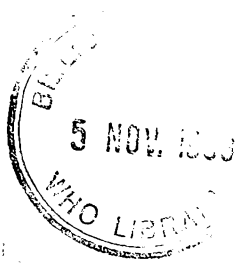


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INSECTICIDE RESISTANCE IN SOME ANOPHELES LARVAE

by

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Introduction

The introduction and use of a new method<sup>1</sup> for the examination of the response to insecticides of the small tropical Anopheles larvae, especially those of A. gambiae (Elliott 1957, and in press), leads to the question as to whether this method can give useful results with larvae of other species which have developed or may in the future develop specific resistance to insecticides. The present paper gives an account of the results of the application of the method to two species, A. sudaicus and A. stephensi, which have developed strains resistant to DDT, and also to the larvae of A. gambiae melas, A. funestus, and of some West African strains of A. gambiae sensu stricto.

Experimental Method

The method used was that of Elliott (1957) wherein fourth instar larvae are exposed to graded aqueous dilutions of the toxicant for a period of one hour, after which they are removed to clean water, fed, and the mortality counted after a recovery period of a further five hours.

<sup>1</sup> The standard WHO test method utilizing a 24-hour exposure period before the mortality is assessed, has in fact encountered negligible control mortality even in A. gambiae elsewhere [Editor].

Experiments with *A. sundaicus*

Eggs of two strains of *A. sundaicus* were received from the Ross Institute, the Java strain showing DDT-resistance in adult stage (Davidson 1957) and the Malaya strain showing normal susceptibility. Larvae hatching from these were reared to the fourth instar and tested, survivors being killed off; the colonization of tropical insecticide-resistant *Anopheles* being considered an unduly hazardous proceeding in Nigeria.

The numbers available sufficed for the examination of their reaction to DDT only; a limited number of trials with dieldrin suggested that the two strains were similar in their reaction to this toxicant, with an LC50 value of about 0.025 p.p.m.<sup>1</sup> The results with DDT are shown in Table I, and indicate LC50 values of 25 p.p.m. (19-33 p.p.m.) for the Java strain and 0.35 p.p.m. (0.26-0.47) for the Malayan. The relationship of these figures to the LC50 values for adults of the same strains, as determined by the Busvine-Nash method, is of some interest.

Davidson (1957) estimates the ratio of LC50 values for the two strains to be of the order of 1:40; the ratio in the larvae is 1:71 or 1:40 x 1.8. The figure 1.8, therefore, may be taken to be an index of the difference between results with larvae and results with adults of the same strain. In this connexion the results obtained for the adults and larvae of *A. gambiae* showing the three grades of BHC resistance occurring in that species may be compared. These are the homozygous resistant (RR), the heterozygous resistant (RS) and the homozygous susceptible (SS).

<u>Adults</u>		<u>Larvae</u>	
(Davidson 1956)		(Elliott 1958a & b)	
$\frac{\text{LC50 RR}}{\text{LC50 RS}}$	$= \frac{0.21}{0.033} = 40$	$\frac{3.7}{0.06}$	$= 62 \quad (40 \times 1.5)$
$\frac{\text{LC50 RS}}{\text{LC50 SS}}$	$= \frac{0.033}{0.007} = 7.6$	$\frac{0.6}{0.045}$	$= 13.3 \quad (7.6 \times 1.75)$

<sup>1</sup> p.p.m. stands for parts per million. The figures in brackets refer to confidence limits for 95% probability.

The existence of this fairly constant relationship between LC50 values for adults (expressed as per cent. toxicant in oil solution) and values for larvae (expressed as parts per million of toxicant in water) suggests that the same physiological mechanism is at work in both stages, and it is a welcome confirmation of the validity of the alternative approach to the problem.

