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# Dehydrochlorination and DDT-Resistance in *Culex* Mosquitos *

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*The mechanism of resistance to DDT was investigated in* Culex *mosquitos to ascertain whether it was associated with detoxication to DDE as in houseflies and* Aedes aegypti. *Resistant larvae of* C. tarsalis *were found to effect this dehydrochlorination, whereas the susceptible larvae did not; resistant larvae of* C. fatigans *completely converted all the absorbed DDT to the metabolite DDE. Enzyme assays* in vitro *showed that the resistant* C. fatigans *had 10 times the dehydrochlorinating activity, and resistant* C. tarsalis *four times the dehydrochlorinating activity, of their susceptible counterparts. The DDT-dehydrochlorinase enzyme of* C. fatigans *resembled that of* Aedes aegypti *more than that of the housefly, though differing from it in at least one respect.*

DDT-resistance has developed in 13 species of culicine mosquitos, including the vectors of filariasis, yellow fever and several encephalitis diseases. In houseflies this resistance is due to a detoxication of DDT to DDE through a dehydrochlorinating enzyme, and the question is whether the resistance mechanism is the same in mosquitos. DDT-resistant adults of *Culex fatigans* were found to produce more DDE than normal (Bami, Sharma & Kalra, 1957) while DDT-resistant larvae completely converted DDT to DDE, which the normal larvae did not;[4] but these differences could have been an effect of the resistance, due to the insects' living and metabolizing for a longer period of time, rather than its cause. The dehydrochlorinating enzyme has been demonstrated in *Aedes aegypti*, and the DDT-resistance levels of various strains of this mosquito were found to be proportional to their content of this enzyme (Kimura & Brown, 1964), while the only DDT metabolite produced *in vivo* and *in vitro* was DDE (Abedi, Duffy & Brown, 1963). The purpose of this study was to ascertain whether a similar resistance mechanism existed in *Culex fatigans* and *C. tarsalis*.

## MATERIAL AND METHODS

The normal strain of *C. fatigans* originated from Rangoon, Burma, in 1963 and was somewhat DDT-tolerant ($LC_{50}$, 0.1 ppm); from it a DDT-resistant substrain ($LC_{50}$, 25 ppm) was developed in seven generations of laboratory selection. The normal strain of *C. tarsalis* was obtained from Corvallis, Oregon, USA (having originated in Hamilton, Montana), while the DDT-resistant strain originated from Oakridge, Oregon. The strains were reared in the laboratory and exposed to artificial dawn and dusk at 12-hourly intervals, the adults being fed on white mice and the larvae on a 1:10 mixture of blood albumin and yeast powder. The biochemical assays were performed on larvae in the early fourth instar.

For studies *in vivo* with *C. fatigans*, up to 2580 larvae were exposed for 24 hours in 250 ml of an

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aqueous suspension of radioactive DDT ; with *C. tarsalis* the larvae were divided into lots of 100 larvae exposed to 250 ml of the suspension. The $^{14}C$ labelling of the DDT was either at the terminal carbon-1 or the middle carbon-2 of the aliphatic chain. After removal to clean water for one hour, they were homogenized in 25 ml of 0.5% trichloroacetic acid, and the homogenate was extracted with two 25-ml portions of chloroform. Aliquots of 1 ml of the chloroform extract were taken and evaporated in planchettes for radioactive assessment of the DDT absorbed. In order to proceed to paper chromatography, the chloroform was then evaporated and the residue dissolved in 5 ml of petroleum ether, and this solution was passed through a column of Woelm alumina (water content 3 %) with the aid of 50 ml of petroleum ether. The solvent was evaporated from the eluate, and the residue dissolved in 0.5 ml of acetone, which was then chromatographed on paper by the descending method of Robbins et al. (unpublished). In this method, the immobile phase was 10% paraffin oil in chloroform, and the mobile phase was an 80:20 mixture of dimethyl-formamide and water. The chromatograms were air dried and then sprayed with a chromogenic agent (10 ml of 10% aqueous $AgNO_3$:10 ml of concentrated $NH_4OH$:80 ml of methanol) and dried under ultraviolet light until the spots became visible. The chromatograms were scanned with a TG-C14 gas-flow carbon counter (Tracerlab) connected to a Recti-Riter (Texas Instrument Co.), and the amounts involved in the peaks were measured by planimeter.

