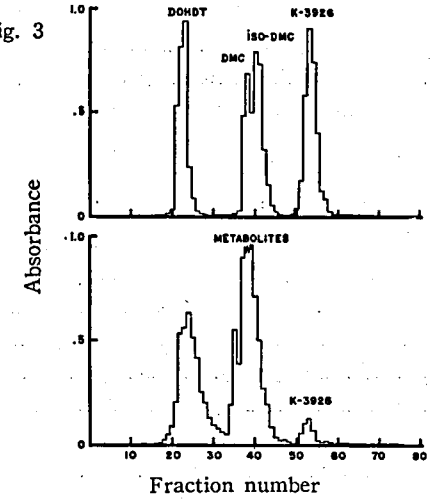



Fig. 4

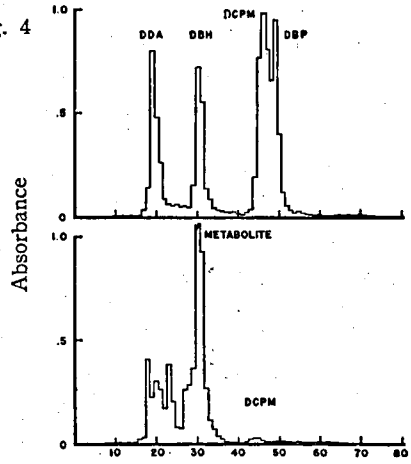



Fig. 5

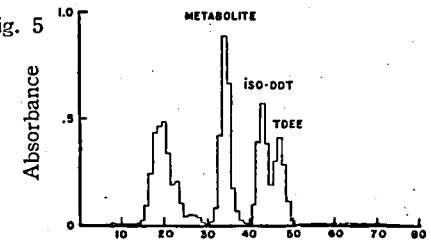



Fig. 6

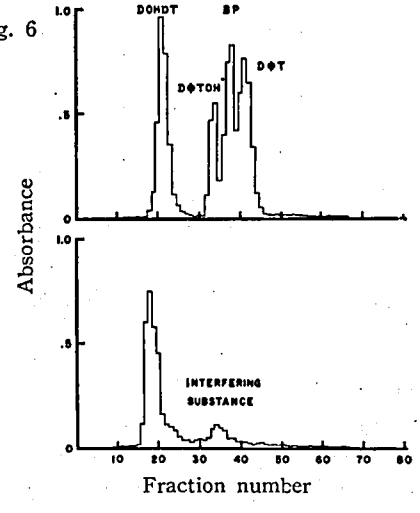


Fig. 7

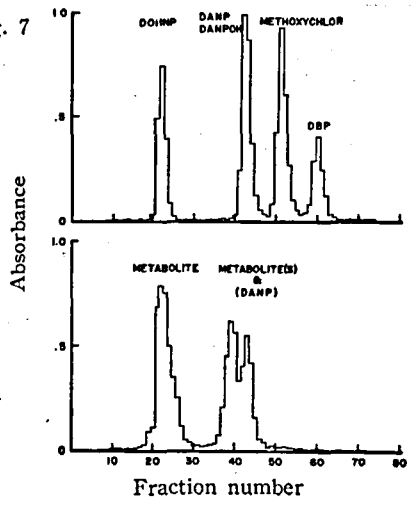
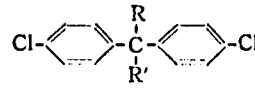



Fig. 1. Chromatographic separation of DDA, Kelthane, DDT, DBP and interfering substances from untreated *Drosophila* pupae in black histogram (upper), and metabolite of DDT in *Drosophila* (lower) on the Duolite CS-101 A-column.
Fig. 2. Chromatographic separation of PCBA, FW-152 and TDE (upper), and metabolite of TDE in *Drosophila* (lower) on the Duolite CS-101 A-column.
Fig. 3. Chromatographic separaton of DOHDT, DMC, *iso*-DMC and K-3926 (upper), and metabolites of K-3926 in *Drosophila* (lower) on the Duolite CS-101 B-column.
Fig. 4. Chromatographic separation of DDA, DBH, DCPM and DBP (upper), and metabolite of DCPM in *Drosophila* (lower) on the Duolite CS-101 A-column.
Fig. 5. Chromatographic separation of *iso*-DDT, TDEE and metabolite of *iso*-DDT in *Drosophila* on the Duolite CS-101 A-column.
Fig. 6. Chromatographic separation of DOHDT, DØTOH, BP and DØT (upper), and ether-extractable substances from pupae of *Drosophila* bred on DØT-containing media (lower) on the Duolite CS-101 A-column.
Fig. 7. Chromatographic separation of DOHNP, DANP, DANPOH, methoxychlor and DBP(upper), and metabolites of DANP in *Drosophila* (lower) on the Duolite CS-101 B-column.

