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RECENT EXPERIMENTS ON POSSIBLE RESISTANCE TO DDT BY ANOPHELES ALBIMANUS IN PANAMA

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SYNOPSIS

Following the observation of a change in the effectiveness of DDT residual house-spraying for *Anopheles albimanus* control at two experimental villages on the Chagres River in Panama, laboratory experiments were made to measure possible physiological resistance in this mosquito.

Blood-engorged *albimanus* from (*a*) a stock colony which had never had contact with DDT or any other chlorinated-hydrocarbon insecticide, (*b*) a stock-colony strain which had been exposed to DDT for more than 70 generations, and (*c*) two villages where 5% DDT in kerosene had been frequently applied, were exposed for 12 minutes to 0.5% *p,p'*-DDT in mineral oil.

The results showed that both the wild *albimanus* and the exposed stock-colony strain had essentially the same susceptibility to DDT as the unexposed stock-colony mosquitos. Any difference in the effectiveness of the insecticide for *albimanus* is therefore considered to be due to a behaviour change rather than to a change in the intrinsic toxicity of DDT for this mosquito.

The term "resistance" has been used in recent years to mean several different things. The chlorinated-hydrocarbon residual insecticides have been shown to be of variable effectiveness against different species of anophelines, depending on the habits and behaviour of these insects and on their intrinsic susceptibility to the toxicants. In addition, one species may initially respond in different ways in different places, as in the case of *Anopheles darlingi*. In British Guiana this species is reported to have been virtually eradicated from a large coastal area by DDT residual house-spraying alone (Giglioli [2]), while in parts of Brazil this technique has been of very much more limited effectiveness (Pinotti [5]).

In this communication the writer confines himself to the particular point of whether or not there is conclusive evidence that intensive use of DDT over a period of years has or has not brought about a change in the response of anophelines to this toxicant, with particular reference to *A. albimanus*. Such changes (true *acquired* resistance) are now well known

in the housefly and in several culicine mosquitos. What evidence do we have that this has happened or is happening in anophelines ?

One great difficulty in attempting to assess the situation has been the lack of a simple standard technique for measuring the minimum or median lethal dose (MLD or LD_{50}) of the newer insecticides against larval and adult stages of mosquitos. More work of this sort has been done with larvae than with adults, probably at least in part because it is easier to arrange a continuous contact between larvae and toxicant in a liquid medium. The work of various authors, summarized in part by Trapido,[6] indicates that there exist significant differences in the initial susceptibility of various anophelines to DDT. While there may be hidden pitfalls in the method of exposing anopheline larvae to acetone or alcoholic suspensions of DDT in water, to establish MLDs, the problem of readily exposing workable numbers of adult anophelines to uniform deposits of DDT has been greater. The series of papers by Hadaway & Barlow [1, 3, 4] has clearly illustrated the importance of such factors as the nature of the surface, the physical state of the toxicant, and the size and form of the deposited crystals (or particles in the case of wettable powders) in determining the mortality rate.

The writer has recently reported on a change observed in the effectiveness of DDT residual house-spraying for the control of *A. albimanus* at two experimental villages on the Chagres River in Panama (Trapido [7]). One of these villages had been sprayed 20 times over a period of eight years and the other 13 times in five years. Comparison of house catches following spraying in 1952 with those after the early sprayings in the period 1945-47 demonstrated that there was no longer the drastic reduction in numbers of *albimanus*, as had been initially observed, nor did there appear to be a selective mortality of engorged *albimanus*. The large reduction in the proportion of *albimanus* which were successful in becoming engorged with blood was an effect that persisted. It was considered possible that this apparent change might be due to acquired physiological resistance to DDT, or to the selection of a population hyperirritable to DDT, or to a combination of these factors. It was necessary at this point to measure the possible contribution of each of these factors in the laboratory. Since the publication of these observations, experiments to measure possible physiological resistance have been completed, and the results are given here.

A simple technique suitable for measuring the toxicity of DDT to adult anophelines, which provides for the contact of the insects with the toxicant in a uniform and easily duplicated physical state, was suggested to the writer by Dr. James Busvine of the London School of Hygiene and Tropical Medicine. This technique consists of dissolving the DDT in a non-volatile vehicle such as clear mineral oil, diluting this with ether, and applying the resultant solution to filter-paper disks. The ether evaporates off rapidly and the filter-paper disks, which are then uniformly impregnated with DDT in mineral oil, may be used after 10 or 15 minutes. In practice 1 ml of the

DDT—mineral-oil solution was diluted with 3 ml of ether, and this mixture was dripped on to filter-paper disks 125 mm in diameter, supported on a bed of pins. After the ether had been allowed to evaporate, a segment 3 cm from the edge of the filter-paper was cut, and the paper was then rolled and inserted into shell vials 25 mm × 80 mm. Approximately 30 blood-engorged mosquitos were then introduced into each paper-lined vial by means of an aspirating-tube ; the tube was withdrawn ; and the paper projecting over the edge of the shell vial was crimped to imprison the mosquitos in the paper-lined vial. Only the bottom of the vial, 25 mm in diameter, was an untreated surface, and it was found that the mosquitos preferred to rest on the filter-paper walls rather than on the small glass surface at the bottom of the test-vials. Following exposure, the mosquitos were released into clean holding-cages, and the mortality was subsequently determined at 24 hours. Various dilutions of pure *p,p'*-DDT in mineral oil were tried in calibration runs, using a stock colony of *A. albimanus* which had never been exposed to DDT. It was found that 0.5% DDT with an exposure time of 12 minutes produced a kill approximating 90% after 24 hours.