For studies *in vitro*, 10 g of larvae were homogenized in 25 ml of 0.018M glutathione in distilled water at 2°C and under nitrogen. For enzyme assay, 2.0 ml of this homogenate were added to 0.5 ml of phosphate buffer at pH 7.4 and 0.5 ml of 0.018M glutathione, and incubated with 200 μg of DDT (or analogous substrate) adsorbed on glass beads in 10-ml stoppered glass bottles under nitrogen at 37°C. Each assay consisted of four replicates, and each experiment consisted of five such assays. At the end of an incubation period of one hour for *C. fatigans* or three hours for *C. tarsalis*, the reaction was stopped with concentrated $H_2SO_4$, and the products were extracted with 15 ml of cyclohexane. The amounts of metabolites produced and of substrate remaining were measured in the Beckman spectrophotometer at the following wave-lengths in millimicrons :

| | |
|---|---|
| DDT and deutero-DDT | 241 |
| *o*-Chloro-DDT | 243 |
| DDD | 235 |
| DDE | 260 |
| *o*-Chloro-DDE | 260 |
| MDE | 260 |

The results were calculated on the assumption of a two-component system. Enzyme assays were also carried out with ring-labelled $^{14}C$-DDT and paper chromatography as in the *in vivo* experiments.

## RESULTS

When larvae of *C. tarsalis* were exposed to 0.5 ppm DDT *in vivo* (Table 1), the DDT-susceptible strain was unable to convert DDT to DDE, while the resistant strain could convert about one-third of the dose to DDE. On the other hand, the normal strain of *C. fatigans*, somewhat DDT-tolerant, could convert about 85% of the 1-ppm dose to DDE, while the resistant strain could convert almost all of the DDT. In *C. tarsalis*, the resistant larvae absorbed about eight times as much DDT as the susceptible larvae, owing to the latter becoming paralysed. On the other hand, the resistant *C. fatigans* absorbed no more DDT than the susceptible larvae.

The results obtained with homogenates *in vitro* (Table 2) show that the resistant strain of *C. tarsalis* produced about four times as much DDE as the susceptible strain. On the other hand, the resistant *C. fatigans* produced about 10 times as much DDE as the susceptible material, the spectrophotometric results being confirmed by the radiometric figures.

The chromatograms of the larval extracts of *C. tarsalis* exposed to $^{14}C$-DDT *in vivo*, as compared to those of an equal mixture of DDT and DDE (Fig. 1), indicate that DDE was the only chloroform-soluble metabolite produced by the resistant larvae; no water-soluble metabolite material could be found either in the larvae or in the water in which they had been exposed. A similar picture was given by the extracts of DDT-exposed larvae of *C. fatigans*, in which the normal larvae as well as the resistant larvae produced DDE (Fig. 1). The chromatograms of the larval homogenates incubated with $^{14}C$-DDT *in vitro* (Fig. 2) indicate that DDE was the only metabolite produced not only by resistant *C. tarsalis*, but also by susceptible or

TABLE 1

PERCENTAGE OF $^{14}$C-DDT CONVERTED TO $^{14}$C-DDE *IN VIVO* BY LARVAE OF RESISTANT AND SUSCEPTIBLE STRAINS OF *C. TARSALIS* [a] AND *C. FATIGANS* [b]