another polar metabolite of K-3926 was also eluted from the column in the Kelthane-region. The absorption spectrum of this unknown metabolite was almost identical with that of *iso*-DMC. Aqueous and non-aqueous systems of paper chromato graphy[16] showed the presence of an unknown polar metabolite, *iso*-DMC, DMC and K-3926 in ether extracts of *Drosophila* bred on media containing K-3926. It is of interest that *iso*-DDT was also metabolized to an unknown metabolite which eluted in the Kelthane-region (Fig. 5). The sample of *iso*-DDT used contained small amounts of TDEE as an impurity. Based on its chromatographic position in the elution profile, on its absorption spectrum (Fig. 12) and on the chemical structure of the parent *iso*-DDT, this metabolite is suspected to be 2,2-bis (*p*-chlorophenyl)-1,1,2-trichloroethanol or *iso*-Kelthane. At present, however, no reference compound is available for comparison and more conclusive identification is not possible. No Kelthane formation from DDE or Cl-DDT in *Drosophila* was observed.

These results indicate that the presence of the unsubstituted hydrogen atom in the alkane moiety is essential for the DDT-Kelthane type metabolism in *Drosophila*. The chemical structures of the identified and proposed metabolites of the diphenyl alkanes are summarized in Table 1.

Table 1. Bis (*p*-chlorophenyl)-methane derivatives and their identified (or proposed) metabolites in *Drosophila*.




| Parent Compound | | | Metabolite | | |
|---|---|---|---|---|---|
| Name | R | R' | Name | R | R' |
| DDT | H | $CCl_3$ | Kelthane | OH | $CCl_3$ |
| TDE | H | $CHCl_2$ | FW-152 | OH | $CHCl_2$ |
| K-3926 | H | $CH_3$ | DMC | OH | $CH_3$ |
| | | | *iso*-DMC | H | $CH_2OH$ |
| DCPM | H | H | DBH | OH | H |
| Cl-DDT | Cl | $CCl_3$ | (No metabolite was observed) | | |
| *iso*-DDT | Cl | $CHCl_2$ | (*iso*-Kelthane Cl $COHCl_2$) | | |

(3) Variations in the ring component: It is generally said that the replacement of the *p*-chlorine of DDT by other substituents causes a decrease in insecticidal activity. For example, DOHDT and DØT are almost non-toxic to insects. The effects of substitution at the *para*-position of DDT on the metabolism by *Drosophila* have therefore been investigated. If DØT is metabolized to its ethanolic DØTOH *in vivo*, the latter would be expected to give a positive color reaction in the Fujiwara test, and, under alkaline conditions, to be converted to the ketone BP which is also

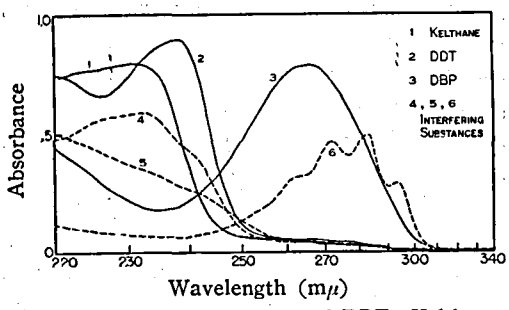



Fig. 8. Absorption spectra of DDT, Kelthane, DBP and interfering substances extracted from untrated *Drosophila*: Nos. 4 and 5 eluted from the column into the DDA-region and No. 6 into the Kelthane-region (see Fig. 1).

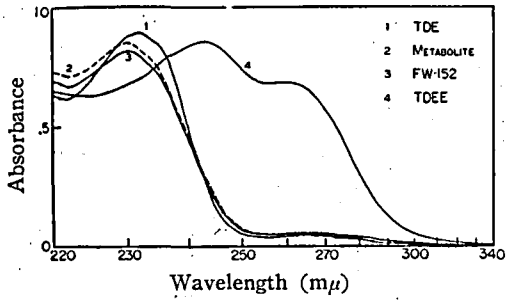



Fig. 9. Absorption spectra of TDE, FW-152, TDEE and metabolite of TDE in *Drosophila*.

