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The following are some of the abbreviations in use :—

Amer. J. Hyg.
Ann. Trop. Med. Parasit.
Brit. Med. J.
Bull. Ent. Res.
Bull. Soc. Path. Exot.
C. R. Soc. Biol.
Geneesk. Tijd. Ned.-Ind.
Ind. J. Mal.
Ind. J. Med. Res.
J. Mal. Inst. Ind.
Jl. R. A. M. C.
J. Trop. Med. Hyg.
Meded. Volk. Ned.-Ind.
Rec. Mal. Surv. Ind.
Rev. App. Ent.
Riv. Malariol.
Trans. Roy. Soc. Trop. Med. Hyg.
Trop. Dis. Bull.
U.S.A. Pub. Hlth. Rpts.

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PRELIMINARY OBSERVATION ON VIABILITY OF SUBMERGED EGGS OF MOSQUITOES.

BY

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[November 8, 1957.]

INTRODUCTION.

DUPREE (1905) roughly divided the mosquito eggs in two groups, (*i*) those which are provided with special mechanism for floating, and (*ii*) those without such mechanism. The first group, in his opinion, could not stand any lengthy (more than 4 - 7 hours) desiccation or submergence while the second group could. He (quoted by Mitchell, 1907) further stated that if an egg raft be secured under water, no larvæ would emerge while any egg broken away from the mass would hatch under similar conditions.

Göeldi (1905) (quoted by Howard *et al.*, 1912) stated that eggs invariably perish when submerged. Peryassú (1908) found that eggs would hatch if submerged in water (temperature 26°-29°C.) a day after being laid, but if submerged soon after being laid they would generally perish. Marchoux *et al.* (1903) found that the hatching was somewhat retarded by immersion and sometimes some of the eggs did not hatch, particularly if the temperature of water was low. Bacot (1916) showed that immersed eggs remain dormant for 5 months. These studies refer to observations made on *Aedes ægypti* (*calopus*, *fasciatus*) and therefore fall under category (*ii*) of Dupree (1905).

However, experience with colonies of *Aedes ægypti*, *Anopheles stephensi* and *Culex fatigans*, maintained at this Institute, has shown repeatedly that eggs immersed under water remain viable. This seemed to be contrary to the general belief that eggs provided with floating mechanisms, once sunk, would perish. To find the precise effects of sinking on mosquito eggs with regard to their viability and its ultimate effects on the life history, the following preliminary experiments were

conducted with eggs of *Aedes ægypti*, *Anopheles annularis*, *Anopheles culicifacies*, *Anopheles stephensi*, *Anopheles subpictus*, and *Culex fatigans*.

MATERIALS AND METHODS.

For the present investigations, eggs of colony-bred *A. ægypti*, *A. stephensi* and *C. fatigans* were used. The eggs of *A. annularis*, *A. culicifacies* and *A. subpictus* were obtained from wild-caught gravid females.

After experimenting with a variety of methods to submerge the fertile eggs for desired periods of time without injury, it was found that mosquito eggs could be kept at the bottom of bowls containing water when placed on circular discs of filter paper and put under a wet muslin cloth fastened by thread to an iron ring with diameter slightly larger than the filter paper disc. The iron ring used for these experiments measured 2 to 2½ inches in diameter and acted as weight to keep the eggs under water within its circumference. The muslin was wetted before placing it into the eggs to ensure quick immersion by excluding air bubbles. The eggs that came off the filter paper and floated away in the act of immersion, were not considered for these studies.

Eggs were submerged in water for desired period of time after which the rings were removed and the eggs allowed to refloat. Those eggs which remained stranded at the bottom were disturbed with a brush to help them refloat. In controls, the eggs were allowed to float constantly on water surface and allowed to hatch.

It was found that eggs, submerged soon after being laid, behaved like those that were many hours old. Therefore, no distinction was maintained as to the age of eggs in these experiments. However, the bulk of experiments were carried with 8 - 12 hours' old eggs.

The water temperature in these experiments varied from 18°- 30°C., depending on the prevalent climatic conditions. However, some experiments were conducted in ice boxes where the water temperature was maintained at 20°C.

OBSERVATIONS.

The eggs of *C. fatigans* (unbroken rafts), *A. ægypti*, *A. annularis*, *A. culicifacies*, *A. stephensi* and *A. subpictus* were submerged for varying period of time under water at temperature between 18° and 30°C. The eggs of all species withstood submerging to different degrees with regard to their viability*.

In the case of *C. fatigans* the controls (without submerging) for experiments gave 77·5 to 92·5 per cent (Table I) hatch when the water temperature was from 24°- 30°C., and 96·9 per cent when the water temperature was 20°C. The per cent hatch in eggs submerged for 3 and 6 hours in water, with temperature from 24°- 30°C., was 65·3 to 87·2 and 54·4 to 55·2 respectively and the hatch in eggs submerged for 12 hours was 0 to 23 per cent only. There was no emergence of larvæ from eggs submerged for 24, 48 and 96 hours. When the water temperature was 20°C., the egg rafts submerged for 3, 12 and 24 hours gave a hatch of

*This was determined by the per cent first instar larvæ obtained after submergence.

92·8, 94·0 and 92·4 per cent respectively. There was no hatch in eggs submerged for 48 hours. In both cases (high and low water temperature), all the eggs that hatched into larvæ refloated (positive refloating ability), whereas those eggs which did not hatch did not refloat (negative refloating ability).

TABLE I.

Viability of Culex fatigans *eggs (rafts) after being submerged under water from* 0 - 96 *hours at different water temperatures.*

Serial number	Age of eggs (Hours)	Period of submergence (Hours)	Number of replicates	Number of eggs	Number of 1st instar larvæ	Per cent hatch	Refloating ability	Time taken for hatching (Hours)	Water temperature
					A				
1.	1 - 8	0	25	2,905	2,686	92·5	Positive	24	24° - 30°C.
2.	1 - 3	3	2	180	157	87·2	Positive	24	24° - 26°C.
3.	8	6	8	915	505	55·2	Positive	24	25° - 29°C.
4.	1 - 8	12	3	320	..	..	Negative	..	24° - 30°C.
5.	8	24	3	345	..	..	Negative	..	24° - 30°C.
6.	8	48	4	400	..	..	Negative	..	25° - 30°C.
7.	8	96	5	400	..	..	Negative	..	25° - 30°C.
					B				
1.	8	0	3	485	376	77·5	Positive	24	25° - 29°C.
2.	8	3	2	255	166	65·3	Positive	24	25° - 29°C.
3.	8	6	2	285	155	54·4	Positive	24	25° - 29°C.
4.	8	12	3	455	155	23·0	Positive	24 – 48	25° - 29°C.
5.	8	24	3	425	..	..	Negative	..	25° - 29°C.
6.	8	48	3	445	..	..	Negative	..	25° - 29°C.
					C				
1.	8	0	1	130	126	96·9	Positive	24 – 48	20°C.
2.	8	3	1	140	133	92·8	Positive	24 – 48	20°C.
3.	8	12	1	135	127	94·0	Positive	24 – 48	20°C.
4.	8	24	1	145	134	92·4	Positive	24 – 48	20°C.
5.	8	48	1	135	..	..	Negative	..	20°C.

The eggs of *A. ægypti* (Table II) when submerged under water for 24, 48 and 96 hours gave a hatch of 51·0, 68·0 and 43·5 per cent respectively while the hatch in the control was 86·6 per cent. It was found that often eggs submerged for more than 24 hours hatched before the expiry of the time intended for submergence, and the larvæ came to surface. No eggs could be submerged beyond 96 hours as they always hatched before this time*. In these experiments, it was found that majority of eggs did not refloat after the ring was removed and generally hatched later than the control.

TABLE II.

Viability of eggs of Aëdes ægypti *after being submerged under water from* 0 - 96 *hours.*

Serial number	Age of eggs (Hours)	Period of submergence (Hours)	Number of replicates	Number of eggs	Number of 1st instar larvæ	Per cent hatch	Refloating ability	Time taken for hatching (Hours)	Water temperature
1.	8 - 12	0	5	500	343	86·6	Positive	24 - 120	20° - 27°C.
2.	8 - 12	24	4	400	204	51·0	Largely negative	48 - 140	20° - 27°C.
3.	8 - 12	48*	5	500	340	68·0	Largely negative	48 - 140	20° - 27°C.
4.	8 - 10	96*	2	200	87	43·5	Negative	120 - 240	26° - 27°C.

*Replicates, where larvæ hatched before the ring was removed, were discarded.

A. subpictus eggs (Table III) were submerged from 0 (control) to 96 hours at three temperature ranges. At 18°- 20°C. water temperature, the eggs, when submerged for 6, 12, 24, 48 and 96 hours, gave a hatch of 73·5, 62·7, 43·0, 63·0 and 67·5 per cent respectively while the hatch in the control was 80·0 per cent. When the water temperature was higher, *i.e.*, 29°- 30°C., the eggs submerged for 6, 12 and 24 hours gave 33·0, 46·7 and 56·0 per cent hatch respectively while the hatch in control was 82·7 per cent. At 29°C. water temperature, the eggs of this species submerged for 24 and 48 hours gave a hatch of 87·4 and 71·3 per cent respectively while the control gave a hatch of 92·9 per cent. The refloating ability in all trials was positive except in the case of eggs submerged for 48 hours at 29°C. water temperature where it was negative. In these experiments, it was found that eggs submerged beyond 24 (Table III-*B*) or 48 (Table III-*C*) hours at 29°- 30°C., hatched before the ring (confining the eggs to the bottom of the bowl) was removed. However, the eggs submerged at 18°- 20°C. behaved differently and eggs remained submerged till 96 hours and refloated after the ring was removed.

Experiments with *A. subpictus* revealed that eggs submerged immediately after being laid, did not lose viability. In five such trials, 386 eggs were submerged for 48 hours, of which 276 (71·5 per cent) hatched into larvæ while the hatch in the control was 92·9 per cent.

*All experiments, where eggs hatched before the removal of rings, were discarded.

TABLE III.

Viability of eggs of Anopheles subpictus *after being submerged under water from 0 - 96 hours at different temperatures.*

Serial number	Age of eggs (Hours)	Period of submergence (Hours)	Number of replicates	Number of eggs	Number of 1st instar larvæ	Per cent hatch	Refloating ability	Time taken for hatching (Hours)	Water temperature
					A				
1.	8	0	3	300	240	80·0	Positive	48 - 72	18° - 20°C.
2.	8	6	2	200	147	73·5	Positive	48 - 96	18° - 20°C.
3.	8	12	3	300	188	62·7	Positive	48 - 120	18° - 20°C.
4.	8	24	3	300	149	43·0	Positive	48 - 120	18° - 20°C.
5.	8	48	3	300	189	63·0	Positive	48 - 120	18° - 20°C.
6.	8	96	2	200	135	67·5	Positive	96 - 120	18° - 20°C.
					B				
1.	8	0	3	300	248	82·7	Positive	24	29° - 30°C.
2.	8	6	2	200	66	33·0	Positive	24 - 72	29° - 30°C.
3.	8	12	3	300	140	46·7	Positive	24 - 72	29° - 30°C.
4.	8	24*	2	200	112	56·0	Positive	24 - 72	29° - 30°C.
					C				
1.	0	0	3	182	169	92·9	Positive	48	29°C.
2.	12 - 36	24	4	482	421	87·4	Positive	48	29°C.
3.	0 - 6	48*	8	578	412	71·3	Negative	48 - 72	29°C.

*Eggs submerged for longer periods before the ring was removed.

Period of submergence.	Per cent hatch.	
0 hours.	81·4	Combined results of A and B above.
6 "	53·3	
12 "	54·7	
24 "	49·5	

Eggs of *A. stephensi* (Table IV) when submerged for 3, 6, 12 and 24 hours under water at 23°- 25°C. (water temperature) gave a hatch of 68·0, 70·0, 62·0 and 26·5 per cent respectively while the hatch in the control was 80·0 per cent. It was observed that eggs kept submerged beyond 24 hours hatched while still underneath the ring. Eggs submerged for 3 to 24 hours had positive refloating ability and the hatching was completed within 24 to 36 hours in both submerged and unsubmerged eggs (control).

A. annularis and *A. culicifacies* eggs (Table V) were submerged for 24 hours giving a hatch of 83·0 and 90·0 per cent respectively. Eggs of *A. annularis* could

not be submerged beyond 24 hours as they hatched while they were still under the rings. It was found that *A. culicifacies* eggs, when submerged immediately after being laid, did not lose viability. Eggs of both these species showed positive refloating ability after expiry of the period of submergence.

TABLE IV.

Viability of eggs of Anopheles stephensi *after being submerged from* 0 - 24 *hours.*

Serial number	Age of eggs (Hours)	Period of submergence (Hours)	Number of replicates	Number of eggs	Number of 1st instar larvae	Per cent hatch	Refloating ability	Time taken for hatching (Hours)	Water temperature
1.	12	0	2	200	160	80·0	Positive	24	23°- 25°C.
2.	12	3	1	100	68	68·0	Positive	24	23°C.
3.	12	6	1	100	70	70·0	Positive	24	23°C.
4.	12	12	1	100	62	62·0	Positive	24	23°C.
5.	12	24*	2	200	53	26·5	Positive	24 - 36	23° - 25°C.

* Eggs submerged beyond 24 hours, hatched before time allowed for this treatment.

TABLE V.

Viability of eggs of Anopheles annularis *and* A. culicifacies *after being submerged from* 0 - 24 *hours.*

Serial number	Age of eggs (Hours)	Period of submergence (Hours)	Number of replicates	Number of eggs	Number of 1st instar larvae	Per cent hatch	Refloating ability	Time taken for hatching (Hours)	Water temperature
					A. annularis				
1.	12	0	1	100	89	89·0	Positive	24 - 48	25°C.
2.	12	24*	1	100	83	83·0	Positive	24 - 48	25°C.
					A. culicifacies				
1.	0	24	1	50	45	90·0	Positive	24 - 36	29°C.

*Eggs intended to be submerged longer, hatched before ring was removed.

DISCUSSION.

The viability of submerged eggs is of great practical importance. According to Dupree (quoted by Horsfall, 1955), mosquitoes with eggs that remain viable after periods of submergence could hibernate as eggs and those in which eggs perish

(lose viability) on submergence could hibernate as larvæ and adults. In the plains of India, there is no true hibernation of mosquitoes. However, in colder mountainous and foot-hill regions it is extremely likely that mosquitoes hibernate in some stage of their life cycle. Beside this consideration, the eggs that remain viable when submerged could result in continuous breeding of mosquitoes in unsuspected and undetected places, thus causing great frustration to the public health workers engaged in anti-mosquito campaigns.

The problem of viability of submerged eggs has continued to draw the attention of biologists from early times. The main conclusions derived from earlier works can be summed up as follows:—

(*a*) The eggs with floating mechanisms lose viability on being submerged for more than 7 hours (Dupree, 1905);

(*b*) The eggs without floating mechanisms do not lose viability on being submerged (Dupree, 1905);

(*c*) Eggs submerged just after being laid, became inviable (Perysasú, 1908);

(*d*) Hatching is retarded if eggs are submerged, particularly at low temperatures (Marchoux *et al.*, 1903).

In the present series of experiments with the eggs of *Anopheles annularis*, *A. culicifacies*, *A. stephensi*, *A. subpictus*, *Ædes ægypti* and *Culex fatigans*, representing eggs with and without floating mechanisms, it was found that both types of eggs could stand, being immersed under water to varying degrees, and that in all cases there is a progressive loss of viability with the increase in period of submergence.

In case of the eggs of *C. fatigans*, although the individual egg has no floating mechanism yet because of the arrangement of a single brood of eggs into a raft, the raft behaves like the eggs provided with floats when submerged. It has been stated (Dupree, quoted by Mitchell, 1907) that if an egg raft be secured under water, no larvæ would emerge. The present investigations show that this is not the case. In water, with relatively higher temperature (24°- 30°C.), the egg rafts of this species could be submerged up to 12 hours without total loss of viability and the per cent hatch decreased with increase in period of submergence of eggs. Graph I shows that eggs submerged for 0, 3, 6, 12 and 24 hours gave a per cent hatch of 77·5, 65·3, 54·4, 23·0 and 0·0 respectively. However, when the water temperature was low (20°C.), the tolerance of eggs to submergence increased and the eggs gave 92·4 per cent hatch after 24 hours of immersion. There was no hatch beyond this period of submergence. It seems, therefore, that low temperature tends to increase the tolerance to longer immersion. There was no emergence of larvæ while eggs were under water and all eggs that hatched showed positive refloating ability.

Aedes ægypti is believed to stand lengthy submergence without loss of viability (Dupree, 1905). In the present experiments, it was found that eggs could stand submergence for longer periods than other species investigated, there being progressive decrease in hatching ability with increase in period of submergence (Table II). The study of the extent to which eggs of this species in particular, and those of anophelines in general, could stand submergence was not possible because of the fact that eggs hatched while still submerged. Such a study could be possible only under fairly low water temperatures when the normal (without submerging) hatching period is prolonged.

GRAPH 1.

Per cent hatch of 1st instar C. fatigans *larvæ from eggs submerged at two different temperatures (data in Table I).*

In case of anophelines at 23°C. to 30°C. water temperatures, it was observed that the eggs could stand immersion mostly up to 24 hours except in case of *A. subpictus* where the eggs could be kept submerged for 48 hours. Eggs invariably

hatched under water in experiments where they were immersed longer than 24 hours (Tables III, IV and V). In controls also, the eggs started hatching after 24 hours and submergence, therefore, did not seem to delay the beginning of larval emergence. However, in case of immersed eggs the completion of hatching took longer than in controls. At low temperatures (18°- 20°C.), the process of hatching in control was prolonged (Table III-*A*) and the eggs could remain under the confinement ring up to 96 hours, resulting in 67·5 per cent hatch after this period

GRAPH 2.

Per cent hatch of 1st instar A. subpictus *larvæ from eggs submerged at two different temperatures (data in Table III).*

of submergence. As already mentioned, it was observed that hatching time was prolonged in this case. Whereas the control hatched between 48 and 72 hours, the eggs submerged for 96 hours hatched between 96 and 120 hours. From experiments with different species of anophelines, it would seem that: (*i*) Submerged eggs do hatch. This was evident by the fact that eggs confined under water beyond 24 hours hatched even when the ring was not removed, and also those submerged for various periods of time hatched after the ring was removed. (*ii*) Certain proportion of eggs could stand prolonged period of submergence at

GRAPH 3.

Per cent hatch of 1st instar A. stephensi larvæ from eggs submerged from 0 - 24 hours (data in Table IV).

lower temperatures when the normal (as indicated by control) hatching time would also increase. This would depend on the extent of decrease in temperature. (*iii*) There is a progressive loss of viability of eggs with increase in the period of their submergence. Graphs 2 and 3 show that eggs of *A. subpictus* and *A. stephensi* when submerged for 0, 6, 12, and 24 hours and 0, 3, 6, 12 and 24 hours gave a per cent hatch of 81·4, 53·3, 54·7, 49·5 and 80·0, 68·0, 70·0, 62·0, and 26·5 respectively.

In case of anophelines, it was found that after the rings confining the eggs to the bottom of the bowl were removed, the eggs invariably came to surface and refloated. However, the fact that eggs could hatch even underneath the rings, puts in doubt the importance of refloating ability of eggs *vis-a-vis* hatching.

As has been mentioned above, Peryassú (1908) found that eggs, submerged soon after being laid, generally perished. However, the present studies revealed that preconditioning ' time factor ' of eggs before submergence is not important for viability. Eggs of *A. culicifacies*, just after being laid, were submerged for 24 hours resulting in a hatch of 90·0 per cent (Table V). Similarly freshly laid eggs of *A. subpictus* gave a hatch of 71·5 per cent when submerged for 48 hours (Table III).

Bacot (1916) has mentioned that lower temperatures arrest the embryonic development resulting in dormancy of the embryo. Perhaps that is why eggs can stand submergence at low temperatures for a longer period than at high temperatures. However, even at low temperatures the hatch, after submergence, is less in proportion to the hatch without submergence. Therefore, it is likely that the embryo is affected in some way to give such results. Further experiments are designed to furnish data on the condition of embryo after the eggs are submerged under water for different periods at different temperatures.

SUMMARY.

Preliminary experiments were undertaken to study the effects of submergence (under water) on viability of the eggs of *Aëdes ægypti*, *Anopheles annularis*, *A. culicifacies*, *A. stephensi*, *A. subpictus* and *Culex fatigans*.

It was observed that:

1. The eggs of the above mentioned species, with and without floating mechanisms, retained viability to varying degrees on being submerged for different periods (0 - 96 hours) when the water temperature ranged from 18°to 30°C. *C. fatigans* proved to be the least tolerant of the species at 23°C. and higher water temperatures.

2. The hatching ability of the eggs decreased with increase in the period of submergence. Eggs did not seem to be able to withstand indefinite period of submergence at the temperatures, 18° to 30°C., used in the present study.

3. Floating of eggs does not seem always to be necessary for hatching. Submerged eggs of *A. ægypti*, *A. annularis*, *A. stephensi* and *A. subpictus* could hatch while still under water whereas eggs of *C. fatigans* could not do so. In case of the

last mentioned species, only those eggs hatched that refloated after the ring was removed.

4. The time lapse between laying and submergence of eggs did not seem to effect the viability. Eggs of *A. culicifacies* and *A. subpictus*, submerged for 24 and 48 hours immediately after being laid, resulted in a hatch of 90 0 and 71·5 per cent respectively.

5. Viability of eggs, submerged under water, seems to increase with decrease in temperature.

The ability of mosquito eggs to withstand sinking may be a cause of these insects appearing in unsuspected places, has been mentioned. Further experiments are envisaged to study the effects of submergence more precisely by varying temperatures. The effects of immersion under water on embryo, life history and biology of mosquitoes, will also be studied.

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SEASONAL PREVALENCE OF ANOPHELINES NEAR DELHI.

BY

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[December 24, 1957.]

AMONG the anopheline fauna of Delhi area, with the exception of *A. culicifacies*, very little has been published regarding the seasonal prevalence of different species.

Afridi and Puri (1940) reviewed the literature on *A. culicifacies*. Other works relating to the seasonal prevalence of this species are those of Afridi *et al.* (1940) for Karnal, and Rajinder Pal (1945) for Delhi. Russell and Rao (1941) dealt with the prevalence of twelve Anopheles species in South Eastern Madras and Bhombore *et al.* (1954) discussed the seasonal prevalence of twenty-three anophelines in the Western Hill tracts of Mysore State.

The purpose of the present paper is to put on record observations made from February, 1953 to July, 1957 on seasonal variation of Anopheles species found around Delhi by employing a standard technique widely used in such investigations at present.

Of the recorded anopheline fauna for this area (Puri, 1955), only *A. moghulensis*, *A. tessellatus*, *A. theobaldi*, *A. turkhudi* were not collected during these investigations. The ten species recorded are: *A. annularis*, *A. barbirostris*, *A. culicifacies*, *A. fluviatilis*, *A. hyrcanus*, *A. pallidus*, *A. pulcherrimus*, *A. splendidus*, *A. stephensi* and *A. subpictus*.

METHODS.

Three identical villages—Arthala, Bhaunja and Karera—were selected outside the limit of Delhi Antimalaria Operations. These are situated at a distance of 12 - 14 miles off Delhi on the road to Ghaziabad as indicated in Map 1. Arthala

MAP I.

SKETCH MAP SHOWING LOCATION
OF CATCHING STATIONS
N
KARERA
TO DELHI
ARTHALA
TO DELHI
BHAUNJA
TO MEERUT
TO MEERUT
TO HAPUR
GHAZIABAD
TO BULANDSHAHR
R. HINDAN
R.S
TO ALIGARH
LEGEND
SEEPAGE WATER COLLECTIONS
CATCHING STATIONS
TREES

remained under observation throughout the period of investigation. Work in Karera had to be suspended after one year and instead of it Bhaunja was taken up for continuity of observations. These villages are about ¼ to 1½ miles from the Hindan River and have about 60 to 80 huts in each. They are situated on raised ground, but beside each of them, in the proximity of the river, there are low-lying areas where seepage water ensures breeding ground all the year round. In addition to the low lying areas of permanent water collections, many temporary water collections crop up on either side of the road during rains. Extent of breeding ground is always directly in proportion to the rain.

There is scanty vegetation in the nature of shrubs, trees, etc., near and around these villages to provide outdoor shelter.

The catching stations included human dwellings, cattlesheds and mixed dwellings. One hour was allotted for catching adult mosquitoes from mixed dwellings and half-hour each for cattlesheds and human dwellings. The catch was made between 7.0 and 11.0 a.m. once in a week and each village was visited once in a fortnight.

The equipment used in catching mosquitoes was an aspirator tube, a flash light, test tubes and Barraud cages. The classification of specimens collected was done on the spot and verified in the laboratory.

Human dwellings and cattlesheds in these villages, as in most other rural areas around Delhi, are mainly mud huts with thatched or mud roof and one opening serving as a door. Cattle are invariably kept in close proximity to the human dwellings and similarly in the sheds, set aside for cattle, often a human being is found sleeping within it.

The density of mosquitoes is estimated in terms of average numbers caught per man-hour.

Throughout the duration of these observations, the same personnel were employed for making catches, classification and recording of the data.

METEOROLOGICAL DATA.

For the present study, the climatological data as recorded by the Meteorological Department of the Government of India at Delhi is taken into consideration. Table I indicates the monthly averages of the maximum, minimum and mean temperature; maximum, minimum and mean relative humidity and total rainfall from February, 1953 to July, 1957. The relative humidity figures are averages of observations made at 8.30 a.m. and 5.30 p.m. The means for the months were worked from the average maximum and minimum temperatures, and relative humidity at 8.30 a.m. and 5.30 p.m. In the case of rainfall, total for each month was calculated. Averages of the monthly maximum, minimum and mean temperatures and relative humidity and the total rainfall for the five-year period (February, 1953 to July, 1957), during which period the present observations were made, were calculated and are included in the last column of Table I.

From the consolidated data for these years, the month of January with 46·2°F. minimum temperature was the coldest month, and the months of May and June with 104·2°F. were the hottest months. From the mean of averages, June with

TABLE I.

Meteorological data for Delhi; February 1953 to July 1957.

(Temperature degrees in Fahrenheit.)

Months	Years																	
	1953			1954			1955			1956			1957			Average (Feb. 1953 to July 1957)		
	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean.	Max.	Min.	Mean	Max.	Min.	Mean
Jan.	..	..	..	68·8	44·8	[illegible]	[illegible]	46·7	57·9	70·4	[illegible]	58·3	66·7	47·0	56·9	68·8	46·2	58·5
Feb. ..	90·0	53·4	71·7	74·2	54·0	[illegible]	[illegible]	49·9	63·7	78·3	[illegible]	63·7	73·7	46·5	60·1	78·8	50·6	64·7
Mar. ..	93·8	65·2	79·5	85·6	59·5	72·6	90·4	63·7	77·1	85·9	[illegible]	73·4	82·4	59·1	70·8	87·2	61·7	74·5
Apr. ..	98·7	72·2	85·5	99·2	71·2	85·2	94·9	66·8	80·9	99·0	[illegible]	85·7	90·5	68·5	79·5	96·5	70·2	83·4
May ..	104·4	81·9	94·7	107·3	82·0	[illegible]	100·9	76·1	88·5	107·0	81·9	94·5	101·6	77·0	89·3	104.2	79·8	92·0
Jun. ..	104·2	85·2	93·2	105·3	84·6	94·9	105·2	85·4	95·3	102·9	84·2	93·6	103·6	81·6	92·6	104·2	82·2	93·2
Jul. ..	94·6	80·5	87·6	96·8	81·3	[illegible]	97·4	82·2	89·8	93·7	79·6	86·7	95·7	81·0	88·4	95·6	80·9	88·8
Aug. ..	91·3	78·5	84·9	96·3	80·8	88·6	[illegible]	78·5	84·8	92·0	78·7	[illegible]	..	..	..	92·7	79·1	85·9
Sep. ..	94·2	76·3	85·3	94·3	78·6	86·5	[illegible]	76·7	83·9	96·0	77·8	[illegible]	..	..	..	93·9	77·4	85·7
Oct. ..	93·5	65·5	79·5	87·2	62·1	74·7	85·8	[illegible]	76·1	85·2	68·2	[illegible]	..	..	..	87·9	65·6	76·8
Nov. ..	84·0	53·0	68·5	83·3	54·1	68·7	81·8	52·7	67·3	80·1	53·1	[illegible]	..	..	..	82·3	53·2	67·8
Dec. ..	78·8	50·1	64·5	77·0	45·3	61·2	73·8	47·0	[illegible]	73·6	48·4	61·0	..	..	..	75·8	47·7	61·8

TABLE I.—(*Contd.*)

Relative humidity per cent.

Months	Years																	
	1953			1954			1955			1956			1957			Average (1953-1957)		
	8.30 a.m.	5.30 p.m.	Mean	8.30 a.m.	5.30 p.m.	Mean	8.30 a.m.	5.30 p.m.	Mean	8.30 a.m.	5.30 p.m.	Mean	8.30 a.m.	5.30 p.m.	Mean	8.30 a.m.	5.30 p.m.	Mean
Jan. ..	..	..	..	89	52	70·5	76	53	64·5	77	48	62·5	87	48	67·5	82·3	50·3	66·3
Feb. ..	60	32	46·0	84	55	69·5	59	30	44·5	54	27	40·5	57	24	40·5	62·8	33·6	48·2
Mar. ..	39	18	28·5	62	40	51·0	49	23	36·0	60	32	46·0	61	36	48·7	54·2	29·8	42·0
Apr. ..	29	16	22·5	33	16	24·5	28	16	22·0	30	16	23·0	42	20	31·0	32·4	14·8	23·6
May ..	35	21	28·0	30	17	23·5	33	18	25·5	38	21	29·5	39	22	30·5	35·0	19·8	27·4
Jun. ..	50	35	42·5	49	31	40·0	47	30	38·5	54	39	46·5	46	30	38·0	49·2	33·0	41·1
Jul. ..	80	66	73·0	73	61	67·0	63	48	55·5	76	63	69·5	75	57	66·0	73·4	59·0	66·2
Aug. ..	82	71	76·5	69	55	62·0	83	74	78·5	76	66	71·0	..	..	..	77·5	66·5	72·0
Sep. ..	69	53	61·0	77	67	72·0	79	67	73·0	64	45	54·5	..	..	..	72·3	53·5	62·9
Oct. ..	47	29	38·0	59	39	49·0	74	57	65·5	76	61	68·5	..	..	..	64·0	46·5	55·3
Nov. ..	46	35	40·5	52	37	44·5	57	41	49·0	63	45	54·0	..	..	..	54·5	39·5	47·0
Dec. ..	73	49	61·0	68	43	55·5	78	48	63·0	75	49	62·0	..	..	..	73·5	47·3	60·4

TABLE I—(*Concld.*).

Total rainfall, inches.

Months	Years					
	1953	1954	1955	1956	1957	Average (1953-1957)
January ..	2·9	1·18	1·29	1·33	2·02	1·46
February ..	0·0	3·8	0·04	0·04	0·0	0·78
March ..	0·0	0·45	0·4	0·79	1·64	0·66
April ..	0·15	0·0	0·81	0·13	0·10	0·24
May ..	0·13	0·03	0·27	0·01	0·34	0·16
June ..	1·78	0·97	2·04	1·12	1·97	1·58
July ..	9·58	9·17	4·45	11·28	10·19	6·93
August ..	9·94	0·58	9·04	8·71	..	7·07
September ..	2·58	2·32	9·8	0·83	..	3·88
October ..	0·09	9·38	3·45	9·30	..	5·55
November ..	0·0	0·0	0·0	0·03	..	0·008
December ..	0·0	0·0	0·0	0·03	..	0·008
Annual ..	27·15	27·88	31·59	33·60		28·326

Average annual rainfall, 1944 to 1956, for Delhi 28·7 inches. (Data not inculded here.)

93·2°F. temperature was the hottest month. The month of May 1954 with 107·3° F. average maximum temperature was the hottest month and January 1954 with 44·8°F. average minimum temperature was the coldest month during the period of investigations (February, 1953 to July, 1957). The average maximum relative humidity of 82·3 per cent (at 8.30 a.m.) was recorded in January and the average minimum of 14·8 per cent (at 5.30 p.m.) was observed in the month of April. The average mean highest relative humidity was reported during the month of August, and the lowest in the month of April, January, July, August, September, October and December were the months with average mean relative humidity higher than 50 per cent. The months of April and May recorded average mean humidity lower than 30·0 per cent.

Delhi and areas around it have three clear cut seasons: (*i*) April to June it is hot and dry, (*ii*) July to September is warm and humid and (*iii*) December to February it is cold and dry. The months of March, October and November do not depict any pronounced trend. The average total annual rainfall during the period of investigation was 28·326 inches of which 25·01 inches fell during June to October.

OBSERVATIONS.

As already mentioned, ten species of anophelines* were collected during the period of the present studies. Table II presents the data, obtained by months and species, for the fifty-four months of study. Table III shows the total consolidated adult collection for each species by months and Table IV records the monthwise per man-hour density of anophelines of Delhi indicating the time spent for collections.

When the consolidated monthly per man-hour density of anophelines (Table IV) of Delhi is plotted along with the average mean temperature, average mean relative humidity and average total monthly rainfall (Graph 1), it is observed that both at low mean monthly temperatures (December to February) and low per cent relative humidity (May) the anopheline density is either low or is on a decline.

GRAPH 1.

Meteorological data (monthly means) for Feb. 1953 to *July* 1957 *and seasonal prevalence of* Anopheles Spp. *and* A. culicifacies *near Delhi.*

*Of the species recorded only *A. culicifacies* is known to be the vector in this area.

TABLE

Adult mosquito collection listed by months and species during the

Month and year	*annularis*		*barbirostris*		*culicifacies*		*fluviatilis*		*hyrcanus*		*palli-*
	T.C.	P.M.H.	T.C.	P.M.H.	T.C.	P.M.H.	T.C.	P.M.H.	T.C.	P.M.H.	T.C.
1953											
Feb.	586	75·6	..	..	10	1·3	..	..	1	0·1	..
Mar.	494	49·4	..	..	76	7·6	1	0.1	..	..	..
Apr.	271	33·8	..	..	63	7·9	..	..	..	..	..
May	171	21·4	..	..	18	2·2	..	..	..	..	..
Jun.	44	4·4	..	..	84	8·4	..	..	2	0·2	..
Jul.	10	2·5	..	..	56	14·0	..	..	..	..	..
Aug.	112	11·2	..	..	271	27·1	..	..	1	0·1	..
Sep.	196	24·5	..	..	165	20·6	..	..	..	..	..
Oct.	166	20·8	..	..	88	11·0	..	..	1	0·1	..
Nov.	338	33·8	..	..	105	10·5	..	..	..	..	..
Dec.	501	62·6	..	..	75	9·4	..	..	..	..	..
1954											
Jan.	194	24·2	..	..	60	7·4	..	..	..	..	..
Feb.	268	33·4	..	..	21	2·6	..	..	1	0·1	..
Mar.	229	22·9	..	..	43	4·3	..	..	1	0·1	..
Apr.	247	30·8	..	..	141	17·6	..	..	..	..	..
May	273	27.3	..	..	119	11·9	..	..	..	..	..
Jun.	54	6·7	..	..	50	6·2	..	..	2	0·2	..
Jul.	33	4·1	..	..	41	5·1	..	..	..	..	..
Aug.	137	13·7	..	..	32	3·2	..	..	..	..	..
Sep.	63	7·8	..	..	18	2·2	..	..	..	..	8
Oct.	23	2·8	..	..	18	2·2	..	..	..	..	26
Nov.	173	17·3	..	..	25	2·5	1	0·1	..	..	82
Dec.	385	48·1	..	..	48	6·0	..	..	..	..	33
1955											
Jan.	466	46·6	..	..	10	1·0	..	..	..	..	2
Feb.	418	52·2	..	..	13	1·6	..	..	..	..	..
Mar.	458	57·2	..	..	15	1·8	..	..	..	..	..

T. C.=total catch.
P. M. H.=per man-hour.

II.
period February 1953 *to July* 1957, *near Delhi.*

dus	*pulcherrimus*		*splendidus*		*stephensi*		*subpictus*		*Culicines*		*Anophelines*
P.M.H.	T.C.	P.M.H.	T.C.	P.M.H.	T.C.	P.M.H.	T.C.	P.M.H.	T.C.	P.M.H.	P.M.H.
..	65	8·6	..	..	205	26·4	..	..	9	1·1	112·0
..	90	9·0	2	0·2	396	39·6	..	..	30	3·0	105·9
..	17	2·1	..	..	491	61·4	..	..	15	1·9	105·2
..	..	..	..	..	59	7·4	4	0·5	1	0·1	31·5
..	..	..	..	..	56	5·6	55	5·5	16	1·6	24·1
..	..	..	..	..	27	6·8	301	75·2	3	0·7	98·5
..	2	0·2	..	..	19	1·9	1503	150·3	20	2·0	190·8
..	19	2·4	..	..	36	4·5	694	86·7	10	1·2	138·7
..	93	11·6	..	..	34	4·2	476	59·2	12	1·5	106·9
..	111	11·1	..	..	17	1·7	247	24·7	6	0·3	81·8
..	89	11·1	..	..	15	1·9	23	2·9	4	0·5	87·9
..	14	1·7	..	..	21	2·6	19	2·3	..	..	38·2
..	7	0·8	1	0·1	10	1·2	..	..	9	1·1	38·2
..	14	1·4	..	..	2	0·2	1	0·1	5	0·3	29·0
..	14	1·7	..	..	25	3·1	9	1·1	35	4·3	54·3
..	12	1·2	..	..	43	4·3	75	7·5	12	1·2	52·2
..	1	0·1	..	..	..	..	111	13·8	14	1·7	27·0
..	1	0·1	..	..	1	0·1	132	16·5	5	0·6	25·9
..	11	1·1	..	..	..	..	354	35·4	12	1·2	53·4
1·0	12	1·5	..	..	..	..	351	43·8	13	1·6	56·3
3·2	1	0·1	..	..	..	..	497	62·1	10	1·2	70·4
8·2	40	4·0	..	..	..	..	537	53·7	34	3·4	85·8
4·1	48	6·0	..	..	..	..	58	7·2	14	1·7	71·4
0·2	41	4·1	..	..	..	..	10	1·0	18	1·8	52·9
..	77	9·6	..	..	..	..	..	..	2	0·2	63·4
..	191	23·8	..	.	..	..	..	..	10	1·2	82·8

TABLE

Month and year	*annularis*		*barbirostris*		*culicifacies*		*fluviatilis*		*hyrcanus*		*palli-*
	T.C.	P.M.H.	T.C.	P.M.H.	T.C.	P.M.H.	T.C.	P.M.H.	T.C.	P.M.H.	T.C.
1955											
Apr.	363	45·3	..	..	83	10·3	..	..	..	..	..
May	278	27·8	..	..	175	17·5	..	..	..	..	..
Jun.	36	4·4	..	..	87	10·8	..	..	..	..	..
Jul.	51	6·3	..	..	72	9·0	..	..	1	0·1	1
Aug.	111	11·1	..	..	138	13·8	..	..	..	..	4
Sep.	62	7·7	..	..	165	20·6	..	..	2	0·2	41
Oct.	60	8·5	..	..	95	13·5	..	..	..	..	13
Nov.	408	51·0	1	0.1	169	21·1	..	..	..	..	1
Dec.	487	60·8	..	..	80	10·0	..	..	1	0·1	68
1956											
Jan.	587	58·7	..	..	60	6·0	..	..	..	..	..
Feb.	820	102·5	..	..	43	5·4	..	..	..	..	4
Mar.	742	92·8	..	..	42	5·3	..	..	1	0·1	..
Apr.	874	87·4	..	..	97	9·7	..	..	1	0·1	..
May	164	20·5	..	..	24	3·0	..	..	..	..	..
Jun.	172	21·5	..	..	12	1·5	..	..	..	..	..
Jul.	36	3·6	..	..	17	1·7	..	..	..	..	..
Aug.	39	4·9	..	..	55	6·9	..	..	..	..	..
Sep.	72	9·0	..	..	124	15·5	..	..	..	..	..
Oct.	52	5·2	..	..	155	15·5	..	..	..	..	6
Nov.	248	30·9	..	..	146	18·2	..	..	..	..	..
Dec.	291	29·1	..	..	183	18·3	..	..	..	..	..
1957											
Jan.	11	1·4	..	..	21	2·6	..	..	..	..	..
Feb.	107	13·4	..	..	43	5·4	..	..	..	..	..
Mar.	359	44·9	..	..	62	7·8	1	0.1	..	..	..
Apr.	687	68·7	..	..	156	15·6	..	..	..	..	..
May	167	20·9	..	..	107	13·4	..	..	..	..	..
Jun.	162	20·3	..	..	65	8·1	..	..	..	..	..
Jul.	12	1·2	..	..	22	2·2	..	..	..	..	..

T. C.=total catch.
P. M. H.=per man-hour.

II—(*Concld.*).

dus	*pulcherrimus*		*splendidus*		*stephensi*		*subpictus*		*Culicines*		*Anophelines*
P.M.H.	T.C.	P.M.H.	T.C.	P.M.H.	T.C.	P.M.H.	T.C.	P.M.H.	T.C.	P.M.H.	P.M.H.
..	173	21·6	..	..	..	..	..	..	9	1·1	77·2
..	2	0·2	..	..	..	..	103	10·3	13	1·3	55·8
..	..	..	..	..	2	0·2	242	30·2	16	2·0	45·6
0·1	1	0·1	..	..	3	0·3	790	98·7	21	2·6	115·6
0·4	1	0·1	..	..	..	..	1000	100·0	26	2·6	93·3
5·1	..	..	..	..	3	0·3	541	67·6	10	1·2	101·5
2·0	..	..	..	..	1	0·1	528	75·4	19	2·7	99·5
0·1	2	0·2	..	..	3	0·3	408	51·0	13	1·6	123·8
8·5	..	..	..	..	1	0·1	155	19·3	17	2·1	98·8
..	3	0·3	..	..	3	0·3	2	0·2	48	4·8	65·5
0·5	3	0·4	..	..	4	0·5	..	..	41	5·1	109·3
..	5	0·6	2	0·3	14	1·8	..	..	60	7·5	100·9
..	8	0·8	..	..	109	10·9	7	0·7	55	5·5	109·6
..	8	1·0	..	..	98	12·3	91	11·4	34	4·3	48·2
..	6	0·8	..	..	32	4·0	217	27·1	49	6·1	54·9
..	..	..	..	..	62	6·2	577	57·7	40	4·0	69·2
..	..	..	..	..	..	..	869	108·6	4	0·5	120·4
..	4	0·5	..	..	..	..	816	102·0	6	0·8	127·0
0·6	15	1·5	..	..	3	0·3	458	45·8	1	0·1	68·9
..	10	1·3	..	..	2	0·3	426	53·3	13	1·6	104·0
..	10	1·0	..	..	11	1·1	220	22·0	13	1·3	71·5
..	..	..	..	..	2	0·3	..	..	26	3·3	4·3
..	1	0·1	..	..	2	0·1	..	..	10	1·3	19·0
..	25	3·1	2	0·3	7	0·9	..	..	59	7·4	57·1
.	63	6·3	..	..	24	2·4	..	..	20	2·0	93·0
.	21	2·6	..	..	58	7·4	40	5·0	25	3·1	49·3
..	1	0·1	..	..	19	2·4	179	22·4	14	1·8	53·3
..	4	0·4	..	..	4	0·4	514	51·4	16	1·6	55·6

TABLE III.

Total adult mosquito collection listed by species and months during the period February 1953 to July 1957, near Delhi.

Serial number	Species	Months												Total
		Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	
	Anophelines													
1.	*A. annularis* ..	1258	2199	2282	2442	1053	468	142	399	393	301	1167	1664	13768
2.	*A. barbirostris* ..	..	..	..	..	..	..	..	..	..	..	1	..	1
3.	*A. culicifacies* ..	151	130	238	540	443	298	208	496	472	356	445	386	4163
4.	*A. fluviatilis* ..	..	..	2	..	..	..	..	..	..	..	1	..	3
5.	*A. hyrcanus* ..	..	2	2	..	1	4	1	1	2	1	..	1	15
6.	*A. pallidus* ..	2	4	..	..	..	..	1	4	49	46	83	101	290
7.	*A. pulcherrimus* ..	58	153	325	275	43	8	6	14	35	109	163	147	1336
8.	*A. splendidus* ..	..	1	6	..	..	..	..	..	..	..	..	..	7
9.	*A. stephensi* ..	26	221	419	649	258	109	97	19	39	38	22	27	1924
10.	*A. subpictus* ..	31	..	1	16	313	804	2314	3726	2402	1959	1618	456	13640
	Culicines ..	92	71	164	134	85	109	85	62	39	42	66	48	997
	Total ..	1618	2781	3439	4056	2196	1800	2854	4721	3431	2852	3566	2830	36144

TABLE IV.