| Mosquito species | No. of larvae exposed | Concentration of DDT (ppm) | DDT absorbed (μg per 100 larvae) | DDT produced (% conversion) | Label position of $^{14}$C |
|---|---|---|---|---|---|
| *C. tarsalis* | | | | | |
| susceptible | 500 | 0.5 | 5.6 | 0 | C-1 |
| | 500 | 0.5 | 6.6 | 0 | C-2 |
| resistant | 500 | 0.5 | 46.5 | 34 | C-1 |
| | 500 | 0.5 | 49.5 | 45 | C-2 |
| *C. fatigans* | | | | | |
| susceptible | 2 580 | 1 | — | 83.3 ± 1.9 | C-1 |
| | 2 580 | 0.1 | — | 88.3 ± 2.5 | C-1 |
| | 430 [c] | 1 | 47.2 ± 1.2 | 86.4 ± 4.0 | C-1 |
| resistant | 2 580 | 1 | — | 96.2 ± 1.0 | C-1 |
| | 430 | 1 | 40.8 ± 5.6 | 99.0 ± 1.0 | C-1 |

[a] 100 larvae per 250 ml.
[b] Up to 2 580 larvae per 250 ml.
[c] Equivalent to 1 g wet weight.

resistant *C. fatigans*, with no sign of a substance with the properties of dicofol.

When resistant homogenates of *C. fatigans* were incubated with C-1-labelled $^{14}$C-DDD (TDE), subsequent chromatography revealed only the peak for the dehydrochlorinated product MDE, with no evidence of the hydroxylated product FW-152. Incubation of resistant homogenates with non-radioactive substrates (Table 3) showed that the greatest increase in activity was for *o*-chloro-DDT, with only slight increases for DDD and still less for deutero-DDT. Exposure of DDT-resistant larvae of *C. tarsalis* to 0.5 ppm radioactive *o*-chloro-DDT resulted in a trace of *o*-chloro-DDE when the C-1-labelled material was used, and no *o*-chloro-DDE with the C-2-labelled material.

TABLE 2

PERCENTAGE DDT CONVERTED TO DDE *IN VITRO* BY LARVAL HOMOGENATES OF RESISTANT AND SUSCEPTIBLE STRAINS OF *C. TARSALIS* IN 3 HOURS AND OF *C. FATIGANS* IN 1 HOUR

| Mosquito species | $LC_{50}$ (ppm) | Spectrophotometric assessment | Radioactive assessment [a] |
|---|---|---|---|
| *C. tarsalis* | | | |
| susceptible | 0.001 | 1.5 ± 0.4 | 2.0 ± 0.6 |
| DDT-$R_1$ | 0.025 | 4.3 ± 0.9 | 5.2 ± 0.5 |
| DDT-$R_5$ | 2.2 | 5.9 ± 0.8 | 8.5 ± 0.3 |
| *C. fatigans* | | | |
| susceptible | 0.1 | 3.8 ± 0.4 | 2.0 ± 0.4 |
| resistant | 25.0 | 40.0 ± 3.3 | 27.0 ± 2.4 |

[a] Using $^{14}$C-DDT labelled at the terminal aliphatic C-1 atom.

## DISCUSSION

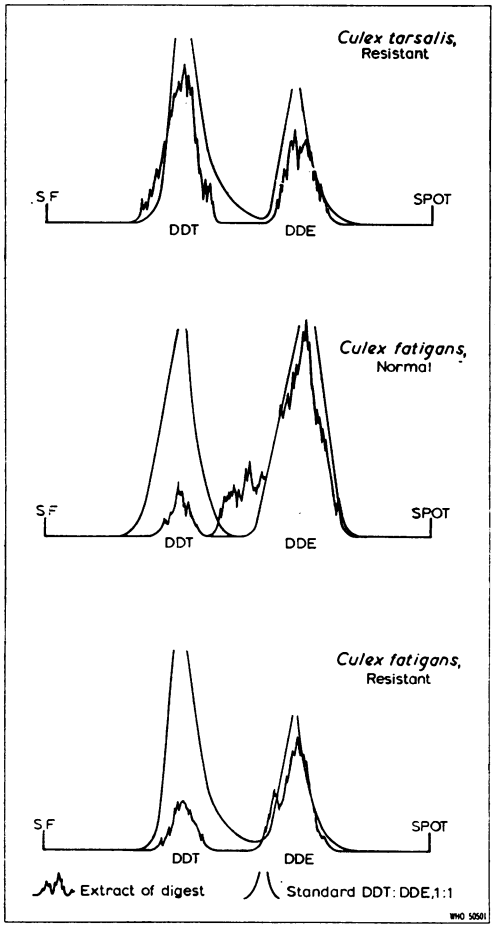
The extent to which DDT-resistant larvae of *Culex tarsalis* and *C. fatigans* dehydrochlorinate