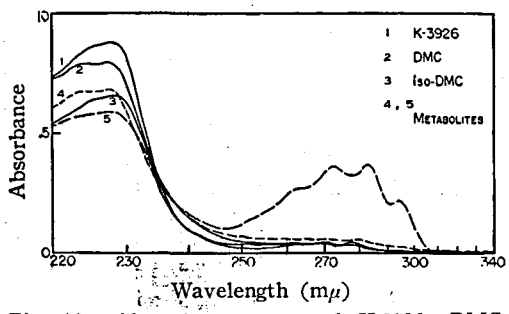



Fig. 10. Absrption spectra of K-3926, DMC, and metabolites of K-3926 in *Drosophila*.

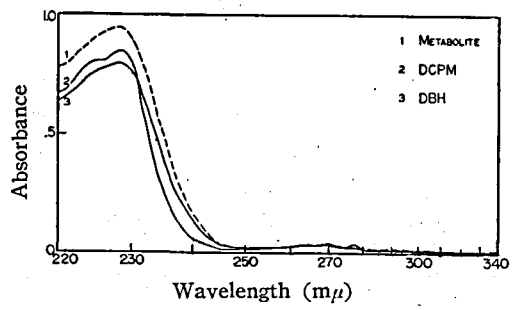



Fig. 11. Absorption spectra of DCPM, DBH and metabolite of DCPM in *Drosophila*.

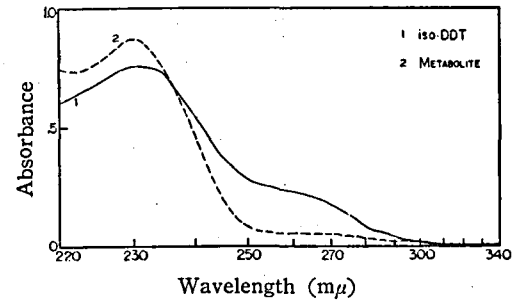



Fig. 12. Absorption spectra of iso-DDT and its metabolite in *Drosophila*.

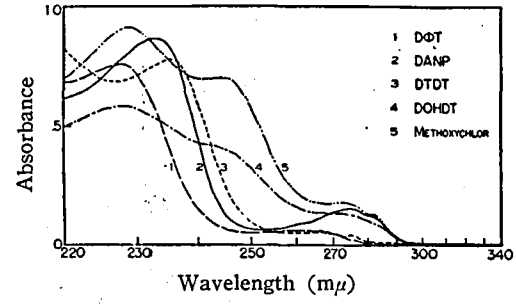



Fig. 13. Absorption spectra of various *para*-substituted analogues of DDT: DØT, DANP, DTDT DOHDT, and methoxychlor.

detectable by the characteristic absorption spectrum of the ketone group in the ultraviolet region. However, when *Drosophila* larvae were bred on media containing 1000-2000 μg of DØT per ml of medium, no appreciable amounts of DØT nor expected DØTOH were detected in the ether extracts of pupal samples (Fig. 6), while the larval samples occasionally contained small amounts of unmetabolized DØT which probably remained in the digestive organ of the larvae. Furthermore, the ether extracts of either larval or pupal samples were negative to the Fujiwara test. Similar negative results were obtained with DOHDT, DTDT and methoxychlor at 1000-2000 μg/ml doses.

These results suggest that the *para*-substituted compounds of DDT are rapidly excreted or converted into much more polar metabolites which are not extractable or are less extractable with ether, probably by aromatic oxidation or simultaneous aliphatic oxidation and conjugation.

(4) Variations in both alkyl and ring moieties: It has been reported that DANP has insecticidal activity against various insect species[6,7]. However, it seems to be practically non-toxic to *Droso-*

*phila*. For example, when *Drosophila* larvae were bred on media containing high doses of DANP such as 1000-2000 μg/ml, there was no significant effect on the rate of emergence. The formation of DANP from DDT is characterized by modification of both the alkyl and aryl moiety of the DDT molecule. The former structure suggests the DDT-Kelthane type metabolism toward its alcoholic derivative(s) and the latter structure suggests methoxychlor-like metabolism probably toward its phenolic derivative (s). When larvae were bred on DANP-containing media and the ether extracts of the pupae were chromatographed through the Duolite CS-101 B-column, some metabolites were eluted into the DDA- and Kelthane-regions (Fig. 7). The ultraviolet absorption spectra of DANP, DANPOH and DOHNP are quite similar to one another. The phenolic compound DOHNP, however, is distinguishable from other related compounds by its chromatographic position at the DDA-region. Both DANP and its alcoholic form DANPOH elute together into the same fraction under the present solvent system thus precluding their discrimination. Based on the characteristic absorption spectrum in the range 260-290 mμ and the chromatographic position at the DDA-region, the first peak in the lower part of Fig. 7 contains at least one metabolite of DANP, probably DOHNP or its derivative. The second peak in the Kelthane-region is unknown but it is suspected to be another alcoholic form of DANP, i.e., 3,3'-dianisyl-neopentanol or *iso*-DANPOH. A direct comparison of the characteristics of the unknown metabolite with a DANP-like spectrum and the reference compound has not been made. The third peak seems to contain either unmetabolized DANP or its metabolite DANPOH but these are indistinguishable as already explained.