Per man-hour density of adult mosquito species and total time spent in collection during February, 1953 to July, 1957.

Serial number	Species	Months												
		Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Total
	Anophelines													
1.	*A. annularis* ..	35·0	55·3	51·8	55·5	23·9	11·1	3·6	10·5	12·2	9·1	32·4	46·0	29·8
2.	*A. barbirostris* ..	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·02	0·0	0·002
3.	*A. culicifacies* ..	4·2	3·2	5·4	12·2	10·0	7·0	5·2	13·1	14·7	10·8	12·3	11·3	9·0
4.	*A. fluviatilis* ..	0·0	0·0	0·04	0·0	0·0	0·0	0·0	0·0	0·0	0 0	0·02	0·0	0.006
5.	*A. hyrcanus* ..	0·0	0·05	0·04	0·0	0·02	0·1	0·03	0·03	0·06	0·03	0·0	0·02	0·03
6.	*A. pallidus* ..	0·05	0·1	0·0	0·0	0·0	0·0	0·03	0·1	1·5	1·4	2·3	2·9	0·6
7.	*A. pulcherrimus* ..	1·6	3·8	7·3	6·2	0·9	0·1	0·15	0·37	1·1	3·3	5·0	4·3	2·9
8.	*A. splendidus* ..	0·0	0·02	0·1	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·02
9.	*A. stephensi* ..	0·7	5·5	9·5	14·7	5·8	2·6	2·4	0·5	1·2	1·2	0·6	0·8	4·2
10.	*A. subpictus* ..	0·8	0·0	0·02	0·3	7·1	19·1	57·9	98·0	75·0	59·4	50·5	13·4	29·5
	Culicines ..	2·5	1·7	3·7	3·0	1·9	2·6	2·1	1·6	1·2	1·3	1·8	1·4	2·2
	Time spent (hours) ..	36	39¾	44	44	44	42	40	38	32	33	36	34	462¾
	Per man-hour density of Anophelines : ..	42·4	67·0	74·2	88·9	47·7	40·0	69·3	122·6	105·8	85·2	103·1	78.7	

The position in case of April where the anophelines show a higher density, inspite of a low percentage of relative humidity, is because of the favourable climatic condition obtained in March resulting in continuation of the build-up of the anophelines into April. The results of dry conditions prevailing in April and May are reflected in low mosquito densities of May and June. The month of January recorded density of 42·4 per man-hour and this was followed by a steady rise till April when the density was 88·9 per man-hour. This would be the 'spring peak' after which there is sudden drop in the population till June with 40·0 per man-hour. After June, with the onset of monsoon, there is a spectacular rise in anopheline population, the peak (122·6 per man-hour) being reached in August. After August, there is a slight decline but high densities are maintained up to November, after which there is a decline till January. From a glance at Graphs 1 and 2, it would seem that enough rainfall, high humidity and temperature between 75°to 90°F., are ideal for high density of anophelines around Delhi.

The fluctuations in the seasonal prevalence of each species are considered separately below:

A. ANNULARIS VAN DER WULP, 1884.

This anopheline was the most dominant species collected and was found throughout the year in good numbers. Both Hodgson and Senior White (Senior White, 1929) reported this species to be essentially a cold weather mosquito in Delhi. The present observations support their assertion in that *A. annularis* is characterised by a relatively low density from June to October and starts to build up from November onwards (Graph 3 and Table IV). However, the present studies show that the highest density, above 50 per man-hour, is recorded during the months of February, March and April, the latter two months being the spring months.

Senior-White (1929) was of the opinion that the low density during fever-season was not a temperature effect alone as the species was found to be prevalent both at 78°F. and 90°F. The present observations seem to indicate that high density of this species is governed by low temperature and low rainfall (Graph 4). In October when the average mean temperature was 76·8°F., the density (9·1 per man-hour) was lower than in April (55·5 per man-hour) with the average mean temperature of 83·4°F. The average total rainfall in October, however, was 5·55 inches as against 0·24 inches in April. Similarly in May when the average total rainfall was only 0·16 inches the density was only 23·9 per man-hour as a result of high average mean temperature (92·0°F).

Around the site of the present observations this species breeds mostly in stagnant swamps of rain water, seepage water in low lying land and borrowpits. It is likely that high rainfall, irrespective of temperature, changes the nature of these breeding grounds to result in low density of this species during June to October.

It has been generally believed that the species rests in much larger numbers in cattle-sheds than in human dwellings (Christophers, 1933). This is, however, not the case in the area under study. Out of 13,768 specimens captured 3,144 were from cattlesheds, 3,554 from human dwellings and 7,070 from mixed dwellings;

GRAPH 3.

Monthly incidence of A. annularis *near Delhi, Feb.* 1953 *to July* 1957.

GRAPH 4.

Temperature and rainfall in relation to density of A. annularis *near Delhi, February* 1953 *to July* 1957.

the per man-hour ratio being 27·1 : 30·7 : 30·6. The only other published data about an area, with a condition approaching this, is about Bengal where Sur and Sur (1929) found an equal number resting in houses and cattle-sheds.

A. BARBIROSTRIS VAN DER WULP, 1884.

This was the least prevalent species present. Only one female specimen was collected from a mixed dwelling during November (1955). *A. barbirostris* is known to be a wild species and needs to be further searched in outdoor shelters. No larval stage has been encountered so far. The species is known to breed

mainly in swamps and has *hyrcanus* as its most frequent associate (Hacker, 1920). The larvæ of the latter species have been observed here occasionally.

A. CULICIFACIES GILES, 1901.

This is the only known vector of malaria in this area and was present throughout the period of study. Afridi and Puri (1940), while reviewing the work on the behaviour of *A. culicifacies*, divided the pre-partition India into seven regions and mentioned that in their South-Eastern region*, which included Delhi, this species occurred in significant numbers throughout the year, with periods of comparatively low densities in January-February and May-June, and had two peaks one in March-April and the other in September. The observations made during the present studies near Delhi differ slightly from those mentioned above. Graph 5 shows that the species has relatively high density during April-May and August to December. The lowest density of 3·2 per man-hour was recorded in February and the highest density of 14·7 per man-hour in September. The spring peak of 12·2 per man-hour was reached in April with the density of 10·0 per man-hour during May. The density in August, September and November was higher than during any other month.

GRAPH 5.

Monthly incidence of A. culicifacies *and* A. pallidus *near Delhi, Feb.* 1953 *to July* 1957.

*Punjab, Sind, Western U.P., South Bihar, Orissa, Jeypore Hill Tracts, Hyderabad State, and parts of Madhya Pradesh.

Afridi *et al.* (1940) observed that the seasonal appearance or disappearance of this species near Karnal (East Punjab) was not correlated with fluctuations in temperature and humidity. They suggested that there was a seasonal element which regulates its activity. The present observations, however, indicate that the most important controlling factor seems to be formation of fresh rain-water pools, devoid of amorphous matter during July, August and September (Graph 2), which agree with the findings of Russell and Rao (1942).

A. FLUVIATILIS JAMES, 1902.

Only three female specimens of this monquito were collected resting indoors during the course of the five years. Two of these were collected in the month of March, 1953 and 1957 and one in November, 1954. None of the specimens was collected inside a cattleshed. *A. fluviatilis* is reputed to breed usually in slow running waters in areas with an altitude of 1,000 to 5,000 feet (Covell, 1944). Its favourite haunts are foot-hill areas and hilly or rocky tracts (Christophers, 1933). These considerations, coupled with the fact that no larval stage of the species has so far been found during the present investigations, make one doubt whether *A. fluviatilis* is indigenous to the area at all. The few adults encountered may perhaps more appropriately be regarded as accidental introductions into the area, probably due to larval drift via. the Hindan River or other agencies such as trains coming from the Dehradun Valley.

A. HYRCANUS VAR. *NIGERRIMUS* GILES, 1900.

This species is generally wild, breeding independently of the proximity of habitations and not commonly found resting indoors (Christophers, 1933; Russell and Rao, 1941). In Thailand, one of the authors* found it frequently resting indoors in large empty straw baskets used for storing food grains. Only 15 specimens were collected indoors during the present study. Specimens were collected throughout the year except during January, April and November which fact seems to indicate that the species may be present throughout the year in relatively low densities. Aquatic stages of the species have also been observed occasionally in this locality.

A. PALLIDUS THEOBALD, 1901.

Russel and Rao (1941) observed that adults of this species seemed to prefer to rest in outdoor places in South-Eastern Madras. However, Bhombore *et al.* (1954), working in Western Hill Tracts of Mysore State, collected good numbers (up to 40 per man-hour) of this species from indoor resting places. The experience of some of the present workers in Bhandara District† in former Madhya Pradesh and Mirzapur District‡ of Uttar Pradesh has been in conformity with the view of latter workers. During the present study, there being no outdoor shelters, it seems that the species is an indoor rester in the areas surveyed in Delhi suburban areas.

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Although this species formed less than one per cent of the total anophelines collected during the present study, there was a pronounced seasonal trend (Graph 5). This species seems to abound in late monsoon and early winter months. Starting in July, the density built up to its peak in December. After December, there is a sudden fall in density in January. No adults could be collected from indoor shelters from March to June. It seems that low temperature, combined with numerous breeding places augmented by monsoons, determines the relative abundance of this species.

A. PULCHERRIMUS THEOBALD, 1902.

This species was present throughout the year and formed about four per cent of the total catch. In this area, it seems to have two seasons of abundance, February to April and October to December (Graph 6). The highest density was recorded in March followed by April and November. May to September were the months of relatively low density. A combination of low temperatures and dry conditions seems to be conducive for high density of this species.

GRAPH 6.

Monthly incidence of A. pulcherrimus, *and* A. stephensi *near Delhi, February* 1953 to *July* 1957.

A. SPLENDIDUS KOIDZUMI, 1920.

Christophers (1933) mentioned that this species is encountered usually in small numbers in indoor shelters. Similar observations were made by Bhombore

et al. (1954) in Western Hill Tracts of Mysore. The present studies confirm these observations. Only seven specimens were collected during the months of February 1953 and 1954 and March 1956 and 1957. No specimens were caught during 1955. In view of the collections being spread over five years, out of which this species was recorded only during February and March, it seems that the species has a seasonal trend in these areas showing a preference for dry spring months. The species has been found to breed in the area.

A. STEPHENSI LISTON, 1901.

Only *A. stephensi* var. *mysoriensis* was encountered and showed erratic behaviour during the present observations. Out of 1,924 specimens collected, 1,355 were caught during 1953 and the species was present throughout the year. In 1954, it was present from January to May and in July, and was not recorded during the other months. In 1955, it did not appear in catches till June and the total catch for that year was only 13. In 1956, this species again re-appeared in higher density and was recorded throughout the year except during July and August. In 1957, the species was recorded continuously till July. The data for seasonal prevalence, when plotted (Graph 6), shows that this species abounds during February to May with the peak in April. In the rural areas, therefore, it appears to be largely a spring mosquito showing preference for periods of low rainfall. In urban areas, *A. stephensi* is known to breed in pools, in seepage water, reservoirs and springs and it is likely that the monsoon rain water washes the natural breeding places to restrict the density of this species. Breeding places are becoming more and more difficult to locate.

A. SUBPICTUS GRASSI, 1899.

This species is known to be a markedly domestic form (Christophers, 1933) and was the second dominant anopheline collected during the present observations. But unlike *A. annularis*, it was not found throughout the year and showed a definite seasonal trend. The per man-hour density during January to April was very low and varied between 0·02 and 0·8 (Graph 7, Table IV). No mosquito of this species was collected during February. The density started building in May (7·1 per man-hour) and rose to its peak in August (98·0 per man-hour). After August there was a gradual decline in the density till November after which there was a sharp decline. The per man-hour density of this species between July and November was above 50·0. *A. subpictus*, therefore, seems to be a typical monsoon species in this area. As mentioned by Christophers (1933), *A. subpictus* breeds especially in very temporary water collections and such type of waters abound during monsoons. That explains the seasonal trend depicted by this species in this area.

CULICINES.

During the present investigations, 997 culicines were also collected from the three types of shelters visited. Since separate studies are being conducted regarding bionomics of this tribe, no attempt was made to organise the data species-wise. From the data presented here, it is evident that culicines were encountered

GRAPH 7.

Monthly incidence of A. subpictus *near Delhi, February* 1953 *to July* 1957.

throughout the year. It is striking to note that, unlike urban areas, the culicines had low density in indoor shelters in these rural areas. The highest density of 3·7 per man-hour was recorded in March and the lowest of 1·2 per man-hour in

GRAPH 8.

Monthly incidence of culicines *near Delhi, February* 1953 *to July* 1957.

September. The months of January, March, April and June showed higher densities than the other months (Graph 8).

TABLE V.

Resting preferences of adult mosquitoes in villages near Delhi; February 1953 *to July* 1957.

Serial number	Species	Dwelling: Cattleshed T.C. : P.M.H.	Dwelling: Human dwelling T.C. : P.M.H.	Dwelling: Mixed dwelling T.C. : P.M.H.	Total TC : P.M.H.
Anophelines:					
1.	*A. annularis* ..	3144 : 27·1	3554 : 30·7	7070 : 30·6	13768 : 29·8
2.	*A. barbirostris*	..	..	1 : 0·004	1 : 0·002
3.	*A. culicifacies* ..	898 : 7·7	1115 : 9·6	2150 : 9·3	4163 : 9·0
4.	*A. fluviatilis* ..	..	2 : 0·02	1 : 0·004	3 : 0·006
5.	*A. hyrcanus* ..	3 : 0·03	5 : 0·04	7 : 0·03	15 : 0·03
6.	*A. pallidus* ..	39 : 0·3	91 : 0·8	160 : 0·7	290 : 0·6
7.	*A. pulcherrimus*	347 : 3·0	385 : 3·3	604 : 2·6	1336 : 2·9
8.	*A. splendidus* ..	1 : 0·009	1 : 0·009	5 : 0·02	7 : 0·02
9.	*A. stephensi* ..	599 : 5·2	583 : 5·0	742 : 3·2	1924 : 4·2
10.	*A. subpictus* ..	2790 : 24·1	3695 : 31·9	7155 : 31·0	13640 : 29·5
Culicines ..		253 : 2·2	297 : 2·6	477 : 1·9	997 : 2·2
Total	T.C. : P.M.H.	8074 : 69·7	9728 : 84·0	18342 : 79·4	36144 : 78·1
Time spent (hours) ..		$115\frac{7}{8}$	$115\frac{7}{8}$	231	$462\frac{3}{4}$

T.C. = total catch. P.M.H. = per man-hour.

ADULT RESTING PLACES.

Table V includes data (total and per-man-hour catch) regarding mosquitoes collected in three types of shelters, namely, (*i*) cattleshed, (*ii*) human, and (*iii*) mixed dwellings, used as catching stations for the present study. Graph 9 represents graphically the preferential resting places of *A. annularis, A. culicifacies, A. pulcherrimus, A. stephensi, A. subpictus* and *culicines* (Species, with density lower than 1·0 per man-hour, are not included in Graph 9.).

As has already been mentioned, in an Indian village it is difficult to make precise distinction between the various types of shelters as man and domestic animals are often found close to each other. However, the data presented here indicate that, so far as daytime resting habits are concerned, *A. annularis, A. culicifacies* and *A. subpictus* seem to have a preference for human and mixed dwellings than

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GRAPH 9.

Preferential indoor resting shelters of adult mosquitoes near Delhi, February 1953 to July 1957.

for cattlesheds. *A. pulcherrimus* seems to prefer human dwellings and cattlesheds to mixed dwellings. In case of culicines, more specimens were caught in human dwellings than cattlesheds and mixed dwellings; however, the overall density (per man-hour) being low the differences may not be significant in this case.

In case of *A. pallidus*, although the density was low, still it seems to prefer human dwelling to cattlesheds. Specimens of *A. barbirostris*, *A. hyrcanus*, *A. fluviatilis* and *A. splendidus* were too few to give any clue about their resting preferences. These species can be considered as rare for this area.

SEX RATIO.

Table VI gives the sex ratio of adults collected from the three types of shelters during the present observations. It is evident that in case of each species, more females than males were caught. Amongst anophelines, *A. pulcherrimus* had the highest female : male ratio of 31·6 to 1, and *A. pallidus* the lowest ratio of 6·8 to 1. *A. culicifacies*, the vector species, had a ratio of 12·8 to 1. More culicine males were caught than in case of anophelines and the ratio was only 2·9 females to 1 male.

TABLE VI.

Sex ratio of mosquitoes collected between February, 1953 *and July,* 1957.

Serial number	Species	Males	Females	Total	Ratio Male : Female
Anophelines:					
1.	*A. annularis* ..	574	13194	13768	1 : 23·0
2.	*A. barbirostris* ..	..	1	1	..
3.	*A. culicifacies* ..	301	3862	4163	1 : 12·8
4.	*A. fluviatilis* ..	..	3	3	..
5.	*A. hyrcanus* ..	1	14	15	1 : 14
6.	*A. pallidus* ..	37	253	290	1 : 6·8
7.	*A. pulcherrimus*	41	1295	1336	1 : 31·6
8.	*A. stephensi* ..	211	1713	1924	1 : 8·1
9.	*A. splendidus* ..	..	7	7	..
10.	*A. subpictus* ..	1381	12259	13640	1 : 8·9
Culicines	..	254	743	997	1 : 2·9
	Total ..	2800	33344	36144	1 : 12·0

SUMMARY AND CONCLUSIONS.

The seasonal prevalence of *Anopheles annularis, A. barbirostris, A. culicifacies, A. fluviatilis, A. hyrcanus, A. pallidus, A. pulcherrimus, A. splendidus, A. stephensi, A. subpictus* and culicines recorded over a period of about five years (February, 1953 to July, 1957) from human dwellings, cattlesheds and mixed dwellings in Arthala, Bhaunja, and Karera, three unsprayed villages near Delhi, has been discussed. It was found that:

1. *A. annularis* is the most dominant species in the area and was present throughout the year. Its density seems to be favoured by a combination of low rainfall and low average mean temperature.

2. *A. culicifacies*, the only known malaria vector in this area, was prevalent throughout the present investigations and showed 'peaks' in April-May, instead of March-April as believed hitherto, and August to December. The highest density of 14·7 per man-hour was recorded in September. The most important factor, conducive to its high density, seems to be formation of fresh rain water pools devoid of amorphous matter.

3. *A. barbirostris*, *A. fluviatilis*, *A. hyrcanus*, and *A. splendidus* seem to be rare species in this area. Of these, only larvæ of *A. hyrcanus* have been encountered. It is likely that *A. fluviatilis* may not be indigenous to this locality, and the specimens encountered may be accidental introductions by larval drifts or passive transmission of adults from distant places (foot-hill areas of Uttar Pradesh).

4. Low temperature and breeding places resulting from monsoons seem to determine density of *A. pallidus*, whereas low temperature and dry conditions appear to favour *A. pulcherrimus* in this area.

5. *A. stephensi* showed erratic behaviour in its seasonal variations during the current studies. However, it formed 4·2 per cent of the collections and it seems that in rural areas (near Delhi) it is largely a spring mosquito, showing preference for low rainfall periods of the year.

6. *A. subpictus*, the second dominant anopheline in the area, was not present throughout the year. It seemed to abound in large numbers during monsoons, which may be due to its preference for breeding in temporary water collections.

7. Culicines generaly showed a low density in indoor shelters in the rural areas investigated.

8. Resting preference of adult mosquitoes in three types of shelters and their sex ratio have been mentioned.

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ON CERTAIN MORPHOLOGICAL ABNORMALITIES NOTED IN *A. PALLIDUS* THEOBALD, 1901 AND *A. STEPHENSI STEPHENSI* LISTON, 1901.

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MORPHOLOGICAL abnormalities are encountered in mosquitoes belonging to most of the species. These abnormalities are usually in the ornamentation of the maxillary palpi, the tarsi of hind legs and of the wings. Most of these variations are of minor importance and are of little consequence in the identification of the species. Two variations, both of which are very unusual, *i.e.*, the lack of dark colouring or hypomelanism in the two adult specimens of *A. pallidus* and the incomplete development of the sixth wing vein in colony bred specimens of *A. stephensi stephensi* (Mettupalyam strain) maintained at Malaria Institute of India, Delhi, are of interest and are reported in this paper.

A. PALLIDUS.

During insecticidal trials in Vindhya Pradesh (now Madhya Pradesh) in the year 1954, two specimens of *A. pallidus*, which differed in colouration from those of typical *A. pallidus*, were collected from a village, Papra, in Satna District. These specimens appear to be hypomelanic forms of *A. pallidus*; the dark colouration, typical of this species, is lacking so that they are uniformly very light-coloured. On the wings, all the dark scales are very pale and the usual pale areas on the hind tarsal segments are indistinct and the tarsi merge into the brownish femora and tibia. Similarly the dark bands on the palpi are more or less completely absent and the whole of the palpi are uniformly very light-brown in colour.

Although the characteristic light and dark colouration is lacking, all other characteristics show that these specimens belong to *A. pallidus*. The only species which they can be confused with is *A. philippinensis*, which is a very closely allied species. However, the presence of scales on the sternopleura and on the dorsum of the last six abdominal segments, conclusively distinguish them from *A. philippinensis*. However, indirect evidence also supported this conclusion. During the whole of the survey of the area, spread over a period of six months, no specimen of *A. philippinensis* was encountered while *A. pallidus* was the most predominant species.

Christophers (1933) reported a case of hypomelanism in *A. pallidus* but neither detailed description nor the locality from where the specimen was obtained, was given. Similar extreme forms of hypomelanism have been noted in *A. subpictus* and *A. sundaicus* (Bonne Wepster and Swellengrebel, 1953); and in *A. annulipes*, *A. meraukensis* and *A. farauti* (Lee and Woodhill, 1944). Similarly *A. stephensi* and *immaculatus* form of *A. vagus* have been described as showing this type of abnormality (Christophers, 1933).

Generally, winter forms of some species are somewhat darker and larger in size as compared to those in the summer. Though melanic forms sometimes appear even at other times of the year also, they are usually common during the winter months. As the two hypomelanic specimens of *A. pallidus*, described above, were collected during the summer months, it appears that the lack of dark pigment is associated with comparatively high atmospheric temperature.

A. STEPHENSI STEPHENSI.

In a number of laboratory-bred *A. stephensi stephensi*, the wing veins, instead of being normal as shown in Plate I (Figure I), showed an abnormality in the incomplete development of sixth wing vein. The abnormality was of four types. In Type I, the sixth vein extended up to the first dark spot, about one-third of its normal length (Plate I, Fig. II). In Type 2, the vein was about half the length of the normal and had a few black scales at the end just below the fork of the fifth vein (Plate I, Fig. III); while in Type 3 also the vein was about half the normal length but ended abruptly a little before the fork of the fifth vein, to be continued again near the wing fringe leaving a blank space about one-third the normal length in between (Plate I, Fig. IV).

In all the three types mentioned above, a fringe spot was discernible at the termination of Vein 6, as in the normal wing, but in Type 4, fringe spot was not present, though the sixth vein extended about one-third the normal length, as in Type I (Plate I, Fig. V).

These variations were found on both the wings with various combinations of the types described above but quite often only one wing showed them, the other being normal. These variations were noted both in males and females.

In all, a total of 11,664 specimens of both males and females were examined. Out of these, 1,923 were with the abnormal wings, giving an overall percentage of 16·4.

Eggs, obtained both from the normal and abnormal forms, were carefully examined to see if any difference existed among them. No difference was, however, observed.

PLATE I

NORMAL AND ABNORMAL WINGS OF A. STEPHENSI STEPHENSI LISTON, 1901.

LEGEND

Fig. I Normal wing

Fig. II-V Wings showing different types of variation in V.6

Offsprings of abnormal females had both normal and abnormal wings and *vice versa*. The proportion of normal to abnormal was 10 : 1.

Dissections of larvæ, pupæ and adults did not reveal any abnormality nor was any parasitic infection found in them, which could possibly account for the attenuation of the sixth wing vein.

This abnormality in the wing was noted only in the laboratory-bred specimens of the Mettupalyam strain. More than a thousand specimens in the collections of the Malaria Institute of India, collected from different parts of India, were examined but the sixth wing vein was found to be normal in all of them.

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COMPARATIVE FIELD TRIALS ON THE RESIDUAL EFFECTIVENESS OF D.D.T., MALATHION AND DIAZINON*.

BY

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[November 8, 1957.]

FIELD trials on the comparative efficacy of residual sprays of D.D.T., malathion and diazinon against mosquitoes in general, and culicines in particular, were carried out in Shertallai Taluk of Kerala State during the period February to May, 1957.

AREA SELECTED.

Filaria survey of Shertallai Taluk was carried out by Iyengar (1938) and recently by Jaswant Singh, Krishnaswami and Raghavan (1956). The entire area is highly endemic for filaria and *W. malayi* is the species of filarial infection. *M. annulifera* is the main vector with *M. uniformis* playing a secondary rôle. In 1955, certain villages in this Taluk were sprayed to test the efficacy of D.D.T., B.H.C. and dieldrin (Jaswant Singh, Krishnaswami *et al.*, 1956). For the present studies, a few other villages in the same area were selected. For each type of treatment, convenient blocks were selected and each of them was treated with a particular insecticide. One block was left unsprayed for comparison. Details of the blocks selected for insecticidal application are given in Table I and their locations are indicated in Map 1.

*D.D.T. was obtained from the supplies made available to Malaria Control Unit, Trichur, Kerala State, under the National Malaria Control Programme. Malathion and diazinon were sent for trials by Messrs. American Cynamide Company and Geigy Insecticides, respectively.

The topography, the type of structure*, the socio-economic conditions and other connected details of the area under study are the same as described by Jaswant Singh, Krishnaswami and Raghavan (1956).

MAP 1.

Sketch map of shertallai area showing experimental areas.

*Majority of houses are thatched with plaited coconut leaves, and walls made of the same material or of bamboo matting. A few houses were pucca with plastered walls.

TABLE I.

Details regarding the experimental area and insecticidal application.

Name of the area	Number of houses	Population	Insecticidal application
Pattanakad	428	2,480	D.D.T. 200 mg./sq. ft.
Thannirmukam ..	253	1,399	Malathion 25 mg./sq. ft.
Kalavancode	256	1,523	Diazinon 25 mg./sq. ft.
Kandamangalam ..	275	1,375	Comparison area (Unsprayed).

FORMULATION AND DOSAGE OF INSECTICIDES TESTED.

All the three insecticides, viz., D.D.T. (75 per cent), malathion (25 per cent) and diazinon (40 per cent) were tried in the form of water dispersible powders. The dosages of insecticides employed were D.D.T. 200 mg./sq. ft., malathion 25 mg./sq. ft. and diazinon 25 mg./sq. ft. To apply 200 mg. D.D.T./sq. ft., ½ gallon of 5 per cent suspension was sprayed over 1,000 sq. ft. surface twice.

The spraying operations were carried out with the help of locally hired labour who were given adequate training in the technique of proper application of insecticides. The spraying was done between February 7 and 12 under the supervision of one of the authors (K.S.K.).

DATA COLLECTED.

In each of the experimental villages, six adult mosquito catching stations were established, three of which were left unsprayed. In the comparison village, six stations were selected for parallel observations. Weekly collections by hand were made in all the stations, one collector spending 30 minutes in each of them.

24-hour mortality rates were obtained by keeping the captured mosquitoes, from sprayed and unsprayed shelters, under optimum conditions in the field.

Dissections of *Mansonioides* adults collected from sprayed and unsprayed villages were made regularly to determine the infection in pre and post-spraying periods.

The bulk of mosquitoes consisted of culicines among which *Mansonioides* formed a very small proportion. The species collected in order of preponderance were *Culex gelidus*, *Culex vishnui*, *Anopheles subpictus*. The other species encountered were *C. sitiens*, *A. vagus*, *A. jamesi*, *A. hyrcanus*, *A. barbirostris*, *M. annulifera*, *M. uniformis* and *M. indiana*. *Mansonioides spp.* formed only about 5 per cent of the total collections of mosquitoes.

For purposes of analysis, the number of mosquitoes collected have been expressed as average per catching station. *Mansonioides* have not been separately shown as their numbers were very small.

RESULTS.

The weekly densities of mosquitoes from sprayed and unsprayed catching stations and comparison villages are shown in Table II and the 24-hour survival rates in Table III.

TABLE II.

Weekly adult collections (average of three stations) from sprayed and unsprayed stations and from comparison areas (average of six stations).

Week	200 mg. DDT/sq. ft.		25 mg. Malathion/sq. ft.		25 mg. Diazinon/sq. ft.		Comparison
	S.	U.S.	S.	U.S.	S.	U.S.	
Pre-spraying (average of 4 weeks)	29·35	20·75	36·6	23·4	35·0	37·2	27·8
0	49·6	34·9	47·7	23·4	60·0	31·0	24·4
1	11·4	25·3	10·7	23·6	11·3	13·1	40·4
2	5·3	10·4	20·7	14·0	7·7	15·0	22·3
3	8·7	42·6	17·6	13·6	10·0	19·4	31·1
4	19·0	55·7	31·3	12·7	13·0	20·0	49·1
5	12·0	36·0	20·3	11·6	22·0	14·6	56·9
6	23·7	56·3	42·3	21·0	26·3	29·6	50·5
7	17·0	74·7	53·0	30·7	22·7	21·7	51·6
8	20·3	65·3	53·6	31·0	44·0	26·7	30·2
9	20·3	66·0	37·0	28·0	28·0	32·0	27·7
10	20·0	67·3	39·0	24·6	29·0	31·0	38·3
11	32·6	97·7	32·3	23·7	32·3	25·3	35·3
12	46·6	36·7	33·7	25·3	21·0	18·7	18·8

S=Sprayed. U.S.=Unsprayed.

D.D.T. AREA.

The density of mosquitoes came down after spraying, and continued to be low for five weeks. Significant rise in density was noted in the sixth week. Twentyfour-hour survival rates of mosquitoes showed progressive increase from the third week (46·2), and by the sixth week it rose to 77·4 per cent.

MALATHION AREA.

The density of mosquitoes dropped during the first week after spraying but it got built up from the second week and by the fourth week there was significant increase. The survival rate in the fifth week was as high as 91·8 per cent.

TABLE III.

Survival rates of mosquitoes from experimental and comparison villages.

Weeks after spraying	D.D.T. 200 mg./sq. ft.			Malathion 25 mg./sq. ft.			Diazinon 25 mg./sq. ft.			Comparison		
	Total number of mosquitoes	Number dead after 24 hrs.	Survival rate	Total number of mosquitoes	Number dead after 24 hrs.	Survival rate	Total number of mosquitoes	Number dead after 24 hrs.	Survival rate	Total number of mosquitoes	Number dead after 24 hrs.	Survival rate
1	35	35	0·0	32	32	0·0	69	69	0·0	242	44	81·8
2	16	16	0·0	77	77	0·0	23	23	0·0	234	17	87·3
3	26	14	46·2	52	38	26·9	29	28	3·4	186	28	90·3
4	57	38	33·3	94	64	31·9	39	8	69·2	295	19	93·5
5	36	21	41·6	61	5	91·8	66	0	100·0	341	21	93·8
6	71	16	77·4	92	13	85·8	79	0	100·0	303	18	94·05
7	51	18	64·7	159	20	87·4	68	4	94·1	310	0	100·0
8	61	13	78·7	161	34	78·8	132	26	80·3	181	13	92·8
9	61	16	73·8	111	30	72·9	84	20	76·1	166	30	81·9
10	84	9	89·3	99	6	93·9	87	20	77·0	230	19	91·7
11	98	18	81·6	102	10	90·2	97	16	83·5	211	26	87·6
12	140	9	93·5	98	13	86·7	63	5	92·0	113	11	90·3

DIAZINON AREA.

During the first four weeks after spray the density of mosquitoes was low and significant increase in their numbers was observed in the fifth week after spraying. Survival rate showed a sharp rise in the fourth week, and in the fifth week the rate was 100·0 per cent.

MOSQUITO DISSECTIONS.

The results of dissections are shown in Table IV.

In D.D.T. area before insecticidal treatment, out of 40 *Mansonioides annulifera* dissected 9 were positive, giving a percentage infection of 22·5. In the post-spraying period, out of 11 *Mansonioides annulifera* dissected from this area one was found infected but that too was from an unsprayed station. In the other experimental area, no infection was encountered in *Mansonioides annulifera* in the post-spraying period though 2·0 and 5·7 per cent infections were noted, respectively, in malathion and diazinon areas before spraying.

In the comparison area, *Mansonioides annulifera* showed infections in varying numbers throughout the observation period (Table IV).

TABLE IV.

Number of Mansonioides spp. *dissected before and after spray.*

Insecticidal treatment	Before treatment			After treatment			Remarks.
	Number dissected	Number positive	Percentage positive	Number dissected	Number positive	Percentage positive	
D.D.T. ..	40	9	22·5	11	1	9·0	From unsprayed catching station in the seventh week after spray.
Malathion ..	50	1	2·0	27	0	0·0	
Diazinon ..	35	2	5·7	11	0	0·0	
Comparison area (unsprayed)	58	8	13·8	164	9	5·4	

DISCUSSION.

Jaswant Singh, Krishnaswami *et al.* (1956) reported that D.D.T. sprayed at 200 mg./sq. ft. was effective in Shertallai Taluk for about eight weeks, but in the present studies the same dosage was observed to be effective for about five weeks. Total absence of mosquitoes during the two weeks, following the application of this insecticide, was not noted as reported by Jaswant Singh, Krishnaswami *et al.* (1956). There was only a marked reduction in the density (Table II).

Diazinon and malathion, each sprayed at 25 mg./sq. ft., do not seem to have any prolonged residual toxicity. Perhaps a higher dosage might show better results.

CONCLUSION.

Judging from the density of mosquitoes and their survival rates, D.D.T. sprayed at 200 mg./sq. ft. was effective for about five weeks and the organophosphorous compounds for about three weeks.

SUMMARY.

Field trials were undertaken in Shertallai Taluk, Kerala State, to test the efficacy of D.D.T., malathion and diazinon.

D.D.T. sprayed at 200 mg./sq. ft was effective for five weeks. Malathion and diazinon, each sprayed at 25 mg./sq. ft., were effective for three weeks.

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STUDIES ON PARTICLE SIZE DISTRIBUTION IN RELATION TO DISPERSIBILITY.

Part II. 75 per cent D.D.T., 50 per cent B.H.C. and 50 per cent dieldrin water dispersible powders.

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In a previous communication, Bami and Cheema (1957) have reported particle size distribution of D.D.T. and clay constituents of several 50 per cent D.D.T. water dispersible powders. It was observed that size of insecticide particles was major factor governing the stability of the ultimate aqueous suspension, the other important factors being fineness of clay, quality and quantity of wetting and dispersing agents used and *p*H of the final aqueous suspension. Other insecticide water dispersible powders have identical requirements and are also formulated in a more or less similar manner. It was, therefore, considered worthwhile to study the relationship of dispersibility with composition and particle size distribution of various constituents of 75 per cent D.D.T., 50 per cent B.H.C. and 50 per cent dieldrin water dispersible powders which are also being widely used in the malaria and filaria control programmes.

MATERIALS AND METHODS.

Two samples, each of 75 per cent D.D.T., 50 per cent B.H.C. and 50 per cent dieldrin water dispersible powder, were selected for the present study. D.D.T. and the dieldrin formulations were of foreign origin while the B.H.C. samples represented the indigenous production. Composition as well as physical and chemical characteristics of all the samples were determined according to World Health Organization Specifications (1956) and other methods already detailed (Bami and Cheema, 1957). It may be pointed out that samples with minimum of 50 per cent dispersibility, as required by specifications (Indian Standards Institute, 1955; World Health Organization, 1956), only were selected for the purpose.

Particle size distribution of clay and insecticide constituents of all the formulations, both before and after accelerated tropical storage, was determined by sedimentation method (Thomas, 1956) as adopted by Bami and Cheema (1957). In each case, the clay constituent was also separated and its particle size determined separately. Chemical estimation of D.D.T. and B.H.C. involved in above procedure, was accomplished through the hydrolysable chlorine method (Bami, 1957) while dieldrin was analysed by the total chlorine method suitably modified (World Health Organization, 1956; Bami, 1957). Densities of clay, D.D.T., B.H.C. and dieldrin were also determined for these studies.

RESULTS.

Results of analysis of 75 per cent D.D.T. (Samples *A* and *B*), 50 per cent B.H.C. (Samples *A* and *B*) and 50 per cent dieldrin (Samples *A* and *B*) water dispersible powders, as well as their physical and chemical characteristics, are given in Table I. It was observed that amount of wetting and dispersing agents (all water soluble constituents) were nearly the same in all the cases and the products were also below 74 microns particle size. Excepting 50 per cent B.H.C. powder (Sample '*A*') which was slightly alkaline, all other products were neutral

TABLE I.

Analysis report on 75 per cent D.D.T., 50 per cent B.H.C. and 50 per cent dieldrin water dispersible powders.

Property tested	75 PER CENT D.D.T. WATER DISPERSIBLE POWDER.		50 PER CENT B.H.C. WATER DISPERSIBLE POWDER.		50 PER CENT DIELDRIN WATER DISPERSIBLE POWDER.	
	Sample 'A'	Sample 'B'	Sample 'A'	Sample 'B'	Sample 'A'	Sample 'B'
1. *Composition percentage*						
(*a*) Insecticide ..	74·9	75·2	51·4	49·8	49·9	50·4
(*b*) Clay	14·9	14·5	38·6	40·2	40·0	39·6
(*c*) Dispersing agents etc.	10·2	10·3	10·0	10·0	10·1	10·0
2. *Physical properties:*						
(*a*) Dispersibility percentage ..	73·7	68·8	54·0	56·3	56·4	64·7
(*b*) Dispersibility after accelerated storage, percentage	60·1	54·1	52·6	54·8	54·8	58·4
(*c*) Percentage passing 200 mesh (74 microns) sieve.	100	100	100	100	100	100
(*d*) do. (after accelerated storage).	100	100	100	100	100	100
(*e*) Acidity or alkalinity	Neutral	Neutral	Alkalinity 0·33 per cent as Na_2CO_3	Neutral	Neutral	Neutral
(*f*) Density of clay	2·54	2·54	2·54	2·54	2·54	2·54
(*g*) Density of insecticide	1·45	1·45	1·88	1·88	1·7	1·7

in reaction. In the case of B.H.C. and dieldrin samples, fall in dispersibility, subsequent to accelerated storage treatment, was generally very negligible as compared to those of D.D.T. water dispersible powder samples (*A* and *B*). However, in no case the dispersibility values fell below 50 per cent limit as required.

Results of particle size analysis of the six samples have been plotted on logarithmic graphs (Graphs 1 to 6). In case of 75 per cent D.D.T. dispersible powder Sample '*A*' (Graph 1) and Sample '*B*' (Graph 2), the fineness of pure clay was identical in both cases (80 per cent of clay below 10 microns particle size). However, clay in the formulations in both cases was found to be slightly coarser, probably due to agglomeration with D.D.T. particles during grinding. However, tropical storage pretreatment seemed to have little influence on the particle size distribution of clay components. Particle size distribution of D.D.T., both before and after accelerated storage, however, indicated increase in particle size due to some agglomeration in both cases. In the case of Sample '*A*' (Graph 1), 67 to 72 per cent of the D.D.T., both before and after accelerated tropical storage, was below 10 microns particle size while in case of Sample '*B*' these values varied from 50 to 64 per cent (Graph 2).

GRAPH 1.

75 per cent D.D.T. water dispersible powder: Sample 'A'.

1. Pure clay.
2. D.D.T. in the formulation.
3. Clay " " "
4. D.D.T. after accelerated tropical storage.
5. Clay " " " "

Analysis of 50 per cent B.H.C. samples '*A*' and '*B*' (Graphs 3 and 4), indicated that pure clay in case of Sample '*B*' was slightly finer (87 per cent

GRAPH 2.

75 per cent D.D.T. water dispersible powder: Sample 'B'.

1. Pure clay.
2. D.D.T. in the formulation.
3. Clay " " "
4. D.D.T. after accelerated tropical storage.
5. Clay " " " "

below 10 microns). However, particle size determination of clay in both the formulations gave nearly identical results both before and after subjecting the materials to accelerated tropical storage. Particle size distribution of B.H.C. in these samples indicated that at least 55 per cent of the material was below 10 microns particle size. The increase in B.H.C. particle size, subsequent to accelerated storage treatment, was negligible, a fact which was also indirectly supported by dispersibility values (Table I).

Fineness of pure clay in case of both the 50 per cent dieldrin water dispersible powders (Graphs 5 and 6) was nearly the same (75 per cent below 10 microns size). However, in the formulation, clay was found to be slightly coarser in case of Sample '*A*' (Graph 6). About 60 per cent of dieldrin in both cases was found to be below 10 microns particle size. The effect of accelerated tropical storage on the particle size of both clay and dieldrin components was very slight as reflected in slight differences in the two dispersibility values (Table 1).

DISCUSSION.

Considering that dispersibility, after accelerated tropical storage in all these cases, was above 50 per cent as required by the specifications (World Health

Organization, 1956; Indian Standards Institute, 1955), there are certain common characteristics of these insecticide formulations which are largely supported by the particle size distribution data. Fineness of pure clay in almost all cases is very high (nearly 75 to 85 per cent below 10 microns size), although these clays in formulations appeared to be coarser due to slight degree of agglomeration with insecticide particles. Insecticides were also ground to nearly identical fineness and above 50 per cent of the insecticide content was generally below 10 microns particle size. The data mentioned above supported the previous observations (Bami and Cheema, 1957) that water dispersible powders, meeting the specifications for dispersibility, have at least 50 per cent of their insecticide content ground to below 10 microns particle size coupled with very fine clay, adequate amount of dispersing and other auxilliary agents (about 10 per cent in the present cases) and nearly neutral *p*H.

GRAPH 3.

50 *per cent B.H.C. water dispersible powder: Sample 'A'.*

1. Pure clay.
2. B.H.C. in the formulation.
3. Clay " " "
4. B.H.C. after accelerated tropical storage.
5. Clay " " " "

Effect of accelerated tropical storage pre-treatment, on the particle size of clay and insecticide components of the formulations, was negligible excepting in case of D.D.T. in sample *A* and *B* (Graph 1 and 2). Slightly higher tendency to agglomerate in case of D.D.T. particles could be attributed to low setting point of the D.D.T. itself and this increase in particle size also led to lowering of actual

dispersibility values in case of D.D.T. formulations (Table I). Fine particle size of clay and insecticide components greatly retarded such agglomeration while adequate amount of dispersing agents also help ultimately to disperse the particles effectively in the aqueous phase.

GRAPH 4.

50 per cent B.H.C. water dispersible powder: Sample 'B'

1. Pure clay.
2. B.H.C. in the formulation.
3. Clay " " "
4. B.H.C. after accelerated tropical storage.
5. Clay " " " "

Previous results with 50 per cent D.D.T. dispersible powders (Bami and Cheema, 1957) and the present findings on 75 per cent D.D.T., 50 per cent B.H.C. and 50 per cent dieldrin dispersible powders have made it clear that in case of products meeting the specifications (World Health Organization, 1956; Indian Standards Institute, 1955) at least 50 and 70 per cent of their insecticide contents were likely to be below 10 and 20 microns particle size, respectively, apart from other requirements. Considering the importance of fine insecticide particles as regards initial and residual biological efficacy as well as high possibility of sorption of such fine particles into mud surfaces (Hadaway and Barlow, 1952*a*:1952*b*; Sharma *et al.*, 1957), it was likely that a large proportion of the active insecticide in quality powders could be lost through sorption. Thus the advantage of higher suspension stability, achieved mainly through extra fine grinding of the insecticide, might be largely counter-balanced by excessive loss of the active toxicant through penetration into porous surfaces.

GRAPH 5.

