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RESISTANCE PATTERNS IN DDT-RESISTANT *AËDES AEGYPTI*

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SYNOPSIS

Colonies of three strains of *Aëdes aegypti* resistant to DDT were obtained from Trinidad, Haiti and Malaya and reared beside a normal colony. From their relative resistance to a series of compounds analogous to DDT, characteristic resistance " spectra " were obtained. The two colonies from the West Indies showed a similar type of resistance, rather different from the (smaller) resistance of the Malay strain. No resistance to methoxychlor or dieldrin was found.*

Instances of insecticide resistance are becoming increasingly common and are far outstripping our understanding of the phenomenon. Obviously, the first requirement is to find satisfactory methods of detecting and measuring resistance. The next step would seem to be an attempt to classify different types; this should indicate whether the mechanism which protects resistant strains varies widely in different species, or even between different strains of the same species.

A convenient way of defining resistance characteristics is by the measurement of relative degrees of resistance to a range of different poisons. Several deductions may be made from this information. In the first place, it should be possible to distinguish " vigour tolerance ", which is likely to be *general*, from " true " or pharmacological resistance, which is *specific* for a particular type of poison. Secondly, a genuine physiological resistance can be rapidly assigned to the main forms: DDT-resistance, cyclodiene-resistance, or organophosphorus-resistance. Thirdly, there may be varieties of these main forms; this can be demonstrated by measuring resistance towards a group of rather closely related insecticides. Then, it would be expected that strains relying on the same defence mechanism would show the same pattern or " spectrum " of resistance towards a group of analogues, although of course they might not necessarily be all at the same level. Further, it is conceivable that, by relating the relative resistance levels to some chemical

* Since the completion of this work it has been reported from the US Army Chemical Center, Md, (Craig, G. B. (1958) *J. econ. Ent.* (in press)) that methoxychlor-resistance has been produced in a subcolony of the Trinidad strain mentioned in this paper.

or physical property of the analogues, a clue to the defence mechanism might be found.

With these ends in view, we have studied the relative resistance of three DDT-resistant strains of *Aëdes aegypti* to various compounds similar to DDT.

Strains used

" *Trinidad* "

DDT-resistance was first reported from Trinidad in 1954 (Gillette [4]) where it had presumably arisen as a result of several years' residual spraying as an antimalarial measure. Our colony was kindly supplied in March 1956 by Mr Quarterman of the Communicable Disease Center, Georgia, USA, where this strain has been under investigation. Larval resistance is about 120 times that of the normal strain.

" *Haiti* "

DDT-resistance was found by Sautet & Vuillet [5] in a colony obtained from Port au Prince, Haiti. Some eggs were kindly sent to us in March 1956. Larval resistance is 340 times the normal.

" *Malay* "

A slightly resistant colony derived originally from a couple of females in Sijangkang in 1952, was maintained in London by Shidrawi.[6] He further raised the resistance by laboratory selection. Larval resistance is only 6 times the normal.

All these colonies have been maintained in laboratory culture ever since without selection, and with no evidence of change in resistance. None of them shows any resistance to dieldrin or gamma-BHC. In view of the persistence and specificity of the resistance in all cases, it seems that a true "pharmacological" form of resistance is involved, rather than "vigour tolerance".

Insecticides used

pp'-DDT—1 : 1 : 1-trichloro-2 : 2-di-(*p*-chlorophenyl) ethane
pp'-DBrDT—1 : 1 : 1-trichloro-2 : 2-di-(*p*-bromophenyl) ethane
pp'-DFDT—1 : 1 : 1-trichloro-2 : 2-di-(*p*-fluorophenyl) ethane
*pp'*DDD—1 : 1-dichloro-2 : 2-di-(*p*-chlorophenyl) ethane
Methoxychlor—1 : 1 : 1-trichloro-2 : 2-di-(*p*-methoxyphenyl) ethane
DANP—1 : 1 : 1-trimethyl-2 : 2-di-(*p*-methoxyphenyl) ethane (also known as dianisyl neo-pentane)
HEOD—1 : 2 : 3 : 4 : 10 : 10 : -hexachloro-6 : 7-epoxy-1 : 4 : 4a : 5 : 6 : 7 : 8 : 8a-octahydro-exo-1 : 4-endo-5 : 8-dimethano-naphthalene (a technical grade containing 85% of this is known as dieldrin)

Methods of measuring Resistance

Practically all the investigation concerned the larval stage. A few tests were made with adults, using the method of Busvine & Nash.[3] These indicated a very much lower degree of resistance in the adults than in the larvae. Thus in the Trinidad strain the median lethal dose (LD_{50}) was 4 times the normal for adults whereas the larval resistance was 118 times. The significance of this is not clear. Either it reflected a much more active defence mechanism in the larvae, or the difference in degree was due to the different methods of measuring resistance in larvae and adults.