### C. Kelthane-Production by Various Insect Species

Since the first study with the large milkweed bug by Ferguson and Kearns in 1949[10], the metabolism of DDT in insects was investigated with numerous insect species. The presence of the red color complex in the Schechter-Haller test has been considered initially to be suggestive of the DDE formation in these insects. The presence of DDE and various non-DDE metabolites in tissues or excreta of DDT-treated insects has recently been demonstrated by means of radiometric and chromatographic techniques[11,12,14,21]. Not only DDE but also some of the non-DDE metabolites are positive to the Schechter-Haller test, but with the exception of Kelthane in *Drosophila*[31] all these non-DDE metabolites still await identification. This previous work, however, did indicate the possibility of Kelthane formation as the metabolite of DDT in other insects than *D. melanogaster*, and several insects have been examined for this possibility.

(1) *Drosophila melanogaster*.——As reported in previous papers of this series, Kelthane is the principal metabolite of DDT in this species, and administered DDT is almost completely converted into Kelthane (Fig. 1). The presence of a trace of a DBP-like compound was occasionally indicated by the elution profile as described previously[31]. If DBP is a true metabolite of DDT, Kelthane may be an intermediate. Kelthane is easily converted into DBP under mild alkaline conditions, but no detectable amount of DBP was found *in vivo* when *Drosophila* larvae were bred on Kelthane-containing media. On the other hand, most of the DBP was not recovered from pupal extracts when larvae were bred on DBP-containing media, suggesting further metabolism or rapid excretion of DBP. In mammals BP is reduced to benzhydrol[34], but in *Drosophila* no DBH was detected chromatographically when larvae were bred on DBP-containing media. Conclusive identification of DBP as a true metabolite of DDT is therefore not possible at present.

(2) *Drosophila virilis*.——A DDT-resistant strain of *D. virilis* was chosen as experimental material since this species belongs to the same genus as *D. melanogaster* and the related genetic control of DDT-resistance has been investigated[17]. Following the breeding of larvae on DDT-containing media, the metabolite was extracted with ether from larvae and pupae. The presence of Kelthane was demonstrated by the Fujiwara test, by the paper chromatographic systems described by Mitchell[16] and by the column chromatography on the Duolite CS-101 resin.

(3) *Musca domestica*.——In the housefly it has

been well demonstrated that the principal metabolite of DDT is DDE and that an unknown water-soluble metabolite is also detected[30].

When a DDT-resistant strain of the housefly was bred on a medium containing 1000-1500 μg of DDT per ml the ether extractable metabolites from larvae, pupae and adults were feebly but always positive to the Fujiwara test, whilst the ether extracts from untreated control produced no pinkish color in the test[38]. This apparently indicates the presence of the oxidative metabolite Kelthane even in the housefly, whereas DDE is the major metabolite of DDT. Therefore, the metabolism of the ethane-type derivative K-3926 was studied as an indicator of the possible oxidation of DDT in insects. This compound obviously cannot undergo dehydrochlorination. When susceptible and DDT-resistant adults of the housefly were topically treated with K-3926 at 10-40 μg/fly, appreciable amounts of its ethanolic metabolite DMC were detected within 24 hours after treatment, which strongly suggests the DDT-Kelthane type conversion in the housefly *in vivo*. Fig. 14 illustrates the presence of such an oxidative metabolite DMC from the non-chlorinated alkane K-3926 in tissues of a susceptible laboratory strain.

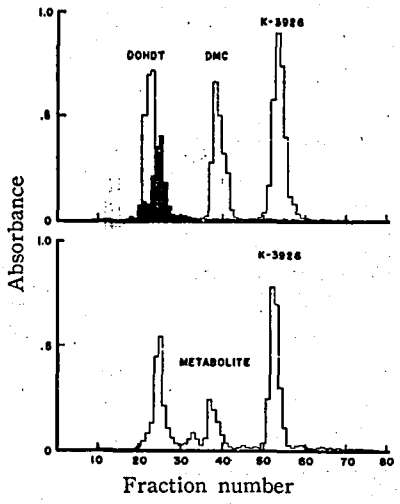



Fig. 14. Chromatographic separation of DOHDT, DMC, K-3926 and interfering substances (in black) of untreated houseflies(upper), and ether-extractable metabolite(s) from the housefly after topical application of K-3926 (lower). The Duolite CS-101 B-column was used.