Experiments with *A. stephensi*

Larvae of three strains of *A. stephensi* were tested, (1) an "S" strain from India, (2) a selection from this made by breeding from larvae surviving exposure to DDT in each generation, and (3) an "R" strain from Iraq, all three being supplied by the Ross Institute. The selected strain had been subjected to DDT pressure for 20 generations (Davidson 1958) and then bred for another 12 generations without further pressure. Results are given in Tables II, III and IV, and the estimated LC50 values, with 19/20 confidence limits, are as follows:

<u><i>A. stephensi</i></u>	DDT	Dieldrin	BHC
Indian "S"	11.5 p.p.m. (7.7 - 17.25)	0.084 p.p.m. (0.058 - 0.122)	-
Selected strain	22.5 p.p.m. (15.0 - 33.5)	0.17 p.p.m. (0.094 - 0.31)	-
Iraq "R"	150 p.p.m. (202 - 111)	0.04 p.p.m. (0.029 - 0.055)	0.088 p.p.m. (0.073 - 0.105)

It will be noted that the susceptibility of all three strains to DDT is low as compared with *A. gambiae*, or with the susceptible strain of *A. sudaicus*, suggesting that even before the development of resistance *A. stephensi* larvae might have been somewhat refractory to control by DDT. The selected strain is about twice as resistant to DDT as the Indian strain from which it was derived, while the Iraq strain is about 13 times as resistant. It seems probable that some of the tolerance

acquired by the selected strain has been lost during the 12 generations since pressure was withdrawn; during the selection period the LC50 (on a 24-hour exposure basis) increased from 0.49 p.p.m. to 3 p.p.m., i.e. sixfold.

The results with dieldrin show barely significant differences between the three strains, but the highest LC50 value is that of the selected strain, which suggests that the result of selection may have the separation of a strain showing a generalized "vigour tolerance" to other insecticides, as well as to DDT.

If the correlation between adult and larval results applies to this species as well as to A. gambiae and A. sudaicus, an expected value for the ratio between LC50 values for resistant and susceptible A. stephensi adults would fall between 1:7.2 and 1:8.6, giving a DDT concentration of between 11.5 per cent. and 13.7 per cent. for the usual one-hour exposure period. These concentrations are not in fact obtainable; results quoted by Davidson (1957) obtained by extrapolation suggest a rather lower value, although his figure for Qatif Oasis of 11.4 per cent. reaches this estimate,

#### Experiments with A. gambiae melas

Larvae of this species were obtained from a colony which was maintained at Yaba during part of 1957. The colony was never entirely self-perpetuating so that the material tested is really a sample of the local wild population. Results of testing with DDT and dieldrin are given in Table V and estimates for LC50 values were obtained as follows:

DDT	0.27 p.p.m. (95 per cent. confidence intervals 0.175 and 0.25)
Dieldrin	0.13 p.p.m. (0.10 and 0.17)

When these figures were obtained, the only A. gambiae material available for comparison was the Lagos (SS) and the Ambursa (RR) colonies. The figures for A. gambiae melas showed an LC50 for dieldrin rather higher than that of the Lagos strain, though much lower than that of the Ambursa strain. The possibility of some kind of connexion between the lower susceptibility to insecticides of A. gambiae melas and its resistance to salt was suspected; the Ambursa material proved equally as susceptible to salinity

as the Lagos, however. When results for A. gambiae tested against dieldrin from Ibadan are taken into consideration, and some other DDT results to be considered later, the figures for A. gambiae melas are seen to fall well within the range of non-resistant A. gambiae.