50 per cent Dieldrin water dispersible powder: Sample 'A'.

1. Pure clay.
2. Dieldrin in the formulation.
3. Clay " " "
4. Dieldrin after accelerated tropical storage.
5. Clay " " " "

SUMMARY.

Two samples, each of 75 per cent D.D.T., 50 per cent B.H.C. and 50 per cent dieldrin water dispersible powder, were completely analysed as regards their composition and were also found to be up to World Health Organization (1956) and Indian Standards Institute (1955) specifications as regards dispersibility, sieve test, *p*H, etc. Particle size distribution of pure clay, as well as insecticide and clay components of all the formulations, was determined by sedimentation method (Bami and Cheema, 1957). Clay was very fine in each case while at least 50 per cent of the insecticide was below 10 microns particle size. Both clay and insecticide particles in these formulations were generally very little prone to agglomeration when subjected to accelerated tropical storage treatment. The amount of dispersing agents in each case was nearly 10 per cent while the final suspensions were neutral or nearly neutral. All these factors, coupled mainly with fine particle size of the constituents, contributed towards the high dispersion stability achieved in case of these formulations both prior and subsequent to accelerated tropical storage treatment.

GRAPH 6.

50 *per cent Dieldrin water dispersible powder: Sample 'B'.*

1. Pure clay.
2. Dieldrin in the formulation.
3. Clay " " "
4. Dieldrin after accelerated tropical storage.
5. Clay " " " "

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SOME GENERAL PHARMACOLOGICAL AND ACUTE TOXICITY STUDIES ON 1-*p*-BROMOPHENYL-2 : 4-DIAMINO-1 : 6-DIHYDRO-6 : 6-DIMETHYL-1 : 3 : 5-TRIAZINE HYDROCHLORIDE (M.I.S.11*).

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AMONGST several analogues of proguanil, antimalarial activity of bromoguanide (N^1-p-bromophenyl-N^5-isopropyl-biguanide) was almost comparable to that of proguanil when tested against avain and simian malarias (Bami and Guha, 1948; Curd *et al.*, 1948; Jaswant Singh *et al.*, 1950). However, this drug was inferior to proguanil as regards acute toxicity and showed no advantage over proguanil in clinical trials (Chaudhuri, 1952). Studies on degradation of proguanil *in vivo* in rabbits and human-beings, led to isolation of metabolite, viz., 1-p-chlorophenyl-2:4-diamino-1:6-dihydro-6:6-dimethyl-1:3:5-triazine, which was even more active than the parent compound (Carrington *et al.*, 1951; Crowther and Levi, 1953). Similar studies with bromoguanide in monkeys, led to the isolation of 1-p-bromophenyl-2:4-diamino-1:6-dihydro-6:6-dimethyl-1:3:5-triazine (M.I.S. 11) which was even more active than proguanil metabolite (Bami, 1953; Jaswant Singh *et al.*, 1954). Further chemical studies led to the synthesis of M.I.S. 11 (Carrington *et al.*, 1954; Bami, 1955) and detailed evaluation of its activity and toxicity.

1-p-bromophenyl-2:4-diamino-1:6-dihydro-6:6-dimethyl-1:3:5-triazine hydrochloride (M.I.S. 11) had a quinine equivalent of 512 and 17 against *P. gallinaceum* (Bami, 1955; Jaswant Singh *et al.*, 1954) and *P. cynomolgi* (Ray *et al.*, 1954), respectively. It was clinically tried against *P. falciparum* and *P. vivax* infections and the findings were comparable to those observed in case of established drugs like chloro-

* M.I.S represents Malaria Institute Survey number for the antimalarial screened.

quin and amodiaquin (Ray *et al.*, 1956). High antimalarial activity and low toxicity, associated with M.I.S. 11, led to the investigation of some of its pharmacological properties to evaluate its possible therapeutic usefulness. Consequently, results of some acute toxicity studies in rats and mice as well as some pharmacodynamic properties of M.I.S. 11 are reported in this paper.

Acute toxicity.—Acute toxicity studies were carried out on albino rats and white mice. The albino rats, originally obtained from the Haffkine Institute, Bombay, were bred in the laboratory and weighed 120 - 150 gm. White mice selected weighed 18 - 22 gm. The drug was easily soluble in water and had a base content of 84.5 per cent. The route of infection, dose in terms of base administered and percentage mortality are shown in Table I. The LD 50 of the drug by various routes, was calculated by plotting the log dose against the probit of the mortality percentage and were found to be as follows:—

(*a*) Rats (Intraperitoneal) ... 130 mg./kg. body weight.
(*b*) Rats (Oral) ... ... 1,500 mg./kg. body weight.
(*c*) Mice (Intravenous) ... 116·9 mg./kg. body weight.

TABLE I.

Acute toxicity studies on 1-*p-bromophenyl*-2:4-*diamino*-1:6-*dihydro*-6:6-*dimethyl*-1:3:5-*triazine* (*M.I.S.* 11).

Species	Route of administration	Dose in mg. base/kg. body weight	Percentage mortality (10 animals used in each case)
Rats	Intraperitoneal	150	100
	"	130	50
	"	100	20
	"	80	0
	Oral	2000	100
	"	1500	50
	"	1200	20
	"	1000	10
	"	800	0
Mice	Intravenous	200	100
	"	150	80
	"	120	60
	"	110	40
	"	100	20
	"	80	0

By oral route, convulsive seizures and death was observed within a few minutes at 2 g./kg. b.w. dosage. At lower doses, the convulsions were followed by coma and death in 3 to 4 hours' time. The surviving rats exhibited no toxic symptoms. Intense scratching of the face and the body was observed in a few animals. The reactions after intraperitoneal administration were slightly different. Irritability as shown by sudden jumpings on slightest movement, was followed by toxic convulsive seizures resulting in death. Prior to the seizures, scratching of the face was observed in almost all animals.

Acute toxicity of the drug (LD 50) to mice, when given intravenously, was found to be 116·9 mg/kg. and the hyper-excitability and convulsive seizures observed were similar in nature to those seen in rats.

Cardio-vascular system.—In dogs, anæsthetized by seconal sodium (30 mg./kg. intraperitoneal), intravenous injections of M.I.S. 11 caused a lowering of the blood pressure. In lower doses, 0·5 mg./kg. and 1·0 mg./kg., an initial transient rise in pressure was observed prior to the lowering (Fig. 1). At higher doses, immediate fall in the carotid blood pressure was seen and the recovery was slow and incomplete. The effect on carotid occlusion was not in any way modified but the drug exhibited slight inhibition of adrenaline activity (Fig. 2), indicating that the *locus* of action of the drug was probably more peripheral than central in nature.

Respiration.—During the hypotensive phase, the respiration rate was found to be accelerated but its depth diminished. This was probably a reflex response to depressor action since the respiration returned to almost the original level with the blood pressure.

Effect on mammalian heart in situ.—The cardiovascular effect was further elucidated by the study of the drug action on the musculature of the mammalian heart *in situ* in seconal sodium anæsthetized dogs under artificial respiration, by intravenous administration of different doses of the drug prior and subsequent to atropinisation (Figs. 3, 4 and 5). At lower doses (0·5 mg. to 2·0 mg./kg.), increased amplitude, associated with an increased rate, was observed in both the auricles and verticles. At higher dosages (5 to 25 mg./kg.), the relaxation was incomplete. Further increase in dose (25 to 50 mg./kg.) caused slowing and diminished contractile action of the heart. Trend of results was similar even after atropinisation (Fig. 5) and vagotomy. The drug probably has a direct stimulatory effect on the musculature of the heart at low dilutions and an inhibitory effect at higher concentrations.

Isolated smooth muscle organs.—The drug in concentrations of 1/1,50,000 inhibited the spasm of the guinea-pig ileum induced by acetylcholine (Fig. 6) and barium (Fig. 7) by about 80 per cent, while the antihistaminic action was markedly less (Fig. 8). In a concentration of 1/30,000 the inhibition was complete against all the three spasmogens and a slight relaxation of the musculature was also noticed. The state of contraction, induced by these spasmogens, was also relieved by the drug, the effect being significant at a higher dilution on the acetylcholine and barium spasms.

Effect of M.I.S. 11 *on neuromuscular transmission: (Rat phrenic nerve diaphragm).*—The neuromuscular action of the drug was investigated by the isolated

rat diaphragm technique (Bulbring, 1946) using a 80 ml. bath containing tyrode solution (with double the amount of glucose) maintained at 36°±0·5° C. with continuous oxygenation. The stimulii were applied at the rate of 9 per minute.

The effect of nerve stimulation on the contractile force of the diaphragm is seen in Fig. 9. A decrease in the height of contraction, and a quick recovery after removal of the drug by washing, was noticed at 50 r/ml. Reduced contractility becomes more marked with increasing concentration of the drug 100 r/ml. and 200 r/ml., but the recovery after washing was still fairly quick.

Direct stimulation of the diaphragm, under similar conditions, was studied to note whether the depressant action is due to a neuromuscular block or a direct effect on the musculature (Fig. 10). Absence of any depressant action, even at concentrations up to 500 r/ml., indicated the *locus* of action of the drug to be one of the neuromuscular junction.

DISCUSSION.

Studies on acute toxicity of M.I.S. 11 in rats revealed that oral LD 50 dose was nearly ten times that of intraperitoneal. The results mentioned above were more akin to those obtained with quinine and mepacrine (Chen and Anderson, 1947) and differed from proguanil in the respect that oral LD 50 dose for proguanil was only three times that of intraperitoneal (Butler *et al.*, 1947). Toxic symptoms like hyper-excitability and convulsive seizures were common to both rats and mice. Similar symptoms were observed in case of chloroquin and other drugs of the 4-amino-quinoline series.

Dihydrotriazine metabolite of bromoguanide (M.I.S. 11) caused lowering of the blood pressure and the recovery was slow and incomplete. The respiration rate was increased probably as a reflex response to the depressor action. Studies on mammalian heart *in situ* also indicated an inhibitory effect on the musculature of the heart, resulting in fall of blood pressure. However, slight antagonisation of adrenaline reaction by M.I.S. 11, indicated that the locus of drug action was only peripheral in nature. The drug not only inhibits the action of spasmogens like acetylcholine and barium but also neutralized the contractions induced by histamine. This could mean a non-specific type of spasmolytic activity. Studies on the effect of M.I.S. 11 on neuromuscular transmission, revealed the locus of action to be neuromuscular junction.

Previous studies on antimalarial activity and toxicity of proguanil, its active dihydrotriazine metabolite and M.I.S. 11 (Ray *et al.*, 1954:1956; Bami, 1953) had indicated that their performances were qualitatively similar though differing quantitatively. Comparison between the above pharmacodynamic properties of M.I.S. 11 with that of the parent drug, proguanil, also confirmed a similarity in mode of action which only differed in magnitude (Sirsi, 1957). M.I.S. 11 was thus found to have no marked advantage in the above mentioned respects over the established antimalarials like proguanil, chloroquin, etc.

FIG. 1. The effect of M.I.S. 11 on blood pressure (carotid artery) and respiration.
Dog: Female, 3·2 kg. Sodium-amytal anaesthesia (30 mg./kg. intraperitoneal).
M.I.S. 11:
Ad. Adrenaline tartrate.
The quantities of drug injected are shown in the kymograph.

FIG. 2. Cardio vascular action of M.I.S. 11.
C.O.: Carotid occlusion. Other particulars same as in Fig. 1.

FIG. 3. Effect of M.I.S. 11 on mammalian heart *in situ* (Myocardiogram). *Dog: Male, 5·9 kg. Sesonal sodium anaesthesia (30 mg./kg. intraperitoneal). Figures in brackets indicate the rate per minute.*

FIG. 4. Myocardiogram.

FIG. 5. *Dog: Female, 5·9 kg. Intr. Atropine sulphate. Interval between the recordings in the figures - 15 minutes.*

FIG. 6. Isolated organ studies. *Schultz-Dale bath*: 15 *ml*. *Perfusion fluid*: *Tyrode*. *Temp.* 35°±0.5°C. *Continuous oxygenation.*
At the arrows, drugs were injected into the bath and allowed to act for 20 seconds and then washed.
Effect of M.I.S. II on the contractions induced by acetylcholine:
Ach: acetylcholine chloride.

FIG. 7. Effect of M.I.S. II on spasmogenic action of barium.
Ba: barium chloride. *Other particulars same as in Fig. 6.*

FIG. 8. Effect of M.I.S. II on histamine-induced contractions.
H: histamine hydrochloride.

Fig. 9. Contractions of the isolated rat diaphragm in response to stimulation of the phrenic nerve 4-min.
Bath volume 100 ml. continuous oxygenation.
↑ *indicates addition of the drug to the bath.*
↓ *indicates wash.*

Fig. 10. Contractions of the isolated rat diaphragm in response to direct stimulation of the musculature. *Details same as in Fig. 9.*

SUMMARY.

1-p-bromophenyl-2:4-diamino-1:6-dihydro-6:6-dimethyl-1:3:5-triazine hydrochloride (an active metabolite of p-bromo analogue of proguanil) has an LD 50 of 1,500 mg./kg. body weight for rats by the oral route and 130 mg./kg. by the intraperitoneal route. Intravenous toxicity in mice (LD 50) is found to be 116·9 mg./kg. body weight.

The drug lowers the blood pressure with an acceleration of rate and diminution of amplitude in respiration. The fall in blood pressure is probably due to a direct effect on the mycocardium.

Though more active against acetylcholine and barium induced contractions, the spasmolytic activity exhibited by the drug is likely to be non-specific in nature since the contractions induced by histamine are also neutralized. It exhibits neuromuscular blocking action of a low degree.

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A RAPID STAINING TECHNIQUE FOR MICROFILARIA.

Part I. Sheathless microfilaria.

BY

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[November 29, 1957.]

SEVERAL techniques have been described for staining of microfilaria in blood. In all cases, the process includes dehæmoglobination with water, fixation in alcohol after drying and staining with various preparations like Mayer's hæmalum, Delafield's hæmatoxylin or Romanovsky stains like Leishman and Giemsa (Blacklock and Southwell, 1935; Belding, 1952; Chatterjee, 1952). Other stains, like J.S.B. (Jaswant Singh and Bhattacharji, 1944) which is used extensively against blood protozoa, have also been found to be quite useful for staining of microfilaria (Raghavan and Krishnan, 1949; Jaswant Singh *et al.*, 1953). However, most of these appear to be time-consuming or as in some, like the technique adopted with J.S.B. (Solution I), though quite useful for routine staining programme the structures are not so well defined.

In the present communication, an attempt has been made to modify and improve the rapid staining procedure using both J.S.B. I and II solutions. Other modifications made are in the technique of dehæmoglobinisation and also in the fixative.

MATERIALS.

Parasite.—Microfilariæ of *Dirofilaria repens* and *D. immitis* were utilised for the studies. These were obtained from dogs harbouring natural infection and maintained in the laboratories.

Fixative.—The fixative used comprised a freshly prepared mixture of nine parts of 70 per cent ethyl alcohol, 0·5 part of formaldehyde and 0·5 part normal hydrochloric acid as described by Narayan Das (1958).

Stain.—Both J.S.B. I and II solutions (Jaswant Singh and Bhattacharji, 1944) were used for staining.

TECHNIQUE.

Three fresh drops of blood were taken on a clean slide and spread over an area approximately 1¾ inch long and ¾ inch wide, and dried in air. The smear was then dehæmoglobinised in a freshly prepared 20 per cent ethyl alcohol instead of distilled water. Complete dehaemoglobinisation was indicated by the disappearance of reddish colour, and this was observed in fresh specimens within two minutes. The time was somewhat longer for old smears depending on age.

Dehæmoglobinisation was followed immediately by fixation of the smear with the new fixative for at least two minutes. The smear was then washed in running tap water for about one minute to remove the excess of fixative.

The slide was then dipped for 30 seconds in J.S.B. II, washed in buffer water (pH 6·4 to 6·8) and then immersed in J.S.B. I for about 40 seconds. After this, the slide was again washed in buffer water. In this respect, the technique was somewhat similar to that adopted for staining malaria parasites as described by Jaswant Singh *et al.* (1953).

In case of over staining, better results were obtained by dipping the slide for a few seconds in a saturated solution of potassium dihydrogen phosphate. Subsequently it was found necessary to wash the smear again in buffer water.

The smear was then dried in air and examined.

For comparison, blood smears were stained with J.S.B. I and II similar to that described above but the fixative used was 95 per cent alcohol or methyl alcohol. Smear was also stained in the same way as indicated by Raghvan and Krishnan (1949).

RESULTS.

Blood smears, treated according to the technique described in the present paper, showed the general outline and the inner structure of the parasite somewhat more clearly than in those preparations used for comparison purposes. The body wall of the microfilaria took up a lighter colour in contrast to the dark blue coloration of the nuclear granules. The disposition of the nuclear column, down to the tip of the tail, was well demonstrable. The column appeared to be quite discrete and much clearer than observed in the comparison series.

Further the various gaps in the nuclear column representing the cephalic space, nerve ring, excretory and anal pores, which serve as land marks for identification, were clearly demonstrable. Repeated trials indicated that the staining characteristics observed under the present technique were fairly consistent and every time brought out distinctly the important anatomical land marks.

DISCUSSION.

When the standard methods which are described in text books are followed meticulously, the results are invariably satisfactory. But most of these are time-consuming and present some degree of problem during mass staining as is often

required in the field. In view of this, Raghvan and Krishnan (1949), while carrying out filaria survey, evolved the staining technique with J.S.B. and the method is fairly rapid. However, quite often the nuclear columns appear to be somewhat indistinguishable and some of the gaps in them cannot be clearly defined in many instances. This could very well be due to the particular type of fixative used and possibly due to the absence of counter-staining with J.S.B. II.

As to the use of alcohol as a fixative, the two grades of alcohol, absolute alcohol and weak alcohol, are commonly used. As a fixing agent, the former kills and hardens the structures so rapidly that they have hardly time to get deformed in the process. Very weak alcohol, on the other hand, possesses a sufficiently energetic coagulating action but yet contains enough water to have a feeble dehydrating action. The intermediate grades of alcohol do not fulfil these conditions and, therefore, should not be employed alone as a fixative. But according to Gatenby and Beams (1950) they may be very useful in combination with other fixing agents by enhancing their penetrating power. It may be mentioned in this connection that the fixative which was used during the present studies, consists of 70 per cent alcohol in mixture with hydrochloric acid which may have enhanced the fixative property in combination with formaldehyde. Formaldehyde in itself has a fast penetrating power and has also the convenient compatibility with the most varied stains. Further, it may be mentioned that 20 per cent alcohol, which was used for dehæmoglobinisation, also acted at the same time as a mild fixative, thus ensuring better attachment of the microfilaria to the slides than what would normally happen with ordinary water.

It is believed that these techniques may have helped considerably in the staining process. It is also likely that the use of the rapid staining technique with J.S.B., similar to that adopted for malaria parasites, may be responsible for the appearance of the nucleus in discrete form particularly in view of the use of the counter-stain (J.S.B. II). This method of staining has also cut short the staining time considerably similar to that adopted in case of plasmodia.

SUMMARY.

A technique has been described for rapid staining of unsheathed microfilaria.

The process consists of dehæmoglobinisation with 20 per cent alcohol, use of a new fixative and staining with J.S.B. solutions I and II. The staining process is almost similar to that used for malaria parasites.

The results have been found to be consistently satisfactory in that not only the nuclear columns appear discrete but there is also differentiation between the different parts of the parasites.

Similar studies are in progress with sheathed microfilaria, particularly *W. bancrofti* and *W. malayi*, and if the observations show equally satisfactory results the technique would be of considerable use.

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FURTHER STUDIES ON PHYSICO-CHEMICAL FACTORS IN RELATION TO *ANOPHELES SUNDAICUS* BREEDING.

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[November 25, 1957.]

INTRODUCTION.

PHYSICO-CHEMICAL constituents of water, such as sodium chloride, carbonates, pH, albuminoid and free ammonia associated with the breeding of *Anopheles sundaicus*, one of the most virulent transmitters of malaria in the southern tidal zones of Bengal, have been discussed by the author in some previous communications (Sen, 1938: 1957*a*: 1957*b*). The importance of a few more factors such as absorbed oxygen of the water, alkalinity and nitrates in relation to the *sundaicus* breeding is discussed now.

METHODS.

The water from the sites where *A. sundaicus* had been known to have bred at one time or another was collected at frequent intervals in stoppered bottles during morning hours and brought to the laboratory direct. Along with this, a collection of the larvæ, prevailing in the breeding places at the time, was also made in the usual way for examination in the laboratory the same day. In this way, a set of observations was available when a particular sample was known to be from places with actual breeding of the species and also when the same place was not having the species either immediately before or after the positive finding.

The methods followed in estimating the different factors reported in this paper, are mentioned in the appropriate places and have been taken from Thresh, Beale and Suckling (1933).

RESULT OF OBSERVATIONS.

Absorbed oxygen.—Altogether 41 analyses of this factor, from samples of water showing *sundaicus* breeding, were made. Out of these, only nine had data corresponding to the three eventualities. The result obtained is given in Table I.

TABLE I.

Absorbed oxygen content in A. sundaicus *breeding waters (p.p.m.).*

Before breeding	During breeding	After breeding
6·32	6·73	6·75
3·58	4·7	4·68
4·25	4·56	6·66
5·79	5·94	8·5
23·07	8·72	10·86
8·55	10·85	18·08
4·13	4·55	4·25
5·66	7·83	7·21
8·74	8·36	12·9

The data available show that absorption of oxygen regularly increased on the days when actual breeding was detected except in one or two instances, and as with chloride content (Sen, 1957*b*), this factor was not always reduced after the cessation of the breeding. In fact, in many instances the content increased even further.

The estimation was done by the Winkler's method.

Alkalinity.—In all, 34 breeding places of *sundaicus* were examined for this factor and quite a large number of observations made. Out of the observations, only nine readings satisfied the three eventualities for the purpose of comparison with the actual breeding conditions of the species (Table II).

TABLE II.

Alkalinity in A. sundaicus *breeding waters (p.p.m.).*

Before breeding	During breeding	After breeding
140	150	190
130	130	140
170	160	220
380	310	150
410	460	510
150	150	120
200	250	160
370	170	170
360	140	200

This factor was tested by titrating against N/50 H_2SO_4 using methyl orange as indicator.

The data obtained varied on different occasions and were not always constant even if the samples belonged to the same breeding places. The rise or fall of this factor had no correlation with the breeding of *sundaicus*.

Nitrites and Nitrates.—Both nitrite and nitrate contents of the breeding places, which were subjected to the analysis of the other factors dealt with in this paper, had been recorded and the result is shown in Table III.

TABLE III.

Nitrite and nitrate contents in A. sundaicus *breeding waters* (*p.p.m.*).

Before breeding		During breeding		After breeding	
Nitrite	Nitrate	Nitrite	Nitrate	Nitrite	Nitrate
Nil	Very minute trace	Nil	Nil	Nil	Nil
,,	Very minute trace	,,	Very minute trace	Nil	Very minute trace
,,	Nil	,,	Very minute trace	2·0	Present
,,	,,	,,	Very minute trace	Nil	Very minute trace
,,	Very minute trace	,,	Nil	,,	Very minute trace
,,	Nil	,,	Trace	,,	Nil
,,	,,	,,	Nil	,,	,,
,,	Very minute trace	Minute trace	Trace	,,	Very minute trace
,,	Very minute trace	0·02	Nil	,,	Very minute trace

The data presented in Table III show that the samples examined were remarkably free from nitrites, excepting on one occasion each, both during breeding of *sundaicus* and after the breeding. It appears, therefore, that this factor has no relationship with the mosquito breeding, neither do the nitrates appear an absolute necessity and the species had been found to breed in presence of minute trace of the content or in its absence.

Nitrite was estimated colorimetrically by matching against standard colours with Ilosvay's solution, while nitrate by reduction with zinc-copper couple.

Reduction of the various chemical factors with time.—In this area, the average salinity recorded was 164·3 (Sen, 1938) and the study during the last few years since then (Sen, 1957*b*), however, shows that this factor had been gradually coming down as time passed (Table IV).

The figures indicate that free ammonia also had an ultimate reduction but this fluctuated very widely in the different years, while the fall in albuminoid ammonia content was slow and somewhat steady. There was no apparent correlation between the two factors. These two factors have been discussed in more details elsewhere (Sen, 1957*b*).

TABLE IV.

Gradual decline of salinity and ammonia contents in A. sundaicus *breeding waters around Calcutta (p.p.m.).*

	1936	1937	1938–1942
Sod. chloride ..	1713	1185	1005
Free NH_3 ..	0·14	0·26	0·01
Alb. NH_3 ..	0·59	0·44	0·28

The other factors of absorbed oxygen, alkalinity and pH did not, however, show appreciable fluctuation and remained almost steady during the period.

SUMMARY.

1. The absorption of oxygen in breeding waters of *Anopheles sundaicus* regularly increased on the days of actual breeding. But there was no corresponding decrease of this factor after the breeding stopped.

2. The data for alkalinity did not show any correlation with the breeding of *A. sundaicus*, and there was considerable variation of this factor on different occasions.

3. The *A. sundaicus* breeding water was remarkably free from nitrites while nitrates were usually present, although absence of the latter was not detrimental to its breeding.

4. The average salinity of the *A. sundaicus* breeding water in the vicinity of Calcutta has been decreasing steadily during recent past. The albuminoid ammonia was more steady in this respect and decreased rather slowly.

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REVIEWS.

Vectors of Malaria in India, 1957, 1st Edition. Published by the National Society of India for Malaria and Other Mosquito-Borne Diseases, 22-Alipore Road, Delhi, 177 pages. Price Rs. 3·00 plus postage.

Based on the reviews of work carried on *A. annularis*, *A. philippinensis*, *A. stephensi*, *A. sundaicus*, *A. fluviatilis*, *A. minimus*, *A. varuna*, *A. jeyporiensis* var. *candidiensis*, *A. leucosphyrus*, and *A. culicifacies*, the ten Indian malaria vectors, published in different volumes and numbers of the *Bulletin of the National Society of India for Malaria and other Mosquito-borne Diseases*, this publication brings together for the first time a comprehensive account of the written word about the malaria transmitting anophelines of India. In addition, there are two articles, 'Pictorial account of malaria vectors in India' and 'Dissection record of wild-caught anopheline mosquitoes other than the reputed vectors of malaria' which have not been published before and have been included in this volume.

6″×5″ size figures of ten vectors are given for easy identification of the species and each article includes a Map showing the distribution of the particular vector in India. Besides, there is a list of references appended separately to every review.

This book is welcome and will serve a useful purpose as a ready reference for workers in malaria on systematics, bionomics, and control of the vector species in India. In addition, the dissection record of all the thirty-nine Indian anophelines will serve as a guide while undertaking future dissections in any area in India. The publishers are to be congratulated for bringing out this volume on the eve of launching the malaria eradication programme in this country.

B. Ananthaswamy Rao.

***Expert Committee on Malaria, Sixth Report. World Health Organization: Technical Report Series,** 1957, No. 123, 84 pages. Price £-/3/6 d. $0·60. Sw. fr. 2. Also available in French and Spanish.

The *Sixth Report of the World Health Organization Expert Committee on Malaria* is devoted to the technical and administrative principles, as well as the practical aspects of malaria eradication. It may be regarded as a guide for drawing up programmes and selecting methods, intended for the use of the malaria control services of the continually increasing number of countries which have adopted the eradication policy advocated by the World Health Assembly.

* Due acknowledgment is made to the Review by the World Health Organization, which has been reproduced here.

After defining malaria eradication, which should not be confused with eradication of the disease vector, and showing that it differs radically in many respects from mere malaria control, the Committee proceeds in this report to an analysis of the theory of eradication, dealing, in particular, with the organizational problems raised by the detection of residual foci, the rôle of immunity in the development of epidemics, the epidemiological factors which influence the re-introduction of the disease or its renewal from small origins (origin of the epidemic, "multiplication rate" of cases and potential severity of the epidemic), the criteria of malaria eradication, and the requirements to be met by a malaria control programme in order to be recognized as a step towards an eradication campaign.

With current techniques and the considerable funds made available to governments by international organizations, the cost of an eradication programme is generally not beyond the financial possibilities of most countries. It seems, therefore, that efficient management at all levels is the factor which requires the greatest emphasis. Consequently, the report devotes considerable space to the various administrative aspects—previously somewhat neglected—encountered in any eradication programme, e.g. legislation, organization of national malaria services, budget and management of finances, functions of personnel of all grades, training of professional and non-professional groups, consolidation, maintenance.

The same desire to provide a detailed guide, based both on experience and on the most recent advances, is shown in the discussion of the other phases and aspects of the programme, e.g. the spraying campaign, prevention of breeding, drug treatment, anopheline resistance to insecticides and measures to be taken when it appears, survey techniques—in particular measurement of transmission—and surveillance processes. A special section deals with problems which may in theory interfere with the success of malaria eradication, namely those arising from the behaviour of the anopheline vectors, those connected with population movements and those related to the living conditions of the people.

Finally, malaria eradication is considered in relation to other public health activities and from the view point of international co-operation.

Annexes to the report include a form for collection of information on personnel employed in antimalaria campaigns, a guide for the presentation of malaria eradication programmes to governments and international organizations, and a form for data to be included in reporting the results and costs of antimalaria programmes.

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NOTES ON THE SEASONAL VARIATIONS IN THE DENSITY OF *CULEX FATIGANS* AND FILARIAL INFECTIONS IN THEM, IN MANGALORE TOWN, MADRAS STATE.

BY

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[September 27, 1957.]

INTRODUCTION.

THIS paper reports the results of observations on the seasonal variations in the density of *Culex fatigans* and filarial infections noted in them for over two years in Mangalore Town, Madras State, during 1954-1955 and part of 1956. The studies have been carried out by a section of the staff of the Municipal Antifilariasis Scheme. The control of filariasis consisted of antilarval measures with larvicides, supplemented by minor engineering methods like filling, canalising and draining.

Mangalore Town has been included for filariasis control under the National Filaria Control Programme since 1955-1956.

PREVIOUS FILARIA SURVEYS.

Periodical check-up surveys to assess the efficacy of the antilarval measures show that during 1950-1955, the disease rate ranged from 2·2 to 4·7 while the infection rate varied between 3·8 and 9·9 per cent (Reports of Regional Malariologist, unpublished).

A comprehensive filaria survey was conducted during March-April, 1954 (Krishnaswami, 1954) covering all the municipal wards and 6·3 per cent of the total population. This survey revealed filarial disease rates varying from 4·7 to 17 per cent and filarial infection rates from 6·7 to 21·0 per cent in the 25 municipal wards. The filarial endemicity rates in the different wards varied between 13·4

and 37·5 per cent while the gross endemicity rate for the whole town was 24·2 per cent. No ward of the town was free from filarial infection or disease.

TOPOGRAPHY.

Mangalore, a coastal town on the West Coast is the Headquarters of South Kanara District of the former Madras State. It has a population of 1,19,000 over an area of 8·15 square miles and aggregated more towards the coast. The principal languages spoken are Kanarese, Konkani and Tulu. Chief occupations are minor industries like manufacture of tiles and potteries, beedies, cashew-nut industry, coffee curing and agriculture.

The town is bounded on the west by the Ullal River and the Arabian Sea while on the east are the hilly terrain contiguous with the western ghats. The town is uneven with a number of elevations and depressions, giving it a picturesque appearance. The lie of the land in general is from east to west, with very few level pieces of land. Because of the numerous elevations and depressions, there are a number of valley lines draining the town and finally entering the sea.

These valley lines carry storm water during monsoon months and at other times function as drains and receive sullage. The numerous depressions within the built-up areas of the town, extensive and low lying as they are, have been converted into paddy fields. Thus the town in general is studded with elevated areas where houses are put up with depressions in between where paddy is grown and with valley lines which drain the areas and feed the fields. Towards the coast, these natural water courses carrying the town sewage, broaden and flatten out and finally enter the sea.

WATER SUPPLY, DRAINAGE AND HOUSING.

The soil is laterite throughout the town and semipervious. Subsoil water level is high near the coastal areas with active springs during the monsoon and immediate post-monsoon months. In other areas, it is fairly low. A system of piped water supply is under execution but people now entirely depend on wells for drinking water. Almost every house has a well, with good potable water. During summer, however, in certain areas, acute scarcity of drinking water is felt.

There is no regular system of drains. As mentioned before, the several natural valley lines which act as storm water courses, during monsoon months, also receive sullage. These enter the sea in five or six different places. There are a few built-up drains in certain areas, but as they are constructed of crude laterite blocks with poor gradient, flow in these drains is either nil or sluggish during summer months. There is a comprehensive drainage scheme for the town under contemplation.

Houses are mostly isolated and discrete, except those close to the sea coast, situated in separate compounds on the pattern of Malabar and West Coast area in general. Houses are constructed of laterite blocks and plastered and roofed with ' Mangalore tiles '. Huts are conspicuously absent.

METEOROLOGICAL CONDITIONS.

The town has an average annual rainfall of 140 inches, contributed by the south-west and the north-east monsoons, the former being responsible for the bulk of the rainfall. It is one continuous rainy season, generally from the last week of May till the end of October. The south-west monsoon becomes definitely set in either towards the end of May or quite early in June. The months of June and July bring in torrential rains. The north-east monsoon becomes continuous with the south-west and rains cease after October. The period November to May is free from rains.

TABLE I.

Rain fall in inches—Mangalore Town.

Month	1949	1950	1951	1952	1953	1954	1955	1956
Jan. ..	0·00	0·00	0·00	0·00	0·00	0·33	0·00	0·00
Feb. ..	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00
Mar. ..	0·00	0·00	0·00	0·00	0·00	0·20	0·03	0·00
Apr. ..	0·00	0·20	2·04	2·09	0·62	4·14	0·82	1·69
May ..	23·15	4·41	3·89	4·40	0·50	5·71	14·76	26·24
Jun. ..	44·99	43·63	43·48	42·51	17·35	46·16	49·20	
Jul. ..	50·69	57·01	35·98	27·18	66·05	46·29	26·42	..
Aug. ..	20·82	12·34	14·72	20·34	14·11	26·65	25·62	..
Sep. ..	16·93	23·07	10·88	1·48	5·91	12·97	20·11	..
Oct. ..	2·94	6·63	5·45	12·16	9·28	5·23	11·46	..
Nov. ..	0·19	2·84	3·31	0·00	0·06	0·18	1·77	..
Dec. ..	0·00	0·00	0·00	0·70	0·00	0·28	0·00	..
Total ..	159·71	149·13	119·75	110·86	113·88	148·14	150·19	..

TEMPERATURE AND HUMIDITY.

The climate of the area is not subject to extremes of heat and cold. The monthly mean maximum and minimum temperatures and relative humidity are given in Table II. The maximum temperature does not go above 90°F. and ranges generally between 82° and 90°F. and the minimum rarely goes below 72°F., ranging between 72 and 80.

As a rule, humidity is very high reaching over 90 per cent and remains persistently high during June to October. Atmospheric humidity drops in November to about 70 per cent and continues so till the outbreak of south-west monsoon again next June.

TABLE II.

Temperature and humidity—Mangalore Town.

Month	1951			1952			1953		
	(1)	(2)	(3)	(1)	(2)	(3)	(1)	(2)	(3)
Jan. ..	88·9	71·3	73	89·5	71·3	73	88·1	71·0	72
Feb. ..	87·8	72·8	81	88·1	75·1	78	88·8	72·3	75
Mar. ..	89·1	76·3	76	88·3	75·6	75	89·8	75·7	75
Apr. ..	88·9	78·2	74	90·5	78·7	73	90·2	79·4	76
May ..	90·1	80·0	75	90·1	80·2	76	90·1	80·8	72
Jun. ..	85·4	74·5	87	84·8	74·4	92	87·5	77·9	84
Jul. ..	83·3	73·9	91	84·1	74·9	90	82·3	73·0	93
Aug. ..	84·3	75·3	88	83·1	74·6	92	84·8	75·5	87
Sep. ..	84·9	75·5	87	84·4	74·0	85	84·4	75·0	89
Oct. ..	87·2	76·0	86	84·1	74·8	88	86·6	75·5	86
Nov. ..	89·2	76·4	83	85·8	71·4	79	89·3	73·8	73
Dec. ..	88·8	69·5	66	89·2	72·6	71	90·5	70·7	62
	1954			1955			1956		
	(1)	(2)	(3)	(1)	(2)	(3)	(1)	(2)	(3)
Jan. ..	89·2	72·7	72	89·5	71·9	69	78·3	70·2	71
Feb. ..	89·0	72·9	75	88·6	75·1	76	87·6	72·5	73
Mar. ..	89·7	76·0	75	89·4	76·5	74	89·4	77·9	75
Apr. ..	89·8	78·6	76	90·0	78·6	70	91·1	80·0	72
May ..	89·9	78·7	79	88·7	77·7	81	86·3	76·3	84
Jun. ..	84·6	75·0	92	84·0	74·6	93	..	..	..
Jul. ..	82·2	73·1	95	83·5	74·4	91	..	..	..
Aug. ..	82·5	74·4	91	82·8	73·8	92	..	..	..
Sep. ..	83·9	73·7	88	84·7	75·1	88	..	..	..
Oct. ..	84·7	74·3	84	85·4	74·8	87	..	..	..
Nov. ..	88·6	72·1	69	86·4	73·0	80	..	..	..
Dec. ..	88·6	71·8	70	88·7	70·8	64	..	..	..

Col. (1) = Mean maximum temperature, degree F.
Col. (2) = Mean minimum temperature, degree F.
Col. (3) = Mean relative humidity at 08.30 hours.

MOSQUITO FAUNA.

The following species of mosquitoes were recorded by Krishnaswami (1954):—

C. fatigans
C. sitiens
C. (ficalbia) minima
C. (lophoceratomyia) sp.
Armegeres obturbans
Aedes (stegomia) ægypti
Anopheles barbirostris
Anopheles jamesi
Anopheles subpictus
Anopheles vagus and
Mansonia (mansonioides) sp.

The breeding places of *Culex fatigans*, the local vector of filariasis, are separately dealt with below. *Armegeres obturbans* have been very heavily breeding in septic tank latrines where there were crevices, however small, in the septic chamber or where the ventilating shaft was not tightly covered over with wire gauze of suitable mesh. It was also found in concentrated cow-dung water and in lead off drains from cattlesheds. *Aedes ægypti* were encountered in artificial containers. The few tanks in the town, mostly used for irrigation purposes, accounted for anopheline mosquitoes. Usually anophelines appear early during monsoon when rain water inundates all low lying areas. Along the coastal areas of the town, where the subsoil water level is very high, are a chain of shallow ponds and pits containing *Pistia stratiotes* which, during summer months, breed *Mansonioides* mosquitoes in scanty numbers.

BREEDING PLACES OF VECTOR (*C. FATIGANS*).

Culex fatigans breed almost ubiquitously in all the wards and at all times of the year. Though antilarval measures adopted have effected considerable improvement, the density of *Culex fatigans* continued to be fairly high.

(*a*) *Valley lines.*—It has already been mentioned how the several natural valley lines, which act as storm water courses during monsoon months, are converted into open sewers resulting in stagnations, causing prolific breeding of *Culex fatigans* during non-monsoon months. It is a common feature in this town that a cowshed or latrine is located at the margin of such channels and the washings from these, received in the channels, increase the organic content and favour culicine breeding. During rainy months, the entire waste matter gets washed away but when rains stop, stagnations occur and mosquito breeding goes up.

These water courses widen out as open drains, meander near the coastal areas and finally enter the sea at a few points.

(*b*) *Drains.*—Pucca drains are few and far between. But the few drains that have beds and sides constructed of laterite blocks, are mostly uncemented.

These drains have to carry 140 inches of rainfall during monsoon and very scanty water during summer and usually these have been designed for the former with the result that stagnation occurs in drains all along their course during summer, forming most favourable places for mosquito breeding.

(*c*) *Paddy fields.*—Mention has been made of the presence of numerous wet fields practically in all the wards of the town, except very near the coast. These form formidable sources of mosquito breeding, especially after the monsoon. The fields are densely surrounded by houses, favouring establishment of closer breeding-feeding units for mosquitoes.

(*d*) *Disused ponds.*—There are a few disused or misused domestic ponds, generally used for soaking palmleaf or coconut husks and these add to the mosquito nuisance.

(*e*) Besides, there are a good number of peridomestic breeding places. Most of the houses have compounds, and breeding takes place in cess pools, lead-off drains from latrines, cattlesheds, wells, etc. Breeding of mosquitoes also occurs in artificial containers, flower pots, ornamental fountains, neglected pits and the like. Almost every compound contains some coconut palms, and all waste water from washing of clothes and cleaning of utensils is allowed to accumulate in pits providing prolific breeding grounds for mosquitoes.

SEASONAL VARIATIONS IN THE DENSITY OF *C. FATIGANS* AND FILARIAL INFECTION IN THEM.

The density of *C. fatigans* (females only), the local vector of filariasis, expressed as number per man-hour and the filarial infection in them are furnished in Table III. Dissections have proved that developmental stages of microfilaria are found in *C. fatigans* only. Graph 1 shows the seasonal distributions of *C. fatigans* and filarial infection in them, with special reference to the meteorological conditions. Graph 2 shows filarial infections in *C. fatigans* and how it varies with atmospheric humidity.

Mosquito collections were made at random in all wards of the town. Though all mosquitoes were dissected for study, as only *C. fatigans* showed infection, females of this species alone are included. The mosquitoes were dissected the same day for evidences of filarial infection.

DISCUSSION.

The filarial surveys, so far conducted, have established that:

(*i*) The species of microfilaria encountered at Mangalore was *W. bancrofti* only, and

(*ii*) *C. fatigans* alone plays a rôle in the transmission of filarial infection in this town.

Density of C. fatigans.—The average density per man-hour of female *C. fatigans* for all the 25 wards has been furnished. Generally the density has been

very high in areas towards the coast where the density of population is also very high and where the drains, which constitute the main breeding grounds, are numerous. The centre of the town and peripheral areas are not so densely populated as near the coast and the mosquito density also is comparatively low but nevertheless high enough. The town has been under continuous antilarval measures with larvicides but the density of *C. fatigans* females has never reached lower than 12 per man-hour even during the height of the monsoon. The highest density recorded has been 40 per man-hour of *C. fatigans* female during October.

TABLE III.

Seasonal density of C. fatigans *and filarial infection rates in them and relative humidity—Mangalore Town.*

	Density of *C. fatigans* (female) per man-hour	Number dissected	Number positive for filarial infection	Infection rate, per cent	Relative humidity at 08.30 hours, per cent
1954					
January ..	..	124	4	3·2	72
February ..	..	281	34	12·1	75
March ..	19·2	1,742	223	12·8	75
April ..	18·0	1,073	159	14·8	76
May ..	17·0	850	164	19·3	79
June ..	15·0	599	139	23·2	92
July ..	11·2	922	144	15·5	95
August ..	14·9	926	190	20·5	92
September ..	18·8	1,227	163	13·5	88
October ..	19·7	1,204	153	12·7	84
November ..	39·7	784	78	9·9	69
December ..	15·9	212	20	9·4	70
1955					
January ..	..	..	..	..	69
February ..	..	..	..	..	76
March ..	14·0	267	30	11·3	74
April ..	14·6	292	32	10·1	70
May ..	..	..	..	..	81
June ..	..	..	..	..	93
July ..	19·0	262	34	12·9	91
August ..	17·6	361	54	14·9	92
September ..	18·3	628	91	14·4	88
October ..	19·0	38	6	15·7	87
November ..	28·1	486	73	15·0	80
December ..	21·6	81	11	13·2	64
1956					
January ..	31·1	179		6·6	71
February ..	23·8	149	12	5·4	73
March ..	21·3	149	8	6·0	75
April ..	15·2	187	9	3·7	72
May ..	17·6	112	7	9·0	84
			10		

GRAPH 1.

Broadly, the breeding grounds of *C. fatigans* here can be classified as (*i*) Peridomestic areas in and around the numerous garden-houses like cess pools, lead-off drains from latrines and cattlesheds, dung pits, flower pots, artificial containers and the like, and (*ii*) the public breeding grounds like natural valley lines receiving sewage within the town, open drains, paddy fields, etc. During the rainy months between June and October, all the latter breeding grounds get a good and complete washing with almost daily rainfall amounting to a total of about 140 inches during this period. The peridomestic areas alone during this season, constitute the only breeding grounds and consequently the density is comparatively low. Whatever antilarval measures with larvicides are carried out, are prone to be rendered ineffective by continuous and incessant rainfall, and rains also hamper the regular house visits of the spraying mazdoors and also of the supervisory staff.