TABLE 3

SPECTROPHOTOMETRIC ASSESSMENT OF SUBSTRATE SPECTRUM OF DDT-DEHYDROCHLORINASE IN *C. FATIGANS*, EXPRESSED AS PERCENTAGE CONVERTED TO DEHYDROCHLORINATED PRODUCT IN 1 HOUR BY HOMOGENATES OF NORMAL AND RESISTANT STRAINS

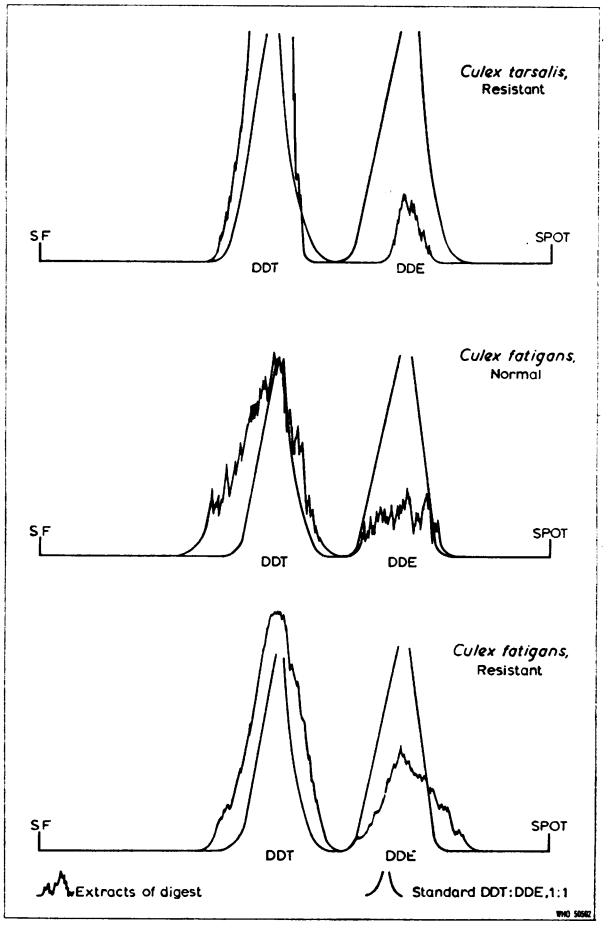
| Product | Normal | Resistant |
|---|---|---|
| DDT | 3.8 ± 0.4 | 40.0 ± 3.3 |
| DDD | 2.4 ± 0.2 | 3.6 ± 0.4 |
| Deutero-DDT | 0.8 ± 0.2 | 2.9 ± 0.8 |
| *o*-Chloro-DDT | 5.9 ± 0.9 | 14.1 ± 1.0 |

FIG. 1
CHROMATOGRAMS OF EXTRACTS OF LARVAE EXPOSED FOR 24 HOURS TO DDT *IN VIVO* [a]




[a] Resistant *C. tarsalis*: 0.5 ppm.
Normal *C. fatigans*: 1 ppm.
Resistant *C. fatigans*: 10 ppm.

FIG. 2
CHROMATOGRAMS OF LARVAL HOMOGENATES INCUBATED WITH 200 μg DDT *IN VITRO* [a]




[a] *C. tarsalis*: 3 hours. *C. fatigans*: 1 hour.