The technique of measuring resistance in the larvae was as follows:

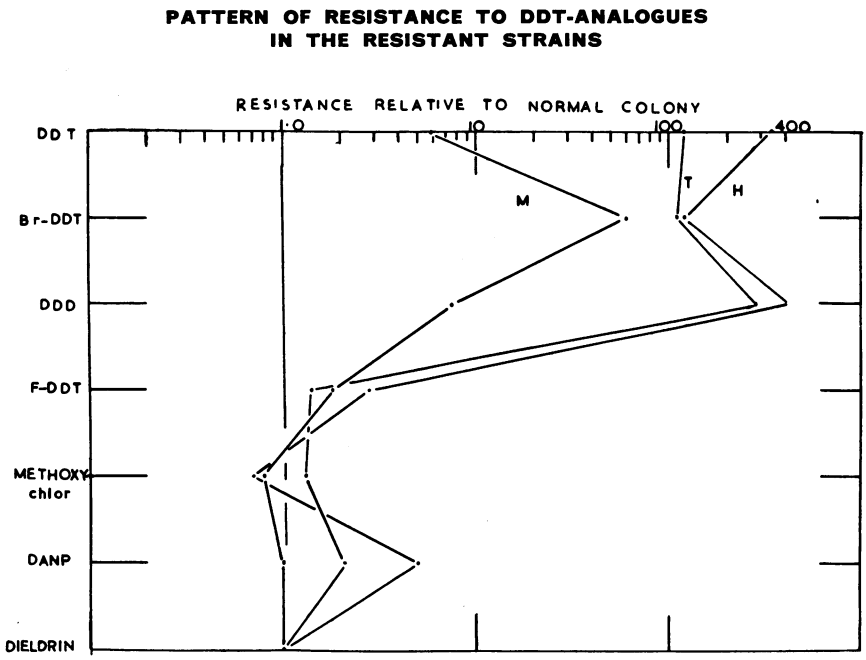
The insecticides were dissolved in alcohol to form solutions ranging from 0.001 % to 1.0 %. Aqueous suspensions were prepared in large beakers containing 500 ml water by adding calculated quantities of the appropriate solutions from a 1-ml graduated pipette. After the suspensions had been stirred with a clean glass rod, a batch of 25 early fourth-stage larvae was added to each. Mortality counts were made 24 hours later, moribund larvae (unable to swim off the bottom) being included with the dead.

Percentage mortalities, based on at least eight replicates, were converted to probits and plotted against log concentrations. The regression lines appeared to be straight but not parallel, since the slope generally became less with greater resistance.

RESISTANCE OF VARIOUS *AËDES AEGYPTI* STRAINS TO DDT-ANALOGUES

Insecticide	LC_{50} (p.p.m.) and relative resistance			
	Laboratory strain	Malay strain	Trinidad strain	Haiti strain
DDT	0.016	0.11 (×5.9)	2.0 (×118)	5.8 (×341)
Br-DDT	0.024	1.5 (×60)	2.5 (×108)	3.0 (×120)
DDD	0.052	0.4 (×7.5)	> 14 (× > 280)	> 20 (× > 400)
F-DDT	0.18	0.32 (×1.8)	0.25 (×1.4)	0.5 (×2.8)
Methoxychlor	0.19	0.16 (×0.8)	0.24 (×1.3)	0.125 (×0.66)
DANP	1.1	1.0 (×0.9)	2.1 (×1.9)	5.0 (×4.5)
Dieldrin	0.025	0.026 (×1)	0.025 (×1)	0.026 (×1)

The LC_{50}'s were estimated graphically, and degrees of resistance were calculated by comparison with the figure for the laboratory colony for each compound. The results are shown in the accompanying table and figure.



Results

The relative resistances of the various strains of *Aëdes aegypti* to the DDT-analogues are indicated in the table, and are shown as " resistance spectra " in the figure. The following conclusions may be drawn. It is evident that the resistance patterns as well as the general levels of resistance are very similar in the Trinidad and Haiti strains (resistance levels are highly correlated, $r = 0.97$). The pattern in the Malay colony is somewhat different. These findings are not surprising in view of the geographical origins of the strains. Additional information on the genetical relations of the three strains, which will be published later by W.Z.C., points to the conclusion that the factor for resistance in the Trinidad and Haiti strains is identical. The Malay strain appears to depend on a different factor.