The months, following the monsoon, present a different picture. The beneficial effects of the monsoon on mosquito density is felt till the end of October only. When the rains stop, the flow in all the valley lines and drains, which was full and fast, now slows down. All the drains which are heavily silted up due to the very uneven nature of the country, are desilted and cleaned up. At this stage, usually in November, a phenomenal increase in *C. fatigans* occurs and this sudden increase during the immediate postmonsoon months, is due to the following causes:—

GRAPH 2.

REFERENCE
INFECTION RATE IN CRATIGANS
RELATIVE HUMIDITY AT 08.30 HRS
1954
1955
1956

(*i*) The drains and natural valley lines which were being flushed, now slow down, and organic contents in them also get highly concentrated. The slowing of the drains and the increase in organic contents in them are most favourable for culicine breeding. These conditions could be tackled by canalisation and larvicidal measures unlike the two other conditions mentioned below, which are more difficult to tackle.

(*ii*) The town abounds in a large number of paddy fields, scattered in all the wards wherever there are natural low-lying areas and depressions. Rains cease in October and the standing crops in the fields now need water. The farmers, therefore, put up bunds across the sewage-laden valley lines which drain the town, raise up the water-level and irrigate the fields. These bunds across the undulating laterite channels, obstruct the flow and cause considerable stagnation of sewage to occur, with resultant mosquito breeding.

(*iii*) The paddy fields themselves, fed by the sewage water, become highly prolific and formidable sources of mosquito breeding. Unlike *A. culicifacies*, the larvæ of which are reported to disappear as the paddy plants grow (Russell and Rao, 1940), culicines are found till the harvest is almost ready, due to the differences in the egg-laying habits of the two mosquitoes.

Whatever be the direct effect of temperature and humidity on mosquito life in general, in the case of Mangalore Town the high rainfall, the uneven nature of the country and extensiveness of the breeding grounds caused by drains and paddy fields, determine the output and the seasonal distribution of *C. fatigans*. The density is lowest during the monsoon months when peridomestic areas alone constitute the only breeding grounds and is highest during the immediate post-monsoon period when drains and paddy fields pose an acute problem.

Filarial infection in Culex fatigans.—The gross filarial infection rates, as seen on dissection, are furnished in Graph 1 together with the corresponding density of female *C. fatigans* and the relative humidity. No month was free from evidences of filarial infection in *C. fatigans*. The minimum infection rate of 3·1 was seen in January while a maximum rate of 23·2 was recorded in June, the average for the town for the period being 13·8 per cent.

There is a negative correlation between the density of the mosquitoes and filarial infection in them (Graph 3). The season and conditions which are good enough for largescale breeding of *C. fatigans*, appear not so suitable for filarial infection in them. As already discussed, the density of *C. fatigans* is low during monsoon months—the peridomestic areas alone contributing to the output—while during the immediate post-monsoon period, the extensiveness of the man-made breeding grounds and the organic concentration in them, cause considerable rise in the density of *C. fatigans*. Thus while the paucity or extensiveness of the breeding grounds determine the mosquito density, filarial infection in them seem to be influenced by other factors.

The filarial infection rate in *C. fatigans* appear to follow the humidity curve directly (Graph 2). The atmospheric humidity is comparatively low (about 70 per cent) during the first four or five months and the last two months in a year, while with the rains the humidity goes to 80-90 per cent and remains so till the rainy months are over. The filarial infection rate in *C. fatigans* is very high during

GRAPH 3.

Density of mosquitoes and filarial infection in them.

the humid, rainy months and it drops to low level as the atmospheric humidity also falls below 80 per cent with the cessation of the monsoon. Thus high humidity appears to favourably influence the filarial infections in *C. fatigans* (Iyengar, 1938) and as this period of high humidity at Mangalore lasts for about six months, the chances of transmission of filariasis to human population are very high. A few urban and semi-urban areas, having similar local meteorological conditions as Mangalore in the West Coast of Madras State, have *bancroftian* filariasis problem. Sometimes it is said by the local authorities, administering these areas and conducting antilarval work as part of their antifilariasis programme, expressing opinions as to why the antilarval staff could not be disbanded or at least a minimum of staff maintained during monsoon months, as a measure of economy. The above findings bring out how unwise it would be to give up or even relax the antilarval measures during monsoon months, if filariasis is to be really controlled in these areas.

SUMMARY.

Observations on the seasonal variations in the density of *C. fatigans* and filarial infection in them, in Mangalore Town of Madras State, have been reported.

While the density of *C. fatigans* remains high during the immediate post-monsoon period caused by the extensiveness of the breeding grounds, the filarial infection rate in them is very high only during the monsoon months when the humidity is constantly over 80 per cent.

While transmission of filariasis is possible throughout the year in Mangalore, the humidity being very high for nearly six months during the wet rainy months, the chances of transmission of filariasis to human population are also very high during this season as the infection rates in the mosquitoes during the monsoon months are high.

The high filarial infection rate all over the town is accounted for by the nature of the country and by the numerous breeding conditions interspersed throughout the town.

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A NEW MICROFILARIA IN THE DOG.

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MONNIG (1950) described eight types of filarial infections in the dog. One or more of them had been noted in dogs in different parts of the world including India. The following types of microfilariæ were recorded: *D. immitis*, *D. repens*, *dracunculoides*, *ochmanni*, *reconditum*, *auquieri*, *lewisi* and *grassii*. The diagnosis of such infections had been based chiefly by examination of the microfilariæ in the peripheral blood, except in the case of microfilariæ of *grassii* which is noted in the subcutaneous lymph spaces. All the microfilariæ are unsheathed except that of *M. ochmanni*. The commonest of these infections are *Dirofilaria immitis* and *D. repens* which have been recorded from many parts of India (Bhale Rao, 1935; Raghavan *et al.*, 1952; Malaria Institute of India, 1956). Recently *W. malayi* like infection was noted in dogs from this area (Malaria Institute of India, 1956).

This note sets out the finding of a new type of microfilaria in addition to microfilariæ of *D. repens* and *immitis** in a dog maintained at this Institute.

METHODS AND MATERIAL.

The dog was obtained from Sakhigopal, Orissa (India), an area endemic for *W. bancrofti* in the local population and for *D. repens* and *D. immitis* in dogs. The studies now reported were carried out on this dog at Delhi.

The air-dried thick smears of blood, obtained from the lobe of the ear of the dog, were dehæmoglobinised with tap water, fixed in acid alcohol (2 per cent acetic acid in methyl alcohol) and stained in J.S.B. I (Jaswant Singh and Bhattacharji, 1944) for 45 seconds.

The smears were examined after drying. The measurements of the important anatomical points, taken in six each of the three types of microfilariæ noted in this dog, are presented in Table I.

*Dr. Willard H. Wright of the National Institute of Health, Bethesda, Md., U.S.A., who visited Delhi in May, 1957, kindly studied the slide and confirmed the currently reported microfilaria as a new one. In this context, attention is drawn to the report of Newton and Wright (1956) who had described *Mf. reconditum* in a dog in U.S.A. with *D. immitis*.

TABLE I.

Measurement (in microns) of the fixed points in percentage of total length from anterior end.

Number	Beginning of nuclear column	Nerve ring	Excretory pore	Anal pore	Last nucleus	Total length (in microns)
D. repens:						
1.	1·9	17·8	26·7	82·8	89·8	298·3
2.	2·0	20·3	29·7	77·0	87·8	281·2
3.	2·0	19·5	28·2	80·5	88·6	283·1
4.	2·5	20·4	30·6	81·5	91·1	298·3
5.	2·0	19·5	28·8	78·5	89·3	293·1
6.	2·0	20·4	29·9	76·9	90·5	279·3
Average	2·06	19·6	28·9	79·5	89·5	
	Average width = 5·2			Average length = 287·2		
D. immitis:						
1.	4·3	20·4	33·3	86·0	94·6	176·7
2.	2·9	18·3	27·9	86·5	96·1	197·6
3.	3·6	17·4	26·6	72·5	86·2	207·1
4.	3·5	17·5	25·4	77·2	91·2	216·6
5.	3·3	18·0	27·0	74·6	87·7	231·8
6.	2·7	18·0	27·0	73·8	91·0	210·9
Average	3·38	18·2	27·8	78·4	91·1	
	Average width =4·1			Average length = 206·8		
New type of microfilaria:						
1.	4·4	26·4	39·6	78·0	91·2	172·9
2.	4·3	26·9	38·7	80·6	91·4	176·7
3.	4·3	26·1	39·1	80·4	92·4	174·8
4.	3·3	23·3	37·8	76·7	91·1	171·0
5.	4·4	27·5	38·5	72·5	92·3	172·9
6.	3.4	25·0	36·4	77·3	92·0	167·2
Average	4·0	25·8	38·3	77·6	91·7	
	Average width = 8·7			Average length = 172·6		

RESULTS.

Camera lucida drawings of the three types of microfilariæ noted in the dog are appended (Plate II). The important characteristics of the three types of microfilariæ are set below in Table II. No sheath was apparent in any of the three types of microfilariæ noted.

PLATE II.

Camera lucida drawings of the three types of microfilariæ found in the blood of a dog from Orissa

A. The new type of Mf described

B. Mf. *Dirofilaria immitis*

C. Mf. *Dirofilaria repens*

TABLE II.

Important points of difference between the microfilariæ of D. immitis, D. repens *and the new type of microfilaria.* (*Measurements are in microns*).

Points of difference	Mf. *D. immitis*	Mf. *D. repens*	Mf. of the new type
Total length ..	218–329	283–360	167·2–176·7
Total breadth ..	3·9–4·4	6·0	8·7
Cephalic space ..	Long, with no intervening nucleus. Forms 3·38 per cent of total length.	Forms 2 per cent of total length. There are two nucleii in the centre of the space lying beside each other.	Forms 4 per cent of the total length. There is one intervening nucleus lying on one side.
Nucleii of the nuclear column	Small, round and slightly overlapping and smudged.	Thick with considerable overlapping and smudged.	No overlapping. Nucleii round, thick and discrete.
Tail end ..	Tail tapers to a fine thread; nucleii do not reach end of tail.	Tail tapers to a fine thread; nucleii do not reach end of tail.	Tail gradually tapering to a blunt point. Nucleii do not reach end of tail.

The length of microfilariæ of *D. immitis* and *D. repens*, given in Table II, is according to Monnig (1950). The lengths of the microfilariæ of *D. immitis* and *D. repens*, measured in the blood smears of the dog, varied from 176.7 to 231.8 μ (average 206·8 μ) and from 279·3 to 298·3 μ (average 287·2 μ), respectively. The measurements of *D. immitis* coincide with those given by Foley (quoted by Korke, 1923). The width of microfilariæ of *D. immitis* and *D. repens* reported by Webber and Hawking (1955) is 3·9 to 4·4 and 6·0 μ, respectively, whereas the average widths of specimens examined by the present authors are 4·1 and 5·2 μ, respectively, for Mf. *D. immitis* and *D. repens*.

The essential points where the morphology of the new type of microfilaria differs from the other two are: the shorter length, greater width, a characteristic intervening nucleus lying on one side of the cephalic space, discrete and round nucleii of the nuclear column and uniformly tapering tail with a free caudal space. Moreover, the beginning of the nuclear column, the nerve ring and the excretory pore are appreciably farther located from the anterior end in the new type of microfilaria as compared to *D. immitis* and *D. repens* (Table I).

MICROFILARIAL PERIODICITY.

Thick blood smears were made from measured quantities (20 c.mm.) of blood drawn from a puncture in the dog's ear lobe. The slides were taken every two hours for 48 hours, at even hours during the first 24 hours and at odd hours during the subsequent 24 hours, thus obtaining data regarding hourly variations in microfilarial density. The slides were stained and examined as described earlier. The results of the periodicity study of the three types of microfilariæ are presented in Graph 1. The maximum number of microfilariæ of *D. immitis* and

D. repens appeared at 21.00 hours, the counts being 30 and 28 respectively per 20 mm. of blood. Those of the new type encountered were very scanty and it is not possible to state anything definite regarding their periodicity.

GRAPH 1.

Periodicity in dog Number 10.

The occurrence of this infection in scanty numbers, concomitantly with other filarial infections, has added to the difficulty in the investigations, including maintenance of the strain. Detailed search for similar infection in other dogs and a thorough post-mortem of this dog have been planned.

SUMMARY.

A new type of microfilaria together with microfilariæ of *D. repens* and *D. immitis* in a dog from Sakhigopal, Orissa, India, is described.

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NOTE ON THE QUANTITATIVE ESTIMATION OF THE DEGREE OF D.D.T. RESISTANCE IN *A. SUBPICTUS* GRASSI, 1899 IN DELHI STATE.

BY

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[February 3, 1958.]

INTRODUCTION.

A. SUBPICTUS adults in rural areas of Delhi State were observed to be D.D.T. resistant in 1956 (Sharma and Krishnamurthy, 1957*a*:1957*b*) and the degree of resistance was stated to be more than ten times when compared with the susceptibility level of the same species of a nearby unsprayed village of Uttar Pradesh (Sharma *et al.*, 1957). Larvæ of this species from D.D.T. sprayed area were also found to be more tolerant to D.D.T. when compared with those from an unsprayed area (Sharma and Krishnamurthy, 1957*b*). Studies were continued during 1957 to estimate the degree of D.D.T. resistance in *A. subpictus* of Delhi State both in the adult and larval stages and the results are presented in this communication.

MATERIALS AND METHODS.

Susceptibility tests, following Busvine and Nash technique (World Health Organization Report Series 80, 1954), were carried with the blood-fed and gravid mosquitoes collected from sprayed villages of Delhi State (Nelothi, Sultanpur and Tilangpur Kothra which are being sprayed with D.D.T. since 1947 and were last sprayed in November, 1956) and from an unsprayed village Arthala of Meerut District, Uttar Pradesh. The unsprayed village is situated at the fringe of sprayed area, the villages in the sprayed area being located at distances varying from 4 to 6 miles from it. Mosquitoes of the unsprayed village were exposed to D.D.T. deposits for one hour and 24 hours, and of the sprayed villages for 24 hours.

Mortality counts in mosquitoes were made just at the end of exposure period in case of 24 hours exposure, and after 24 hours of observation period in case of one hour exposure.

For measuring the degree of insecticide resistance in the larvæ, tests following World Health Organization technique (using alcoholic solutions of insecticide) were carried out both in the laboratory* and field.

The results of the experiments were analysed by Probit analysis method following the arithmatic technique (Finney, 1952). The final results of the biometric analysis are given in Table I.

RESULTS AND CONCLUSIONS.

It may be seen from Table I that in the unsprayed area, the M.L.Cs. of D.D.T. for *A. subpictus* adults were 2·6 per cent with one hour of exposure and 0·12 per cent with 24 hours of exposure. The M.L.C. values of this species with one hour of exposure were reported to be 0·3 per cent in 1955† and 0·26 per cent (with fiducial limits 0·21-0·28) in 1956. The M.L.Cs. of D.D.T. for *A. subpictus* in sprayed villages after 24 hours of exposure were 3·0 per cent (with fiducial limits 2·8-3·3) in 1956 (Sharma *et al.*, 1957) and 6·0‡ per cent (with fiducial limits 4·6-7·8) in June and July, 1957. The regression co-efficients of probit upon log concentration in the unsprayed and sprayed areas (after 24 hours exposure) during the present studies were $0{\cdot}8 \pm 0{\cdot}1$ and $1{\cdot}4 \pm 0{\cdot}15$, respectively, and the difference between them was found to be significant. According to Hoskins and Gordon (1956), a higher M.L.C. with steeper slope of *lc-pr*§ line (*i.e.*, higher value of regression co-efficient) is obtained in the case of vigour selection when compared with a homogeneous susceptible population and also in case of 'super resistant' population when compared with heterogeneous or partially resistant population. The comparatively higher value of regression co-efficient, *i.e.*, steeper *lc-pr* line (Chart 1) in the case of *A. subpictus* of sprayed villages and with as high as 50 times M.L.C. value compared to the value obtained in unsprayed village, indicate that such a high increase in insecticide tolerance is due to physiological resistance and not due to vigour selection. This is further supported by the absence of higher M.L.C. for B.H.C. in the mosquito population of sprayed area as compared to unsprayed area (Sharma *et al.*, 1957). Therefore the flatter *lc-pr* line of *A. subpictus* of unsprayed village would mean that the population is heterogeneous and contained a fairly large proportion of individuals with lowered insecticide susceptibility due to its close proximity to the sprayed area. The presence of heterogeneous population in the unsprayed village is also supported by the higher M.L.C. of D.D.T. and significantly lower value of regression co-efficient with one hour of exposure in 1957 than in 1955 and 1956 (Chart 2). The M.L.C. (0·3 per cent × 1) and the regression co-efficient (2·8) values of 1955 for *A. subpictus* adults of unsprayed area are quite in conformity with similar values for

*High mortality in larvæ was observed when kept for conditioning in the laboratory for 24 hours.

†Technical report of the Scientific Advisory Board of the Indian Council of Medical Research for the year 1955.

‡Five per cent sol. of D.D.T. in B.O.C. white oil gave the probit of mortality 5 and the M.L.C. value by arithmetic calculation was found to be 6·0.

§Log concentration probit regression line.

TABLE I.

Summary of the biometric analysis of susceptibility tests data of A. subpictus *adults and larvæ.*

Place of test	Species, with stages	Sprayed or unsprayed	Insecticide	Exposure period (Hours)	Observation period (Hours)	Regression equation Y =	M.L.C. with 95 per cent fiducial limits	Regression co-efficient	x=Log(z)	X^2 (degree of freedom)*
Field	*A. subpictus* (adults)	Unsprayed	D.D.T.	1	24	3·0541+1·4x	2·6 per cent (2·16 - 3·14)	1·4 ±0·1	x10	(28)=8·1
,,	,,	,,	,,	24	..	4·3808+0·78x	0·12 per cent (0·09 - 0·17)	0·78±0·09	x100	(60)=10·3
,,	,,	Sprayed	,,	24	..	2·5294+1·4x	6·00 per cent (4·63 - 7·78)	1·4±0·15	x10	(26)=5·3
,,	(larvæ)	,,	,,	24	..	1·5541+1·7x	1·2 p.p.m. (1·1 - 1·4)	1·7±0·1	x100	(35)=6·2
,,	,,	Unsprayed	,,	24	..	3·8696+0·8x	0·28 p.p.m. (0·20 - 0·37)	0·8±0·1	x100	(36)=0·5
Laboratory	,,	Sprayed	,,	24	..	0·0441+1·77x	0·63 p.p.m. (0·54 - 0·75)	1·77±0·09	x1000	(32)=3·7
,,	,,	Unsprayed	,,	24	..	2·4015+1·03x	0·34 p.p.m. (0·27 - 0·43)	1·03±0·1	x1000	(45)=0·9
,,	,,	Sprayed	B.H.C.	24	..	1·4133+2·53x	0·26 p.p.m. (0·20 - 0·33)	2·5±0·3	x100	(4)=8·5
,,	,,	Unsprayed	B.H.C.	24	..	1·2423+2·83x	0·21 p.p.m. (0·17 - 0·26)	2·8±0·3	x100	(10)=19·8

*Degree of freedom = Total number of batches in all concentrations minus two.

susceptible anopheline mosquitoes in general obtained by the Busvine-Nash Technique (Busvine, 1956; Sharma *et al.*, 1957). These values can, therefore, be used as a base line data for estimating the degree of resistance of this species. On this criterion, the degree of resistance in *A. subpictus* adults of the sprayed area in 1957 may be expressed as much more than 50 times when compared to the base line M.L.C. value obtained in 1955.

CHART I.

Slopes of the fitted log probit lines for A. subpictus *adults of sprayed and unsprayed areas (24 hours exposure).*

The M.L.Cs. of D.D.T. for resistant *A. subpictus* larvæ, collected from the sprayed area and tested in the laboratory and field, were found to be 0·63 p.p.m. and 1·2 p.p.m. respectively with 24 hours exposure. The corresponding values for the unsprayed area were found to be 0·34 p.p.m. and 0·28 p.p.m. respectively.

From the above mentioned data, it may be seen that the larvæ of the sprayed area were two to four times more tolerant to D.D.T. than those of the un-sprayed area. From Table I, it may be seen that like the adults, the regression co-efficient values for the larvæ of the sprayed area were also more than those of the unsprayed

CHART 2.

Slopes of the fitted lc-pr lines for A. subpictus *(adults) of unsprayed area (1 hour exposure).*

area which showed that the heterogenity in the population of the unsprayed area was also reflected in the larval tests (Chart 3). The larvæ of the sprayed and unsprayed villages were found to be equally susceptible to B.H.C. and there was no difference between the regression co-efficient values.

In the insecticide susceptibility tests of larvæ, the mortality was recorded at the end of 24 hours of exposure to insecticide and again after 24 hours of observation period, and the M.L.C. and regression co-efficient values were calculated separately; the values are given in Table II. From this table, it may be seen that there is no significant difference, except in one experiment, in M.L.C. and regression co-efficient values as assessed after 24 hours exposure or after a further 24 hours recovery period for both D.D.T. and B.H.C. in the case of laboratory tests. This is in conformity with the observation of Parker (1957) for *Aedes ægypti* larvæ. In the case where significant difference in M.L.C. values was noted (in the larvæ of unsprayed area), a high pupation rate, *i.e.*, to the extent of about 20 per cent was observed during the 24 hours of observation period. The M.L.C. value,

CHART 3.

Slopes of the fitted lc—pr *lines for* A. subpictus *larvae of sprayed and unsprayed areas.*

when calculated in this case after excluding the pupæ from the total number of larvæ exposed, was found to be significantly less when compared to the M.L.C. obtained after 24 hours of exposure though there was only slight increase in larval mortality during observation period of 24 hours. But when the pupæ were not excluded from the calculation, the difference in M.L.C.s was found to be not significant. Unlike the laboratory tests, in field tests significantly lower M.L.C. values were found after observation period of 24 hours when compared with the M.L.C. value obtained just after the 24 hours exposure. In the field tests, locally procured hard water was used and wide differences in maximum and minimum temperatures were recorded.

From the present studies, it may be concluded that the population of *A. subpictus* in the unsprayed village was not the true susceptible population. The heterogenity of the population, with respects to its insecticide tolerance, may be

TABLE II.

Values of M.L.C. and regression co-efficient of susceptibility tests of A. subpictus (*larvæ*).

Sprayed or unsprayed	Place of test	Insecticide	After exposure of 24 hours		After exposure of 24 hours and then allowed 24 hours recovery period		Temperature (°C.)	Water temperature (°C.)
			M.L.C. with 95 per cent fiducial limit	Regression co-efficient	M.L.C. with 95 per cent fiducial limit	Regression co-efficient		
Sprayed	Laboratory	D.D.T.	0·63 p.p.m. (0·54 - 0·75)	1·8±0·1	0·53 p.p.m. (0·46 - 0·62)	2·3±0·2	25°-26°	25°-26°
Unsprayed	,,	D.D.T.	0·34 p.p.m. (0·27 - 0·43)	1·0±0·1	0·16 p.p.m. (0·13 - 0·20)	1·2±0·1	25°-26°	25°-26°
Sprayed	,,	B.H.C.	0·26 p.p.m. (0·20 - 0·33)	2·5±0·3	0·20 p.p.m. (0·14 - 0·25)	2·5±0·3	25°-26°	25°-26°
Unsprayed	,,	,,	0·21 p.p.m. (0·17 - 0·26)	2·8±0·3	0·15 p.p.m. (0·12 - 0·20)	2·6±0·3	25°-26°	25°-26°
Sprayed	Field	D.D.T.	1·2 p.p.m. (1·1 - 1·4)	1·7±0·1	<0·1 p.p.m.	..	18°-25°	16°-21°
Unsprayed	,,	D.D.T.	0·28 p.p.m. (0·20 - 0·37)	0·8±0·1	<0·1 p.p.m.	..	18°-25°	16°-21°

due to the inflow of individuals with lowered D.D.T. susceptibility from surrounding sprayed area. However, the degree of D.D.T. resistance in *A. subpictus* adults of the sprayed area was estimated to be 50 times when compared with the heterogeneous population of the unsprayed village in 1957, but when compared with the M.L.C. values of relatively homogeneous susceptible population of the unsprayed village in 1955, it was considered to be much more than 50 times. The degree of resistance was found more in the adult stage than in the larval stage.

SUMMARY.

Studies on the susceptibility of *A. subpictus* adults and larvæ to D.D.T. and B.H.C. were carried out during 1957 in sprayed and unsprayed villages near Delhi. In the case of *A. subpictus* adults of unsprayed area, rise in M.L.C. of D.D.T. was observed in 1957 when compared to M.L.C. values obtained in 1955 and 1956 which meant that the mosquito population in the unsprayed village in 1957 contained a fairly large proportion of individuals with low D.D.T. susceptibility due to its close proximity to sprayed villages. The M.L.Cs. values obtained in 1957 showed that *A. subpictus* adults from D.D.T. sprayed area were much more than 50 times resistant to D.D.T. when compared to that obtained in 1955 from an unsprayed village. *A. subpictus* larvæ from D.D.T. sprayed villages were two to four times more tolerant to D.D.T. than those from the unsprayed village.

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URBAN, SEMI-URBAN AND RURAL FILARIASIS IN BALLIA DISTRICT, UTTAR PRADESH.

BY

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[July 17, 1957.]

A REPORT on investigation of the filariasis problem in Ballia Town (Uttar Pradesh) was published by Rahman, Singh and Gujral (1957). Surveys have since been conducted in selected rural and semi-urban areas of Ballia District from the period May, 1956 to August, 1956. Ballia is the eastern-most district of Uttar Pradesh, bordering on Saran and Shahabad districts of Bihar. Geographical limits are the parallels of 25° and 26° north latitude and 83° and 80° east longitude. River Ghagra forms the north-eastern boundary while River Ganges the soluth-eastern; the confluence of the two rivers being situated at a point about 30 mives east of Ballia City, the district headquarter. Both the rivers are liable to severe floods year after year, inundating vast areas of land in the district. The area is deltaic and the subsoil water level is very high. Water for irrigation during previous years has been obtained only from rains and manually-operated wells. State tube-wells and canals are now being laid. In addition to these, there are nu merous ponds and lakes. The most important lake is the great 'Sburaha Tal', which, when full, covers an area of 8,500 acres. Man-made breeding places such as borrow-pits, brick kilns, pools under culverts etc., which form potential breeding places of culicines, abound in the district.

Total area of the district is 1,240 square miles or 7,97,708 acres. Population of the district is about 11 lacs, majority being agriculturists with small holdings. Present density of population is 887 per square mile whereas it was 864 per square mile in 1945. Average yearly rainfall for the last three years (1953-1955) was 41·93 inches. Rainy season extends from mid-June to the end of August.

Literacy in comparison to other parts of the State is very high (70 per cent) and people are politically very conscious. But their attitude towards health is that of apathy.

The biggest city in the district is Ballia with a population of 30,000 only. There is one notified area with a population of 9,500 and five town areas with an average population of about 7,000 each. Rail communication exists between Ballia and Chhapra (Bihar). One National Extension Service Block and one normalized block are located in the district. One control unit under the National Filaria Control Programme was established in Ballia towards the end of 1955.

For purposes of discussion in this paper, municipalities have been classified as urban; notified town areas as semi-urban; and villages as rural areas. Results of survey, conducted in the following areas, have been incorporated.

1. *Urban.*—Areas included in the limits of Ballia Municipality.

2. *Semi-urban.*—Areas included in the limits of Notified Area Committee, Rasra; and the Town Area Committee, Bansdih.

3. *Rural.*—Twenty-three randomly selected villages named as under and numbered in Map 1:—

1. Amhar Patti Dakshin
2. „ „ Uttar
3. Asan
4. Bedua
5. Beruar Bari
6. Bhojpura
7. Bisukia
8. Chhotki Sarai
9. Chilkahar
10. Garwar
11. Khajuri
12. Kharsara
13. Madhopur
14. Mithwar
15. Mubarakpur
16. Phephna
17. Piyaria
18. Ratanpura
19. Ratsar
20. Sarai Bharti
21. Sikandarpur
22. Subhai Bandh
23. Sukhpura

Survey was conducted at night between 20.30 and 24.00 hours. From each individual, a thick smear was prepared by withdrawing 20 c.mm. (roughly 3 drops) of finger blood, airdried and stained by J.S.B. Stain I (Jaswant Singh and Bhattacharji, 1944) and examined.

RESULTS OF SURVEY.

The type of infection in all the areas, whether urban, semi-urban or rural, is exclusively *Wuchereria bancrofti* and the vector *Culex fatigans.* One fact was brought out very conspicuously: not a single village, out of the 23 randomly selected, was free from infection. So, it could perhaps be reasonably assumed that filariasis is prevalent almost throughout the district.

ANALYSIS OF DATA FROM DISPENSARIES.

There are 13 allopathic dispensaries scattered in the district, besides the district and police hospitals in Ballia City. Location of the dispensaries is shown in the map of the district (Map I). Dispensary attendance of filaria cases, for the last five years, is indicated in Table I.

Although not a true index of the prevalence of this disease, yet if the morbidity statistics from hospitals and dispensaries have any significance, an analysis of Table I as well as of Graph 1 indicates that the incidence of the disease has probably been gradually increasing during the last five years. Judging from the very slight increase in density of population from 864 per square mile in 1945 to 887 per square mile at present, it appears that there has not been any large-scale immigration into the district. Since the data has been collected only from

MAP OF BALLIA DISTRICT.

those dispensaries which were existing throughout the five year period from 1951 to 1955, the only possible factors that could account for the increase in the dispensary figures are that:—

(*i*) on account of increased literacy and health consciousness, more persons reported at hospitals to seek relief;

(*ii*) on account of posting of a more popular surgeon at the district hospital, more persons reported for hydrocoele operations;

(*iii*) increase in the dispensary figures might have been due to a genuine increase in the incidence of the disease.

Whatever the cause may be, increase in the incidence of the disease from 1951 to 1955 is 11·84, 2·68, and 2·55 times in urban, semi-urban and rural areas, respectively. (Unexplained sudden rise of cases in Rasra dispensary in 1953 is not understandable). On the whole, the trend in the increase of incidence of the disease in rural areas indicates a uniform rate of increase (Graph 1).

The detailed analysis of data, regarding the three types of areas, is presented in Table II.

TABLE I.

Filaria cases attending dispensaries during five years (1951-1955).

Serial number and type	Name of dispensary	Number of filaria cases reported				
		1951	1952	1953	1954	1955
1. Urban	District and Police hospitals ..	203	314	332	1047	2405
2. Semi-urban	Rasra ..	16	29	246	56	57
	Bansdih ..	9	5	5	5	10
	Total ..	25	34	251	61	67
3. Rural*	Ratsar	12	13	10	36	45
	Siar	0	7	5	9	2
	Sikandarpur ..	24	21	56	32	47
	Naubarar-koderva ..	2	2	3	4	2
	Maniar	7	4	1	5	4
	Dubhar ..	..	..	..	12	4
	Bairia	5	16	15	19	27
	Kharsara ..	2	1	3	0	2
	Total	52	64	93	117	133

*The remaining three rural dispensaries at Ratanpura, Narehi and Nawanagar started functioning late in 1954 and hence not included.

TABLE II.

Details of filariasis in the urban, semi-urban and rural areas surveyed.

Particulars	Data in respect of		
	Urban area	Semi-urban area	Rural area
Number of persons examined	3,179	1,764	2,813
Number with microfilaria	383	193	273
Microfilaria rate, per cent	12·05	10·9	9·8
Average infestation per 20 c.mm. ..	28·45	14·31	14·08
Number with disease	1,096	478	559
Disease rate, per cent	34·47	27·09	19·87
Number with disease-cum-infection ..	86	45	47
Endemicity rate per cent	43·81	35·44	27·9

GRAPH 1.

Number of patients with filarial symptoms attending dispensaries from 1951 to 1955 in urban, semi-urban and rural areas of Ballia district (U. P.)

Infection rate.—The infection rate is high in all types of areas. But the highest percentage of persons showing circulating microfilariæ in their blood are found in urban areas (12·05 per cent); next in frequency being semi-urban (10·9 per cent) and finally rural areas (9·7 per cent).

Average infestation.—Microfilaria load in the community is also highest in the urban areas as illustrated by the average infestation rate (28·45). It is lesser in semi-urban areas (14·31) and the least in rural areas (14·08).

Disease rate.—The community shows the same trend with regard to disease manifestations also. Disease rate is the highest in urban areas (34·47 per cent), decreasing in semi-urban (27·09 per cent) and rural areas (19·87 per cent).

Mixed rate.—Percentage of persons showing both the disease manifestations as well as circulating microfilariæ in their blood was 2·7 per cent in urban areas, 2·55 per cent in semi-urban areas and 1·67 per cent in rural areas.

Endemicity rate.—Consequently, endemicity rate is highest in urban areas (43·81 per cent), decreasing in semi-urban and rural areas, being 35·44 and 27·90 per cent, respectively.

From the bar diagram, (Graph 2), it will appear that the infection, disease and endemicity rate are highest in urban, lesser in semi-urban and least in the rural areas.

GRAPH 2.

Filarial infection, disease and endemicity rates in urban, semi-urban and rural areas.

Infection and disease rates, according to age-groups, in urban, semi-urban and rural areas are depicted in Table III.

TABLE III.

Infection and disease rates according to age-groups.

Type of area	Up to 2 years		Above 2 to 5 years		Above 5 to 10 years		Above 10 to 20 years		Above 20 years	
	Infection rate	Disease rate	Infection rate	Disease rate	Infection rate	Disease rate	Infection rate	Disease rate	Infection rate	Disease rate
Urban	0	0	6·06	6·06	6·30	6·28	12·2	24·51	12·63	37·73
Semi-urban	0	0	3·03	6·06	7·3	7·29	11·3	21·48	12·46	36·65
Rural	0	0	2·89	2·89	6·13	4·80	10·01	16·72	11·76	29·24

It will appear from Table III as well as Graph 3 that both the infection and disease rates in urban areas are higher than the corresponding rates in the rural areas in all the age-groups. However, the difference is not appreciable except in the age-group 3-5 years. In this age-group of pre-school children, infection rate in urban areas is double than that of the pre-school children of semi-urban and rural areas. This may be attributable to the greater preponderance of culicine mosquitoes in the cities where no drainage schemes exist, higher density of population in cities and higher microfilaria load in the urban community; all these factors being conducive to produce infection at a relatively early age. In all areas, increase in both the infection and disease rates shows a close correlation till the ten years age-group is reached. Beyond that age, whereas disease rate increases very steeply, increase in infection rate is very slight.

GRAPH 3.

Filarial infection and disease rates according to age-groups in urban, semi-urban and rural areas of Ballia District, U.P.

The youngest children showing infection in urban and non-urban areas were three and four years old, respectively.

Entomological data.—During the period from May to August 1956, culicine density in urban areas was 56 per man-hour, whereas it was only 23·9 per man-hour in non-urban areas. *Culex fatigans* alone were found to harbour infective larvæ. 4·1 per cent mosquitoes were found infected in urban areas and only 1·6 per cent in non-urban areas.

Thus, quantum of transmission in urban areas was more than in other areas.

SUMMARY.

1. Results of a filaria survey, conducted separately in urban, semi-urban and rural areas of Ballia District in Uttar Pradesh, are incorporated in this paper.

2. The entire district appears to be filarious and the problem is not confined merely to isolated pockets.

3. Prevalent infection is exclusively *Wuchereria bancrofti* in urban as well as rural areas and the vector responsible for its spread is *Culex fatigans*. The quantum of transmission in the mosquitoes in urban areas is more than in non-urban areas.

4. The disease has probably gradually been increasing during the last five years 1951-1955; 11·84 times in urban, 7·68 times in semi-urban and 2·55 times in rural areas.

5. Infection, disease and endemicity rates are highest in urban, lesser in semi-urban and least in the rural areas.

6. Average infestation (microfilaria load) in the community is also distributed in the same manner, being 28·45, 14·31 and 14·08 microfilaria per 20 c.mm. of blood in urban, semi-urban and rural areas, respectively.

7. Infection rate in the pre-school children in urban areas is twice that in the rural areas.

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ADVANCES IN THE STUDY OF CULICINE MOSQUITOES OF HAINAN, SOUTH CHINA.

BY

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[November 20, 1957.]

MANY studies have been made of mosquitoes in Hainan Island, South China. The investigation has been centered, largely, on the tribe Anophelini, while the members of the other two tribes, i.e., Megarhinini and Culicini, have not yet been thoroughly investigated. According to the reports made up to 1946, only 16 species distributed in five genera of Megarhinine and Culicine mosquitoes were recorded. In recent years, measures for controlling mosquito-transmitted diseases have been taken by the government and many papers on Hainan mosquitoes have been published (Chang Pen-hwa, 1954; Meng Ch'ing-hwa, 1955; Chu Feng-i, 1957, etc.).

The present paper is an up-to-date list of the Culicine and Megarhine fauna of Hainan Island and includes 71 species and varieties representing nine genera. The list is as follows:—

Genus *Megarhinus* Robineau-Desvoidy, 1827
M. splendens Wiedemann, 1819

Genus *Aedes* Meigen, 1921
A. (*Aedes*) *dux* Dyar and Shannon, 1925
A. (*Aedimorphus*) *caecus* Theobald, 1901
A. (*Aedim.*) *mediolineatus* Theobald, 1901
A. (*Aedim.*) *taeniorhynchoides* Christophers, 1911
A. (*Aedim.*) *vexans* Meigen, 1830
A. (*Banksinella*) *lineatopennis* Ludlow, 1905
A. (*Finlaya*) *albolateralis* Theobald, 1908
A. (*F.*) *greeni* var. *kanaranus* Barraud, 1924
A. (*F.*) *formosensis* Yamada, 1921
A. (*F.*) *saxicola* Edwards, 1922
A. (*F.*) *togoi* Theobald, 1907
A. (*F.*) *sp.*
A. (*Stegomyia*) *ægypti* Linnaeus, 1762
A. (*S.*) *albolineatus* Theobald, 1904

A. (*S.*) ***albopictus*** Skuse, 1895
A. (*S.*) ***scutellaris*** Walker, 1859
A. (*S.*) ***subalbopictus*** Barraud, 1931
A. (*S.*) ***vittatus*** Bigot, 1861
A. (*S.*) ***w-albus*** Theobald, 1905

Genus *Armigeres* Theobald, 1901
A. (*Armigeres*) ***obturbans*** Walker, 1860
A. (*Leicesteria*) ***magnus*** Theobald, 1908

Genus *Culex* Linæus, 1758
C. (*Culex*) ***bitæniorhynchus*** Giles, 1901
C. (*C.*) ***bitæniorhynchus*** var. ***ambiguus*** Theobald, 1903
C. (*C.*) ***bitæniorhynchus*** var. ?
C. (*C.*) ***fuscocephalus*** Theobald, 1907
C. (*C.*) ***fatigans*** Wiedemann, 1828
C. (*C.*) ***gelidus*** Theobald, 1901
C. (*C.*) ***mimulus*** Edwards, 1915
C. (*C.*) ***mimeticus*** Noe, 1899
C. (*C.*) ***orientalis*** Edwards, 1921
C. (*C.*) ***sitiens*** Wiedemann, 1828
C. (*C.*) ***tritæniorhynchus*** Giles, 1901
C. (*C.*) ***vagans*** Wiedemann, 1828
C. (*C.*) ***vishnui*** Theobald, 1901
C. (*C.*) ***whitmorei*** Giles, 1904
C. (*C.*) ***sp.***
C. (*Culiciomyia*) ***nigropunctatus*** Edwards, 1926
C. (*Culi.*) ***shebbearei*** Barraud, 1924
C. (*Culi.*) ***pallidothorax*** Theobald, 1905
C. (*Lophoceratomyia*) ***cinctellus*** Edwards, 1922
C. (*Loph.*) ***fraudatrix*** Theobald, 1905
C. (*Loph.*) ***fraudatrix*** var. ***annulata*** Taylor, 1916
C. (*Loph.*) ***minor*** var. ***bengalensis*** Barraud, 1934
C. (*Loph.*) ***uniformis*** Theobald, 1905
C. (*Lutzia*) ***fuscanus*** Wiedemann, 1821
C. (*L.*) ***halifaxi*** Theobald, 1903
C. (*L.*) ***vorax*** Edwards, 1921
C. (*Mochthogenes*) ***castrensis*** var. ***foliatus*** Brug, 1932
C. (*Moch.*) ***hinglungensis*** Chu, 1957
C. (*Moch.*) ***malayi*** Leicester, 1908
C. (*Neoculex*) ***brevipalpis*** Giles, 1902

Genus *Ficalbia* Theobald, 1903
F. (*Mimomyia*) ***chamberlaini*** Ludlow, 1904
F. (*Etorleptiomyia*) ***luzonensis*** Ludlow, 1905

Genus *Mansonia* Blanchard, 1901
M. (*Mansonioides*) ***uniformis*** Theobald, 1901
M. (*M.*) ***longipalpis*** Vander Wulp, 1892

Genus *Orthopodomyia* Theobald, 1904
O. ***anopheloides*** var. ***maculata*** Theobald, 1910

Genus *Tripteroides* Giles, 1904
T. aranoides Theobald, 1901
T. powelli Ludlow, 1909
T. similis Leicester, 1908

Genus *Uranotænia* Lynch Arribalzaga, 1891
U. annandalei Barraud, 1926
U. bicolor Leicester, 1908.
U. campestris var. *zelena* Barraud, 1934.
U. edwardsi Barraud, 1926
U. luteola Barraud, 1934
U. macfarlanei Edwards, 1914.
U. maxima Leicester, 1908
U. obscura Edwards, 1915
U. recondita Edwards, 1922
U. stricklandi Barraud, 1926
U. testacea Theobald, 1904

Of the species listed above, the subgenus *Aedes* and *Aedes* (*Aedes*) *dux*, *A.* (*Aedim.*) *mediolineatus*, *A.* (*Aedim.*) *tæniorhynchoides*, *A.* (*F.*) *greeni* var. *kanaranus*, *A.* (*F.*) *saxicola*, *A.* (*S.*) *subalbopictus*, *Culex* (*C.*) *bitæniorhynchus* var. *ambiguus*, *C.* (*Loph.*) *cinctellus*, *C.* (*Loph.*) *fraudatrix*, *C.* (*Loph.*) *fraudatrix* var. *annulata*, *C.* (*Loph.*) *minor* var. *bengalensis*, *C.* (*Moch.*) *hinglungensis*, *Ficalbia* (*M.*) *chamberlaini*, *Orthopodomyia anopheloides* var. *maculata*, *Tripteroides similis*, *Uranotænia bicolor*, *U. campestris* var. *zelena*, *U. edwardsi*, *U. luteola*, *U. recondita*, *U. stricklandi* are new records in China. (Figs. 1 to 5).

A new species, *Culex* (*Mochthogenes*) *hinglungensis*, found in Hainan Island, was described in Chinese (Chu, 1957), a translation of which is given below:—

Male.—It is a small-sized dark mosquito, closely related to *Culex* (*Mochth.*) *pluvialis*, but differs from the latter in male terminalia. The lateral plate of phallosome has no minute teeth or spines.

Head.—There is a large area of flat scales with some narrow, curved and upright scales on the vertex and the nape. Antennæ, palpi and proboscis are dark brown in colour, and the palpi are about 1/6th of the length of the proboscis.

Thorax.—Mesonotum and scutellum are covered with narrow, curved and dark brown scales. Pleaura are dark brown, without scales. Wings with dark-scales, are about 2 cm. long. Legs are dark brown.

Abdomen.—Dark brown dorsally, paler ventrally.

Hypopygium.—Subapical lobe of coxite has three leaf-like processes, two bristles and two rods. Style is slender, curved, with a terminal finger-like appendage and a small hair in the anterior 1/3 rd. Lobes of phallosome have no minute teeth or spines.

The above is described from a single male specimen caught in the crevice of rocks near mountain streams at Hing-lung, Hainan Island, in May 1954. The type specimen, consisting of a single male, is preserved in the Department of Parasitology, Second Military Medical University, China.