#### Experiments with A. funestus

A limited amount of material of this species was available from the colony whose maintenance commenced at Yaba in 1957 (Service 1957); results of tests with DDT are given in Table V, the LC50 value obtained being 0.3 p.p.m. (0.21-0.44). Figures for other insecticides were obtained but are based on small numbers only; in general they are higher than those for A. gambiae, although there is no reason to suppose that the strain is other than a normally susceptible one, and the adults seem, also on the basis of a few tests only, to be comparable to those studied by Ramakrishna and Elliott (1957) from northern Nigeria. The LC50 values then obtained were 0.32 per cent. for DDT and 0.0033 per cent. for EHC. (In the original publication the DDT figure was, due to an error for which the present writer is solely responsible, given as 1.32 per cent.). Thus it seems that a relatively resistant larva may produce a susceptible adult; the sluggish, long-lived A. funestus larva requires more toxicant to irreversibly upset its metabolism in the space of an hour than the active, fast-growing larva of A. gambiae, but the corresponding adult is more susceptible, as its smaller size and equally active behaviour would suggest. The correlation between resistance in larvae and in adults seen in the other three species cannot be extended to other species. Similarly in A. stephensi, although the adult is 1.4 times (EHC) to 3.3 times (DDT) as resistant as A. gambiae, the larva has disproportionate advantage over the A. gambiae larva in its resistance to DDT of 18 times, though its EHC resistance is only double. With DDT some of the discrepancies may be due to different feeding habits, since in the aqueous phase used in the testing method this toxicant is mainly in suspension and must be ingested to have its effect, but both dieldrin and EHC are in true solution, so that A. funestus and A. stephensi must be protected by slower absorption or by more stable metabolic processes.

Experiments with *A. gambiae*

The occurrence of DDT resistance in this species would be a most serious matter for the whole future of malaria control in Africa; let it be said at once then that no evidence of any true specific physiological resistance to DDT has so far been forthcoming. Nevertheless, as might be expected, some degree of variation in the level of susceptibility to this toxicant has been observed in different areas, and a knowledge of the normal range of susceptibility will be an important factor in the detection of DDT resistance should it ever arise. Material from the following areas has been examined: Lagos, western Sokoto, Kano and Ibadan in Nigeria, and Freetown, Sierra Leone. LC50 values of p.p.m. are listed as follows, in descending order:

Ibadan "A"	3.8	(2.5 to 5.7)	
Freetown	1.8	(1.2 to 2.7)	
Ambursa colony	0.8	(0.66 to 0.96)	(Elliott 1957)
Kano colony	0.68	(0.45 to 1.02)	
Ibadan "C"	0.56	(0.40 to 0.78)	
Ibadan "B"	0.20	(0.11 to 0.36)	
Western Sokoto	0.094	(0.069 to 0.127)	(Elliott & Ramakrishna 1958)
Lagos wild	0.070	(0.56 to 0.87)	
Lagos colony	0.064	(0.056 to 0.073)	(Elliott 1957)
Ibadan "D"	0.030	(0.023 to 0.038)	

There is a remarkably wide range of variation, and it will be noted that the highest and the lowest LC50 both refer to material from the town of Ibadan. Batch "A" from Ibadan was mentioned as having also a high dieldrin resistance by Elliott (in press): they were all the offspring of one female. Batches "B" and "C" from Ibadan may have contained representatives of the same type, which appears to have a high tolerance to insecticides in general rather than a specific resistance. Batches "A", "B" and "C" were obtained between March and May 1957; subsequent samples of larvae

from Ibadan have been unusually low in their resistance to DDT, though normal to dieldrin, and are consolidated as group "D". The use of insecticides at Ibadan has been confined to a limited amount of DDT larvaciding.

The second highest figure comes from Freetown, where the use of DDT larvacides for 12 years may have selected out a relatively tolerant population. This is followed by some figures from northern Nigeria, for the Ambursa and Kano colonies. The sporadic occurrence of a relatively tolerant strain in this area seems to be established also on the basis of results with adults (Elliott & Ramakrishna 1956 and Armstrong et al. 1956), but it is not universal and is not always associated with the dieldrin and EHC resistance factor also present in the area.

It will be noted that no A. gambiae sample showing an LC50 value approaching those of DDT-resistant A. stephensi and A. sudaicus, or even of normal susceptible A. stephensi, has been found, so that the general reversion to the use of DDT in control schemes against A. gambiae appears to be on a sound basis; it seems that all of the larvae examined in the present study would be controllable with the normal application rate of a DDT larvacide; the response of the corresponding adults to imagocidal treatments is less certain.