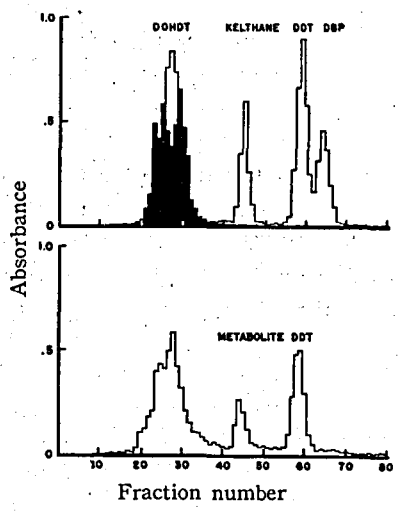



Fig. 15. Chromatographic separation of DOHDT, Kelthane, DDT, DBP and interfering substances (in black) of untreated German cockroaches (upper), and ether-extractable metabolite(s) from adult roaches treated topically with DDT (lower). The Amberlite CG-50 resin column was used.

(4) *Blattella germanica.*——When 50 μg DDT were topically applied to the ventral thorax of female roaches, the elution profile of the ether extractable metabolite through the Amberlite CG-50 resin column indicated the presence of a Kelthane-like metabolite (Fig. 15). The Fujiwara test was also positive to all the samples examined. Similar results were also obtained indicating the oxidative metabolism of TDE to FW-152, though the chromatographic profile is not illustrated here. From this evidence it is concluded that the German cockroach can convert DDT to Kelthane.

Using $C^{14}$-labeled DDT, Hoskins *et al.*[11] have demonstrated that both resistant and susceptible strains of the German cockroach convert DDT to four major metabolites, "Nos. 1, 2, 3 and 4", and that the metabolite "No. 4" is converted to DBP under alkaline condition and "Nos. 3 and 4" give pink color reactions respectively in the Schechter-Haller test. Chromatographic behaviour and their chemical nature are consisted with the possibility that the Hoskins' metabolite "No. 4" of the German cockroach is Kelthane.

(5) *Periplaneta americana.*—— Robbins and Dahm[21] have reported that in the feces of the American cockroach DDE and five metabolites with differont $R_f$ values were detected on paper

chromatograms using ethane-labeled and ring-labeled radioactive DDT. However, no metabolite was identified except DDE.

In order to elucidate whether Kelthane is produced from DDT in the American cockroach, 60 μg DDT were topically applied to the ventral thorax of adult females. The tissues and their feces were extracted with ether within 4-8 days after the treatment. The ether extract occasionally showed a pink color which interferes with the analysis by the Fujiwara color test. Therefore, this specific method is not applicable for detecting the Kelthane formation. Chromatographic profiles from the Amberlite CG-50 resin with the 3:2 isopropanol-water system indicated the presence of traces of Kelthane in both tissues and feces.

D. Differential Toxicity of Kelthane against Insects

Kelthane is less toxic against both DDT-resistant and susceptible strains of *D. melanogaster*. Both sexed adults of a laboratory strain of the German cockroach are also resistant to Kelthane. For example, topically applied Kelthane give no mortality within a range of 1-100 μg/male, whereas 10-15 μg DDT per male cause almost 100% mortality within 24 hours. On the other hand, Kelthane is more toxic than DDT against a DDT-resistant strain of the housefly in which Kelthane is a minor metabolite of DDT. For example, 5-100 μg doses of DDT cause only 2-12% mortalities within 24 hours when each female fly of a DDT-resistant strain was topically treated, but only 5 μg Kelthane give 100% mortality in the simiar topical application. In a highly DDT-susceptible strain of the housefly, Kelthane, at doses of 0.05-2.0 μg per female, does not show any insecticidal activity, whereas 1-2 μg of DDT causes 100% mortality of females within 24 hours. K-3926 is also less toxic than DDT to a DDT-susceptible strain of the housefly but is more toxic than DDT to a DDT-resistant strain. These results on differential toxicity of DDT and its derivatives in the housefly are in accordance with a previous finding of Reuter and Ascher[20].

## Discussion

Experience has suggested two method for investigating the relation between resistance and metabolism of the absorbed insecticide. The first and most direct one is determine the enzymatic conversion of the insecticide *in vitro*. The second and rather indirect one is the use of a non-toxic analogue of the insecticide *in vivo*.