FIG. 1-2. The male hypopygium of *Culex* (*Moch.*) *hinglungensis* Chu.
3. The male hypopygium of *Aedes* (*Aedes*) *dux* Dyar and Shannon.
4. The female hypopygium of *Aedes* (*Aedes*) *dux* Dyar and Shannon.

FIG. 5. *Pelagia* *sanhoptus* Fr., one of the natural breeding places of *Anles* (*F.*) *gravi* Ward *Sonnanus* Barraud in the rainy season.

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FILARIASIS SURVEY IN THE LACCADIVE, MINICOY AND AMINDIVI ISLANDS—MADRAS STATE.

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INTRODUCTION.

THIS paper briefly summarises the results of filariasis survey conducted in Laccadive, Minicoy and Amindivi islands, as part of the general health and medical survey, during the period December, 1954 to February, 1955. The Laccadive Archipelago forms a long narrow belt, extending due north and south from the level of the South Kanara District in latitude 14°N. to Adu Atoll in latitude 40°S. with Maladive islands, in the Arabian Sea.

There are ten inhabited islands in the Laccadive Archipelago—Minicoy, Kalpeni, Androth, Kavarathi, Agathi, Chetlat, Kiltan, Bitra, Ameni and Kadamath, besides a few uninhabited sand banks (Map 1). The first 5 islands of this group were under the control of the Collector of Malabar and the rest under the Collector of South Kanara District. Their administrative headquarters were at Mangalore and Kozhikode of erstwhile Madras State. After reorganization of the States in 1956, these islands have come under the administrative control of the Union Government.

TOPOGRAPHY, SOIL AND HYDROGRAPHICAL FEATURES.

The islands which are long and narrow, lie generally from north to south, except Androth which lies east to west. The inhabited portion is generally in the broadest part of the island. Every island has a lagoon excepting Androth, on its western side and a storm beach on its eastern side. The lagoon which is shallow,

MAP I.

N
MANGALORE
CHETLAT
BITRA
KILTAN
KADAMATH
AMINI
AGATHI
ANDROTH
KAVARATHI
CANNANOOR
INDIA
KOZHIKODE
PONNANI
SANALIPAR
KALPANI
COCHIN
WELLINGTON
COMERIN
MINICOY.
REFERENCE
FILARIAL
NON-FILARIAL
UNINHABITED
MAP
SHOWING THE LACCADIVE ARCHIPELAGO
NOT TO SCALE

with its bottom filled with sand and coral and semi circular in shape, protects the island from the fury of the sea during the south-west monsoon.

The land in all the islands is almost flat and about 10-15 feet above mean sea level. The soil in the middle strip of the island is undulating and somewhat rich. The surface is sandy elsewhere and is poor. The top layer—about 4-5 feet in depth—consists mainly of finely disintegrated coral with scanty admixture of surface humus. Under this top layer, there is a compact but porous layer of lime stone varying from six inches to two feet in thickness. Beneath this lime stone stratum, there is a layer of sand containing potable water which is abundant and perennial.

The hydrographic conditions may be briefly summarised and enumerated as follows:—

(*i*) Natural formations of water sources in the form of swamps and marshes are totally absent.

(*ii*) *Tanks.*—These may be classified as of two kinds, namely, the mosque tanks and the domestic tanks—the former being used for religious and lay ablutions by men only, while the latter used solely by women for bathing, washing and ablutions. They are coloured, turbid and invariably with high degree of pollution. While the mosque tanks are few in number; the number of the so called women bathing tanks is rather formidable, every three or four houses having one. The entire inhabited areas of the islands are practically littered with these tanks and step wells. All these tanks have their sides pitched with coral stones and with vegetations grown to provide privacy.

(*iii*) Except in Minicoy and partly in Androth, the rest of the islands have a number of step down wells or mere sand pits which provide drinking water.

(*iv*) Some of these islands have nursery plots for raising coconut seedlings. They are invariably low-lying shallow depressions with the top soil scooped out. A deep trench is made around the nursery bed and owing to the high subsoil water table, these trenches hold up water. Similar trenches are cut in the house gardens where betel vine and other crops are raised.

(*v*) Pits, for soaking coconut husk, are found in most of the inhabited islands. Their number and extent vary in different islands. Though coconut soaking is done in the beach because of the superior quality of the coir yielded thereby, yet during monsoon months when sea is rough, they have perforce to do it in inland pits. On the islands of Kiltan and Chetlat, no husk soaking is done anywhere inland.

The sub-soil water level is high but there is no water logging. Due to the porous nature of the soil, and drainage being easy there are no open stagnations. There are practically no sewage or sullage collections around houses, as people use very little water, bathing being done in tanks and sea by women and men respectively.

POPULATION—HOUSING—OCCUPATION.

The total area of all the ten inhabited islands is about ten square miles—the largest being Minicoy with 1·75 square miles in area while the smallest, Bitra,

is only 26 acres in extent. The population of all the ten islands put together is 20,902 (1951 census). The density of population varies from island to island—the maximum being 3,200 and the average about 2,000 per square mile.

Houses are usually constructed of coral stones and with thatched roofs. Terraced buildings are less common except in Minicoy where houses are mostly of pucca materials. Ventilation in houses is uniformly poor and rooms are dark, more especially in Androth. Sewage or sullage accumulations or need for disposal of same does not arise at all, as soil is sandy and any little spilt water is immediately absorbed. There are no sanitary conveniences of any type in any of the islands and people invariably use the sea shore for answering calls of nature.

The islanders are all Muslims. Except in Minicoy, the people talk Malayalam with slightly different accent, while the inhabitants of Minicoy talk a language called Mahal. The economic level of the islander is very low and on the verge of poverty, except in Minicoy where it is slightly better. The chief occupation of the islanders is copra trade with the mainland and coir making is a cottage industry. Fishing is done purely for local consumption except in Minicoy, where "*Bonito*" or "*Mas*" fishing forms an important and valuable industry.

There are frequent intercommunications between island to island through "*odams*" or sail boats, except with Minicoy—the southern-most. For purposes of trade, the islanders visit the coastal towns of Cochin, Kozhikode and Mangalore between November and April, after the monsoon months are over.

CLIMATE AND RAINFALL.

The winds experienced are those of the two monsoons. The south-west monsoon definitely sets in towards the end of May and becomes continuous with the north-east monsoon. Sea voyage by the islanders is impossible between May and October. The islands, though fed by both the monsoons, obtain the main bulk of the rainfall through the south-west monsoon. The amount of rainfall decreases as one goes to the northern islands. The average annual rainfall in Maladives—about 80 miles south of Minicoy—is 150 inches while at Minicoy it ranges round about 100 inches. Kalpeni, which is north of Minicoy, receives only about 60-65 inches; while in Ameni, the northern-most of the Laccadive group, the rainfall is just below 60 inches.

The islands are not subjected to extremes of temperature. They maintain a steady temperature in shade between 70°F. to 90°F. The northern islands are said to be warmer than the southern islands. Agathi has the reputation of being the hottest. It is said that during the pre-monsoon months, the increasing moisture content of the air creates a rather heavy oppressive climate.

METHODS AND MATERIALS.

The filarial survey was generally rapid, commensurate with the period of stay in each island, varying from a few hours to a few days. Sampling was random, representative and unbiassed, collected by a house to house visit. People were generally very co-operative. The population sampled varied from a minimum of 10 per cent in Kalpeni to a maximum of 37 per cent in Kiltan, the overall percentage being 17·4. Examination was carried out at night between the hours

9 p.m. and 1 a.m. People, who gave clear history of attacks of filarial lymphangitis, were included under filarial disease manifestations and classified as filarial lymphangitis. Quantity of blood taken was approximately 10 c.mm. Entomological survey included collection of larvæ and adult mosquitoes and dissection of the latter, the same day. As the different stages of the developing filariæ in the mosquito were not recorded, the results have been entered only in relation to the location of the larvæ in the mosquito.

PARASITOLOGICAL FINDINGS.

Of the ten islands, only nine were surveyed. The other island, Bitra, with a population of 46, was not surveyed as the stay in this island was only just a few hours by day. Filariasis was totally absent in the three islands of (*i*) Minicoy (*ii*) Kavarathi and (*iii*) Ameni. Only *W. bancrofti* type of infection was noticed in all the remaining six islands surveyed. No case showed *W. malayi* infection during the survey.

The survey covered a total population of 20,856 of all the islands combined. Table I shows the area of each island, population, percentage of population surveyed and degree of filarial endemicity.

TABLE I.

Area of each island, population, percentage of population surveyed and degree of filarial endemicity.

Name of island	Area in sq. miles	Population (1951 census)	Percentage of population examined	Results of survey		
				Filarial infection rate	Filarial disease rate	Filarial endemicity rate
(1)	(2)	(3)	(4)	(5)	(6)	(7)
Islands with filarial endemicity.						
Kalpeni ..	1·02	2,269	10·0	9·7	16·29	25·9
Androth ..	1·67	3,651	26·1	18·19	29·70	47·8
Agathi	1·08	2,038	25·0	4·2	0·80	5·0
Chetlat ..	0·39	994	30·0	13·0	13·40	26·4
Kiltan	0·62	1,252	37·0	8·4	5·50	13·9
Kadamath ..	1·17	1,642	20·0	14·0	7·00	21·0
Islands without filarial endemicity.						
Minicoy ..	1·75	3,447	9·5	..	..	..
Kavarathi ..	1·35	2,404	10·0		..	..
Ameni	0·95	3,159	10·0	..	..	..
Total for all islands where filariasis is endemic ..		11,846		11·4	15·5	26·9

In the islands endemic for filariasis, the filarial infection rate varied from 9·7 per cent in Kalpeni to 18·19 per cent in Androth, the average being 11·4 per cent. The lowest filarial disease rate of 0·8 per cent was noted in Agathi, while Androth recorded a high disease rate of 29·7, the average filarial disease rate being 15·5. The filarial endemicity varied from 5 per cent in Agathi to 47·8 per cent in Androth.

Appendix I shows the incidence of types of disease manifestations like filarial lymphangitis, elephantiasis of limbs, genital lesions and combined afflictions. A total of 2,780 persons, representing nearly 23·4 per cent of the total population in the six islands with filariasis, were examined. Four hundred and thirty-one cases of filarial disease were recorded and are classified as below:—

Type of disease	Number recorded	Percentage to total with disease
(*i*) Elephantiasis of limbs	281	65·2
(*ii*) Elephantiasis of limbs combined with genital afflictions	23	5·3
(*iii*) Genital afflictions	73	16·9
(*iv*) Lymphangitis	54	12·5

The overall disease rate was 15·5.

Appendices II and III respectively show the distribution of filarial disease and infection in relation to age and sex. In the age group 1-5 years, the filarial disease recorded was only of lymphangitis and no case of elephantiasis was noticed. The incidence of the disease was greater amongst men than in women—238 men and 193 women, while the infection rate was almost equal in both the sexes—162 men and 163 women.

Appendix IV shows the difference in infection rate between persons with elephantiasis and those without elephantiasis.

ENTOMOLOGICAL DATA.

The mosquito fauna and vector.—During the investigation of filariasis in all the islands, the following species of mosquitoes were recorded, both from adult catches and larval collections:—

C. fatigans	*A. subpictus*
C. vishnui	*A. vagus*
C. tritaenorhyncus	*A. hyrcanus*
C. cornutus	*A. barbirostris*
Armegeres obturbans	*Aedes albopictus*
A. varuna	*Aedes edwardsi*

The mosquito fauna was, however, limited to three or four species only in each island. Minicoy recorded the largest number of culicine species, while anophelines were absent. *C. vishnui* and *A. varuna* were the commonest and were

recorded in most of the islands surveyed. *C. fatigans* constituted over 95 per cent of the total adult mosquito catches. Distribution of the different species of mosquitoes is shown in Appendix V.

C. fatigans was the only species that was found naturally infected. The overall mosquito infection rate of *C. fatigans* in all the islands during the months of survey was 12·5. Appendix VI shows the filarial infection rate in *C. fatigans* in the different islands. The infection in mosquitoes ranged between 8·6 and 18·2 per cent in five out of the six islands surveyed. Kalpeni island recorded an endemicity rate of 26·0, but peculiarly enough the infection rate in mosquito was nil during the present inquiry. Probably a longer stay might have enabled one to incriminate the vector. In view of the fact that the man-hour catch of *C. fatigans* worked out to be as high as 84·5 and this species constituted 98 per cent of the total adults caught, and this species being the only vector in all the adjoining islands, it may be presumed that this is the vector in this island also.

Anopheline breeding was confined to drinking water step wells and fresh water stagnations in trenches and pits in the low-lying "*thottams*" ("*Thottams*" are the places where paddy was attempted to be cultivated by scooping out the top layer of sand). *C. fatigans* had a marked preference for coir soaking pits and women bathing tanks with a very high degree of organic pollution, both vegetable and human. *C. vishnui* preferred to breed in mosque tanks and pits and trenches with lesser degree of organic pollution. *A. subpictus* was sometimes breeding in association with *C. fatigans*.

C. fatigans was totally absent from the islands where filariasis was not recorded, namely, (*i*) Minicoy, (*ii*) Kavarathi and (*iii*) Ameni. This fact, in all probability, accounts for the absence of filarial infection also in these three islands. In the islands with no filariasis, *C. vishnui* was the preponderating species of mosquitoes just like *C. fatigans* in the other islands with filariasis. In Minicoy, anophelines were also absent.

DISCUSSION.

Filariasis is believed to be of recent introduction in the Laccadive islands, the first island to have noticed this affliction being Androth. The first case of elephantiasis was traced in a woman who had lived in Cannanore—a filarial coastal town in the mainland—for a few years after her marriage with a mainlander and returned to her native island of Androth with elephantiasis of the lower limbs. With remarkable unanimity of opinion, the islanders testified the above as the first case of such a disease. Thereafter, during the course of a decade, the disease became well established and today within the course of about 25 years, Androth is the worst afflicted of the Laccadive islands group. The disease has gradually spread to other islands by intercommunication, though three of the islands are still completely free. In many islands with filarial disease, the history of occurrence of the first case can be traced back to over a decade.

The incidence of filarial disease in both the sexes is highest in the age group 16 - 30 years with the age group 31 - 60 years coming next. The disease rate is not high either in children less than five years old or in old people.

The filarial infection rates correspond very close with the disease rate in the islands in the several age-groups.

While the filarial infection rate appears to be nearly equal in both the sexes, the incidence of the disease is slightly higher in men than in women. The difference may be due to increase in the number of genital afflictions, more common in case of men.

As against 9·1 per cent of people with elephantiasis who showed microfilaria in night-blood, 13 per cent of rest of people with no elephantoid swelling showed microfilaria on blood examination by night.

Only *bancroftian* type of filarial infection was noticed and quite likely, infection got introduced from the highly filarious coastal towns of Cochin, Mangalore, Cannanore and Kozhikode with which, as pointed out earlier, there is free movement of local people for trade purposes. *W. malayi* infection was not noticed in any of the islands; nor were *mansonoides* mosquitoes recorded, though in the adjoining mainland, both *W. bancrofti* and *W. malayi* are prevalent. *Pistia stratiotes* is totally absent in all the islands.

C. fatigans was the sole carrier of filriasis in the islands where filariasis is endemic. They were found to breed in only one or two types of water collections with heavy organic pollution, i.e., they had special preference for tanks where women bathe and pits where coir is soaked. Programmes of eradication of all mosquitoes is quite a feasible proposition in all the islands in view of the small extent of breeding grounds with no possibility of increase to any great degree even during the rainy months. The eradication project may comprise an all-out measure—antilarval (both permanent and temporary) and anti-adult measures, and can be limited to a period of five or six months from November to April, the driest part of the year.

The three islands of Minicoy, Kavarathi, and Ameni did not show evidences of prevalence of filariasis either clinically or on blood examination nor was *C. fatigans* recorded in any of these three islands. In the Laccadive islands, the output of *C. fatigans* would seem to determine filarial endemicity. The epidemiology of filariasis in the islands may be stated as " No *C. fatigans*—no filariasis ". Why *C. fatigans* is found only in certain islands and are totally absent in certain others and what are the factors which govern its presence or absence, are questions which appear difficult to explain. The island of Kadamath is separated from Ameni island by a distance of only six miles. No two inhabited islands are so close to each other, as these two in the whole of the Laccadive groups. During the non-monsoon months, there is almost daily country craft traffic between these two islands, the time taken for a trip being only slightly over one hour. While Kadamath is highly endemic for filariasis with *C. fatigans* as the vector, the island of Ameni is completely free from filariasis, *C. fatigans* also being completely absent. The favourite habitats of *C. fatigans* larvæ, viz., pits for fibre soaking and the women bathing tanks, exist to a greater degree in Ameni which is free from filariasis than at Kadamath. The density of population also is twice as high in Ameni as that in Kadamath. It is difficult to say why there is no filariasis and no *C. fatigans* in Ameni, while filariasis is endemic and *C. fatigans* preponderates the mosquito fauna in Kadamath? The hydrogen ion concentration of the breeding water or the quantity of dissolved salts may be some factors, one does not know. In Kavarathi, one of the islands free from filariasis and *C. fatigans*, a few school going children were noticed with mottled enamel. Drinking water from three different

wells from that island was got chemically analysed and was reported to contain 0·4 p.p.m. of flourides in each of the three different sources. Whether the rest two filaria-free islands also show the same phenomenon, and if this has anything to do with the absence of *C. fatigans* and filariasis, is not known.

The island of Bitra is the smallest, where only 46 persons who have migrated from Chetlat island were staying. A filaria survey by night could not be done, but general examination showed a few cases of elephantiasis amongst them. Rapid entomological survey did not reveal the presence of *C. fatigans*. It will be of interest to note whether filariasis naturally gets extinct in that island in course of time, if the absence of *C. fatigans* is confirmed.

SUMMARY.

A filariasis survey was carried out in the Laccadive group of islands with a population of about 20,000. The islands of Kalpeni, Androth, Agathi, Kiltan, Chetlat and Kadamath were endemic for filariasis, while Minicoy, Kavarathi and Ameni were found to be free from filariasis.

The island of Bitra could not be surveyed.

W. bancrofti was the only type of infection noticed. *W. malayi* infection, *mansonioides* mosquitoes and *Pistia stratiotes* were not encountered.

The overall filarial disease rate and infection rate were 15·5 and 11·4 respectively for all the islands where filariasis was endemic.

C. fatigans was the only vector in the areas surveyed as proved by dissections.

ACKNOWLEDGEMENT.

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APPENDIX I.

Classification of filarial disease in the Laccadive islands.

Name of island	Number examined	Number with disease manifestation	With lymphangitis		With elephantiasis of lower limbs		With elephantiasis of upper limbs		With genital lesion		With elephantiasis of both upper and lower limbs		Limbs and genital lesion	
			Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Kalpeni	227	37	2	0·88	24	10·56	1	0·44	8	3·52	2	0·88	..	..
Androth	963	287	40	4·15	145	15·05	16	1·66	46	4·77	21	2·18	19	1·96
Agathi	559	5	2	0·32	3	0·48	..	..	..	..	..	..	..	..
Kiltan	472	26	2	0·42	16	3·39	1	0·21	7	1·48	..	..	..	..
Chetlat	269	56	5	1·18	34	7·94	..	..	8	1·63	5	1·16	4	0·93
Kadamath	290	20	3	1·00	11	3·79	2	0·69	4	1·40	..	..	..	..
Minicoy	330	No filarial diseases were recorded.												
Kavarathi	220													
Ameni	309													

APPENDIX II.

(*i*) *Age and sex distribution of filarial disease—Laccadive islands.*

Age group (years)	Kalpeni	Androth	Agathi	Kiltan	Chetlat	Kadamath
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
1 – 5	M, F: Nil	M 0·7; F Nil	M, F: Nil	M, F: Nil	M 5·3; F 8·9	M, F: Nil
6 – 15	M 16·2; F 2·7	M 6·9; F 3·1	M, F: Nil	M 7·6; F 15·3	M 21·4; F 17·8	M, F: Nil
16 – 30	M 16·2; F 27·2	M 25·4; F 18·1	M; F 80·0	M 15·3; F 26·9	M 19·6; F 25·0	M 15·0; F 10·0
31 – 60	M 21·6; F 16·2	M 24·3; F 20·2	M; F 20·0	M 23·1; F 11·5	M; F 1.7	M 50·0; F 25·0
Over 60	M, F: Nil	M 0·7; F 0·1	M, F: Nil	M, F: Nil	M, F: Nil	M, F: Nil

M=Male. F=Female.

APPENDIX II—(*Contd.*)

(*ii*) *Age and sex distribution in all the islands.*

Age group (years)	Males	Females	Total
1 – 5	5	5	10
6 – 15	40	24	64
16 – 30	97	89	186
31 – 60	94	74	168
Over 60	2	1	3
Total ..	238	193	431

APPENDIX III.

(*i*) *Age and sex distribution of filarial infection—Laccadive islands.*

Age group (years)	Kalpeni		Androth		Agathi		Kiltan		Chetlat		Kadamath	
		Per cent		Per cent		Per cent		Per cent		Per cent		Per cent
1 – 5	M	Nil	M	3	M	0	M		M	2·9	M	
	F	Nil	F	1·2	F	3·8	F	2·4	F		F	5·0
6 – 15	M	4·5	M	11·0	M	26·9	M	9·7	M	11·7	M	5·0
	F		F	9·8	F	23·1	F	9·7	F	11·7	F	10·0
16 – 30	M	31·8	M	22·1	M	11·5	M	7·3	M	14·7	M	15·0
	F	13·6	F	16·3	F	19·2	F	31·7	F	14·7	F	30·0
31 – 60	M	22·7	M	17·7	M	7·6	M	12·1	M	14·7	M	20·0
	F	22·7	F	15·8	F	7·6	F	26·8	F	23·5	F	15·0
Over 60	M	4·5	M	2·4	M		M		M	2·9	M	
	F		F	0·6	F		F		F	2·9	F	

M=Male. F=Female.

(*ii*) *Age and sex distribution in all the islands.*

Age group (years)	Males	Females	Total
1 – 5	6	6	12
6 – 15	36	34	70
16 – 30	60	65	125
31 – 60	54	56	110
Over 60	6	2	8
Total ..	162	163	325

APPENDIX IV.

Infection rates amongst persons with and without elephantiasis—Laccadive islands.

Name of island		Number examined	Positive for microfilaria	Percentage
Kalpeni	With elephantiasis ..	35	2	5·7
	Without elephantiasis ..	192	20	10·4
Androth	With elephantiasis ..	247	29	11·7
	Without elephantiasis ..	716	134	18·7
Agathi	With elephantiasis ..	5	Nil	Nil
	Without elephantiasis ..	608	26	4·2
Kiltan	With elephantiasis ..	24	Nil	Nil
	Without elephantiasis ..	448	40	9·0
Chetlat	With elephantiasis ..	34	1	3·0
	Without elephantiasis ..	235	33	14·1
Kadamath	With elephantiasis ..	17	1	6·0
	Without elephantiasis ..	267	39	14·6

APPENDIX V.

Distribution of different species of mosquitoes in Laccadive islands.

Species of mosquitoes	Minicoy	Kalpeni	Androth	Kavarathi	Agathi	Kiltan	Chetlat	Bitra	Ameni	Kadamath
C. tritænorhyncus ..	+	—	—	—	—	—	—	—	—	—
C. vishnui	+	—	+	+	+	—	+	+	+	+
C. fatigans	—	+	+	—	+	+	+	—	—	+
C. cornutus	—	—	—	+	—	—	—	—	—	—
Aedes albopictus ..	+	—	—	—	+	—	—	—	—	—
Aedes edwardsi ..	+	—	—	—	—	—	—	—	—	—
Armigeres obturbans ..	+	+	—	—	—	+	—	—	+	—
A. subpictus ..	—	+	+	+	—	—	+	—	+	+
A. vagus	—	+	+	—	—	—	—	—	—	—
A. varuna	—	—	+	+	+	+	+	—	+	—
A. barbirostres ..	—	+	—	—	—	—	—	—	—	—
A. hyrcanus ..	—	+	—	—	—	—	—	—	—	—

APPENDIX VI.

Infection rate in Culex fatigans *and stages of parasites found.*

Name of island	Number of *C. fatigans* dissected	Number found infected	Stage of parasite			Infection rate
			A.F.	T.F.	P.F.	
Kalpeni ..	140	Nil	..	..	..	Nil
Androth ..	147	26	17	8	1	10·8
Agathi ..	93	8	8	..	..	8·6
Kiltan ..	107	18	6	7	5	16·8
Chetlat ..	84	13	12	1	0	15·4
Kadamath ..	115	21	15	4	2	18·2

A.F.=Abdominal form. T.F.=Thoracic form. P.F.=Probosis form.

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THE BEHAVIOUR OF *A. SUNDAICUS* RODENWALDT IN RELATION TO THE APPLICATION OF RESIDUAL INSECTICIDES IN TJILATJAP, INDONESIA.

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[January 2, 1958.]

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INTRODUCTION.

THIS paper records the entomological observations carried out during the period 1952 through 1956 in the malaria control project, Tjilatjap, mid-Java, Indonesia, sponsored jointly by the Government of Indonesia and the World Health Organization. These observations relate to the period before and after spraying residual insecticides in Tjilatjap and later in other areas of mid-Java. The author has been connected with the project as Entomologist, World Health Organization, throughout the period under report during which he was associated

with three Senior Officers of World Health Organization, Dr. L. F. Gunaratna, Dr. P. C. Issaris and Dr. F. Ronnefeldt and with national counterparts Mr. R. M. Suroto and Mr. M. Siran.

DESCRIPTION OF THE AREA.

The project area is situated between 108° 57′ and 110°E. longitude and 7° 41′ and 7° 47′ S. latitude. It is mainly confined to the coastal belt, about 120 kilometers long (extending eastward from Tjilatjap harbour) and six kilometers wide and is generally flat with a gradient of not more than approximately one meter per kilometer. The area is intersected by a number of rivers running from north to south and by a network of irrigation channels. In addition, there are many lagoons along the coast close to the estuaries of the rivers. Most of these rivers and lagoons are constantly subject to tidal action and consequently up to a distance of nearly ten kilometers inland, pockets of brackish water are encountered on either side of the rivers.

The soil which is of volcanic origin is very rich and fertile with abundant rice cultivation. Other crops like coconut, tapioca and groundnut are also extensively cultivated. The population is chiefly rural in character and agriculture is the main occupation. Fishing in the sea and lagoons is an additional occupation of those living on the coasts. The houses are situated largely in the midst of rice-fields or coconut groves and made of a temporary or semipermanent material such as wood or bamboo for the structural frame work and dried leaves of the '*nipa*' palm or bundles of '*alang*' grass for the roof. More permanent materials, such as bricks and tiles, are used by the well-to-do. The houses are generally large in size and have a number of compartments used as living or bed rooms. Kitchens form separate annexes of considerable size. The walls of the houses are lime-washed once a year. Cattle are usually tied in the open or in sheds with roofs but all sides open.

The area is subject to heavy rainfall. Humidity remains high all the year round. The average annual rainfall in this area is 3800 mm. over a period of 63 years 1879-1941 and the average number of rainy days in a year 190*. The rainfall is distributed throughout the year but the highest precipitation occurs from October to December. The rainfall figures, recorded at different points in the project area during the years 1952 to 1956, did not differ appreciably from these data. From records maintained at Tjilatjap, the yearly average minimum temperature during these five years was 23·0°C. and the average maximum temperature 36·0°C. The relative humidity varied from 70 to 90 per cent all the year round.

DETAILS OF SPRAYING RESIDUAL INSECTICIDES FROM YEAR TO YEAR (*VIDE* TABLE I AND MAP 1).

1952.—The first spraying operations were carried out during April to June in the area (Zone B1 in the Map) lying east of Tjilatjap town. The extent of this area was 118 sq. kilometers and the total population approximately 65,000. DDT 75 per cent wettable powder was used as 5 per cent concentration in water

*From "*Rainfall in Indonesia*" by H. P. Berlage, Meteorological Department, Djakarta.

MAP 1.

Tjilatjap, Indonesia.

TABLE I.

Details of spraying 1952 -- 1956 *in the project area.*

Zone	Extent (sq. km.)	Population	Particulars of spraying				
			1952	1953	1954	1955	1956
Banjumas Residency							
B - 1 ..	118	65,000	DDT (April-June)	DDT	DDT	DDT	Dieldrin
B - 2 ..	40	30,000	No spray	DDT	DDT	DDT	Dieldrin
B - 3 ..	35	15,000	DDT (October)	DDT	DDT	DDT	Dieldrin
B - 4 ..	30	20,000	No spray	No spray	No spray	No spray	Dieldrin
Kedu Residency							
K - 1 ..	280	82,000	No spray	No spray	DDT	DDT	Dieldrin
K - 2 ..	50	16,000	No spray	No spray	No spray	DDT	Dieldrin
K - 3 ..	30	11,400	No spray	No spray	No spray	Dieldrin	Dieldrin
K - 4 ..	30	15,000	No spray	No spray	No spray	No spray	No spray

NOTE.—Spraying during the year 1953 was carried out in August-October while spraying in the subsequent years was carried out in September-November.

at the rate of 2 gm./sq. meter of DDT (Tech.). Walls were sprayed only up to a height of two and a half meters from the floor. Kitchens and cattlesheds were left unsprayed. The adjoining area (Zone B2 in the Map), about 40 sq. km. in extent and with a population of approximately 30,000 was left unsprayed for comparison.

During the same year, by the middle of October, DDT spraying was extended to an area (B3 in the Map) of about 35 sq. km. and population 15,000. Kitchens and cattlesheds were also sprayed but otherwise there was no change in the spraying procedure.

1953.—The spraying operations were carried out from August to October including the area (B2 in the Map) which remained unsprayed till then. The total extent of the sprayed area (B1, B2 and B3) was 200 sq. km. and total population 1,10,300. During this operation, the roofs and the full height of the walls were sprayed. The area lying eastward (Zone B4), with an extent of 30 sq. km. and population approximately 20,000 served as comparison area.

1954.—In addition to areas (B1, B2 and B3) in Banjumas Residency, the area (K1) in the adjoining Kedu Residency received DDT spray at the same dose of 2 gm./sq. meter during the period September to November. The extent of the sprayed areas was 480 sq. km. and total population estimated as 192,000. The unsprayed areas (B4, K2, K3 and K4) are 160 sq. km. in extent with a population of approximately 63,000.

1955.—All the areas (B1 to B4) in Banjumas Residency and the area (K1) in the Kedu Residency making a total of about 500 sq. km. and including a total

population of about 241,000 were sprayed between the months September and November with DDT at 2·0 gm./sq. meter. Thus the zones B1 and B3 received their fourth round, B2 the third round, K1 the second round and B4 the first round of spray with DDT.

During this year the insecticide dieldrin was also tried for the first time in the area (Zone K3) which was about 30 sq. km. in extent and had a population of 11,400. Dieldrin 50 per cent wettable powder was used as 1·3 per cent concentration at the rate of 0·5 gm./sq. meter of dieldrin (Tech.) in January 1955.

The unsprayed zones were K2 and K4.

1956.—No area was sprayed with DDT this year. All the zones B1 to B4 and K1 to K3 were sprayed with dieldrin between the months September and November at the dose of 0·5 gm./sq. meter, leaving only K4 at the extreme end for comparison observations.

Note:—Each year only one spraying was carried out.

PRE-OPERATIONAL AND POST-OPERATIONAL SURVEY.

ENTOMOLOGICAL DATA.

Anopheline catches in Tjilatjap (Banjumas Residency) Area (vide Table II).

Table II represents the daytime catches of anophelines in the sprayed and comparison areas from July 1952 through June 1956 in Tjilatjap (Banjumas) areas. (Graph 1).

Table II.

Anopheline catches in Tjilatjap area from July 1952 to June 1956 from indoor daytime resting places.

Year and months	Sprayed area*		Unsprayed area	
	Density per man-hour		Density per man-hour	
	A. sundaicus	All other species	*A. sundaicus*	All other species
1952				
July ..	0·1	4·2	2·0	28·0
August ..	0·1	0·6	3·0	28·0
September ..	0·1	1·0	2·0	25·0
October ..	0·1	1·0	1·0	23·0
November ..	0·0	1·6	2·0	7·0
December ..	0·1	0·2	1·0	10·0
1953				
January ..	0·5	0·5	8·0	15·0
February ..	0·1	0·8	9·0	21·0
March ..	0·1	1·5	16·0	35·0
April ..	0·5	3·0	8·0	31·0
May ..	0·0	2·4	3·0	27·0
June ..	0·0	3·8	1·0	35·0

TABLE II—(*Concld.*).

Year and months	Sprayed area*		Unsprayed area	
	Density per man-hour		Density per man-hour	
	A. sundaicus	All other species	*A. sundaicus*	All other species
July ..	0·2	5·0	2·0	58·0
August ..	0·1	3·1	4·0	43·0
September ..	0·5	1·3	3·0	16·0
October ..	0·0	0·0	4·0	5·0
November ..	0·0	4·7	2·0	13·0
December ..	0·1	5·1	4·0	23·0
1954				
January ..	0·1	4·1	7·0	12·0
February ..	0·0	1·0	5·0	11·0
March ..	0·0	1·1	7·0	16·0
April ..	0·0	1·9	25·0	15·0
May ..	0·0	1·4	6·0	8·0
June ..	0·0	1·8	6·0	11·0
July ..	0·1	1·9	4·0	11·0
August ..	0·0	3·0	6·0	15·0
September ..	0·1	1·0	14·0	11·0
October ..	0·1	0·8	21·0	16·0
November ..	0·0	0·8	34·0	26·0
December ..	0·0	0·0	0·0	0·0
1955				
January ..	0·0	0·2	0·0	1·0
February ..	0·0	0·4	0·0	2·0
March ..	0·0	0·6	2·0	2·0
April ..	0·0	1·0	7·0	4·0
May ..	0·0	1·8	30·0	11·0
June ..	0·0	1·8	16·0	16·0
July ..	0·0	3·1	3·0	15·0
August ..	0·0	1·1	1·0	1·0
September ..	0·0	0·4	This area was also included for spraying during the campaign in 1955.	
October ..	0·0	0·2		
November ..	0·0	0·0		
December ..	0·0	0·2		
1956				
January ..	0·0	0·3		
February ..	0·0	0·5		
March ..	0·0	0·1		
April ..	0·2	0·4		
May ..	0·2	0·7		
June ..	0·1	0·5		

*Period of spray: 1952, April-May. DDT at 2 gm./sq. meter.
1953, August-October. " " " "
1954, September-November " " " "
1955, September-November " " " "

GRAPH 1.

Monthly prevalence of A. sundaicus and other species in Tjilatjap project area—July 1952 to June 1956.

The daytime catches of *A. sundaicus* remained low in the sprayed area throughout the period 1952 to 1956. In the unsprayed area, there was wide fluctuation with high densities in some months (16 per man-hour in March 1953, 34 in November 1954 and 30 in May 1955). From these data, one is likely to infer that DDT has brought about a considerable reduction in the density of *A. sundaicus.* However, the author's impression is that the number of mosquitoes caught during the night in sprayed areas did not appear to be materially lower than those in unsprayed areas. Some definite evidence to this effect is furnished in subsequent sections of this paper.

Anopheline catches in Kedu area.—Table III shows the daytime catches of anophelines from March 1954 to August 1956 in the adjoining Kedu area which was sprayed with DDT 75 per cent wettable powder once in November-December 1954 and again in the same months in 1955 (*Vide* also Graph 2).

In the sprayed area the pre-spray density of *A. sundaicus* is much smaller than in the unsprayed area during the corresponding months. While in sprayed area there have been no catches of *A. sundaicus* during day-time after the first spray in November and December 1954, in the unsprayed areas also the densities in 1955 and 1956 were far less than during the last four months of 1954 except in July 1956 when the density rose to 16 per man-hour. In this area while the absence in daytime catches of *A. sundaicus* in sprayed areas may, even if it betokens an actual reduction in its density, be due in great part to natural causes such as appear to have operated in the unsprayed area, further studies showed that there was appreciable contact between man and this species during the night in the sprayed areas.

Anopheline findings in dieldrin sprayed areas and unsprayed comparison area in Kedu (Vide *Table IV and Graph 3*).—The density of *A. sundaicus* in the sprayed area is less than in the unsprayed area before spraying was undertaken in the former area. The maximum density in the area sprayed later was only 22 per man-hour in November 1954 while it was 54·5 in the unsprayed area in October 1954. In 1955, the daytime densities rose in the sprayed area to 5·4 in October while in the unsprayed area it rose to 39 per man-hour in July and remained as high as 30 per man-hour in September 1955.

The second spray was carried out in November-December 1955 and in the remaining eight months the daytime catches in the sprayed areas remained nil while in the unsprayed areas the daytime density varied from 2·4 to 30·0. An essential difference in the behaviour of *A. sundaicus* after spraying with DDT and dieldrin respectively is that, while, after the application of the former insecticide the daytime densities remained low or nil all through the period subsequent to spraying, after the application of dieldrin zero or low daytime densities were found only for about 9 or 10 months and later there was an appreciable increase. This difference is important and will be discussed later.

Anopheline findings in areas sprayed with DDT for four years and with dieldrin in the fifth year.—In Tjilatjap area where four sprayings had been done with DDT since 1952, dieldrin was applied at the dose of 0·5 gm./sq. meter in September 1956. The anopheline findings up to June 1956, following the four sprayings with DDT, are already furnished in Table I. In Table V are given the findings in the fifth year subsequent to the dieldrin spray.

TABLE III.

Anopheline catches in Kedu area from March 1954 to July 1956, from indoor daytime resting places.

Year and months	Sprayed area*		Unsprayed area	
	Density per man-hour		Density per man-hour	
	A. sundaicus	All other species	*A. sundaicus*	All other species
1954				
March ..	0·2	4·0	8·0	62·0
April ..	0·1	4·0	5·0	35·0
May ..	0·1	6·0	1·0	21·0
June ..	0·3	10·0	1·0	33·0
July ..	0·3	13·0	2·0	44·0
August ..	0·0	8·0	7·0	48·0
September ..	0·4	5·0	13·0	27·0
October ..	0·2	3·0	35·0	54·0
November ..	0·0	2·0	30·0	60·0
December ..	0·0	0·0	10·0	36·0
1955				
January ..	0·0	0·0	5·0	21·0
February ..	0·0	0·0	2·0	17·0
March ..	0·0	0·0	1·0	11·0
April ..	0·0	0·0	2·0	13·0
May ..	0·0	0·0	3·0	15·0
June ..	0·0	0·0	2·0	39·0
July ..	0·0	0·0	6·0	28·0
August ..	0·0	0·0	2·0	15·0
September ..	0·0	0·0	6·0	18·0
October ..	0·0	0·0	8·0	21·0
November ..	0·0	0·0	4·0	7·0
December ..	0·0	0·0	1·0	10·0
1956				
January ..	0·0	0·0	6·0	48·0
February ..	0·0	0·0	1·0	10·0
March ..	0·0	0·0	1·0	13·0
April ..	0·0	0·0	1·0	50·0
May ..	0·0	0·0	3·0	35·0
June ..	0·0	0·0	7·0	28·0
July ..	0·0	0·0	16·0	37·0

*Periods of spray: 1954, November-December DDT at 2 gm./sq. meter.
1955, November-December DDT at 2 gm./sq. meter.

It has already been pointed out in Table II that little or no daytime resting was found on surfaces sprayed with DDT for four years. This absence of daytime resting in indoor places continued to occur even after DDT was replaced by dieldrin (Table V). There was also no material reduction in malaria. In fact there was no difference whatsoever as regards either daytime resting or nocturnal contact between man and mosquito (of this species) after dieldrin had been substituted for DDT.

TABLE IV.

Anopheline catches in dieldrin sprayed area and unsprayed comparison area in Kedu from March 1954 *to December* 1956, *from indoor day-time resting places.*

Year and months	Sprayed area*		Unsprayed area	
	Density per man-hour		Density per man-hour	
	A. sundaicus	All other species	*A. sundaicus*	All other species
1954				
March ..	8·5	57·0	8·8	42·0
April ..	2·2	33·5	7·7	49·4
May ..	1·0	14·0	1·5	27·0
June ..	0·2	25·0	1·0	30·6
July ..	0·5	73·0	2·5	32·0
August ..	1·0	25·0	5·1	64·0
September ..	2·0	27·0	24·0	56·0
October ..	10·0	73·0	54·5	133·5
November ..	22·0	97·0	46·5	137·0
December ..	10·0	74·0	18·5	87·0
1955				
January ..	0·0	0·2	8·3	22·7
February ..	0·0	3·0	7·0	34·0
March ..	0·0	0·0	8·0	25·0
April ..	0·0	0·0	7·5	14·0
May ..	0·0	5·5	26·2	30·0
June ..	0·0	9·0	15·7	21·7
July ..	0·0	6·5	39·0	42·0
August ..	0·0	9·5	12·0	17·0
September ..	1·6	47·0	30·0	37·0
October ..	5·4	20·0	28·0	30·0
November ..	1·3	8·4	11·0	13·0
December ..	0·0	0·0	13·0	14·0
1956				
January ..	0·0	0·0	30·0	37·0
February .	0·0	0·0	3·5	7·0
March ..	0·0	0·0	5·0	9·0
April ..	0·0	0·0	2·4	10·0
May ..	0·0	0·0	12·0	41·5
June ..	0·0	0·0	6·5	27·0
July ..	0·0	5·0	15·0	51·0
August ..	0·0	0·0	5·0	12·0
September ..	1·0	1·2	3·4	16·0
October ..	1·0	2·3	7·9	12·2
November ..	3·0	4·1	4·2	17·3

*Periods of spray: I spray January 1955, dieldrin at 0·5 gm./sq. meter.
II spray November-December 1955, same dose.

TABLE V.

Anopheline findings in Tjilatjap area following the dieldrin spray in the fifth year; from indoor daytime resting places.

Month and year	Sprayed area	
	Density per man-hour	
	A. sundaicus	All other species
September 1956 ..	0·0	0·0
October „ ..	0·0	0·0
November „ ..	0·0	0·1
December „ ..	0·0	0·0
January 1957 ..	0·0	0·5
February „ ..	0·0	0·4
March „ ..	0·1	0·2

RESULTS OF DISSECTIONS.

The data regarding dissections of anophelines carried out in Tjilatjap and Kedu areas during the period 1952 to 1956 are summarised in Table VI.

In Tjilatjap unsprayed area, the annual mosquito infection rates in *sundaicus* were 0·27 per cent in 1952, 0·16 per cent in 1953, 0·27 per cent in 1954 and nil in 1955 (the number dissected were too small, only 250 as compared with thousands in previous years due to the transfer of most of the available technicians from Tjilatjap area to other areas).

In Kedu unsprayed area, these rates were 0·46 per cent in 1954, 0·28 per cent in 1955 and 0·28 per cent in 1956. In the Kedu area, included for spraying with DDT, the pre-spray infection rate was 0·35 per cent.

In Tjilatjap area, *A. subpictus* also showed infections in 1953 (0·8 per cent) and in 1954 (0·2 per cent) and in Kedu area this species was found infected once in 1954 (0·03 per cent).

In DDT sprayed area in Tjilatjap, infections in *A. sundaicus* were met with in 1 out of 31 in 1952 (3·2 per cent), 1 out of 340 in 1953 (0·29 per cent), 2 out of 2,227 in 1954 (0·09 per cent) and nil out of 456 in 1955. No infections were met with in the few dissections carried out in *A. subpictus*. No dissections were made in Kedu area after DDT spray as the field stations in that zone had to be closed for unavoidable reasons.

GRAPH 2.

Monthly prevalence of A. sundaicus *and other species in Kedu area—March 1954 to July 1956.*

Unsprayed area.

GRAPH 3.

Density of A. sundaicus *and other species in Kedu area (March 1954 to August 1956).*

Unsprayed area.

Dieldrin trial area.

(0·5 gm./sq. meter)

TABLE VI.

Summary of dissections of anophelines.