#### Summary

1. The method devised in 1957 for examination of the response to insecticides of Anopheles larvae has been applied to larvae of A. sudaicus, A. stephensi, A. funestus and A. gambiae gambiae and A. gambiae melas. (Elliott, 1957)
2. In two strains of A. sudaicus the resistance of the larvae to DDT was correlated with that of the adults. The ratio between LC50 values for adults of a susceptible and resistant strain was found to be simply related to that for those of the larvae. A numerically similar value connects the same ratios between the LC50 values of adults and larvae of three genetic types of A. gambiae, the toxicant in this case being EHC.

3. In A. stephensi the larvae of three strains were tested and all found to be relatively resistant to DDT as compared with A. gambiae. The comparison between adults and larvae of a susceptible and a resistant strain showed that the relationship found to apply in the case of A. gambiae and A. sudaicus probably applies to this species also.
4. In A. gambiae melas the susceptibility to DDT and dieldrin is well within the range of variation of non-resistant strains of A. gambiae. In A. funestus the larvae appear to be more susceptible than might have been expected from the susceptibility of the corresponding adults. The relationship between susceptibility of adults and larvae, therefore, applies only between strains within a species, and not between different species.
5. In A. gambiae from different parts of West Africa a wide variation in LC50 value for DDT is found, but the most resistant larvae studies fall far below DDT-resistant A. sudaicus or even susceptible A. stephensi in their tolerance of this toxicant, and true specific resistance to DDT is not considered to have been seen.

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TABLE I. EFFECTS OF DDT ON LARVAE OF TWO STRAINS OF A. SUNDAICUS

Concentration of DDT (p.p.m.)	Percentage mortality in 4th - instar larvae	
	Java strain	Malaya strain
100	89	-
60	84	-
40	53	100
20	49	-
10	25	100
6	14	-
4	0	100
2	-	86
1	-	83
0.6	-	66
0.4	-	49
0.2	-	35
0.1	-	16
0.06	-	14
0.04	-	7
0.02	-	0
Average number exposed at each concentration	33	25

TABLE II. EFFECTS OF DDT ON LARVAE OF THREE STRAINS OF A. STEPHENSI

Concentration of DDT (p.p.m.)	Percentage mortality in 4th - instar larvae		
	Indian 'S' strain	Selected strain (LXXL)	Iraq 'R' strain
200	-	-	60
100	-	-	30
60	-	-	30
40	71	53	13
20	72	56	2
10	39	42	0
6	48	28	-
4	19	10	-
2	20	7	-
1	13	0	-
0.6	0	-	-
Average number exposed at each concentration	30	40	40

TABLE III. EFFECTS OF DIELDRIN ON LARVAE OF THREE STRAINS OF A. STEPHENSI

Concentration of dieldrin (p.p.m.)	Percentage mortality in 4th - instar larvae		
	Indian 'S' strain	Selected strain (LXXL)	Iraq 'R' strain
2	100	100	-
1	100	100	-
0.6	88	100	-
0.4	75	55	100
0.2	63	17	100
0.1	44	75	74
0.06	47	25	60
0.04	33	19	50
0.02	31	23	47
0.01	17	0	12.5
0.006	5	-	-
0.004	0	-	-
Average number exposed at each concentration	20	20	30

TABLE IV. EFFECTS OF BHC ON LARVAE OF A. STEPHENSI

Concentration of BHC (p.p.m.)	Per cent. mortality in 4th instar larvae of the Iraq 'R' strain
4	100
1	100
0.4	100
0.2	81
0.1	56
0.06	27
0.04	20
0.02	5
0.01	0
Average number exposed at each concentration	40

TABLE V. EFFECTS OF DDT AND DIELDRIN ON A. GAMBIAE MELAS AND A. FUNESTUS

Concentration of toxicant (p.p.m.)	Per cent. mortality in 4th - instar larvae		
	<u>A. g. melas</u> (dieldrin)	<u>A. g. melas</u> (DDT)	<u>A. funestus</u> (DDT)
4	100	-	100
2	100	100	97
1	90	87	75
0.6	79	77	68
0.4	80	66	51
0.2	70	56	40
0.1	48	39	30
0.06	30	14	25
0.04	30	0	18
0.02	0	-	-
0.01	-	-	0
Average number exposed at each concentration	50	80	63