DDT *in vivo* is approximately proportional to their DDT-resistance levels, and is in line with the results previously obtained with *Aedes aegypti* (Abedi, Duffy & Brown, 1963)—namely:

| *Species* | $LC_{50}$ *(ppm)* | *DDT dose (ppm)* | *Conversion (%)* |
|---|---|---|---|
| *C. tarsalis* | 2.2 | 0.5 | 34-45 |
| *A. aegypti* | 0.7-9.0 | 0.5 | 50-72 |
| *C. fatigans* | 25 | 1 | 99 |

However, the normal strain of *C. fatigans*, with an $LC_{50}$ of 0.1 ppm, could dehydrochlorinate twice as much DDT as the resistant strain of *C. tarsalis*.

The dehydrochlorinating enzyme of *C. fatigans* resembles that of *Aedes aegypti* (Kimura & Brown, 1963) in being one-third as active on *o*-chloro-DDT as on DDT, and in being inactive against deutero-DDT. But it differs with respect to DDD as a substrate; whereas the enzyme of *Aedes aegypti*, like that of *Musca domestica*, is more active on DDD than DDT, in *C. fatigans* it is only one-tenth as active on DDD as on DDT.

In *C. tarsalis*, *o*-chloro-DDT cannot be dehydrochlorinated by resistant larvae, only traces of *o*-chloro-DDE being found under conditions where *Aedes aegypti* effected 10% conversion (Abedi, Duffy & Brown, 1963). The addition of DMC to DDT in 1 : 1 mixture had the same effect on *C. tarsalis* as on *Aedes aegypti*—namely, an insignificant decrease in DDE production. It was strange to find that the malathion-resistant Fresno strain of *C. tarsalis*, which is scarcely cross-resistant to DDT ($LC_{50}$, 0.02 ppm), nevertheless effected a 30% conversion of DDT *in vivo*.

When DDT-resistant strains are compared with susceptible strains, it is seen that they have enhanced powers of dehydrochlorination both *in vivo* and *in vitro*. DDT-resistant *C. tarsalis* larvae dehydrochlorinate one-third of the dose of DDT that susceptible larvae do not degrade at all, and contain four times as much dehydrochlorinase enzyme as the susceptible strains. DDT-resistant *C. fatigans* larvae completely dehydrochlorinate a dose of DDT that susceptible larvae convert to the extent of 85%, and contain ten times as much dehydrochlorinase enzyme as the normal larvae. It is therefore evident that in both species of *Culex* an enhanced dehydrochlorinative detoxication is a causal factor in DDT-resistance.

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## RÉSUMÉ

Le but du présent travail était de déterminer si, chez les culicinés, le mécanisme de la résistance au DDT implique un processus de détoxication de l'insecticide en DDE, analogue à celui qui intervient chez *Musca domestica* et *Aedes aegypti*.

On a constaté que des larves d'une souche de *Culex tarsalis* résistante au DDT détoxifient le DDT en DDE, alors que des larves d'une souche sensible au DDT ne le font pas. Des larves d'une souche normale, mais pas tout à fait sensible, de *C. fatigans* produisent une grande quantité de DDE, tandis que des larves d'une souche résistante transforment tout le DDT absorbé en son métabolite DDE. *In vitro*, l'examen spectrophotométrique montre que les souches résistantes de *C. fatigans* et de *C. tarsalis* produisent respectivement 10 fois et 4 fois plus de DDE que les souches homologues sensibles au DDT.

La DDT-déshydrochlorinase de *C. fatigans* ressemble à celle de *A. aegypti* et diffère de celle de *Musca domestica*, en ce sens qu'elle agit sur le *o*-chloro-DDT, mais non sur le deutéro-DDT ; en revanche, à l'opposé des enzymes de détoxication existant chez *A. aegypti* et *Musca domestica*, la DDT-déshydrochlorinase de *C. fatigans* est beaucoup moins active sur le DDD.

Il apparaît que, chez ces deux espèces de *Culex*, la résistance au DDT résulte non d'une diminution de l'absorption de l'insecticide, mais bien d'un accroissement du taux de détoxication du DDT en DDE par la DDT-déshydrochlorinase.

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