Year	Zone	I Quarter		II Quarter		III Quarter		IV Quarter		Total		Other species
		A. sundaicus	*A. subpictus*	*A. sundaicus*	*A. subpictus*	*A. sundaicus*	*A. subpictus*	*A. sundaicus*	*A. subpictus*	*A. sundaicus*	*A. subpictus*	
	1. Tjilatjap area.											
1952	Unsprayed ..	112 (2, 1)	1115 (0. 0)	130 (1, 0)	272 (0, 0)	1598 (2, 0)	559 (0, 0)	721 (1, 0)	516 (0,0)	2561 (6, 1)	2462 (0, 0)	8700 (0, 0)
	Sprayed ..	..	..	..	..	11 (0, 0)	4 (0, 0)	20 (0, 0)	7 (0, 0)	31 (1, 0)	11 (0, 0)	48 (0, 0)
1953	Unsprayed ..	744 (1, 1)	602 (2, 0)	397 (0, 0)	677 (1, 0)	422 (0, 0)	530 (0, 0)	367 (0, 1)	401 (1, 0)	1930 (1, 2)	2210 (4, 0)	4038 (0, 0)
	Sprayed ..	207 (0, 0)	27 (0, 0)	51 (0, 0)	39 (0, 0)	34 (0, 0)	60 (0, 0)	48 (1, 0)	553 (0, 0)	340 (1, 0)	679 (0, 0)	1138 (0, 0)
1954	Unsprayed ..	1705 (2, 9)	1142 (1, 7)	2184 (0, 5)	1025 (0, 1)	3805 (0, 7)	1255 (0, 0)	2934 (0, 6)	981 (0, 0)	10628 (2,27)	4403 (1, 8)	2853 (0, 0)
	Sprayed ..	270 (0, 1)	62 (0, 0)	677 (0, 1)	38 (0, 0)	917 (0, 0)	79 (0, 0)	363 (0 ,0)	33 (0, 0)	2227 (0, 2)	212 (0, 0)	1329 (0, 0)
1955	Unsprayed ..	25 (0, 0)	69 (0, 0)	175 (0, 0)	56 (0, 0)	50 (0, 0)	8 (0, 0)	0 (0, 0)	0 (0, 0)	250 (0, 0)	133 (0, 0)	1200 (0, 0)
	Sprayed ..	1 (0, 0)	2 (0, 0)	404 (0, 0)	191 (0, 0)	81 (0, 0)	15 (0, 0)	0 (0, 0)	0 (0, 0)	486 (0, 0)	208 (0, 0)	480 (0, 0)
	2. Kedu area.											
1954	Unsprayed ..	355 (0, 9)	197 (0, 0)	273 (0, 1)	854 (0, 0)	992 (0, 3)	1322 (0, 0)	3309 (0,10)	2187 (0, 0)	5029 (0,23)	4560 (0, 0)	4562 (0, 0)
	DDT area Pre-spray ..	287 (0, 2)	541 (0, 0)	463 (0, 3)	554 (0, 0)	1006 (0, 1)	1327 (0, 0)	483 (0, 2)	100 (0, 0)	2239 (0, 8)	2522 (0, 0)	2403 (0, 0)
	Dieldrin area Pre-spray ..	287 (0, 3)	208 (0, 1)	368 (0, 0)	787 (0, 0)	734 (0, 2)	910 (0, 0)	2581 (0, 7)	1724 (0, 0)	3970 (0,12)	3629 (0, 1)	2965 (0, 0)
1955	Unsprayed ..	804 (0, 1)	1127 (0, 0)	1167 (0, 5)	341 (0, 0)	1035 (0, 4)	31 (0, 0)	966 (0, 1)	85 (0, 0)	3972 (0,11)	1484 (0, 0)	7621 (0, 0)
	DDT area Post-spray ..	0	0	0	0	0	0	0	0	0	0	0 (0, 0)
	Dieldrin area Post-spray ..	0	4 (0, 0)	3 (0, 0)	3 (0, 0)	29 (0, 0)	22 (0, 0)	110 (0, 0)	80 (0, 0)	142 (0, 0)	109 (0, 0)	1138 (0, 0)
1956	Unsprayed ..	1543 (0, 3)	84 (0, 0)	954 (0, 1)	86 (0, 0)	1808 (0, 8)	4 (0, 0)	..	..	4305 (0,12)	174 (0, 0)	8625 (0, 0)
	DDT area ..	0	0	0	0	0	0	0	0	0	0	0
	Dieldrin area	0	0	54 (0, 0)	0	0	0	0	0	54	0	86 (0, 0)

NOTE.—The first figure in brackets shows number found with oocysts and second figure number with sporozoites.

In Kedu area, sprayed with dieldrin as the first insecticide itself, the pre-spray infection rate was 0·3 per cent in *A. sundaicus* and 0·03 per cent in *A. subpictus* (1954). After spraying, only a few dissections were made due to paucity of mosquitoes and no infection was met with.

Day-time and night-time catches compared (Vide Table VII).

Day-time catches from resting surfaces in unsprayed houses and cattlesheds and through window traps show that about 80 per cent of the endophagous mosquitoes are also endophilic. After DDT spray for two seasons, the endophily is reduced to 8·0 per cent though endophagy perhaps continued to occur nearly to the same extent. This may be due to (1) the insecticide having irritated and excited the mosquitoes when they rested on the sprayed surfaces and driven them out; (2) the mosquitoes having altered their behaviour so as to avoid having any contact with sprayed surface, or (3) a combination of the above two phenomena, or (4) the first phenomenon in the first instance followed by the second at a later stage (Issaris and Sundararaman, 1954).

Table VII.

Day-time and night-time catches of A. sundaicus *in DDT sprayed* and in unsprayed comparison areas during the period March* 1954 *to December* 1955.

Area	Day-time catches						Night-time catches†					
	Total time spent in man-hours	Total *A. sundaicus*	Resting on walls		In window traps‡		Total time spent in man-hours	Total *A. sundaicus*	Resting on walls		On human baits	
			Number found	Per cent	Number found	Per cent			Number found	Per cent	Number found	Per cent
DDT sprayed ..	1,240	338	26	7·8	312	92·2	86	1,110	90	8·1	1,020	91·9
Unsprayed ..	1,240	4,887	4,008	82·0	879	18·0	86	2,728	1,410	51·7	1,318	48·3

Note.—During March-December, 1954, there were 9 traps in the DDT sprayed area and the total number of attempts was 378; and from January to September, 1955, there were 18 traps and the total number of attempts was 988. In unsprayed area, there were 5 traps throughout and the number of attempts was 850.

*First spray in April-July 1952, second in September-November 1953 and third in September-November 1954.

†The collections on each occasion are for 20 minutes at hourly intervals from 18.00 to 6.00 hours.

‡Window traps inspections at 6.00 a.m.

In unsprayed areas, the number caught per man-hour during the night on resting surfaces was about equal to that caught in the act of biting man but in areas sprayed with DDT only 8·0 per cent were found resting on sprayed surfaces and

the rest actually biting man. Judging from the man-hour catches, while there was some decrease in sprayed areas in night-time catches (daytime catches being almost nil), the proportion biting man during the night showed no absolute increase in sprayed areas but the number resting on sprayed surfaces showed both an absolute and relative reduction. This shows that a certain proportion that has been diverted from endophily to exophily, probably leave the house without a feed, having been excited by contact with sprayed surface.

In areas sprayed with dieldrin in 1956 (September) after four earlier annual applications of DDT, the daytime catches during the subsequent months continued to be nil or very few (Table V). But in the night catches six months after spray, 92 per cent of *A. sundaicus* were found in the act of biting human baits and only 8 per cent resting on the walls, which is exactly what was observed in the DDT sprayed areas (Table VIII).

TABLE VIII.

Night-time catches of A. sundaicus *during January and February,* 1957, *in areas that had been sprayed annually with DDT since* 1952 *and with dieldrin in September,* 1956, *and in unsprayed comparison area.*

Area	Night-time catches				
	Total *A. sundaicus* caught	Resting inside on walls		On human baits	
		Total	Per cent	Total	Per cent
At Selok and Ketapang sprayed with DDT until 1955 and with dieldrin in 1956 ..	795	62	8·0	733	92·0
In exclusively unsprayed area Pasir Gedangan	496	235	47·3	261	52·7

On the other hand, results of a different nature were observed in an area where in January 1955, dieldrin was sprayed as the first insecticide at a dosage of 0·5 gm./sq. meter. Following the dieldrin spray, the daytime collections remained as shown in Table IV, nil for seven months, followed by a slight increase thereafter. The catches during the nights during the period from February to December, 1955, were almost nil as shown in Table IX.

It is thus quite evident that for nearly nine to ten months, following the spray, there were practically no vectors in houses either at night or during daytime.

LETHAL EFFECTS OF DDT ON *sundaicus*.

In these excitory contacts, apparently the insecticide is not picked up to the extent of having lethal effect on a majority of the mosquitoes as Table X would show.

TABLE IX.

Night-time catches of A. sundaicus *during February to December* 1955 *in area that had been sprayed with dieldrin as the first insecticide at* 0·5 *gm./sq. meter in January,* 1955.

Area	*NIGHT-TIME CATCHES				
	Total *A. sundaicus* caught	Resting inside on walls		On human baits	
		Total	Per cent	Total	Per cent
Pasir Malang area sprayed with dieldrin at 0·5 gm./sq. meter in January, 1955, for the first time	1	1	100·0	0	0·0
In unsprayed area Pasir Gedangan during the same period ..	680	357	52·5	323	47·5

*Twelve nights; the collection on each occasion for 20 minutes at hourly intervals from 18.00 to 6.00 hours.

TABLE X.

Mortality observations among A. sundaicus *captured during daytime (morning) inside DDT sprayed structures and in window traps.*

(March, 1954 to September, 1955).

Months after spray	ON WALLS INSIDE HUTS		IN WINDOW TRAPS		
	Number observed	24 hour mortality. Number dead	Number observed	24 hour mortality	
				Number dead	Per cent
1 – 4 ..	15	14	191	62	32·0
5 – 8 ..	13	3		None found	
9 – 12 ..	5	2	94	12	13·0
Controls ..	Not observed		740	89	12·0

NOTE.—From March to December, 1954, there were 9 traps in the DDT sprayed area and the total number of attempts was 378 and from January to September, 1955, there were 18 traps and total number of attempts was 988. In unsprayed area, there were 5 traps throughout and the number of attempts was 850.
This is further shown in Table XI.

Table XI shows that the mosquitoes that feed on man in houses sprayed with DDT, do not suffer any appreciable mortality and hence no reduction in transmission may be expected.

TABLE XI.

Mortality observations among A. sundaicus *captured during night-time on human baits and on walls inside DDT sprayed structures.*

(March, 1954 to September, 1955).

Months after spray	Resting on walls		On human baits		
	Number observed for mortality	24-hour mortality. Number dead	Number observed for mortality	24-hour mortality	
				Number dead	Percentage
1	1	1	46	4	9·0
2	0	0	32	1	3·1
3	3	2	52	6	11·5
4	1	1	43	8	18·6
5	1	0	90	4	4·4
6	7	3	140	7	5·0
7	0	0	12	0	..
8	2	1	51	4	7·8
9	11	4	214	11	5·1
10	4	2	94	16	17·0
11	0	0	16	4	..
12	1	0	12	3	..
Total ..	31	14 (45 per cent)	802	68	8·5

CHANGES IN RESTING BEHAVIOUR IN AN EXPERIMENTAL HUT SPRAYED WITH DDT WHEN THE VILLAGE ITSELF REMAINED UNSPRAYED.

TABLE XII.

Night-time catches in an experimental hut sprayed with DDT at Ketapang village when the village itself remained unsprayed.*

(July, 1954 to August, 1955) 16 nights.

Total *A. sundaicus*	Resting on walls		On human baits	
	Total	Per cent	Total	Per cent
1340	525	39·1	815	60·0

* Sprayed on 19th July 1954 at 2·0 gm./sq. meter.

The behaviour is more like in unsprayed villages. As much as 39·0 per cent are found to rest on sprayed walls during the night and 60·0 per cent biting man (compared with 51·7 and 48·3 respectively in unsprayed villages and 8·1 per cent and 92 per cent respectively in villages entirely sprayed).

GUT AND OVARIAN CONDITIONS OF *A. sundaicus* BEFORE AND AFTER SPRAYING.

TABLE XIII.

Gut and ovarian conditions of A. sundaicus.

	Total examined	GUT AND OVARIAN CONDITIONS							
		Freshly fed	Per cent	Half gravid	Per cent	Gravid	Per cent	Unfed	Per cent
				Before spray					
1. Inside huts on walls ..	3,242	1,641	50·6	540	16·6	696	21·4	366	11·4
2. In window traps	664	261	39·3	118	17·8	170	25·6	115	17·3
				After spray					
1. Inside huts on walls ..	142	47	33·2	2	1·4	2	1·4	91	64·0
2. In window traps	254	59	23·2	9	3·6	52	20·4	134	52·8

NOTE.—All collections were done in the mornings.

This shows that in sprayed areas, a much greater proportion of catches, made in the morning, remains unfed. A high proportion leaves the houses before feeding, presumably irritated by contact with sprayed surface.

TIME OF ENTRY OF *A. sundaicus* AND TIME OF FEEDING (UNSPRAYED AREAS).

TABLE XIV.

(i) *Observations on the time of entry of* A. sundaicus *at night.*

Period of collection at night	Number of *A. sundaicus* caught	Percentage out of the total
1st quarter ..	400	17·1
2nd quarter ..	666	28·4
3rd quarter ..	787	33·6
4th quarter ..	489	20·9

TABLE XIV—(*Concld.*).

(*ii*) *Observations on the time of feeding of* A. sundaicus *at night.*

Period of collection	Total examined	Number found unfed	Percentage out of total unfed	Number found fed	Percentage out of total fed
1st quarter ..	137	93	26·4	64	13·8
2nd quarter ..	199	102	36·9	97	20·9
3rd quarter ..	212	57	20·6	155	33·4
4th quarter ..	192	44	16·1	148	31·8
	740	276	100·0	464	100·0

The above findings show that entry takes place all through the night and in larger proportions before and after midnight. Feeding also takes place all through the night but relatively more after midnight.

OUT-DOOR RESTING BY *A. sundaicus.*

TABLE XV.

(*i*) *Distribution in different types of out-door shelters.*

Time spent man-hours	Rock crevices		Hollows in sand banks		Plant bushes		Other types		Total number
	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	
1,000	805	54·4	331	22·4	48	2·7	298	20·5	1,482

(*ii*) *Abdominal condition of* sundaicus *in out-door shelters.*

Unfed		Freshly fed		Half gravid		Gravid		Total number
Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	
427	42·1	270	26·7	117	11·5	199	19·7	1,013

The findings in Table XV show that there is very definite evidence of exophily.

Hosts of predilection.

Results of precipitin tests on *sundaicus* blood are as shown in Table XVI (*i*).

Table XVI (*i*).

Period	Where collected	Where tested	Number tested	Positive for		Anthropophilic index
				Man	Ox	
September, 1951 to April, 1954	Houses	Eykman Institute, Djakarta	453	217	..	48
June, 1954 to November, 1954	Houses	Malaria Institute, Delhi	2,487	1,210	428	74
September, 1951 to April, 1954	Outdoor shelters	Eykman Institute, Djakarta	135	34	..	25
June, 1954 to November, 1954	Outdoor shelters	Malaria Institute, Delhi	357	74	234	24

(Note.—Results from Eykman Institute, Djakarta, indicated only whether the samples tested were positive for human or not. Results from Malaria Institute, Delhi, indicated whether positive for human or bovine).

Further tests at Lister Institute, London, on blood samples sent there between July, 1955 and December, 1955 gave the results as shown in Table XVI (*ii*).

Table XVI (*ii*).

Place collected	Number tested	Number positive for						Anthropophilic index (per cent)
		Man	Ox	Fowl	Pig	Mixed	Total	
Houses	496	328	118	1	2	6	455	72

The specimens collected from houses are shown to be predominantly anthropophilic, being over 70·0 per cent in a majority of cases and those collected from outdoor shelters contained appreciable numbers with human blood.

Susceptibility of *sundaicus* to DDT.

During the period April, 1955 to December, 1955 a series of tests on the susceptibility of *A. sundaicus* to DDT was carried out using mostly engorged female *sundaicus* collected exclusively from the unsprayed sectors of Tjilatjap and Kedu areas*. The results of the tests are summarized in Table XVII.

*The method adopted was that of Busvine and Nash as described in the Fifth Report of the World Health Organization Expert Committee on Malaria (1954).

TABLE XVII.

Summary of results of susceptibility tests to DDT on A. sundaicus *from unsprayed areas in Tjilatjap and Kedu during April to December,* 1955.

Concentration of DDT used in test	Number tested	Dead in 24 hours	Observed mortality per cent	Corrected* mortality per cent
2·0 per cent ..	961	936	97·4	97·2
1·0 „ „ ..	922	768	83·4	82·5
0·75 „ „ ..	672	410	61·0	58·9
0·5 „ „ ..	835	430	51·5	48·9
0·25 „ „ ..	454	90	19·8	15·5
Controls ..	859	44	5·1	..

*Correction applied by ABBOTT'S formula.

This shows that the median lethal concentration of *A. sundaicus* to DDT is 0·5 per cent and that this species is highly susceptible to DDT.

MALARIOMETRIC DATA—SPLEEN AND PARASITE SURVEYS.

The results of spleen and parasite surveys in children (2-9 years) in DDT sprayed and comparison areas in Tjilatjap (Data taken from periodical reports submitted by Senior Officers), are given in Table XVIII.

These data do not show any substantial benefits of DDT spraying. Spleen rates have risen in sprayed areas in 1954 and 1955 though relatively by smaller proportions than in unsprayed areas. Parasite rates, instead of showing proportionate rise with spleen rates both in unsprayed and in sprayed areas, actually show a reduction which is significantly larger in sprayed areas. But as spleen rates have shown a considerable rise in sprayed areas, it has to be recognized that active transmission has taken place despite DDT spray for 4 consecutive years.

In Kedu area also where the pre-DDT spleen rates in 1954 were 33 per cent in sprayed and comparison areas, after spray the spleen rates in 1956 were 43 per cent in sprayed areas and 46 per cent in comparison areas. Parasite rates dropped to 0·2 per cent in 1956 from 11·7 per cent in 1954 in sprayed areas but they also showed a reduction from 16·5 per cent to 0·5 per cent in 1956 in comparison area.

In another area in Kedu, after dieldrin spray the spleen rate dropped from 100 per cent in 1954 to 75 per cent in 1956 while in comparison area the reduction was from 100 per cent in 1954 to 83 per cent in 1956. Likewise parasite rate came down to 2·5 per cent in 1956 from 16·1 per cent in 1954 in dieldrin sprayed area while in unsprayed area the reduction was from 22·2 per cent to 8·3 per cent in 1956.

TABLE XVIII.

Spleen and parasite surveys in children (2 - 9) years in DDT sprayed and comparison areas in Tjilatjap. (Data taken from periodical reports submitted by senior officers of the World Health Organization).

Period of survey	Sprayed area					Comparison area				
	Spleen survey			Parasite survey		Spleen survey			Parasite survey	
	Number examined	Spleen rate per cent	Average enlarged spleen	Number examined	Parasite rate per cent	Number examined	Spleen rate per cent	Average enlarged spleen	Number Examined	Parasite rate per cent
Oct. – Dec. 1951 and Jul. – Aug. 1952 ..	4706	44·0	2·1	4724	7·0	3918	30·2	2·0	3382	11·0
Jul. – Sep. 1953 ..	5715	17·2	1·9	3243	1·9	4564	12·6	1·8	2466	1·1
May – Jul. 1954 ..	6854	67·6	1·4	6656	2·0	4659	58·0	1·5	4477	3·8
Apr. – Jun. 1955 ..	6239	61·0	1·4	6239	0·5	3004	60·0	1·4	3004	0·9
Apr. – Jun. 1956 ..	6079	48·0	1·5	6079	0·2	1580	44·0	1·5	1580	0·4

INFANT PARASITE RATES.

This is further confirmed by infants who were negative on previous monthly examinations showing parasites after spraying as shown below:

	Sprayed area	Comparison area
December, 1953	4 new infections in infants negative in previous monthly examinations (out of 391).	10 new infections (out of 243).

New infections continued to occur in 1954; among 35 infants in sprayed areas against 41 infants in unsprayed areas, and in 1955 among 9 infants in sprayed areas against 3 in unsprayed area.

In Kedu area, the infant parasite rates showed such a natural decline even in comparison area (from 12 - 14 per cent to 0·8 per cent) that nil rates in 1956 out of more than 200 infants examined in areas sprayed with DDT, do not necessarily betoken any beneficial effect. But in dieldrin sprayed areas, the difference between sprayed and comparison areas is marked. In the latter area, infant parasite rates in some months in 1956 were as high as 11·3 and 16·2 per cent. In the sprayed area, only in January 1956 two infants out of 149 were positive. In all other months, no infant was positive out of more than 50 examined each month. In 1954, both in sprayed and unsprayed area the infant parasite rates were between 40 and 50 per cent.

DISPENSARY FIGURES.

These do not show any material change during the 3 years 1953, 1954 and 1955. Fever cases (17,886) accounted for 7·7 per cent of total admissions in 1953, 5·8 per cent in 1954 (17,265 cases) and 7·4 per cent in 1955 (12·631 cases).

DISCUSSION.

The data presented in this paper lead to certain conclusions listed below in sequence.

DEGREE OF STABILITY OF MALARIA.

The area under study is liable to considerable fluctuations in malaria transmission as evidenced by childhood spleen rates in the comparison villages. There are no grossly detectable changes in climate to account for the instability of malaria transmission. But as shown by Macdonald (1952) in the case of Ceylon, it is possible that minute changes do occur in Tjilatjap area and may themselves account in part or whole for the instability. The local vector, *sundaicus*, shows a high anthropophylic index (72·0 per cent) and is relatively long-lived (P or daily survival rate—0·80 as shown by Davidson, 1955) and should, therefore, normally be associated with stable malaria as postulated by Macdonald (1952, *loc. cit.*). The childhood parasite rates show such divergence from spleen rates—a fall in the former rate with a conspicuous rise in the latter—that one hesitates to draw any valid conclusions therefrom. Infant parasite rates have reached as high as 50·0 per cent in some areas in some years. On the whole, one should associate mesoendemicity with this area in normal years with a definite liability to epidemic exacerbations and post-epidemic hyperendemicity in certain years when there may be extremely favourable conditions for transmission either climatically, though not grossly detectable, or due to certain not fully recognized conditions favourable for *sundaicus* breeding and output. Changes in climatic conditions, bringing about increased transmission, are associated with an increase in (*a*) mosquito densities, (*b*) the degree of their association with man and (*c*) infection rates, (Covell, 1939). Mosquito infection rates do not show gross fluctuations in the areas under report but densities do exhibit gross variations. On the whole, therefore, the habits of *sundaicus* normally tend to stability due to endophagy, high endophily and anthropophilic index but its output is liable to changes on account of its special saline habitats in the coast being subject to gross influences and variations. Thus a good element of instability is super-imposed upon a substratum of stable malaria transmission at the level of mesoendemicity. Such happenings tend to make it difficult to draw conclusions from experimental control limited to short periods of time as natural causes may themselves account for a good part of the variations in malariometry.

THE VECTOR SPECIES.

A. sundaicus is the principal vector species along the coast. *A. subpictus* may play a subsidiary role along with *sundaicus* in some areas but it has not been found infected by itself. The highest infection rate met with is about 0·5 per cent.

Such a low infection rate, in spite of a high anthropophilic index, betokens a very low infective gametocyte rate as the longevity of the vector does not appear to be too short.

FEEDING AND RESTING HABITS.

The host of predilection for *sundaicus* is man much more than cattle or other hosts. Adult females of this species enter into houses all through the night but principally around midnight. Feeding, which is largely of the endophagous type, occurs also all through the night, more after than before midnight. Ordinarily a very large proportion of the mosquitoes rests on wall surfaces before and after feeding and during the day. There is, however, an appreciable proportion seeking outdoor shelter by day.

VULNERABILITY TO DDT.

The vector species is as vulnerable to DDT as most other vectors. Even after 3 or 4 years of application of DDT, the MLC is about 0·6 per cent as compared with 0·5 per cent before spraying commenced. Further, habits of the mosquito, such as a great degree of endophily and endophagy and a high anthropophylic index, should render malaria transmission by this species more readily amenable to control and interruption with the use of DDT.

INTERRUPTION OF MALARIA TRANSMISSION.

The observations recorded in this paper do not, however, show that malaria transmission has been appreciably reduced by spraying DDT. Spleen rates have gone up during the spraying period. Parasite rates have declined but they show mostly the same order of decline in comparison areas. New infections have occurred in infants after one, two and even three years of application of DDT. Nocturnal association of *sundaicus* with man continued to occur in sprayed areas. Mosquito infections are met with after spraying for two or three years. It is thus evident that when DDT was sprayed once a year at the dosage of 2 gm./sq. meter the objective was not achieved.

POSSIBLE REASONS FOR FAILURE OF DDT TO INTERRUPT TRANSMISSION.

RESISTANCE.

Grandell (1954) and Chow and Suparmo (1956) have shown that in certain parts of Java, *sundaicus* has acquired true physiological resistance. But in the present area where DDT has failed to interrupt transmission even after 4 annual sprays, *sundaicus* continues to be nearly as vulnerable to DDT as before spray. There is, therefore, no question of physiological resistance nor of any morphological resistance so far as this area is concerned.

BEHAVIOURISTIC CHANGE.

Muirhead Thomson (1947) and Davidson (1953) have described changes in *A. gambiae* after DDT spray to the extent that the mosquitoes are irritated by

short contacts with DDT sprayed surfaces, that they do not pick up lethal dose of the insecticides, that they continue to bite man and transmit malaria and the utility of DDT in controlling transmission is thus very largely restricted. This phenomenon has been described as excitorepellency by Viswanathan and Rao (1947) but in their areas it does not interfere with interruption of transmission. In the present observations, there is reason to believe that the phenomenon of 'excitorepellency' does occur, at all events in the early stages. But it cannot explain all the present observations. When only one hut is sprayed but not the rest of the village, *sundaicus* continues to rest on sprayed walls of the hut though in slightly smaller proportions. It is only when the whole village is sprayed and the bulk of the anopheline population is exposed to the residual insecticides that the females of their offspring avoid having more than a short period of contact with sprayed surface. Presumably such a characteristic—to fly away from irritating contacts—is an inherent characteristic of some variable proportion of the total mosquito population and when all available surfaces are sprayed, only this proportion survives and produces subsequent broods exhibiting this characteristic. That this behaviour is exhibited by all survivors and their offspring, is shown by the fact that, even when DDT is replaced by dieldrin, exactly similar happenings continue, viz., *sundaicus* caught in plenty in the act of biting man at night, very few resting on walls at night and practically none by day. When dieldrin is sprayed as the first insecticide, there is reduction in density both by day and at night and there is interruption of transmission. The capacity for all offspring to avoid any contact with resting surfaces probably takes a few years to develop but not more than four. Thus the behaviouristic change is two-fold—an initial excitorepellent phenomenon followed by survivors consisting only of that proportion of the original population which avoid contact with sprayed surfaces and their offspring also exhibiting the same characteristics. There is no gross alteration in behaviour such as from endophagy to exophagy. But the degree of exophily is increased after DDT spray and probably associated with it there is also a slightly increased exophagy. There is no evidence to show that this change is specifically induced by the application of insecticide. It may well be explained by the survival of one fraction of the original population which escaped lethal action on account of poor or absence of contact with sprayed surface. This proportion, though small in the first instance, may soon build itself to pre-spraying densities so long as there was available biotic potential for the species for larval breeding. Thus, while DDT may not have induced a change in behaviour acquired as a result of stress and critical pressure against survival, it has helped in making a small proportion of *sundaicus* having the characteristic of avoiding resting on surfaces, build up densities at the pre-spray level consisting solely of individuals exhibiting this characteristic. This is associated with the progressive disappearance of individuals not exhibiting this characteristic at all or to the same extent on account of contact with sprayed surfaces, their continued susceptibility to the insecticides and consequent lethal action.

FUTURE CONTROL OF MALARIA.

In the areas under report, substitution of dieldrin for DDT does not hold out any hopes of interruption of transmission for the reasons already stated. Either

no kind of control, except drug therapy, should be adopted for some yearsi n order to see if there is a regression in the habits of the vector (if there is, control can later be established by dieldrin spray), or antilarval methods of control have to be resorted to. Or yet again, space spraying of contact insecticides like pyrethrum has to be adopted during the night which is highly impracticable.

SUMMARY OF FINDINGS AND CONCLUSIONS.

The observations described in this paper were made during the assignment of the World Health Organization Team in Tjilatjap-Kedu coastal areas in the south coast of mid-Java province from 1952 through 1956.

The area was liable to considerable fluctuations in malaria transmission with meso-endemicity in normal years but with a definite liability to epidemic exacerbations and post-epidemic hyperendemicity.

A. sundaicus is the principal vector species along the coast. *A. subpictus* is also found to play a subsidiary rôle along with *A. sundaicus* in some areas.

Studies on the habits of *A. sundaicus* showed that the species was highly anthropophilic, that it was largely endophagous and feeding occurred all through the night but more after than before midnight. Ordinarily the mosquitoes rested on wall surfaces before and after feeding and during the day. Apparently a few avoided contact with wall surfaces. Though largely endophilic, there is some degree of exophily.

Following the DDT spray once every year, there was a virtual disappearance of *A. sundaicus* in the daytime catches but its nocturnal association with man continued. After four years of spraying, when DDT was replaced by dieldrin in the fifth year, exactly similar happenings continued, viz., abudnant *sundaicus* in the act of biting man at night, very few resting on walls at night and practically none by day.

It became obvious that DDT spray failed in its objective as spleen rates went up, new infections continued to occur in infants afte, one, two or even three years of application of DDT and mosquito infections were met with after spraying for two or three years.

When dieldrin is used as the first insecticide, there is reduction in density both by day and at night and there is interruption of transmission.

Even after four sprays with DDT, susceptibility tests showed that *A. sundaicus* in this area continued to be nearly as vulnerable to DDT as before spray and that there was no physiological resistance in this area as was the case in some other parts of Java.

The failure of DDT in this area is believed to be the result of an altered behaviouristic manifestation in *A. sundaicus*. There is good lethal action. But certain proportion avoid contact with sprayed surfaces in full or in part. They and their survivors continue to exhibit this characteristic, escape lethal action and build up densities as high as during the pre-spray period, but consisting almost entirely of individuals manifesting the characteristic of avoiding contact with surfaces by day or by night. This is possible so long as there is available biotic potential for larval breeding. There is no evidence to show that the species has

acquired this characteristic by mutation on account of the stress and critical pressure of insecticidal application.

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MALARIA CONTROL ON TEA ESTATES IN ASSAM, 1947-1956.

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INTRODUCTION.

This account of ten years of malaria control on tea estates in Assam refers to a sample of more than 3,00,000 persons resident on those estates that are members of the Indian Tea Association and that are advised by the Ross Institute of Tropical Hygiene.

The sample is 38 per cent of the total population of member estates, which itself forms about nine per cent of the population of Assam.

Since tea estates were first planted, now over 100 years ago, it has been necessary to bring in labourers and their families from Central India because Assamese are not land-hungry and do not need, or care for, manual work on estates. The estate population is now permanently domiciled and only a small proportion ever return to their original homes.

Despite their long residence in Assam, it is probable that their immunity to malaria is even now less complete than that of Assamese. Certainly in pre-control days, confronted with a vector as formidable as *Anopheles minimus* and with a climate more humid and more enervating than they had known, they felt the effects of malaria far more severely than did their indigenous neighbours in rural villages.

MEDICAL SERVICES.

Estates must by law provide hospitals whose standards of staff, equipment and construction are specified by Government. The estate hospital has a very important place in malaria control both because the control organisation is based on it and because the records of cases of malaria provide an independent check on the efficiency and success of control.

Workers and their families take full advantage of medical facilities because they have come to appreciate treatment and because a sick worker must be medically certified to qualify for paid sick leave. Malaria morbidity rates are

probably exceptional for most tropical countries in that treated cases and " actual " cases closely correspond and in well over 90 per cent the diagnosis is confirmed microscopically.

Groups of estates combine to engage Principal Medical Officers. The average population of a medical practice is 28,000, a convenient unit for health returns and one used in this review.

The entire costs of the medical services, including malaria control, are borne by the estates.

HOUSING.

The majority of labourers' houses have thatch roofs and walls of mud plaster laid on a bamboo frame. There are generally a verandah and two rooms, in one of which cooking is done. Though there are shuttered windows, they are invariably kept closed, day and night, so that the rooms are very dark. The verandah is frequently enclosed to provide an additional room.

Each householder usually builds a cow-shed with a thatch roof and bamboo walls. The sheds are light and airy and are not suitable as daytime shelters for the main vector, *Anopheles minimus*.

Measurements of a large number of houses give an average sprayable surface of 1,000 square feet. This includes all internal wall surfaces and the undersurface of the thatch roof but does not include the cow-shed. If the verandah is enclosed, it adds about 350 square feet to this total. Both cow-sheds and verandahs are sprayed.

A residual insecticide programme is considerably affected by the custom of replastering walls, inside and outside, with a mixture of mud and cow-dung. Replastering is done before certain festivals and on occasions of births, deaths or visits. It varies from estate to estate and appears to be related to the housewife's pride in her home rather than to caste or tribe. Often as many as 20 per cent of the houses may be replastered in the interval between insecticide applications. Neither admonitions nor appeals have any effect and it is necessary to have replastered houses reported so that they may be resprayed.

VECTORS OF MALARIA IN ASSAM.

The main vector is *A. minimus*. There are a limited number of estates where *A. leucosphyrus* is a probable subsidiary vector but there is no known instance where it is the only vector and generally its importance is still not certain.

Typically, *A. minimus* breeds in grassy-edged streams and drains which are open to the sun and where the water is clear and the current velocity is low. Less often this species breeds in shallow saucer-shaped wells and in crab holes in the banks of streams. On tea estates, a large proportion of the favoured breeding places are man-made.

In the dry season, when the stream volume and velocity are greatly reduced, breeding occurs at the edges of large rivers though the cycle from egg to adult is lengthened by the low temperatures then prevailing.

THE MALARIA TRANSMISSION SEASON.

Depending on the date of spring rains, vectors can be found in small numbers in houses from March or April. In the absence of control, the numbers increase each month, reaching a peak in late October and early November. Thereafter, disappearance is rapid and house searches in winter seldom produce a single specimen, though vectors in scanty numbers must be sheltering because cold weather breeding in perennial streams is a well established finding.

PAST HISTORY OF ESTATE MALARIA CONTROL.

Larval control was first introduced in 1930. Oiling was used to a considerable extent in the first years but the emphasis then shifted to species control by shading of streams, drains and swamps and by increasing the current velocity by sluice gates and by flushing siphons.

Up to the outbreak of the World War II, a steady reduction of malaria was achieved and juvenile spleen rates of 70 per cent or higher fell to 30 per cent or less. The demands of the war effort on materials and supervisors soon led to a reduction of larval control and then to cessation so that by 1944 the malariometric indices had in some instances regained their pre-control levels.

After the War, D.D.T. became available and was quickly adopted by estates; in addition much use was made of chemo-suppression, mainly with proguanil. As a result of this dual attack, lost ground was soon recovered and by 1947 results had already surpassed the best ever achieved by larval control.

THE POST-WAR REGIME, 1947 TO 1956.

In 1950, research by the Ross Institute showed that insecticide applications at fairly frequent intervals gave better results than heavier concentrations at long intervals, doubtless because replastering, as discussed above, had less chance of covering lethal deposits for long periods of time. It was also found that B.H.C. was more effective than D.D.T., though it was not then realised that this was due to a fumigant effect of the former after it had been adsorbed by mud walls.

In 1951, an investigation by urine tests of the efficiency of proguanil mass administration showed that only 56 per cent of 742 people who claimed to have swallowed their tablets, had in fact done so. It became evident that consumer resistance had steadily increased to a point where continuous and regular drug administration had become impossible and reliance could not be placed on chemotherapy as a major weapon. Accordingly, estates were advised to reserve proguanil for emergencies and to develop an efficient insecticide programme as the sole measure of malaria control.

The reasons for resistance to drug administration were never uncovered. As tea estate labour is well-disciplined and relatively co-operative, this failure must raise doubts where the method is used in the general rural population of any malarious country.

From 1952 onwards, it became possible to introduce a uniform regime and estates were advised to apply 11 mg. of gamma B.H.C. per square foot at intervals

not exceeding six weeks. Importance is laid on an early application of the first round which should be completed by the end of March, before the first spring-season breeding of *A. minimus*. The last application is in October so that six rounds are given in the transmission season.

Occasionally the first and even the second applications are given at a concentration of 22 mg. per square foot, especially where past experience has shown that a spring-season increase in malaria morbidity is difficult to control.

To sum up, the period 1947 to 1951 was one where residual insecticides and chemo-suppressives were both used and where D.D.T. gradually gave way to B.H.C. In the second five-year period, B.H.C. has been used as the sole measure of malaria control.

SPRAYING EQUIPMENT.

The stirrup pump alone is used. The choice was early dictated by availability and has remained because of a reluctance to disturb a successful procedure in which teams are now thoroughly trained.

The disadvantage of two men per sprayer is more apparent than real because it is unlikely that the class of men employed would work as well alone. A positive advantage of a man controlling the discharge line unhampered by a shoulder container, is that he can more readily spray under the low beds and shelves and stacks of firewood that are so favoured by *A. minimus* as daytime shelters.

In practice two, less often three, spraying units work in adjacent houses. Each pump requires two men, and one extra man keeps two or three buckets charged with suspension. Each unit, if well trained and supervised, will spray between 25 and 30 houses in a working day.

Workers are reasonably amenable and accustomed to spraying programmes so that very little trouble is caused by locked houses and none at all by refusal of permission to spray. If any locked houses are found, the inspector sends his team back in the late afternoon or early next morning when houses are certain to be open.

All buildings are included in the programme, labourers' houses and cattle-sheds, schools, shops, hospital, staff quarters and the residences of the managerial staff.

The effect of B.H.C. on bed bugs and cockroaches is much appreciated and there was opposition in several estates to an experimental reduction in the number of rounds. When, eventually, spraying ceases there will certainly be organised protests unless, of course, these insect pests have by then become resistant to B.H.C.

ORGANISATION.

The residual insecticide regime having been tested in the field and then recommended by the Ross Institute, its adoption by estates followed. Principal Medical Officers instructed their Malaria Inspectors or frequently sent them to the Ross Institute for training. They, in turn, trained their stirrup pump operators until correct technique became routine. Other important duties of Inspectors are to supervise their spraying teams at work, to maintain equipment in good order

and to keep detailed registers that show the dates of spraying rounds, the number of houses sprayed each day and the quantity of insecticide used.

Technicians from the Ross Institute are available to train spraying teams, to inspect and report on equipment and to investigate results.

Each estate is responsible for ordering its insecticide and equipment, basing the orders on the advice of the Malaria Inspector and the Principal Medical Officer. There are, therefore, no problems with regard to procurement of large quantities nor to distribution of supplies to control units.

MALARIA MORBIDITY AS A CHECK.

At least until malaria has been reduced to a disease of minor importance, monthly returns are not sufficient and estates are advised to complete weekly malaria case reports. If the case incidence per mille is graphed as either an average daily rate or a weekly rate, rises are quickly detected and the necessary adjustments of control can be made.

Checking the spraying programme, by searching for the vector in houses, has not been done to any extent though the methods of window-traps and space-spraying are both used by the Ross Institute in investigating cases where results fall below expectations.

Judged by results, despite the apparent handicap of no central executive authority, the system has worked extremely well and has far more than met the requirements of estate managements, that malaria must not interfere with tea cultivation or manufacture. Indeed it is now negligible as a cause of ill-health and is fast coming as near to disappearance as can be expected until it is controlled in the villages by a Government Programme.

MALARIOMETRIC INDICES.

The only two indices in use are the malaria morbidity rate and the spleen rate in children aged two to nine years. These are depicted in Table I. As has been shown above, the former has a high degree of accuracy both by reason of completeness and by microscopic confirmation in the majority of cases.

TREND OF THE MALARIA MORBIDITY RATE.

From 1947 to 1949, there was a gratifying fall in the morbidity rate. Viewed from the vantage point of 1956 it is now clear that, despite fluctuations, the downward trend was continuous. But in the years 1950 to 1954, this subsequent decrease could not be foreseen and progress seemed disappointingly slow in view of the intensity of the insecticide programme. It is now seen that the rate was halved in each of the years 1955 and 1956.

CONTROLLED ESTATES IN AN UNCONTROLLED ENVIRONMENT.

To the present (1957), the State Malaria Control Programme has reached only a few of the planting districts. Controlled estates, in relation to the

uncontrolled countryside, may be likened to islands in a sea, some small, some large, some close together, some far apart but all, taken together, still make only a small proportion of the area of the State.

TABLE I.

Malaria morbidity per mille. Spleen rate per cent. 1947-1956.

Year.	Malaria morbidity per mille.	Spleen rate per cent.
1947	150·7	18·2
1948	95·1	14·5
1949	73·8	10·0
1950	70·8	12·5
1951	50·7	9·9
1952	62·2	8·5
1953	42·1	6·5
1954	46·0	4·0
1955	22·4	2·0
1956	11·9	1·5

Note.—Population sample of 315,000 including 63,200 children aged 2 to 9 years.

The majority have uncontrolled villages very near their boundaries and there is a considerable movement of estate people, either visiting friends in villages for a night or two or spending their annual leave on small uncontrolled estates or villages where malaria can be readily contracted.

Thus it is quite certain that the estate malaria morbidity will be affected by the prevalence of the disease in villages and that the good years and bad years of uncontrolled regions must be reflected by estate records. Thus in 1952, known to be a year of unduly high malaria in villages, the estate malaria incidence increased by 11·5 per mille over 1951.

This close inter-relationship has made futile attempts to interpret annual estate fluctuations or to relate them to changes in control regimes. Nevertheless certain conclusions can be drawn:—

(1) When the original malaria morbidity rate is high, a rapid reduction can be obtained with ease and by residual insecticides or drug suppression, alone or in combination.

(2) A still further reduction of malaria, to very low figures, can be achieved even though control is restricted in area and though there is movement between controlled estates and uncontrolled villages.

(3) While village malaria transmission undoubtedly affects estate indices, yet their trend is steadily downwards year by year despite the absence of control in villages.

(4) To reduce its incidence still further, until malaria approaches disappearance, required five more years of intensive control, an indication that *A. minimus* is a formidable foe. Any hopes that it will yield as easily as have *A. culicifacies* and *A. fluviatilis* in other States of India, will not be realised and any under-estimate of the difficulties of control of *minimus* malaria may delay a national eradication programme.

THE JUVENILE SPLEEN RATE.

For many years, it has been customary to examine children each year for spleen enlargement. The examinations are made at the end of the transmission season in children aged from two to nine years, born on the estate.

The children in this age-group in the present sample averaged 63,200 each year, the annual fluctuations being very small.

The spleen rates per cent for the years 1947 to 1956 are given in Table I.

The spleen rate fell rather more regularly than the morbidity rate. It did not reflect the 1952 and 1954 rises of the latter though it showed a small and unexplained rise of 1·4 per cent in 1950, i.e., in the period when malaria control had not yet been standardised.

To show the ranges of morbidity incidence that can occur without change or with only small changes, in the Spleen Rate, the rates for the two sub-samples are given in Table II.

TABLE II.

Malaria morbidity per mille per annum. Spleen rate per cent. Sub-samples G and F. (1952-1956).

Year.	Sub-sample G.		Sub-sample F.	
	Morbidity	Spleen rate	Morbidity	Spleen rate
1952	45	4·1	30	2·1
1953	36	2·3	23	2·0
1954	53	2·1	40	3·0
1955	36	1·3	25	2·1
1956	24	1·1	13	0·8

Notes.—

(1) Sub-sample G contained 26,090 persons including 7,305 children aged 2 to 9 years.

(2) Sub-sample F contained 19,820 persons including 5,550 children aged 2 to 9 years.

It is seen that when the spleen rate falls below five per cent, it becomes insensitive and is no longer reliable as an indicator of the amount of malaria still present. Though eventually both rates may reach zero, their downward paths are not necessarily parallel and a spleen rate of two per cent can be associated with a morbidity rate as high as 53 per mille per annum or as low as 25.

In general it may be said that after several years of intensive control a spleen rate of five per cent is clear evidence of unsatisfactory control. Since a Spleen Rate of 1·5 per cent (Table I) is associated with a morbidity rate of 12 per mille per annum, it may be asserted that a rate in excess of one per cent is unacceptable in a control programme aimed at reduction of malaria to vanishing point.

SUMMARY.

A description is given of an intensive programme of malaria control by residual insecticides on tea estates in Assam.