The first method is quite useful when the insecticide is enzymatically metabolized *in vitro*. Unfortunately, however, the application of this method is limited to a few insect species. In the housefly, DDT is easily converted to DDE by the DDT-dehydrochlorinase *in vivo* and *in vitro*[5,28,20], but in mosquitoes, Brown and co-workers met a difficulty with DDT-dehydrochlorination by enzyme preparations under several conditions, whereas DDE was a major metabolite of DDT *in vivo*[5,8]. In *Drosophila melanogaster*, all attempts have failed to demonstrate the presence of DDT-oxidizing and DDT-dehydrochlorinating enzyme systems (Tsukamoto, Wada and Watanabe, unpublished). More recently Hoskins and co-workers[1] have successfully prepared a microsomal enzyme system capable of converting DDT to a Kelthane-like metabolite in the German cockroach, the American cockroach and the housefly.

The second method was, therefore, employed in the present investigation. A single and complete dechlorinations of trichloromethyl moiety of DDT give rise to the derivative TDE and K-3926, respectively. TDE is an effective insecticide as well as DDT against susceptible strains of *Drosophila*, and K-3926 seems to be less toxic than DDT but to be not non-toxic against susceptible strains. The methane-type derivative DCPM is one of the most simple analogues of DDT and is also less toxic to *Drosophila*. Fortunately, these unchlorinated alkane-type derivatives of DDT are metabolized to their alcohols as shown in the various figures. Therefore, these compounds will give a promising tool for confirming the causative relationship between the DDT-resistance and the DDT-Kelthane type conversion in insects.

Bergmann and Kaluszyner[4] have chemically synthesized Kelthane from DDT by the following consecutive stages: dehydrochlorination of DDT, chlorination of DDE, acetoxylation of Cl-DDT and final hydrolysis to Kelthane (Fig. 16), but

all attempts have failed to carry out directly the conversion of Cl-DDT to Kelthane in a single step. Although the mechanism of DDT-Kelthane type conversion by *Drosophila* is still unknown, both DDE and Cl-DDT which are precursors of Kelthane in the chemical conversion are not precursors of Kelthane *in vivo*. From these results it is apparent that the mechanism of the DDT-Kelthane conversion *in vivo* is quite different from and rather simpler than that of the chemical conversion.

Oxidation of alkylbenzenes in mammals is of special interest, since metabolism of DDT and its analogues in *Drosophila* seems to resemble the mammalian metabolism. According to the investigations by Williams, Smith and co-workers[22,23,26,34], ethylbenzene, *n*-propylbenzene, *iso*-propylbenzene, *n*-butylbenzene, *sec*-butylbenzene and *sec*-pentylbenzene are oxidized to their alcohols mostly at the activated carbon atom adjacent to the benzene ring ($\alpha$-oxidation). In *tert*-butylbenzene, however, no replaceable hydrogen atom is present at the $\alpha$-carbon, and $\omega$-oxidation is the major metabolic pathway. On the other hand, neither diphenylmethane nor triphenylmethane are oxidized to their alcoholic forms in mammals, whereas these compounds have a hydrogen atom at the $\alpha$-carbon adjacent to the benzene ring. In DDT, TDE, K-3926 and DCPM, these compounds carry a replaceable hydrogen atom at the $\alpha$-carbon, but these compounds are derivatives of diphenylmethane, suggesting that no hydroxylation of the DDT-analogues will take place at the $\alpha$-carbon in *Drosophila* like mammals.

Despite this argument, however, the $\alpha$-oxidation of these alkane-type analogues of DDT gives rise to their corresponding alcohols in *Drosophila*. TDE and K-3926 have another replaceable hydrogen at the $\beta$-carbon, and this suggests the possible and additional $\beta$-oxidation. The presence of unknown metabolite eluted from the column ahead of the $\alpha$-oxidation products FW-152 is occasionally detected from the chromatographic profile. But amounts of the unknown metabolite are too insufficient for further identifications whether this peak is a true $\beta$-oxidation metabolite of TDE. The ultraviolet absorption spectrum of the metabolite of K-3926 is similar to both that of DMC and that of *iso*-DMC. DMC elutes from the column slightly ahead of *iso*-DMC in reference elution profiles. This evidence indicates that the peak of metabolite in the Kelthane-region is a mixture of DMC, *iso*-DMC and interfering substance from insect tissues. In Cl-DDT or DDE, there is no replaceable hydrogen atom at the $\alpha$- or $\beta$-carbons, and as would be expected therefore no oxidation product was detected as the metabolite in the Kelthane-region or DDT-region of the elution profile. On the other hand, *iso*-DDT carries a replaceable hydrogen at the $\beta$-carbon. Therefore, the major metabolite eluted from the column into the Kelthane-region (Figs. 5 and 12) seems to be the compound 2,2-bis(*p*-chlorophenyl)-1,1,2-trichloroethanol or *iso*-Kelthane.