Discussion

DDT-resistance in the housefly appears to be rather complex, depending on more than one defence mechanism. However, it seems likely that the most important of these is the enzyme DDT-dehydrochlorinase which

degrades the insecticide to the non-toxic compound DDE [1,1, dichloro 2,2-di (*p*-chlorophenyl) ethylene]. This enzyme is said to be present in all resistant flies and absent (or virtually so) in susceptible ones. The degradation of DDT to DDE has been demonstrated in vivo in larvae of the Trinidad strain of *Aëdes aegypti* (Brown [1]), though it was not possible to conduct the reaction in vitro with the extracted enzyme, under the conditions suitable for this operation with dehydrochlorinase from resistant flies. A little DDE was produced by susceptible *Aëdes* larvae at the very much lower dose comparable to their relative LC_{50} (Brown & Perry [2]). The evidence is, therefore, a little inconclusive. Resistant *Aëdes aegypti* larvae definitely degrade DDT to DDE, but it is not certain that they can do so more efficiently than susceptible larvae, at doses that cause no mortality in either.

The results of the present tests with DDT-analogues suggest that all three strains develop highest resistance to three analogues which are easiest to dehydrochlorinate by alkali, namely, Br-DDT, DDT and DDD; they are less resistant to analogues more stable in alkali, namely F-DDT, methoxychlor and DANP. The relative resistance of the Malay strain to the analogues shows a fairly good correlation with alkali dehydrochlorination, $r = 0.84$; the Trinidad and Haiti strains do not show any good correlation with alkali dehydrochlorination rates. Sternburg et al.[7] point out that alkali dehydrochlorination rates are by no means correlated to the rates of degradation by housefly dehydrochlorinase. They do not, unfortunately, show whether or not fly resistance to these analogues parallels the enzymatic breakdown of those compounds in vitro. It seems that there could not be close correspondence with tolerance levels to different analogues in all resistant fly strains, for it is known that their relative resistance patterns are not always the same.

So far as resistant *Aëdes* are concerned, the relative resistance to different analogues is certainly not correlated with rate of degradation by housefly enzyme. (Thus, methoxychlor should be fairly easily degraded, but none of the *Aëdes* colonies was resistant to this compound.) Perhaps this is not surprising, since Brown [1] has shown that the dehydrochlorinase of the Trinidad *Aëdes aegypti* does not function, in vitro, under conditions favourable for housefly enzyme. Furthermore, we have found that DMC, which antagonises the dehydrochlorination protective mechanism in houseflies, has no synergistic effect on DDT used against larvae of the Trinidad or Malay resistant strains.

Unfortunately, no definite conclusions can be reached, at present, regarding the exact mode of DDT-resistance in *Aëdes aegypti*. We believe that our results add a little support for the theory of a dehydrochlorination mechanism, though it seems that the enzyme responsible must be different from that in the housefly. In view of the different " resistance spectrum " of the Malay strain, as compared to the Trinidad and Haiti colonies, it is likely that more than one defence mechanism is involved.

RÉSUMÉ

Des colonies de trois souches d'*Aëdes aegypti* résistant au DDT ont été capturées à La Trinité, à Haïti et en Malaisie; elles ont été élevées dans les mêmes conditions qu'une souche normalement sensible. On a entretenu ces colonies pendant de nombreux mois sans les exposer au DDT: aucun changement important n'a été observé dans leur degré de résistance. Des larves au quatrième instar, exposées à des suspensions de DDT, ont manifesté une résistance assez élevée. Le rapport de la CL_{50} des diverses souches à celle de la colonie normale était le suivant: Haïti, 340; La Trinité, 120; Malaisie, 6. La résistance des adultes n'a pas été aussi forte: la CL_{50} obtenue avec la souche de La Trinité au moyen de l'épreuve de Busvine/Nash ne correspondait qu'à 3 ou 4 fois la CL_{50} normale. La résistance des larves a été mesurée en utilisant une série d'analogues du DDT, ce qui a permis d'obtenir des « spectres de résistance » caractéristiques des souches. La résistance de l'ensemble des souches est maximum à l'égard du DDT, du DDD et du « Bromo-DDT » et minimum à l'égard du méthoxychlore, du dianisyl néopentane et du « Fluoro-DDT »; aucune de ces souches n'est résistante à la dieldrine. Sous l'action d'un alcali, les trois premiers analogues du DDT perdent plus facilement un HCl que les trois derniers, mais il se peut qu'il s'agisse là d'une pure coïncidence. On n'a pas obtenu de réduction de la résistance en ajoutant au DDT le soi-disant synergique qu'est le DMC.

Le type de résistance a été très semblable pour les colonies de La Trinité et de Haïti, mais assez différent pour la colonie de Malaisie. Ces résultats concordent avec l'origine géographique respective des diverses souches et aussi avec les résultats de certains travaux génétiques effectués par l'un des auteurs (W.Z.C.), travaux qui seront publiés par ailleurs.

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