In the past ten years, malaria morbidity has been reduced from 151 per mille per annum to 12 and the child spleen rate from 18 per cent to 1·5 per cent.

Houses are commonly and frequently replastered and to offset the deleterious effect on residual deposits, frequent rounds of insecticide application at relatively low concentrations are used.

B.H.C. is used to the exclusion of D.D.T. because it is less affected by the porous mud walls of the great majority of houses.

Tea estates are islands of malaria control in the sea of rural Assam which is as yet uncontrolled. Despite their limited areas, and despite movements of residents to and from estates, malaria can, even in these unfavourable circumstances, be reduced almost to vanishing point.

When it falls below five per cent, the spleen rate is shown to be an unreliable indicator of the quantity of residual malaria. It is suggested that it should not exceed one per cent in an efficient and prolonged control programme.

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THE INFANT PARASITE RATE ON SOME ASSAM TEA ESTATES.

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[May 17, 1958.]

INTRODUCTION.

In 1957, the infant parasite rate was used to measure the amount of malaria transmission that might still be taking place on a group of Assam tea estates whose malaria morbidity and spleen rates, already low, were steadily falling.

This planting district lies north of the Brahmaputra River, and close to the foot-hills of the North East Frontier Agency. The resident population of the group numbered 27,680 in 1957 and included about 6,000 children aged between two and nine years. Applying the 1956 birth rate of 48 per mille, the expected number of infants would be about 1,300.

The malaria vector is *Anopheles minimus* and the transmission season lasts from April to October or early November.

MALARIA CONTROL.

In 1947 mepacrine was given as a mass suppressive; in 1948 it was replaced by proguanil in a dosage of 100 mg. twice weekly at first, and 300 mg. once weekly later.

D.D.T. was first used in 1948 but was soon replaced by B.H.C. which, after 1954, was the sole method of malaria control. The insecticide regime was to apply a concentration of 10 mg. per square foot (110 mg. per square metre) from March to October with intervals of six weeks between rounds.

Though house spraying in the early years was neither as efficient nor as effective as it became later, yet together with mass-suppressives it resulted in an immediate and dramatic fall in the morbidity and spleen rates.

MALARIA INDICES.

Table I refers to the medical practice of 12 estates from which nine were selected for the infant parasite rate investigation.

In 1946, the morbidity rate was 463 per mille and the spleen rate in children aged two to nine years was 49 per cent.

1947 reflected the first year of control by a morbidity rate almost halved and a spleen rate more than halved. In 1948, further substantial reductions were obtained. Thereafter until 1951, the morbidity rate was fairly stationary though the spleen rate continued to fall each year. After 1954, when B.H.C. replaced D.D.T., the decrease in malaria morbidity was steady (except for a rise in 1954) and by 1957 the rate had fallen to 18 per mille.

TABLE I.

Annual malaria morbidity and spleen rates.

Year	Average population	Malaria cases per mille	Spleen rate per cent
1946	22,400	463	49·2
1947	23,506	283	19·3
1948	23,892	66	—*
1949	24,904	65	10·0
1950	24,974	46	8·7
1951	25,484	64	4·9
1952	25,936	45	4·1
1953	25,229	36	2·3
1954	25,965	54	2·1
1955	27,227	36	1·3
1956	27,410	23	1·1
1957	27,680	18	0·4

*Not recorded.

THE SPLEEN RATE.

As early as 1951 the spleen rate had fallen to five per cent and by 1957, to 0·4 per cent, only 24 of 5,914 children examined having palpable spleens. As it approached zero the spleen rate was no longer a guide to malaria morbidity;

thus a spleen rate of 8·7 per cent in 1950 was associated with a morbidity rate of 46 per mille, while in 1952, though the morbidity rate was unchanged, the spleen rate had fallen to 4·1 per cent.

THE INFANT PARASITE RATE.

Infants born between January 1 and June 30, 1957 were examined fortnightly by thick and thin blood smears. It was realised that examinations starting in the first week of July were too late and that infections early in the transmission season would be missed. Unfortunately, other commitments prevented an earlier start.

Although a few mothers refused permission, it was possible to start the series with 333 infants, all but five of whom completed the tests. Two of these departed with their parents from the district, and three died (the causes of death were recorded as congenital debility, dysentery and pneumonia).

Since the estates are surrounded by villages where there is no malaria control whatever, it was necessary to keep a careful watch for infants who spent one or more nights away from their homes. Altogether 47 infants had to be excluded because of such absences, leaving 286 who never left the estates and who had blood smears taken fortnightly from the beginning of July to the beginning of November.

Of the 286, 14 were infected with malaria (Table II) giving a parasite rate of 4·9 per cent. None of the 14 was positive on more than one fortnightly examination.

TABLE II.

Species of malaria parasites.

Species	Form	Number of infants
P. falciparum	Trophozoites.	5
	Gametocytes.	3
P. vivax	Trophozoites.	2
	Trophozoites and gametocytes.	2
	Gametocytes.	1
P. malariæ	Gametocytes.	1
		14

The dates of finding positive blood smears are given in Table III. As three infants were positive at the first examination, it is more than likely that a number of earlier infections were missed and that the rate throughout the full transmission season would have been considerably higher than 4·9 per cent.

Of the 14 infants positive for malaria, 11 were infected up to August and only three afterwards.

Five of these infants lived on one estate and the remaining nine were distributed between five estates. This is shown in Table IV.

TABLE III.

Dates when blood was positive for malaria parasites.

Week ending	Number of infants positive
1957 July 13 ..	3
July 27 ..	1
August 3 ..	2
August 10 ..	2
August 24 ..	3
September 7 ..	1
September 21 ..	1
September 26 ..	1
	14

TABLE IV.

Places of residence of infants.

Estate	Number of infants completing test	Number positive for malaria
A	28	1
B	39	1
C	42	5
D	19	2
E	13	*Nil*
F	37	*Nil*
I	27	3
K	36	2
L	45	*Nil*
	286	14

The numbers of infants on each estate are too small to establish with any confidence, imperfections in a residual insecticide regime supposedly uniform on

all estates. Estate G is a possible exception although the malaria morbidity for the year, 9·8 per mille, was only half the rate for the group and the spleen rate was 0·2 per cent compared with 0·4 per cent for the group.

CONCLUSIONS.

In this group of estates in 1957, a child spleen rate of 0·4 per cent and a malaria morbidity rate at all ages of 18 per mille per annum indicated very satisfactory malaria control following five years of intensive house spraying with B.H.C. Yet 4·9 per cent of 286 infants were infected with malaria during part only of the transmission season.

The persistence of malaria must, in large part, be attributed to uncontrolled villages close to estate boundaries and it is evident that while malaria control in limited areas can be highly successful, eradication is not possible.

In a malaria eradication programme, no reliance can be placed on the spleen rate, however low, as an indication of the degree of residual transmission and only a zero rate is acceptable.

SUMMARY.

Blood examination of infants on a group of tea estates in Assam showed that there was still a considerable amount of malaria transmission despite low morbidity and spleen rates.

Of 286 infants, 14 were infected with malaria between June and November, 1957.

In this year, the malaria morbidity rate at all ages was 18 per mille and the child spleen rate was 0·4 per cent.

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OBSERVATIONS ON MASS THERAPY WITH DIETHYLCARBAMAZINE (HETRAZAN) FOR FILARIA CONTROL IN BOMBAY STATE.

BY

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[May 21, 1958.]

FILARIASIS is an important mosquito-borne disease prevalent in many parts of the world. The infection runs a chronic course and is very difficult to get rid of. Considerable time (three to six months or even longer) passes between the bite of an infective mosquito and the appearance of infection in blood stream and the disease has a very insidious onset. The earliest stage of infection is, therefore, frequently very difficult to recognize.

Filarial infection in a community has not been correctly assessed so far. The importance of filariasis and the urgency of its control have been increasingly realized recently, but its effective control is a painstaking and difficult task in contrast to rapid effective control achieved in some other arthropod-borne diseases. It is estimated that out of about 180 million people exposed to this disease in the world, about one-third live in filaria endemic areas of our country and though the infection shows a patchy distribution, the overall picture presents a problem of great public health importance.

There is generally a higher incidence of this disease in the coastal regions than in the hinterland because of the conditions of high temperature combined with high humidity obtained in such regions. Wherever ideal climatic conditions exist and breeding conditions for the vector are favourable, micro-filarial state of the community is a very important factor in causing the active spread of this disease.

Detailed filaria surveys have not been carried out in all parts of Bombay State, but it is estimated that a population of about four million lives in areas where

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filariasis is a problem. The areas where this disease is prevalent are (1) Surat Town and Rander, Bulsar, Billimora and other parts of Surat District, (2) Bassein Town and Dahanu, Umbergaon and some other parts of Thana District, (3) Coastal areas of Saurashtra, (4) Urban and rural areas of Vidarbha region specially from Nagpur and Chanda districts. The infection in all these areas is entirely of *Bancroftian* type. Under the National Filaria Control Programme, four Filaria Control Units are functioning in Bombay State, one each at Nagpur and Surat and two in Saurashtra region. Four more control units are to be established in the State soon, one unit to serve the areas of Thana and Surat Districts, one for Chanda District, and one-and-a-two-third unit for Nagpur Town and 1/3 for Bhandara District. Each Control Unit protects a population of about 3,00,000. It undertakes (1) a preliminary survey, (2) selection of special study area within the control area and comparison area outside control area, but adjacent to it, each having a population of about 30,000 and almost identical conditions of filarial endemicity for carrying out detailed clinical, entomological and parasitological observations to assess the effect of control measures, (3) control measures consisting of (*a*) one round of indoor residual spraying with dieldrin at a dosage of 56 mg./square foot in urban areas at the peak season of mosquito densities and three rounds in a year in rural areas, (*b*) intensive anti-larval measures throughout the year in urban areas and (*c*) one round of mass treatment of the entire population with diethylcarbamazine.

The Surat Unit is operating in that town only. Surat is an important industrial and business town of Bombay State having a population of 2,23,182 (1951 census). The city has no underground drainage system. Disposal of domestic and industrial sullage is achieved by directing it into soakage pits. These and many other places like storm water drains, surface drains, unused wells, etc., provide ample breeding places for the culicine mosquitoes. Filariasis is prevailing in this town for over 100 years and it is surmised that the sources of importation of the disease in this town are the coastal towns of Cochin and Mangalore from where country boats are plying since long, undertaking passenger traffic also. A systematic survey to determine the incidence of filariasis in this town was carried out in 1950-51, when it was found that about 12 per cent of the population surveyed showed evidence of infection. The disease was found to be prevalent equally in all wards of the town. The municipality was, therefore, advised to undertake (*a*) anti-larval measures to be carried out throughout the year and (*b*) anti-adult measures by indoor residual spraying with D.D.T. at the peak season of mosquito nuisance. Results of these measures were assessed by surveys from time to time, the position in 1956 being as follows:—

	Year of survey.	
	1951	1956
Number of persons examined	7,803	4,000
Number of persons showing filarial infection	947	431
Number of persons showing disease	259	93
Number of persons with micro-filaria and disease ...	22	15
Infection rate, per cent	12·4	11·2
Disease rate, per cent	3·6	2·7
Endemicity rate, per cent	15·7	13·5

These findings do not reveal any marked reduction after five years of control measures. However, the further progress of the disease had been arrested. Children in the age-group of five to nine years, who may be considered to have received a fair amount of protection from primary infection during this period of control, show a significant fall in the infection rate from 8·8 in 1951 to 5·5 in 1956-57. Since September 1955, the Unit is included under the National Filaria Control Programme and, therefore, first indoor residual spraying with dieldrin 50 per cent wettable powder was carried out in March, 1956 and the second round in May, 1957. Only malariol is being used as a larvicide throughout the year instead of benzene-hexa-chloride 50 per cent wettable powder provided under the National Filaria Control Programme. Mass Hetrazan therapy was carried out during the period from September 24, 1956 to January 31, 1957. The number of persons who availed themselves of this therapy is 73,139 (i.e. about 32·7 per cent only).

NAGPUR UNIT.

This unit was established in 1956 and, after an initial survey, took up control activities in 1957. Infection rate in this area is of the order of 16·1, disease rate 11·4 and endemicity rate 25·9. The unit includes the entire rural and semi-urban areas of Saoner and Katol Tahsils of Nagpur District having a population of 2,83,237. Three rounds of dieldrin spraying have been carried out in the year 1957 in the months of January and February, June and July, November and December. Mass hetrazan therapy, extending over a period of 17 weeks, has been completed from February to June 1957. About 60 per cent of the population of this unit have availed itself of this therapy. Anti-larval work has been taken up in three semi-urban areas of this unit only in the later part of the year 1957.

SAURASHTRA UNIT.

Saurashtra region has two filaria control units. One is located at Rajkot and extends over 80 miles on the south-west coast of Saurashtra including the towns of Porbandar, Veraval, Mangrol and Manavadar, which have an infection rate varying from 10 to 15. It covers a population of 3,18,060, out of which 1,87,652 is urban and 1,30,408 is rural. The first round of indoor residual spraying with dieldrin is over in the entire unit. Anti-larval work is in progress at Porbandar, Veraval, Patan, Mangrol, Bantwa and Kutiyana covering a population of about 1,62,557. Benzene-hexa-chloride 50 per cent wettable powder is used for anti-larval measures. Mass therapy with hetrazan tablets has been completed in Porbandar, Chhaya, Veraval, and Somnath Patan, covering a population of 1,13,763. The percentage of population covered by mass therapy is 59·8, 42·3, 59·0 and 84·0 respectively. The second unit mainly carried out surveys in 48 places of Saurashtra and Amreli districts and has yet to take up control operations.

Control of filariasis is an extremely difficult proposition and does not show a spectacular reduction as in the case of malaria where one or two years of control operations bring about appreciable reduction. The two main methods of control, namely (1) attack on the vector in its larval and adult stage and (2) attack on the

parasite load in human beings, are still in evolutionary stages and need standardization by extensive field trials. The anti-larval and anti-adult measures are essential for vector control but they alone may not suffice to achieve the object of filaria control. These measures have to be supplemented by anti-parasitic measures. Mass distribution of hetrazan tablets is, therefore, an important weapon in the programme of the control of this disease as it markedly helps in reducing the infection in a community to a low level.

Diethylcarbamazine (hetrazan), a piperazine compound, is a colourless crystalline solid with high water solubility. The dihydrogen citrate-salt is stable in warm climates and is marketed in the form of compressed tablets. It is a powerful filaricide, having a specific action on microfilaria in human host. It was first introduced by Santiago-Stevenson *et al.* in 1947. It has been shown that this drug kills the micro-filariæ in blood but does not cure the patient of elephantiasis though the drug is supposed to have some effect in relieving early symptoms of the disease. No direct evidence is yet available that the drug has any action on the adult worm but the sustained reductions of micro-filaria, after treatment, observed at some places, strongly suggest that the adult worms as well as micro-filaria are affected. The exact nature about the action of this drug is still a matter of some controversy but it has been claimed that mass treatment with diethylcarbamazine provides a promising approach for effectively controlling the transmission of the disease as the therapy is effective in appreciably reducing the micro-filarial count. The drug is quickly absorbed from the alimentary canal, rapidly metabolised and excreted in urine within one to two days.

Mass therapy with diethylcarbamazine, though a marked advance in the control of this disease, cannot be relied upon as a single method for complete control and a combination of all the three methods of control, *viz.*, anti-adult, anti-larval and anti-parasitic are essential for the effective control of the disease. The programme of mass therapy is also not a simple or an easy task due to practical difficulties involved in covering the entire community and the drug having very little effect against the established disease, which make the public non-appreciative of this measure and consequently non-cooperative towards it. A complete course of this therapy is spread over a period of five days and every person is administered a dose of diethylcarbamazine tablet (100 mg.) on a full stomach. Pregnant women, children below two years and all chronically ill persons are excluded from the administration of this drug. The tablet is put in the mouth and washed down with water in the presence of a drug distributor to ensure that the drug is actually taken by the individual. The dosage schedule adopted is as follows:—

Age group		Dose
I	(adults)	... A single dose of two tablets for five days.
II	(12 to 18 years)	... A single dose of one and a half tablets for five days.
III	(6 to 12 years)	... A single dose of one tablet for five days.
IV	(2 to 6 years)	... A single dose of half tablet for five days.

Table I shows the percentage of persons who availed themselves of this scheme and the percentage of persons who availed themselves of the full course of

therapy for the three different Units. In Saurashtra Unit, the population of Porbandar, Chhaya, Verawal and Patan, where the mass hetrazan therapy work has been completed, is taken.

TABLE I.

Percentage of persons who availed themselves of mass therapy with diethylcarbamazine and the percentage of persons who availed themselves of the full course of therapy.

Unit	Population	Number who took drug	Percentage to total population	Number who took complete course of therapy	Percentage to total population
Nagpur ..	2,83,237	1,58,400	55·9	60,061	21·2
Surat	2,23,832	73,139	32·7	30,065	13·4
Saurashtra .	1,13,763	60,447	53·0	35,743	31·4

Experience shows that there is a better response to this measure in rural and semi-urban areas than in urban areas as the population in rural areas is mainly dependent on agriculture and the occurrence of reactions does not deter them from taking the drug, specially so when the scheme is executed during non-agricultural season. As against this, people from the urban areas are engaged more in small or large industries and majority of them are daily wage earners. The fear of losing daily wages on account of the occurrence of reactions, therefore, weighs heavily resulting in a poor response towards the scheme. So also rumours with their distorted versions spread more rapidly in urban than in rural areas. This happened in Surat Town and inspite of best propaganda and organization of treatment facilities for persons showing reactions, response from the public though encouraging to start with, was completely lacking in subsequent weeks. Table II in which the information for the first five weeks from Surat Town is compiled, would show that there is a steep fall in the number of persons availing themselves of the drug therapy from the first week to the fifth week though the number of teams engaged for distribution of drug had remained the same and an almost equal population was allotted per week.

As regards reactions occurring as a result of taking this drug, no special observations were made to assess the incidence of these reactions in the micro-filaria positive and negative persons separately. These reactions were found to be more on the second and third day of the therapy and their incidence varied from 12·7 per cent in Saurashtra Unit to 31·4 per cent in Nagpur Unit as is seen from Table III for the entire period of the therapy in each unit.

In a large number of cases, the reactions were of very mild and transitory nature and yielded to the simple remedies provided to each drug distributor for administration to persons getting the reactions. While administering these tablets, a mixture of powder, containing sodabicarb and calcium lactate in equal proportions, was given to every person to reduce the incidence of nausea and vomiting. Other drugs used to treat these reactions were A.P.C. powders, soda salicylas,

magnesium sulphate, diuretic mixture, sulphaguanidine tablets, expectorant mixture, carminative mixture, antistine tablets, etc. Serious reactions like ocular swellings, abscess formations, acute abdominal pains, acute glandular swellings, retention of urine, jaundice, etc., were few and were personally seen by the superior supervising medical staff and treatment was immediately arranged. Four fatal cases (three from Surat Unit and one from Nagpur Unit) occurred during the course of therapy and were alleged to be attributed to it, but there was not sufficient evidence to definitely correlate these fatal results to the hetrazan therapy alone.

TABLE II.

Number of persons who availed themselves of mass therapy with diethylcarbamazine from the 1st week to 5th week in Surat Town.

Week	Population allotted for mass therapy	Number who actually took the drug					Total number who took the drug	Number who did not take the drug
		5 days	4 days	3 days	2 days	1 day		
1st ..	10,751	5,547	1,470	718	610	627	8,972	1,779
2nd ..	10,960	3,135	1,625	1,044	1,268	1,117	8,189	2,771
3rd ..	9,707	1,731	997	751	721	797	4,997	4,710
4th ..	10,092	3,030	1,205	864	643	674	5,416	4,676
5th ..	9,350	954	598	394	398	509	2,853	6,497

TABLE III.

Incidence of reactions occurring as a result of taking diethylcarbamazine.

Unit	Number of persons who took the drug	Number who got reactions	Percentage
Nagpur ..	1,58,400	59,781	31·4
Surat	73,139	17,305	23·8
Saurashtra ..	60,447	7,677	12·7

The reactions observed were as follows:—

General.— Headache, body pain, joint pains, fever, general debility, vertigo, giddiness, drowsiness, muscular weakness.

Gastro-intestinal.— Nausea, vomiting, glossitis, constipation, distension of abdomen, diarrhoea, intestinal colic, bleeding per rectum.

Respiratory.— Sore throat, bronchitis, asthmatic attacks, hemoptysis, cough.

Nervous.— Convulsions, depression.

Urinary.— Irritation of bladder, retention of urine, hematuria.

Gynaecological.— Excessive menstrual bleeding, dysmenorrhoea, irregular periods, stoppage of menstrual flow.

Liver.— Jaundice.

Special organs.— Conjunctivitis, ear-ache, epistaxis, ocular swellings, swellings of breasts, increase in scrotal swellings, orchitis.

Skin.— Urticaria, lymphagitis, lymphadenitis, superficial abscesses.

These cases were enquired into in detail, by the Medical Officers in charge of the Units. Diarrhoea in suckling babies was noticed in some cases of Nagpur Unit. So also delayed reactions of the nature of swellings of legs, arms, lymph nodes, testicles, etc., and continuous fever, ear-ache and deafness after a period of about two weeks were noticed in a few cases by the Medical Officers-in-Charge of dispensaries of Nagpur Unit and it was observed that such reactions were restricted to areas having high endemicity rates of 30 per cent and over.

As per general instructions issued to the drug distributors, the drug had to be administered on full stomach and not on empty stomach. This was, however, not found to be possible in every case and many persons seemed to be taking the drug on practically empty stomach. To judge whether the taking of hetrazan tablets on empty stomach was responsible for many of the minor reactions that were occurring, a limited study, to observe some cases who definitely gave history of having taken the drug either on full or empty stomach, was carried out in Surat Unit. Out of 123 persons who took the drug on full stomach, 29 complained of reactions. Out of 109 persons who took the drug on empty stomach, 25 complained of reactions. The percentage of persons who thus complained of reactions was almost the same (23 per cent) in both these groups and correspond with the overall percentage of 23·8 per cent for the entire period of therapy in this Unit. The nature of reactions, however, showed some differences as stated below:—

	Drug taken on full stomach.	Drug taken on empty stomach
Number of persons observed	123	109
Number who complained of reactions ...	29 (23·5 per cent)	25 (23 per cent)
Symptoms complained of:		
Fever	25	14
Headache	8	2
Body pains	8	3
Diarrhoea	2	2
Constipation	1	6
Vomiting	0	5
Abdominal pain	11	4

Fever, headache and body pains are thus observed to be more in persons who took the drug on full stomach whereas constipation, vomiting and abdominal pain, i.e., symptoms of gastro-intestinal nature are more frequent in persons who took the drug on empty stomach. While no positive conclusion can be drawn from this limited observation, it indicated that whether the drug was taken on full or empty stomach the percentage of persons getting the reactions was not in any way affected although the nature of reactions showed some differences in the two groups.

Mass therapy with hetrazan has an added advantage in that it clears the community of the ubiquitous intestinal parasite- round-worm infection also. Out of 30,427 persons who took the drug in the first five weeks in Surat Town, 2,722 persons or about nine per cent gave history of having passed round worms. Passing of worms was observed more on the third and fourth days after taking the

drug as is seen from Table IV below, and a proportionately large number belonging to the age group 6 - 12 years reported the passing of worms.

TABLE IV.

Number of persons who passed round worms as a result of mass therapy with hetrazan.

Week	Number who took the drug	Number who passed round worms on						Total
		1st day	2nd day	3rd day	4th day	5th day	6th day	
1st ..	8,972	0	109	314	190	176	0	789
2nd ..	8,189	5	117	158	169	130	0	579
3rd ..	4,997	0	94	93	130	132	2	451
4th ..	5,416	2	97	204	161	158	3	625
5th ..	2,853	1	36	90	62	89	0	278
Total	30,427	8	453	859	712	685	5	2,722

A scrutiny of 500 entries in serial order from one register pertaining to the work done in the first week in Ward 1 of Surat Town was carried out. From this sample of 500 persons comprising 269 males and 231 females, 2 males and 4 females, all adults, had filarial disease manifestations, giving a disease rate of 1·2 as against the disease rate of 2·7 (1956 survey) for the entire town. Out of these 500 persons, 400 persons falling in the different age-groups by sexes had taken the drug for one to five days as shown in Table V below:—

TABLE V.

Sample survey of filariasis in Ward 1 of Surat Town.

Number of persons surveyed:—						Males					Females					Total
						269					231					500
Number who took the drug:—						234					166					400
Drug taken for	Number of males, according to age-group, who took hetrazan					Number of females, according to age-group, who took hetrazan										
	I	II	III	IV	Total	I	II	III	IV	Total						
5 days ..	60	16	26	18	120	35	15	15	14	79	199					
4 days ..	23	12	8	9	52	21	6	6	7	40	92					
3 days ..	10	1	5	1	17	8	1	5	2	16	33					
2 days ..	12	1	4	3	20	5	4	4	3	16	36					
1 day ..	11	2	7	5	25	10	1	1	3	15	40					
Total ..	116	32	50	36	234	79	27	31	29	166	400					

Group I = Adults
Group II = 12-18 years
Group III = 6-12 years.
Group IV = 2-6 years.

From Table V, it is observed that the percentage of males who took the drug is slightly higher (87 per cent) than that of females (72 per cent). Out of the 100 persons from this sample who did not take the drug at all, 54 were due to refusals, 10 due to pregnancy, 33 were children below two years, and 3 were cases of chronic illness. Only 51 persons out of these 400 who took the drug, i.e. 12·8 per cent, reported the occurrence of reactions. This group of 51 persons, who complained of reactions, consisted of 27 males (11·1 per cent) and 24 females (14·4 per cent). Table VI below shows the percentage of reactions occurring in different age-groups.

TABLE VI.

Percentage getting reactions, occurring in different age-groups, as a result of taking hetrazan.

Age-group	Males			Females		
	Number who took the drug	Number getting reactions	Percentage	Number who took the drug	Number getting reactions	Percentage
I (Adults) ..	116	14	12·0	79	14	17·7
II (12 to 18 years) ..	32	3	9·4	27	4	14·9
III (6 to 12 years) ..	50	6	12·0	31	2	6·5
IV (2 to 6 years) ..	36	4	11·1	29	4	13·8

The above Table VI shows that, except in age group 6 to 12 years, the proportion of reactions is slightly higher in females than in males. Head-aches, body pains and fevers were complained of by 60 per cent (30 out of 51) and symptoms of gastro-intestinal nature by 40 per cent. No reactions of any serious nature were observed in this group. History of passing round worms was recorded in 36 persons, 18 males and 18 females, and was most common in the age-group 6-12 years as shown in Table VII below:—

TABLE VII.

Number of persons who passed round worms as a result of taking hetrazan.

Age-group	Number who had taken the drug		Number who passed round worms	
	Males	Females	Males	Females
I (Adults)	116	79	2	3
II (12 to 18 years) ..	32	27	3	4
III (6 to 12 years) ..	50	31	9	11
IV (2 to 6 years) ..	36	29	4	0

A limited observation to study the effect of this therapy on the reduction of micro-filaria circulating in blood was carried out in the Surat and the Nagpur Units. The samples included both sexes and individuals of different ages also. In Surat Town, the number of persons observed was 70. The average infestation rate per 20 c.mm. of blood observed before the therapy was 18·4. All these persons took a complete course of hetrazan during the mass therapy scheme. Blood sample was taken three to four months after the therapy when the average infestation rate was found to be 3·7. There was thus a reduction of about 83 per cent in the average infestation rate. Twentyseven persons (38·3 per cent) out of 70 showed complete clearance of micro-filaria after the therapy. In eight persons (11·4 per cent) a slight increase in the micro-filaria count was observed inspite of taking of the drug.

In Nagpur Unit, the average infestation rate for the 97 persons observed from the special study area was 38·1 before the therapy. Blood examination was carried out again two months after the administration of the drug when the average infestation rate dropped down to nine, showing a reduction of about 76 per cent. Complete clearance was observed in 17 persons (17·5 per cent) and an increase in micro-filaria count was observed in seven persons out of 97 (7·2 per cent). Out of these 97 persons, 55 had taken a complete course of therapy for five days, 13 for four days, 10 for three days, 11 for two days and 18 for one day only. The above information is shown in Table VIII below:—

TABLE VIII.

Effects of mass therapy with hetrazan on the reduction of micro-filaria.

Unit	Number of persons observed	Average infestation rate		Percentage reduction after therapy	Complete clearance of micro-filaria after therapy	Increase in micro-filaria after therapy
		Before therapy	After therapy			
Surat ..	70	18·4	3·7	83	27(38·3 per cent)	8(11·4 per cent)
Nagpur ..	97	38·1	9	76·3	17(17·5 per cent)	7(7·2 per cent)

The claim of some observers that hetrazan causes a great reduction of micro-filarial density by its remarkable rapid action in destroying the micro-filaria, is thus fairly substantiated. The drug, however, cannot be regarded as a completely safe compound although no fatal cases, directly attributed to this drug, have been recorded nor any grave complications of the nature of agranulocytosis, aplastic anæmia, exfoliative dermatitis, uraemia, etc., are observed. Only mild toxic symptoms like anorexia, nausea, vomiting, sleepiness, head-ache, etc., and other reactions of allergic type have been observed in quite a large number of persons who availed themselves of this therapy. It is not possible to predict what particular reaction would occur at a particular place. Generally speaking, severe reactions of allergic type occur in micro-filaria positive persons and reactions in children appear less severe than in adults. In majority of cases a combination

of symptoms like fever, head-ache, body pains or nausea, vomiting, abdominal pain, etc., were observed. Whatever may be the nature of these reactions and the causes responsible for their occurrence, inspite of the claims made by some authorities that these reactions are of very minor and temporary nature and do not warrant the discontinuance of the therapy, their impact on the community cannot be lost sight of in a mass therapy programme. The mass hetrazan therapy scheme covered a population of 6,20,832 in Bombay State and out of 291,986 persons who took the drug, 84,763 or 29 per cent complained of reactions of various nature which undoubtedly had its adverse effects on the successful implementation of the scheme. The execution of the scheme especially becomes more difficult if it has to operate over a long period of several months when no amount of efforts and propaganda becomes of any use.

A detailed study of these reactions occurring in different age-groups and sexes, their relation to food and the number of days for which the drug is taken, the dose schedule and the distribution methods, etc., requires to be carried out to allay fears of the public and to arrive at a minimum effective dose which can be safely and effectively used in mass therapy schemes. There are also many gaps in the scientific knowledge of this disease which requires careful research and experimentation. In the control of this disease, the importance of permanent measures cannot be underestimated. Efficient drainage system is essential to prevent breeding and where water supply schemes are being executed, drainage schemes must also be provided. Both these schemes must be instituted simultaneously. Even in rural areas, with or without a Village Panchayat, drainage systems must be improved specially in villages where the disease prevails. As the incidence of the disease depends on repeated infections, all possible precautions must be taken to protect oneself from a mosquito bite. The reduction of mosquito densities by anti-larval and anti-adult measures as well as reducing mosquito breeding by providing efficient drainage, therefore, assumes special significance. Attention must also be paid to proper construction of septic tanks, aqua privies, etc., where profuse mosquito breeding has been detected at some places as a result of faulty construction.

SUMMARY AND CONCLUSIONS.

1. Filariasis forms a very important public health problem in Bombay State. It is estimated that about four million people in the State reside in filaria endemic areas.

2. Although our present methods of control measures have advanced considerably from spectacular researches made in recent times both in regard to vector species as well as specific drug therapy against infective organisms, there is much scope for evolving a safe and suitable method of combined attack against this stealthily advancing dreadful disease. Efficacy of indoor residual spraying with insecticides for culicine control is yet to stand the test of time. Use of insecticides of chlorinated hydrocarbon group for anti-larval measures should be discouraged as there is definite evidence that continuous use of insecticides leads to rapid development of resistance in the insect population. This would not only hamper the progress of control of filariasis, but would also prevent the successful control of malaria from such areas.

3. Filaria control measures do not yield spectacular results and require to be pursued year after year if some tangible reduction in the incidence is to be achieved. Control of filariasis is, therefore, a most difficult task when compared to malaria where spectacular results can be achieved within one or two years of the institution of control measures.

4. Mass hetrazan therapy has been carried out in Bombay State on such a large scale as has not been carried out elsewhere so far. Distribution of this drug covering a population of 6,20,832 was undertaken and out of them 2,91,986 persons availed themselves of this therapy. There are undoubtedly several difficulties in organizing and implementing such a scheme of great public health importance on such a large scale. Several reactions of varying intensity were observed in about 29 per cent of cases which greatly affected the successful implementation of the scheme. The dose schedule also needs some revision as the present method of prophylaxis extends over a period of five days and many people discontinue the therapy after taking the drug for one or two days as a result of occurrence of reactions or other personal inconveniences. Considerable need for flexibility in the dose schedule and distribution arrangements is, therefore, felt. Trials on large scale to arrive at a safe dosage and the necessity for repetition of the dose with minimum period after which the drug should be administered again as a prophylactic measure, are immediately required to be undertaken.

5. Anti-culicine measures, both anti-larval and anti-adult, are required to be vigorously pursued for keeping the vector densities at low levels to arrest further transmission of the disease. In handling the insecticides belonging to chlorinated hydrocarbon group, great personal care must be exercised by the persons engaged in this work. The use of such insecticides for anti-larval measures must be discouraged as there is ample evidence to show that such use brings about the development of resistance in vector species and if such resistance does develop, it might hamper anti-malaria campaign also which might lead to a very serious situation by interfering with malaria eradication programme.

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FILARIASIS IN TRAVANCORE-COCHIN (KERALA STATE).

Part III.

Hill-Tribe Settlements in Quilon and Trivandrum Districts.

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KERALA STATE is bounded on the west by the Arabian Sea and on the east by the Western Ghats. Between the mountainous and coastal tracts lie the foot-hill zone and the extensive undulating region with low hills intercepted by narrow valleys. The entire coastal tract of this State is highly endemic for filariasis. The other areas are generally free from filariasis except among certain small groups of forest tribes living in the mountainous areas in South Kerala, where both malaria and filaria co-exist (Iyengar, 1938). The results of filariasis surveys of some of the forest settlements carried out between December, 1956 and February, 1957 are set out below.

PHYSIOGRAPHY.

The forest settlements surveyed are located in Quilon and Trivandrum districts of South Kerala. Their exact situation in the revenue division of the above mentioned two districts is indicated in Table I.

These settlements are in the midst of dense tropical forest in isolated clearings, with only a few scattered huts situated either on the top of a hillock surrounded on all or some sides by narrow valleys, or on steep precipitous mountain slopes. Almost all settlements have a river or a stream flowing in their neighbourhood.

TABLE I.

Location of the Kani Settlements surveyed.

Settlement	District	Taluk	Pakuthi (Taluk sub-division)	Kara (Village)	Forest range	Settlement group
Cherukara ..	Quilon	Pathanapuram	Anchal	Kulathpula	Anchal	Cherukara
Edathara ..	"	"	"	"	"	
Vattakarikkam	"	"	"	"	"	
Kadmankode	"	"	"	"	"	Kadmankode
Kochukarikkam	"	"	"	"	"	
Muruthimoodu	"	"	"	"	"	
Kuzhaviyodu	"	"	"	"	"	
Villumala ..	"	"	"	"	Shenthurni	Villumala
Randammile	"	"	"	"	"	
Peruvashikala	"	"	"	"	"	
Kocharuppa	"	"	"	"	Kulathpula	Kocharuppa
Edapana ..	"	Kottarakara	Kumil	Chilathara	"	
Vanchiyod ..	"	"	"	"	"	
Kalayapuram	Trivandrum	Nedumangad	Palode	"	"	Kalayapuram

Communication facilities are poor, approachable chiefly on foot through jungle tracks. Wild animals are abundant and visits to these areas involve a certain degree of risk.

METEOROLOGICAL CONDITIONS.

Temperature ranges between 65°F. and 95°F. The annual rainfall of about 100 inches is chiefly due to south-west monsoon between May and September, while occasional showers due to north-east monsoon occur during October to December. January, February and March are the dry months.

TOPOGRAPHY.

The elevation of the settlements is about 1,000 feet above sea level. The number of houses and the population per settlement varies from 4 and 30 to 10 and 60 respectively.

SOCIO-ECONOMIC CONDITIONS.

The settlers belong to the Mala Arayan and Arayar castes. Originally nomadic, since about 60 years or so they have been settled in the present sites. Their chief occupation was hunting and they used to live on jungle tubers but at present they live on the yield from minor cultivation.

The Scheduled Tribes Welfare Department has started schools, mobile dispensaries, etc., which facilities are availed of only by some of the settlements that are nearer to big villages and which have made good progress. Marriages are mostly among their own community, either from the same settlement or neighbouring areas. Registration of births and deaths in the settlements was unsystematic prior to 1955, whereafter, with the establishment of the Welfare Centres, the registration is improving.

HISTORY OF FILARIASIS.

Some of the oldest settlers, aged 73 to 75, stated that when they settled there 55 years back, no one, to their memory, had elephantoid swellings. As the disease is prevalent only among those born and brought up locally, they believe that the infection has originated from the settlement areas.

WATER SUPPLY.

Small ponds and depressions, holding seepage water collection at the foot of the hills and hillocks, serve as the main drinking water sources, though in Kadamankode Settlement a few wells were noted.

PREVIOUS SURVEYS.

Iyer (1901) recorded 27 cases of filariasis from the hilly taluk of Pathanapuram. Between 1931 and 1934, Iyengar (1938) studied the incidence of filariasis in some of the forest settlements in Pathanapuram, Kottarakara and Nedumangad taluks, results of which are set out in Table II.

In the forest settlement areas, a high filarial endemicity occurred in association with hyperendemic malaria (Iyengar, 1938).

Out of several species of mosquitoes dissected, Iyengar (1938) found only *M. uniformis* infected in Pathanapuram and Kottarakara taluks with an infection rate of 7·1 and 23·3 respectively. In the forest settlements, though adult *M. uniformis* were collected from houses, yet their breeding places could not be located by him.

PRESENT SURVEY.

A combined filaria and malaria survey, between 8.30 p.m. and 1 a.m., was carried out of as many of the persons as possible in each settlement visited. Filarial disease manifestations were recorded by enquiry only. Two blood smears were obtained from each individual, a thick and thin film on the same slide for malaria

TABLE II.

Results of a filaria survey of some forest settlements (Iyengar, 1938).

Taluk	Settlement	Number examined	Infection rate (per cent)	Disease rate (per cent)	Endemicity rate (per cent)
Pathanapuram ..	Peruvazhikkele	31	41·9	67·7	80·6
" ..	Villumala	19	36·8	47·4	57·9
" ..	Kadmankode	29	44·8	96·6	100·0
" ..	Cherukara	38	42·1	50·0	78·9
Kottarakara ..	Madathorai	87	35·6	41·7	66·7
Nedumangad ..	Kalayapuram	21	23·8	28·6	47·6
" ..	Kalathu	43	11·6	2·3	14·0
" ..	Peringamala	45	26·7	8·8	33·3

parasite and another, a thick film (about 20 c.mm. of blood), for micro-filariæ. These smears were later stained with J.S.B. and examined. Smears positive for micro-filariæ were examined in detail to diagnose differentially the species of micro-filariæ and their density noted. In two persons, micro-filarial periodicity was studied in four-hourly blood smears of 20 c.mm. of blood.

In each of the settlements surveyed, adult mosquito collections were made from all the houses, cattlesheds, mixed dwellings and possible outdoor resting places in their vicinity on the day following the night survey. In addition, collections from houses up to midnight from Kalayapuram and Villumala settlements, and the whole night from Cherukara Settlement were also carried out. The collections were identified and female mosquitoes dissected for possible filarial and malarial infections. Various water collections in the vicinity of houses were examined for likely breeding of mosquitoes.

RESULTS.

I. PARASITOLOGICAL OBSERVATIONS.

Altogether 471 persons, comprising about 42 per cent of the total population in the five settlement groups, were examined. Only *W. malayi* infection was noted. The infection, disease and endemicity rates were 25·9, 28·9 and 46·3 per cent respectively. 7·9 per cent of the population showed both infection and disease as would be noted from Table III.

Incidence of filarial infection in different age-groups and both sexes is shown in Table IV. It would be noted from Table IV that the infection rate was seen rising more or less steadily up to the age of 30, after which it remained almost

TABLE III.

Endemicity of filaria and malaria in the different groups of Kani settlements surveyed.

Settlement (Group)	Population	Number surveyed	Percentage population examined	Filaria: Infection: Number	Filaria: Infection: Percentage	Filaria: Average infestation	Filaria: Percentage: Disease	Filaria: Percentage: Infection & disease	Filaria: Percentage: Endemicity	Malaria: Spleen rate	Malaria: Malaria parasite rate.
Cherukara ..	225	147	65·3	53	36·1	42·0	15·0	4·5	47·6	0·0	0·0
Kadamankode ..	218	93	43·0	21	22·6	24·5	32·3	5·4	44·1	1·1	0·0
Villumala ..	228	48	21·5	15	31·2	66·5	35·4	16·6	50·0	0·0	0·0
Kocharuppa ..	228	71	31·1	17	23·9	34·5	42·2	12·6	53·5	0·9	0·0
Kalavapuram ..	238	112	47·1	16	14·2	46·7	33·0	5·3	41·9	0·0	0·0
Total/Average	1,137	471	41·4	122	25·9	41·6	28·9	7·9	46·3	0·42	0·0

TABLE IV.

Incidence of filarial infection in different age-groups and both sexes.

Age-group (years)	Male: Number examined	Male: Infection: Number	Male: Infection: Percentage	Male: Average infestation per 20 c.mm.	Female: Number examined	Female: Infection: Number	Female: Infection: Percentage	Female: Average infestation per 20 c.mm.	Both Sexes: Number examined	Both Sexes: Infection: Number	Both Sexes: Infection: Percentage	Both Sexes: Average infestation per 20 c.mm.
0–5 ..	42	1	2·4	1·0	50	3	6·0	10·0	92	4	4·3	7·8
6–10 ..	30	8	26·6	16·4	36	8	22·2	17·5	66	16	24·2	17·0
11–20 ..	35	9	25·7	43·8	42	14	33·3	25·3	77	23	29·9	32·5
21–30 ..	64	27	42·2	45·3	68	21	30·8	41·3	132	48	36·4	43·5
31–40 ..	26	8	30·1	48·1	14	2	14·3	4·0	40	10	25·0	39·3
41–50 ..	12	6	50·0	63·5	15	2	13·3	21·5	27	8	29·6	53·0
51 and above	26	11	42·3	97·1	11	2	18·1	23·5	37	13	35·1	85·8
All ages ..	235	70	29·8	59·8	236	52	22·0	28·7	471	122	25·9	41·6

static. The maximum recorded was 36·4 per cent in the mixed population (both sexes) of this group. Of 24 infants examined, none was positive either for filaria or malaria. The youngest age at which infection was noted was three years. The average infestation per 20 c.mm. of blood, in all age-groups and sexes combined,

was 41·6. The infestation showed a clear cut progressive rise with advancing age in males. This was reflected in the combined population also, but was less evident in females, in whom this rise was not found after the age of 30. Both the infection rate and density of micro-filariæ were less in all age-groups in females as compared with males.

Table V indicates the incidence of filarial disease in different age-groups. In all the age-groups, excepting six to ten years group, the males showed a higher disease rate than females. From the below mentioned Table V, it would be noted that in both sexes, there was more or less a gradual increase with advancing age up to the age of 50. The youngest age at which disease was noted, was six years. No genito-urinary lesions were noted in persons with the disease as would be noted from Table VI setting the incidence and types of disease in the two sexes. The lowest infection rate of 9·1 per cent was in the group having obvious elephantoid swellings. Twenty per cent of the people surveyed, having no disease manifestation, proved to be symptomless carriers. Among the filarial fever cases, 32·3 per cent harboured micro-filariæ.

TABLE V.

Incidence of filarial disease in different age-groups and both sexes.