From the chemical structure of a non-chlorinated insecticide DANP, both $\alpha$- and $\beta$-oxidations are expected in the alkyl moiety. Rogers *et al.*[24] have chemically converted DANPOH to DANP and further to DOHNP. The conversion of the anisyl group to the hydroxyphenyl group is also a common phenomenon in mammalian detoxication. DANP is therefore assumed to be metabolized in *Drosophila* to at least three possible forms, i.e., 1,1-dianisylneopentanol (DANPOH), 3,3-dianisylneopentanol(*iso*-DANPOH)and1,1-bis-(*p*-hydroxyphenyl)-neopentane (DOHNP). The presence of DOHNP or its derivative is indicated from the ultraviolet spectrum for the first peak at the DDA-region of the chromatographic profile. This region is difficult to interpret in the spectrometric analysis especially at shorter wavelengths than 240 m$\mu$ because of the presence of the natural metabolites. Fortunately most of the derivatives of DANP have a characteristic second absorption maximum at 260-290 m$\mu$ region which is not obscured by the presence of the natural metabolites.

The results of variations at the *para*-position of the aromatic moiety of DDT suggest almost complete metabolism or excretion of an administered chemical. Although there is no direct evidence, the evidence from studies of mammalian metabolism suggest that *p*-H, *p*-$OCH_3$ and *p*-$CH_3$ groups in the ring structure may be oxidized to the *p*-OH or the *p*-COOH form, respectively, and more extensively to their conjugated forms. As already mentioned, these polar metabolites, if any, may be less extractable with ether or are