While this technique does not expose the mosquitos to the sort of dry films they would experience in the field, it does assure that the same sort of insecticide contact is duplicated in all tests, and the consistency of the results obtained on different trials confirmed the validity of the test-method. The space in which the mosquitos are confined is small enough for fairly uniform contact with the treated surfaces to be maintained throughout the exposure period. This method is intended, of course, to measure only the ability of the mosquitos to survive contact with the toxicant, not their ability to detect or avoid it.

Following this standard procedure, blood-engorged *albimanus* from four sources were exposed. In all, 2,122 mosquitos were used in these tests.

1. *Stock colony*

These *albimanus* were from the colony established at the Gorgas Memorial Laboratory in 1938, and had never had contact with DDT or any other chlorinated-hydrocarbon insecticide.

2. *Exposed colony*

In 1948 the stock colony of *albimanus* was split and one portion was exposed to DDT as a dry deposit out of acetone solution for a period sufficient to produce a mortality of approximately 80%. The progeny of the survivors were exposed in this manner through more than 70 generations until 1952.

3. *Santa Rosa strain*

The village of Santa Rosa had been sprayed 14 times with 5% DDT in kerosene between 1947 and 1953 ; engorged female *albimanus* from a horse-baited stable trap in the centre of the village were obtained for these tests.

4. *Gatuncillo strain*

Gatuncillo village had been sprayed with the same toxicant 21 times between 1944 and 1953. For these tests *albimanus* taken in the same manner as those from Santa Rosa were used.

TABLE I. RESULTS OF EXPOSING BLOOD-ENGORGED ANOPHELES ALBIMANUS FOR 12 MINUTES TO 0.5% p,p'-DDT IN MINERAL OIL

Source of mosquitos	Number of replications	Mortality after 24 hours (%)	
		mineral oil (control)	0.5% DDT in mineral oil
Stock colony	12	5	92
Exposed stock-colony strain	8	3	85
Santa Rosa village	6	4	95
Gatuncillo village	10	6	97

The results (see table I) indicate clearly that, despite the contact of the wild *albimanus* population with DDT over a period of years, these mosquitos now have essentially the same susceptibility to DDT as those from the laboratory colony with a history of no contact with DDT. Similarly, the stock-colony strain which had been exposed to DDT for more than 70 generations shows no significant decrease in susceptibility. It can only be concluded that any differences in the effectiveness of DDT residual house-spraying for the control of *albimanus* must be due to a behaviour change, and not to any change in the intrinsic toxicity of DDT to this mosquito.

RÉSUMÉ

L'auteur a fait récemment des observations qui semblaient indiquer un changement dans l'efficacité du DDT vis-à-vis d'*Anopheles albimanus*, dans deux villages du Panama, où des pulvérisations de DDT avaient été effectuées pendant 8 et 5 ans respectivement. Comparant les captures de 1945-47 à celles de 1952, on n'observait plus, en effet, ni la réduction massive du nombre des anophèles ni la mortalité sélective des moustiques gorgés de sang; on observait encore, cependant, la réduction du nombre des anophèles qui réussissaient à se gorger de sang. Ces constatations pouvaient faire penser soit à une

résistance physiologique acquise chez les moustiques à l'égard du DDT, soit à la sélection d'une population hyperirritable par le DDT, soit à une combinaison de ces deux facteurs.

Des expériences de laboratoire ont été entreprises pour éclaircir ce point à l'aide d'une technique que l'auteur décrit en détail.

Plus de 2.000 *A. albimanus* gorgés de sang ont été exposés pendant 12 minutes à du DDT. Ces insectes provenaient de trois sources : *a)* d'une colonie élevée en laboratoire depuis 1938, qui n'avait jamais été en contact avec un insecticide chloré; *b)* de la même souche, dont 70 générations, de 1948-52, avaient été exposées au DDT; *c)* de deux villages où du DDT à 5 % dans le pétrole avait été fréquemment appliqué. Les moustiques de ces trois lots se sont montrés à peu près également sensibles au DDT, qui provoquait dans les conditions d'expérience une mortalité d'environ 90% après 24 heures. Il semble donc qu'il faille attribuer les changements observés à une modification dans le comportement du moustique plutôt qu'à une diminution de la toxicité du DDT pour l'anophèle.

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