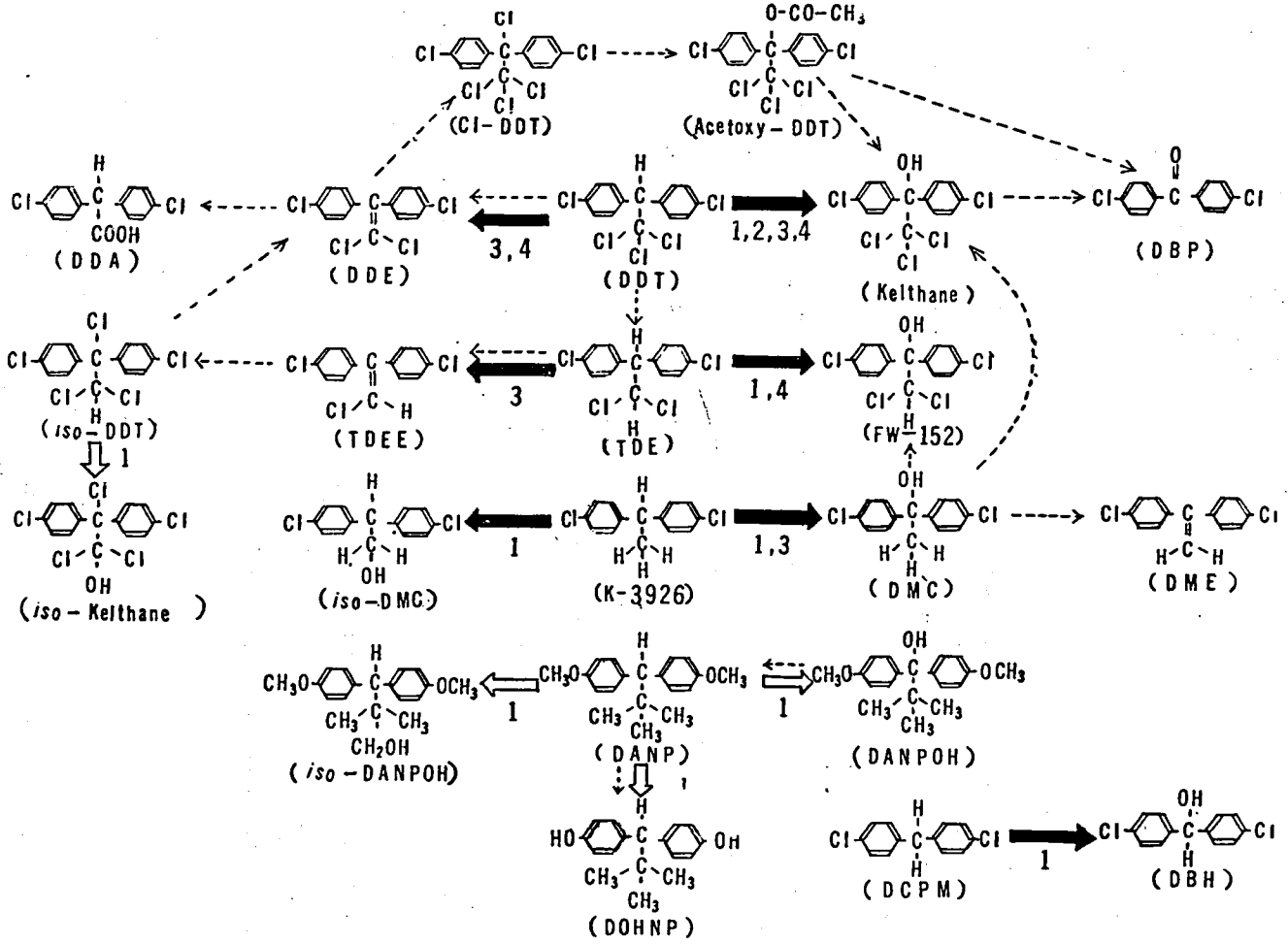



Fig. 16. Scheme for chemical conversions and metabolic pathways of DDT and its derivatives in insects

1. *D. melanogaster*, 2. *D. virilis*, 3. *M. domestica*, 4. *B. germanica*

identified path, proposed path, chemical path

eluted from the column into the solvent front their presence being obscured by the normal ultraviolet-absorbing metabolites from insect tissues. Since identification of these polar metabolites was not a subject of the present investigation and since their fractionation would require another chromatographic system and/or another technique such as radiometry, no further attempt has been made to identify these polar or water-soluble unknown metabolites.

As shown in the present paper the DDT-Kelthane type conversion is not a metabolic pathway peculiar to *D. melanogaster* but also occurs in insects of other order. Apart from the present results obtained *in vivo*, oxidative metabolism of DDT to a Kelthane-like compound *in vitro* has more recently been demonstrated in the housefly, the American cockroach and the German cockroach[1]. It is therefore a likely speculation that insects have originally had the metabolic abilities both to dehydrochlorinate and to hydroxylate DDT, and during the course of evolution of the species, one of the pathways has exceedingly developed in one group of insects such as the housefly and the other group such as *Drosophila*. The selection pressure with K-3926 in an insect population, therefore, may bring the accumulation of genes which control the oxidative metabolism, since this compound has no chlorine atom to be dehydrochlorinated in its chemical formula. It is however still unknown whether several parallel oxidations of the alkanes to the corresponding alcohols are performed by only a single enzyme (or enzyme system) which is produced under the same gene action, or what is the normal substrate of the insect enzyme *in vivo*.

Perry and Buckner[18] have investigated the metabolism of DDT in the human body louse and have shown that DDT-resistant strain metabolized DDT to an unknown water-soluble form which is positive to the Schechter-Haller test. Unfortunately, it is not yet resolved whether the water-soluble metabolite in this species is produced further through the known pathways via DDE or Kelthane or directly from DDT through an unknown or new metabolic path.

Available information on the metabolism of DDT in insects has been considered to be relatively sufficient, a great deal of which has been obtained through works with the housefly. However, responses to pesticides, effects of synergists, metabolic pathways *in vivo*, enzyme activities *in vitro*, etc. are largely different not only from strain to strain but also from species to species, and hence it should be recognized again that much more complete information on the biochemistry, particularly on species specificity, is necessary in each insect pest of medical or agricultural importance.

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**Résumé**

L'auteur a décrit dans cet article des résultats des études comparatives sur le métabolisme du DDT dans des insectes.

La séparation chromatographique sur colonne de résine et la caractéristique sur spectre d'absorption ultraviolette du DDT et des composés voisins du DDT ont été utilisées pour l'identification de leurs métabolites chez la drosophile. Les composés, DDT, TDE, K-3926 et DCPM, ont été métabolisés à leurs dérivés alcooliques correspondants tels que le Kelthane, le FW-152,

le DMC, l'*iso*-DMC et le DBH, respectivement. Toutefois, il a été montré que les alcools correspondants n'ont pas été détectés dans les extraits des drosophiles qui ont été traitées par les dérivés aromatiques du DDT tels que le DØT, le DTDT, le DOIIDT et le méthoxychlore.

En plus, il est très intéressant que le métabolisme du DDT au Kelthane a été aussi démontré chez des souches résistante et sensible de mouche et une souche normale de blatte.

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