Age-group (years)	Males			Females			Both sexes		
	Number examined	Number with disease	Percentage	Number examined	Number with disease	Percentage	Number examined	Number with disease	Percentage
0–5 ..	42	3	7·1	50	1	2·0	92	4	4·3
6–10 ..	30	5	16·6	36	9	25·0	66	14	21·2
11–20 ..	35	10	28·6	42	9	21·4	77	19	24·6
21–30 ..	64	25	38·1	68	21	30·9	132	46	34·8
31–40 ..	26	14	53·8	14	6	42·9	40	20	50·0
41–50 ..	12	7	58·1	15	8	53·3	27	15	55·5
51 and above	26	16	61·5	11	2	18·2	37	18	48·6
All ages ..	235	80	34·2	236	56	23·7	471	136	28·9

2. PERIODICITY.

Four-hourly micro-filarial counts of two persons showed a nocturnal periodicity (Graph 1).

3. MALARIA SURVEY RESULTS.

Only two of the 471 persons surveyed had enlarged spleen, making a spleen rate of 0·41 per cent for the community. No blood smear was positive for malaria parasite (Table III).

TABLE VI.

Percentage incidence of various filarial disease manifestations in both sexes.

	Male		Female		Both sexes	
	Total positive (80)		Total positive (56)		Total positive (136)	
	Number	Percentage	Number	Percentage	Number	Percentage
Filarial fever ..	33	41·3	29	51·8	62	45·6
Swelling glands ..	25	31·3	5	8·9	30	22·1
Swelling one leg ..	9	11·3	5	8·9	14	10·3
Swelling both legs ..	9	11·3	3	5·4	12	8·8
Swelling one hand ..	..	..	6	10·7	6	4·4
Swelling both hands ..	..	..	1	1·8	1	0·7
Swelling hands and legs	4	5·0	7	12·5	11	8·1
All swelling excluding glands	22	27·5	22	39·3	44	32·4

GRAPH 1.

Periodicity studies of micro-filariae

4. Entomological observations.

The following species of mosquitoes were collected either as adults or larvæ:—

Anophelines:—

A. annularis, A. aconitus, A. barbirostris, A. culicifacies, A. fluviatilis, A. hyrcanus, A. jamesi, A. jeyporiensis, A. karwari, A. pallidus, A. subpictus, A. tessellatus, A. vagus.

Culicines:—

C. bitaeniorhynchus, C. fatigans, C. gelidus, C. mimeticus, C. mimulus, C. vishnui, C. whitmorei, Aedes albopictus, M. uniformis, Lutzia fuscanus, Orthopodomyia anopheloides, Tripteroides aranoides, Uranotaenia testacea, Uranotaenia alboannulata.

Of the anophelines, *A. jamesi* was the most predominant, followed by *A. jeyporiensis*, and a solitary specimen of *A. fluviatilis* being collected. *C. vishnui* formed the bulk of culicines collected, while *M. uniformis* was the only species of the sub-genus mansonioides present in the area.

The indoor day collections were generally poor, though from cattlesheds a fair number of mosquitoes were obtained. In a few night collections, using human baits in cattlesheds along the cattle, large number of mosquitoes were caught while feeding on man.

Results of night collections showed that *M. uniformis* entered houses during the early hours of the night and especially between 7 and 8 p.m., as would be noted from Table VII.

Table VII.

*Number of mosquitoes caught during general collection made from human dwellings in the night.**

Species	6 p.m. to 9 p.m.	9 p.m. to 12 night	12 night to 3 a.m.	3 a.m. to 6 a.m.
M. uniformis ..	17†	5	1	0
C. vishnui ..	38	22	8	9
C. fatigans ..	17	1	0	0
C. gelidus	4	0	0	0
A. jamesi ..	1	5	0	0
A. jeyporiensis ..	1	1	0	0
A. tessellatus ..	1	0	0	0
A. hyrcanus ..	2	0	0	0
A. karwari ..	0	0	2	3

* On one night between 6 p.m. and 6 a.m. and on the second day between 6 p.m. and 12 mid-night.
† Most of them were caught between 7 and 8 p.m.

Outdoor collections, from jungle stream banks and tree trunks, yielded *Orthopodomyia anopheloides*, *Tripteroides aranoides*, *Uranotaenia testacea* and *C. mimulus*. A fully-fed specimen of *M. uniformis* was caught from shrubs near a human habitation.

The actual and potential breeding places consisted of ricefields, seepages, seepage channels, ground pools and forest streams.

Pistia stratiotes was totally absent in all the water collections. *Mansonioides* egg clusters were, however, found on the under surface of the blades of grass growing in seepage channels in Kadmankode. The larvæ that hatched out from these egg clusters in the laboratory, were found attached to algæ collected from the breeding places.

The results of mosquito dissections (Table VIII) showed that *M. uniformis* was the vector of *W. malayi* infection. Out of 206 *M. uniformis* dissected,

TABLE VIII.

Record of mosquito dissections.

	First survey (December, 1956)		Second survey (February, 1957)	
	Number dissected	Number positive	Number dissected	Number positive
A. aconitus	1	0	..	..
A. barbirostris	7	0	..	..
A. fluviatilis	1	0	..	..
A. hyrcanus	37	0	2	0
A. jamesi	101	1*	8	0
A. jeyporiensis	86	0	7	0
A. karwari	34	0	8	0
A. pallidus	15	0	2	0
A. subpictus	55	0	..	..
A. tessellatus	40	0	2	0
A. vagus	16	0	..	..
M. uniformis	174	0	32	3†
C. fatigans	209	0	103	0
C. gelidus	1	0	6	0
C. vishnui	273	2‡	124	8*
C. whitmorei	16	0	..	..

*I stage larvæ in abdomen or thorax.
†II and III stage and infective larvæ in thorax. Infective larvæ in head and proboscis.
‡I and II stage larvæ in thorax.

3 showed infective larvæ in the head and proboscis. The infected *uniformis* specimens were collected from Cherukara Forest Settlement of Pathanapuram Taluk. Ten *C. vishnui*, out of 397 dissected, showed developing forms of Stages I and II only and thus this species was not considered of any importance.

DISCUSSION.

Iyengar (1938) surveyed the forest settlements in Pathanapuram, Kottarakara and Nedumangad taluks between 1931 and 1934. During the present survey, excepting for Peruvazhikala, Kalathin and Peringamala, the rest of the settlements surveyed by Iyengar (1938 *loc. cit.*) have been covered. Madathorai settlement in Kottarakara is represented in this survey by Kocharuppa. As against 194 persons examined by Iyengar (1938 *loc. cit.*), in the present survey the number covered was 471. The average infection, disease and endemicity rates recorded by Iyengar (1938 *loc. cit.*) and those obtained during the present survey are given in Table IX.

TABLE IX.

Results of survey of some forest settlements in South Kerala carried out between 1931 and 1934 by Iyengar (1938) and present survey during 1956-57.

Survey	RATE (PER CENT)					
	INFECTION		DISEASE		ENDEMICITY	
	Average	Range	Average	Range	Average	Range
Iyengar (1938)* ..	36·2	23·8 to 44·8	52·8	28·6 to 96·6	70·2	47·6 to 100
Present	25·9	14·2 to 36·2	28·9	15 to 42·7	46·3	41·9 to 53·5

*As calculated from published data.

From the above, it would appear that the incidence of filariasis in these settlements at present has declined. As no pre-control filaria surveys were carried out the extent of collateral filaria control by residual insecticidal application for malaria control during the last four years, cannot be estimated. It can, however, be categorically stated that malaria, which was notorious in these forest settlements, has now been effectively controlled.

However, filarial infection was found to be on the gradual increase up to the age of 30 after which the incidence remained more or less stationary without much fluctuation. The disease rate also showed a gradual increase with advancing age up to the age of 50 after which its incidence showed a tendency to come down (Table IV).

The above findings plus the absence of genital lesions amongst persons with disease as also of relatively less of positives in persons with disease, are in conformity with the findings of Iyengar (1938 *loc. cit.*), Raghavan and Krishnan (1949) and Jaswant Singh *et al.* (1956) in *W. malayi* areas.

Iyengar (1938 *loc. cit.*) found *M. uniformis* infected in nature in Pathanapuram and Kottarakara taluks. It is not clear whether he found the same species infected in any of the other hill-settlement areas in these taluks. In Nedumangad Taluk, Iyengar (1938 *loc. cit.*) reported an endemic *W. malayi* focus in the forest settlements. Though *M. uniformis* could be found in that place, neither infection in them nor their exact breeding places could be detected. The above mentioned scanty information regarding the vector of *W. malayi* in the forest settlements and that malaria used to be highly endemic in those places, gave rise to the doubt to the present authors whether anophelines were playing any role as a vector of filariasis. The present findings, however, indicate *M. uniformis* as the vector of *W. malayi* in the tribal settlements.

An interesting feature is the time of entry of *M. uniformis*, i.e., between 7 and 8 p.m.

Further the presence of *Mansonioides* sp. egg clusters in blades of grass is similar to that of Fraser (1936) who reported breeding of *Mansonioides* sp. in blades of dol grass in Assam. Thus the need for detailed investigations on the bionomics of *M. uniformis* in these forest settlements and in other areas where this species is the main vector, becomes necessary.

SUMMARY.

Some of the forest settlements in Quilon and Trivandrum districts in Kerala State inhabited by hill-tribes were surveyed between December, 1956 and February, 1957 for the prevalence of malaria and filaria.

Incidence of malaria was almost nil at the time of survey and it was due to the activities of the National Malaria Control Programme there.

The filarial infection, disease and endemicity rates were 25·9, 28·9 and 46·3 per cent respectively. The only prevailing species was *W. malayi* and it had nocturnal periodicity.

The vector of filariasis in this region was found to be *M. uniformis* which entered human dwellings mostly during early hours of the night.

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FILARIASIS IN KERALA STATE.

Part IV. Filaria Survey of Eriyad Panchayat, Cranganore Taluk.

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[April 7, 1958.]

PHYSIOGRAPHY.

ERIYAD PANCHAYAT of Cranganore Taluk in Trichur District, Kerala State, is bounded on the north by Edavilanga Village, on the south by Periyar River, on the east by Lokamaleswaram and Methala Villages and on the west by the Arabian Sea (Map 1). It covers an area of 4,180 acres and is connected with Trichur by road and with Ernakulam by road as well as back waters. The nearest railway station, Irinjalakuda, is about 16 miles away.

The soil is sandy and the subsoil water level is very high. Extensive groves of coconut trees are present all round. Main occupation of the people is fishing and coir industry.

Almost every house has a compound with one or more ponds having abundant growth of *Pistia stratiotes*. These ponds are used either for bathing, washing utensils and clothes or for watering the coconut gardens or for soaking coconut husk in connection with coir making.

Wells form the chief source of drinking water. Sewage disposal is unsatisfactory. Barring a few borehole latrines, there are no privies.

Eriyad Panchayat consists of two villages, namely, Azhikode and Eriyad, the former divided into four and the latter into five wards. Total population of the Panchayat, according to the 1951 census, is 19,011, made up of 9,307 males and 9,704 females. The population consists of Hindus, Christians and Muslims, the last being the majority.

MAP I.

MAP OF CRANGANORE
TALUK, KERALA STATE
EXPLANATION
BOUNDARY OF ERIYAD PANCHAYAT
MUKUNDA
PURAM TALUK
NARAYAN MANGALAM
PULLUT VILLAGE
PALLIPURAM VILLAGE
POYYA VILLAGE
MADATHUMPADY VILLAGE
PARUR TALUK
LOKAMALESWARAM VILLAGE
TALUK OFFICE
METHALA VILLAGE
PERIYAR RIVER
PONNANI TALUK
EDAVILANGA VILLAGE
WARD VII (26.6 P.C)
NES BLOCK
WARD VI (25.1 P.C)
ERIYAD VILLAGE
WARD V (13.2 P.C)
SCHOOL
WARD VIII & IX (30.1 (35.6 P.C) P.C)
ARABIAN SEA
WARD I (2 P.C)
WARD III (19.1 P.C)
PANCHAYAT OFFICE
AZHIKODE VILLAGE
WARD IV (11.7 P.C)
WARD II (11.6 P.C)
BOAT JETTY

METEOROLOGICAL CONDITIONS.

Rainfall.—The average annual rainfall is about 112 inches. The rainy season is between May and December, and the main precipitation occurs in June and July.

The annual average maximum and minimum temperature is 93°F. and 83°F. respectively. Relative humidity remains high throughout the year.

THE SURVEY.

There is no record of any past filaria survey of the area. A parasitological and clinical survey of the Panchayat was carried out in December, 1957 by calling at houses, selected at random in the different wards of the Panchayat, between 8.30 p.m. and 00.30 a.m. As far as possible every inmate of the houses visited was included in the survey. From each person, about 20 c.mm. blood was obtained from the finger and made into a thick smear. The blood smears were dehaemoglobinised the next day, and stained with J.S.B. stain I (Jaswant Singh and Bhattacharji, 1944) for five minutes. Number of micro-filariæ in each positive slide was counted and species identified. At the time of survey, the filarial disease manifestations were also noted. Cases giving history of filarial fever or lymphadenitis and/or lymphangitis were also recorded, but not included for working out the disease manifestation and endemicity rates.

Adult mosquito collections were made from human dwellings as well as cattlesheds from various wards, both in the morning as well as at night. The female mosquitoes were identified and dissected to detect developing filarial infection in them.

Larval collections were carried out during the day from different types of breeding places.

RESULTS.

(*A*) HUMAN SURVEY.

A survey of 1,787 persons, forming 9·4 per cent of the total population, showed that *W. malayi* was the only species of infection prevalent, and that the infection was prevalent in all the wards in varying degrees as shown in Table I.

The overall micro-filaria rate was 11·02 per cent for the area.

The various indices for males and females belonging to the different age-groups are tabulated in Table II. The infection rate increased more or less progressively up to the age of 20 after which it remained almost static with minor fluctuations.

Of 16 infants examined, two showed infection, the youngest being a female infant, ten months old, with 9 mf./20 c.mm.

The number of micro-filariæ per positive slide ranged from 1 to 114 and averaged 10·4/20 c.mm.

TABLE I.

Infection, disease and endemicity rates, and average infestation, by wards.

Ward number	Number surveyed	Number with micro-filaria	Percentage with micro-filaria	Number with disease	Disease rate	Number with disease and micro-filaria	Endemicity rate	Average infestation
I ..	151	2	1·33	1	0·7	0	2	16
II ..	155	5	3·22	13	8·4	0	11·6	4·8
III ..	141	14	9·8	14	9·8	1	19·1	19·5
IV ..	77	1	1·16	8	10·4	0	11·7	2
V ..	196	18	9·18	8	4·04	0	13·2	6
VI ..	410	74	18·04	32	7·8	1	25·1	12·2
VII ..	334	54	16·1	38	11·3	3	26·6	8·5
VIII ..	216	21	9·7	54	25·0	10	30·1	3·2
IX ..	107	8	7·5	33	30·8	5	33·6	8·4
Total (including 16 infants) surveyed	1,787	197	11·02	201	11·2	20	21·15	10·4

TABLE II.

Infection and disease rates in the two sexes according to age-groups.

Age-group (years)	Both sexes					Males					Females				
	Total examined	No. with mf.	Infection rate.	No. with disease	Disease rate	Number examined	No. with mf.	Infection rate.	No. with disease	Disease rate	Number examined	No. with mf.	Infection rate	No. with disease	Disease rate
2 – 5 ..	199	19	9·5	3	1·5	97	9	9·3	1	1·03	102	10	9·8	2	1·96
6 – 10 ..	226	26	11·5	2	0·89	144	14	12·2	1	0·86	112	12	10·7	1	0·9
11 – 20 ..	383	48	12·5	37	9·6	173	19	10·9	22	12·7	210	29	13·8	15	7·1
21 – 30 ..	395	41	10·3	54	13·7	168	17	10·1	32	19	227	24	10·6	22	9·7
31 – 40 ..	246	24	9·7	39	15·8	116	12	10·3	25	21·5	130	12	9·2	14	10·7
41 – 50 ..	165	22	13·3	30	18·1	76	13	17·1	13	17·1	89	9	10·1	17	19·1
Above 50 ..	157	15	9·5	35	22·2	78	12	15·4	21	26·9	79	3	3·8	14	17·7
Total ..	1,771	195	11·06	200	11·2	822	96	11·6	115	13·9	949	99	10·53	85	8·95
0 – 1 ..	16	2	12·5	1	6·25	9	0	0	0	0	7	2	28·5	1	14·3
Total including infants ..	1,787	197	11·02	201	11·2	831	96	11·5	115	13·9	956	101	10·56	86	8·99

The filarial disease rate for the whole community was 11·2 per cent and was noted to rise progressively with age, being 1·5 per cent in the two to five years age-group, and 22·2 per cent in the age-group of 51 and above. This general trend was evident in both the sexes.

Out of 16 infants examined, one, aged one year (female), showed swelling of right upper extremity and was also positive for infection.

Two hundred and one persons had filarial disease manifestations. Their relative incidence is given below. Genito-urinary lesions were characteristically absent. Of the above 201, twenty (ten per cent) were also positive for micro-filariæ.

Particulars of limb affected	Number of persons
One leg ...	112
Both legs ...	46
One hand ...	21
Both hands ...	5
Both leg and hand ...	17
	201

Endemicity.—The overall endemicity rate of the community worked out to 21·15 per cent, showing that it is one of high filarial endemicity.

(*B*) ENTOMOLOGICAL SURVEY.

Four hundred and twentythree adult mosquitoes were collected during the survey and consisted of *M. annulifera*, *M. uniformis*, *M. indiana*, *C. fatigans*, *C. sitiens*, *C. gelidus*, *C. vishnui*, *C. lutzia*, *Armigeres obturbans*, *A. subpictus*, *A. vagus*, *A. hyrcanus*, *A. annularis*, *A. jeyporiensis*, *A. jamesi* and *A. karwari*.

The results of dissection of 292 female mosquitoes are given below:—

Species	Number dissected	Number positive	Percentage of infection	Remarks
M. annulifera	77	2*	2·6	
M. uniformis	16	1†	6·2	
M. indiana	4	..	..	
C. fatigans	12	..	..	
C. sitiens	5	..	..	
C. gelidus	17	..	..	
C. vishnui	21	..	..	
C. lutzia	2	..	..	
Armigeres obturbans ..	28	..	..	
A. subpictus	83	..	..	
A. vagus	14	..	..	
A. hyrcanus	2	..	..	
A. annularis	1	..	..	
A. jeyporiensis	1	..	..	
A. jamesi	3	..	..	
A. karwari	1	..	..	

* One mosquito harboured 3rd stage, and another infective larva.
† 2nd stage larva.

From the above, it could be seen that the developing stages of the parasite were present in *M. annulifera* and *M. uniformis* only.

Mansonioides sp. were found breeding in ponds having rotting coconut husks with *Pistia stratiotes.*

DISCUSSION.

Eriyad which, till recently, belonged to the erstwhile Cochin State, now forms part of Kerala State. Topographically the rural areas in the coastal tracts of old Travancore and Cochin States are very much alike. In the former, extensive tracts of *W. malayi* infection are known to occur (Iyengar, 1938) and the same species can be expected to occur in the contiguous areas of Cochin having the same physiographical conditions. The present survey confirms this.

An interesting feature, however, is the high rate of positives for infection among persons with filarial disease as compared to apparently healthy persons, i.e., 10·0 and 11·1 per cent, respectively, which differs generally from the findings of Iyengar (1938) in other *W. malayi* areas in the Travancore State.

In general, high infection rate is accompanied with high infestation and low infection with low infestation. Similar findings were recorded by Buxton (1928) in Santo near Hebrides, and Iyengar (1938) in the former Travancore State. The results of a few surveys conducted in some of *W. malayi* areas in the recent past, including the present one, are given below:—

Name of place	Persons surveyed	Infection rate (per cent)	Average infestation (per positive)	Reference
Shertallay	8,463	20·9	33·7	Jaswant Singh *et al.* (1956)
Kani Settlement	1,137	25·9	41·6	Raghavan *et al.* (1958).
Edapally	631	11·7	12·7	Raghavan *et al.* (1958).
Eriyad Panchayat ..	1,787	11·02	10·04	Nair and Radhagovinda Roy (1958).

SUMMARY.

A filariasis survey of Eriyad Panchayat, a coastal area in Kerala State, was carried out in December, 1957. One thousand seven hundred and eightyseven persons were examined. The infection, disease and endemicity rates were 11·02, 11·2 and 21·15 per cent, respectively. The average infestation was 10·4/20 c.mm. The species of filarial infection prevalent was *W. malayi* only.

Mansonioides annulifera was incriminated as the main vector with an infection rate of 2·6 per cent and *M. uniformis* as the secondary career.

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NOTE ON THE SUSCEPTIBILITY TO D.D.T. OF HOUSEFLIES OF DELHI AND RAJASTHAN.

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INTRODUCTION.

D.D.T. was put into widespread use against insect pests in 1945. Soon after this, reports started appearing regarding *M. domestica* becoming resistant to it (Sacca, 1947 and Wiesman, 1947). Since then, similar reports from almost all over the world have been received (Busvine, 1957). But no change has been reported in the susceptibility of *M. vicina* and *M. nebulo* to D.D.T. in India and Ceylon even after the use of D.D.T. in malaria control for a number of years (Rajindar Pal, 1951*a* and Hess, 1953). D.D.T. resistant *M. vicina*, however, has been reported from Japan (Yasutomi, 1956 and Byers *et al.*, 1956).

Studies were carried out in 1957 (April to July) to elicit change, if any, in the D.D.T. susceptibility of the *Musca* population of the rural areas of Delhi State after the intensive use of the insecticide for ten years in malaria control programme. The results of these investigations are reported in this communication.

MATERIALS.

Adult flies, collected from D.D.T. sprayed village Madanpur of Delhi State, were used in the present studies. This village has regularly been sprayed with D.D.T. twice a year since 1947. It was last sprayed on August 31, 1956 at 100 mg. D.D.T. per sq. ft. For purposes of comparison, flies obtained from an unsprayed village Nathdwara, District Udaipur (Rajasthan), were brought to Delhi for

parallel experiments with Delhi flies under identical conditions. A very high mortality (about 90 - 95 per cent) was observed in flies obtained from the field, both in Delhi and Rajasthan, and consequently wild-caught flies could not be tested for their susceptibility to D.D.T. The surviving insects from both the sources were identified and reared in the laboratory species-wise with a view to determine the insecticide susceptibility of F_1 generation. As there was high mortality of insects in F_1 generation, particularly of *M. nebulo*, insecticide susceptibility tests could only be carried out with *M. vicina* Delhi and Rajasthan strains but not with *M. nebulo*. In the F_2 generation, susceptibility tests were performed with all the strains of *M. nebulo* and *M. vicina*. *M. nebulo* and *M. vicina* from both the sprayed and unsprayed areas were reared and tested separately although these were not considered as two distinct species, and inter grading forms in both *M. nebulo* and *M. vicina* were noted (Sharma *et al.*, 1957). The insect colonies were maintained in the same room under similar conditions of temperature and humidity. Meticulous care was taken to avoid any contamination of the colony with insecticide.

Test Procedure:

Flies were exposed to different dosages of D.D.T. deposits on glass panels in accordance with the technique described by Rajindar Pal (1951*b*). D.D.T. suspension in water (prepared from 75 per cent D.D.T. water dispersible powder) was sprayed on glass panels so as to give deposits of 50, 25, 12·5, 6·25, 3·125 and 1·5625 mg. D.D.T. per sq. ft. Four to five days old flies were exposed to different dosages of D.D.T. for 15 minutes and kept in clean cages, under observation for 24 hours after exposure, for recording the mortality in them. Female flies were exposed to varying dosages of 50, 25 and 12·5 mg. and male flies to 6·25, 3·125 and 1·5625 mg. D.D.T. per sq. ft. Fresh panels were used for each set of experiments. Ten to fifteen flies were exposed on one panel and at least three replicate tests were carried out for each dosage.

BIOMETRIC TECHNIQUE.

The experimental data were analysed following the Probit analysis technique, and the parameters, with their standard errors, were estimated following the maximum likelihood method outlined by Finney (1952). The final results of the analysis are given in Table I. Highly exaggerated ranges of fiducial limits of MLD (obtained by extrapolation) were observed in case of experimental results not showing the critical range of mortality with varying dosages of D.D.T. In such cases (where either much lower or much higher than 50 per cent mortality rates only were observed), therefore, the MLD values were expressed as more than the highest dosage or less than the lowest dosage tried in the experiment.

RESULTS AND DISCUSSION.

From Table I, it may be seen that the MLDs of D.D.T. for *M. vicina* females of F_1 generation of Delhi and Rajasthan strains were 16·22 mg. and 12·88 mg. per sq. ft., respectively. The difference in the MLDs for both the strains was not

TABLE I.

Probit analysis of susceptibility data of houseflies to D.D.T.

Species	Strain	Experimental series numbers	Generations tested	Equation of the fitted 1d-p.r. line	M.L.D. of D.D.T. (mg./sq. ft.)	95 per cent fiducial limits of M.L.D.	Regression coefficient	Remarks
M. vicina (females)	Delhi	1	F_1	$Y=1{\cdot}0923X+3{\cdot}6783$	16·22	26·7–9·9	1·09±0·49	
M. vicina ,,	Rajasthan	1	F_1	$Y=1{\cdot}8749X+2{\cdot}9176$	12·88	17·7–9·4	1·88±0·34	
M. vicina ,,	Delhi	1	F_2	$Y=1{\cdot}966X+1{\cdot}9256$	36·66	50·47–26·61	1·97±0·51	
M. vicina ,,	Delhi	2	F_2	(Y=0·9099+3·031	>50 145·55	776·25–27·29	0·91±0·44)	Corrected mortality rate at the highest dosage, 50 mg. per sq. ft., was 35 per cent.
M. vicina ,,	Rajasthan	1	F_2	$Y=3{\cdot}39X+0{\cdot}9687$	15·45	22·08–10·79	3·39±0·07	
M. vicina ,,	Rajasthan	2	F_2	$Y=2{\cdot}1126X+2{\cdot}5798$	13·96	22·54–8·65	2·11±0·44	
M. nebulo (females)	Delhi	1	F_2	$Y=2{\cdot}0213X+1{\cdot}5207$	52·6	67·76–40·83	2·02±0·44	Corrected mortality rate at the highest dosage, 50 mg. per sq. ft., was 47.1 per cent.
M. nebulo ,,	Rajasthan	1	F_1	(Y=1·4589X+4·2077	<12·5 3·53	15·49–0·13	1·46±0·6)	Corrected mortality rate at the lowest dosage, 12·5 mg. per sq. ft., was 80·4 per cent.
M. vicina (males)	Delhi	1	F_1	Critical range of mortality was not obtained. χ^2 values for each concentration at P.=0·05 showed that the flies of Delhi and Rajasthan did not differ significantly in their susceptibility to D.D.T.				
M. vicina ,,	Rajasthan	1	F_1					
M. vicina ,,	Delhi	1	F_2	..	>6·25	..	..	Corrected mortality rate at the highest dosage, 6·25 mg. per sq. ft., was 13·8 per cent.
M. vicina ,,	Rajasthan	1	F_2	$Y=1{\cdot}01X+4{\cdot}4194$	3·76	6·24–2·26	1·01±0·45	
M. nebulo ,,	Delhi	1	F_2	(Y=2·6963X+1·6104	>6·25 18·07	81·66–3·99	2·7 ±1·7)	Corrected mortality rate at the highest dosage, 6·25 mg. per sq. ft., was 11·2 per cent.
M. nebulo ,,	Rajasthan	1	F_2	..	2·0*	..	..	
M. nebulo (females) and *M. vicina* combined	Delhi	..	F_2	$Y=1{\cdot}7477X+1{\cdot}9645$	54·6	59·7–49·9	1·7±0·27	
,, ,,	Rajasthan	..	F_2	$Y=1{\cdot}8866X+3{\cdot}2169$	8·81	12·9–6·0	1·9±0·37	
M. nebulo (males) and *M. vicina* combined	Delhi	..	F_2	..	>6·25	..	..	Corrected mortality rate at the highest dosage, 6·25 mg. per sq. ft., was 12·6 per cent.
,, ,,	Rajasthan	..	F_2	$Y=0{\cdot}9X+4{\cdot}5246$	3·37	4·04–2·82	0·9±0·4	

*M.L.D. worked out by graphic method.

Figures in brackets were obtained by extrapolation (arithmetical method).

statistically significant at the conventional level (P=0·05). In the case of *M. vicina* males of F_1 generation "critical ranges of mortality" were not obtained in the tests and hence MLDs could not be worked out. However, when the hypothesis of equal susceptibility in the flies of both the strains to different dosages of D.D.T. was tested, it was found to be tenable. This showed that *M. vicina* (males and females) of the two strains did not differ in respect of their susceptibility to D.D.T. in the F_1 generation. MLDs of D.D.T. for *M. nebulo* and *M. vicina* of Delhi strains in F_2 generation did not differ significantly from each other. Similarly no difference could be observed between the MLDs of D.D.T. for *M. nebulo* and *M. vicina* of Rajasthan strain. These observations showed that both *M. nebulo* and *M. vicina* were equally susceptible to D.D.T., besides not being two distinct species morphologically. Hence the results of the susceptibility tests for the two species were combined and MLDs worked out. The combined MLDs of D.D.T. for the female flies in F_2 generation were 54·6 mg. and 8·8 mg. per sq. ft. for Delhi and Rajasthan strains, respectively, and these values differ significantly from each other. Similarly the combined MLDs of D.D.T. for male flies were more than 6·25 mg. and 3·4 mg. per sq. ft., respectively. This showed that the female and male flies of Delhi strains were 6·2 times and 1·9 times more tolerant to D.D.T. when compared with the susceptibility of the female and male flies of Rajasthan strains. Higher tolerance to D.D.T. in *M. nebulo* and *M. vicina* (male and female) was also reflected in the Delhi strains when their MLDs were computed separately and compared with the respective MLDs of *M. nebulo* and *M. vicina* (male and female) of Rajasthan strains in F_2 generation (Table I). It may be noted from the above account that though the flies from the sprayed and unsprayed areas were equally susceptible to D.D.T. in F_1 generation, in F_2 generation flies of Delhi strains (sprayed area) exhibited significantly higher tolerance to D.D.T. when compared with Rajasthan strains (unsprayed area). Decrease in susceptibility to D.D.T. in certain strains of houseflies, during insecticide-free laboratory rearing, has been reported from Netherlands (Meltzer, 1956).* The possible causes for lowered susceptibility of the insects reared in the laboratory could have been, (*i*) the selection of the vigorous insects during colonization, (*ii*) variation in conditions during rearing, (*iii*) variation in conditions influencing the susceptibility tests, and (*iv*) increase in the frequency of individuals with lowered susceptibility in colony populations due to selection. Since the flies of Delhi and Rajasthan strains were reared and tested under similar conditions, there was little chance of causes (*i*) to (*iii*) enumerated above having played any significant role in the observed lowered susceptibility of flies of Delhi strains in F_2 generation. However, this aspect of the problem requires further detailed studies.

It may, therefore, be stated that the increased tolerance to D.D.T. in F_2 generation most probably was due to increase in frequency of individuals with lowered susceptibility in that generation. As flies were not subjected to D.D.T. selection pressure in the laboratory, the presence in greater proportion of individuals tolerant to D.D.T. in Delhi strains, when compared to Rajasthan strains, was due to some selection of insecticide-tolerant flies in Delhi where D.D.T. has been used for malaria control since 1947.

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*Reference obtained by courtesy of Dr. A. W. A. Brown of the World Health Organization.

SUMMARY.

Houseflies (genus *Musca*) collected from a D.D.T. sprayed village of Delhi and an unsprayed village of Rajasthan were colonised in the laboratory and tested for their susceptibility to D.D.T. Flies of the Delhi strains in F_2 generation were found to be more tolerant to D.D.T. than those of the Rajasthan strains.

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NEW FIXATIVES FOR STAINING MICROFILARIAE.

BY

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[December 1, 1957.]

FIXATION of a tissue has very essential effects upon cells such as penetration, coagulation and toughening. These three properties have been considered to be the foundation of the first effective fluid for fixation in bulk originally conceived by Flemming (1882). Later variations in the components of Flemming's fluid have given rise to all the chief fixatives which are in use today. The second line of development arose from the penetrating alcohol fixatives first used by Carnoy (1884) in mixture with glacial acetic acid.

Among the fixatives commonly used for parasitological studies, mention may be made of Schaudinn's, Bouin's, Carnoy's, Gilson's and Zenker's. Of particular importance in fixing and staining of microfilariæ from the blood of vertebrates, the materials employed have been (Belding, 1952);

(1) mixture of equal parts of ether and 95 per cent alcohol;

(2) silver impregnation followed by either Giemsa or Delafield's or Bohmer's hæmatoxylins;

(3) Saisawa-Sugawara silver impregnation technique;

(4) Wilson's technique involving air-dried smear being stained directly in Giemsa diluted with phosphate buffer (pH 7·2);

(5) the technique followed using Leishman stain; and

(6) the technique followed by the Malaria Institute of India, staining microfilariæ with J.S.B. I (polychromed methylene blue) soon after dehæmoglobinisation of the blood smear with water.

This method is simpler and quicker than the technique followed by Iyengar (1938) using aqueous solution of methylene blue.

While following the techniques mentioned so far, the object seems to have been to achieve good results regardless of time factor involved in each technique.

Reduction of glacial acetic acid component to preserve the chondriosomes from solution, replacement of osmic acid by less expensive reagent and inclusion

of reagents to reduce surface tension or raising of osmotic pressure, have been the main objects of recent workers.

In view of the above considerations, and also to facilitate further possible systematic parasitological studies, work was undertaken in this Institute to evolve a new fixative with a view to improving the staining technique by using Giemsa, Delafield's and Ehrlick's hæmatoxylins, J.S.B. I and II and Schiff's reagent following Feulgen's technique. The present communication describes the utility of a new fluid in fixing the microfilariæ from the blood of vertebrates.

MATERIALS AND METHODS.

Fresh, unfixed and unstained human blood smears containing *Wucheraria bancrofti* and *W. malayi*, microfilariæ from the blood of garden lizard (*calotes*) and partridges as well as from dog showing mixed infection, identified in this Institute as *Dirofilaria immitis* and *D. repens*, were available for the present work. Besides these, blood smears containing human microfilariæ which were previously dehæmoglobinised with water and preserved for a time after staining with J.S.B. I (polychromed methylene blue), were also fixed and stained.

While dealing with fresh, unfixed and unstained blood smears containing microfilariæ, it is a common practice to dehæmoglobinise the smear with water. In the present work, freshly prepared 20 per cent alcohol was employed for two to three minutes instead of water for dehæmoglobinisation. Immediately after this, the Fixative I described here was placed over the smear and kept for 10 and 30 minutes respectively. Further procedure was just the same for both preserved smears and fresh ones.

The stains employed for tests were dilute aqueous solution of Delafield's hæmatoxylin (5 c.c. of this stain in 55 c.c. of distilled water) involving progressive type of staining; as well as Giemsa (1 c.c. of Giemsa stain in 49 c.c. of distilled water); Ehrlick's hæmatoxylin, J.S.B. I and II and also Schiff's reagent followed by Feulgen's technique. All these stains were prepared in the laboratory.

Fixative I.—As fixing reagent, a mixture of commercial absolute alcohol and normal hydrochloric acid in the proportion of three to one, was employed. The components of the fixative, that is, absolute alcohol and normal hydrochloric acid were tested out separately for their effect on fixing and staining. The results thus obtained were compared with smears fixed in the mixture of three parts absolute alcohol and one part of normal hydrochloric acid, staining dyes being the same.

Absolute alcohol.—It is preferable to use pure quality of absolute alcohol. For the present tests, locally available absolute alcohol was used. The slides were fixed for 10 and 30 minutes respectively, then they were passed through 90 per cent and 70 per cent alcohols to water and then stained.

Fixation with absolute alcohol presented rather a poor picture because the important cell structures probably did not harden enough to present a satisfactory picture after staining.

Normal hydrochloric acid.—Commerical concentrated hydrochloric acid (Sp. gr. 1·16) was available for experiment. A quantity of normal hydrochloric acid was easily prepared by mixing 5 c.c. of the concentrated acid in 55 c.c. of distilled

water. A set of slides, containing microfilariæ, were fixed in this normal acid for 10 and 30 minutes respectively. After fixation, the slides were immersed thoroughly in water to remove the acid, then stained and examined.

Fixing property of normal hydrochloric acid was not very encouraging. The picture of microfilariæ, presented after staining, was poor.

Mixture of three parts of absolute alcohol and one part of normal hydrochloric acid.—Slides were fixed in the freshly prepared mixture for 10 and 30 minutes respectively. After fixation, the slides were passed through 90 per cent and 70 per cent alcohols to water and stained with all the dyes mentioned above.

The morphological characters of microfilariæ, after fixing in this mixture of absolute alcohol and normal hydrochloric acid, offered quite satisfactory results after staining with all the dyes mentioned above.

For a comparative assessment of the fixing property, batches of slides were also fixed in methyl alcohol for 10 and 30 minutes and then stained in all the dyes mentioned above. But the results of staining of microfilariæ were poor.

The essential step in the fixation-staining process is, in general, one of salt formation of non-specific nature (Gulick, 1941). Feulgen's method of staining is a solitary example of a known chemical reaction being concerned. A word about the picture obtained with Schiff's reagent, after Feulgen's technique, would be equally interesting. The best results were obtained after fixing in the mixture of absolute alcohol and normal hydrochloric acid as compared to those fixed in the individual constituents of the mixture. The nuclei of the microfilariæ and that of the various developmental stages of infective larvæ of *W. bancrofti* gave a very clear picture of Feulgen positive material.

FIXATIVE II.

Yet another fixing fluid, prepared always fresh just before use by mixing 9 parts of seventy per cent ethyl alcohol, 0·5 part normal hydrochloric acid and 0·5 part formaldehyde has been described elsewhere (Narayandas and Ray, 1958). This Fixative II has been tried successfully in respect of sheathless microfilariæ, *Dirofilaria repens*, and *D. immitis* from the blood of dog. The method, involving this Fixative II and the simple re-stainable aqueous stains J.S.B. I and II, is found to be simple, quick and useful for routine work. The stained specimens do not fade away early.

The dyes employed for staining sheathed microfilariæ (man, partridge and *caloles*) and sheathless forms (dog) were just the same. While using dilute solutions of Giemsa and Delafield's hæmatoxylin as well as Ehrlick's hæmatoxylin for a progressive type of staining, much difficulty was not encountered in obtaining the desired depth of colourations in the microfilariæ, but the avidity, with which the structures of microfilariæ stained with J.S.B. I and II solutions, needed vigilance. It was, of course, observed clearly that different species of microfilariæ from different species of hosts required varying amounts of time for staining after fixing the smears in the new Fixative I. The dog microfilariæ required 10 seconds in J.S.B. II solution and about 6 to 8 seconds in J.S.B. Solution 1, and a good differentiation in stain being achieved by immersing the slide in buffer water.

TABLE

Comparative results of fixing and

(1) Wucheraria

Fixatives	Period of fixation (in minutes)	DELAFIELD'S HÆMATOXYLIN			EHRLICH'S HÆMATOXYLIN		
		Nucleus	Bodywall	Sheath	Nucleus	Bodywall	Sheath
I. Absolute alcohol	30	Brick-red: Fairly clear	Light-purple Fairly clear	Light-purple Fairly clear	Purple: Distinct	Light-brown Fairly clear	Very light-brown. Not clear
II. Normal hydrochloric acid	30	Violet: Distinct	Light-purple: Fairly clear	Light purple: Fairly Clear	Not very distinct: Purple	Very light-brown	Very light-brown: Not clear
III. 3 parts absolute alcohol and 1 part normal hydrochloric acid	30	Deep purple: Very clear and distinct	Very light-blue: Very clear	Light-purple: Very clear	Very distinct and clear: Blue	Light-purple Clear	Light purple: Clear
IV. Methyl alcohol	30	Distinct: Brick-red	Light-purple: Fairly clear	Light-purple: Clear	Brick-red: Not clear	Fairly clear: Light-brown	Light-brown: Fairly clear

(2) Wucheraria

3 parts absolute alcohol and 1 part normal hydrochloric acid.	30	Black: Smudged.	Buff: Clear	Buff: Clear.	0	0	0

TABLE

Comparative results of fixing and staining of microfilariæ

Source of micro-filariæ	Fixative	Period of fixation (minutes)	DELAFIELD'S HÆMATOXYLIN			EHRLICH'S HÆMATOXYLIN		
			Nucleus	Bodywall	Sheath	Nucleus	Bodywall	Sheath
Calotes	One part normal hydrochloric acid and three parts absolute alcohol	10	Blue: Distinct	Light-purple	Light-purple	Blue: Distinct	Light-purple	Light-purple
Partridge		10	Blue: Distinct	,,	,,	,,	,,	Unstained, but clear.
					Dog: Dirofilaria repens and D. immitis—			
Dog	,,	10	Blue: Distinct	Light-blue	..	Dark-blue: Distinct	Light blue	..

I.

staining of human microfilariæ.

bancrofti.

Giemsa			J.S.B. I and II			Schiff's reagent with light green		
Nucleus	Bodywall	Sheath	Nucleus	Bodywall	Sheath	Nucleus	Bodywall	Sheath
0	0	0	Violet: Not distinct	Very light-pink	Not clear	Purple: Distinct	Not clear	Not clear
0	0	0	Blue: Not very distinct	Very light-blue	Very light-pink: Not clear	Purple: Distinct	"	"
Blue: Not distinct	Fairly clear	Not clear	Deep blue. Not very distinct	Light-blue Fairly clear	Not clear	Purple: Very distinct	"	"
0	0	0	Blue: Not Not very distinct	Light-blue: Fairly clear	Not clear	0	0	0

malayi.

Blue: Smudged	Pinkish	Not clear	Violet: Smudged	Pinkish: Clear	Pinkish: Not very clear	Purple: Smudged	Not clear	Not clear

0 = Materials not available.

II.

from the blood of vertebrates. Calotes and partridge.

Giemsa			J.S.B. I and II			Schiff's reagent with light green		
Nucleus	Bodywall	Sheath	Nucleus	Bodywall	Sheath	Nucleus	Bodywall	Sheath
Violet: Distinct	Not clear	Not clear	Violet: Distinct	Not clear	Not clear	Purple: Distinct	Not clear	Not clear
"	Light-pink	"	"	Light-pink	"	Purple: Distinct	"	"

Sheathless.

Violet: Distinct	Light-purple	..	Violet: Distinct	Light-pink	..	Purple: Distinct	Not clear	..

Human blood smears required 30 seconds in J.S.B. II solution and about 30 to 40 seconds in J.S.B. I solution. By slight variations in time in each of the solutions II and I and using the buffer water, the body-wall and the nuclear material of the sheathed microfilariæ from the blood of partridge and *calotes*, were brought out clearly. But the sheaths in all these cases were not found to take up J.S.B. stains. A simple technique by which the sheath of the human microfilariæ could be brought out for routine examination, involving the use of the simple aqueous stains J.S.B. I and II, will be published in a subsequent paper.

Blood smears stained with Giemsa and J.S.B. solutions were allowed to dry before mounting in Canada balsam, whereas slides stained with other dyes were upgraded through alcohols, cleared in xylol and mounted in Canada balsam.

DISCUSSIONS.

The objects to be treated with a fixative have certain invariable properties. They exist, however, under various conditions of cell and tissue environment. ' Good fixation is the corner stone of good histology ' (Langeron, 1925). Fixation profoundly influences the subsequent binding of the dye to the proteins of the cells.

The alcoholic fixatives, containing glacial acetic acid, soften cell walls and matrices conveniently and by their own lower surface tension allow of the penetration of the resistant mambranes (Darlington and La Cour, 1947). Acetic acid, with its small ions, penetrates a tissue rapidly, but it swells up the protoplasm and does not toughen it for later treatment or at least not quickly enough (Darlington and La Cour, 1947). This difficulty is got over by combining the stain with an acid for joint action, or by combining alcohol with the acid to fix and harden the protein, other than chromosomes in the cells, so that the chromosomes may be preserved for later staining. Acetic acid does not coagulate proteins, but fixes nucleoproteins whereas chromic and osmic acids contract the protoplasm. Osmic acid vapour gives the best possible fixation but without deep penetration. Carnoy's fixative has a disadvantage in making the cuticle (Nematodes) splinter and disintegrates the underlying tissues, but the nuclear picture is good.

It is well known that ethyl alcohol is a reducing agent, being easily oxidised to acetaldehyde and thence to acetic acid. It must not be used in mixture with chromic acid, potassium dichromate and osmic acid (Coatney and Beams, 1950). Further, alcohol precipitates albumin and globulin instantaneously which are insoluble in water (denaturation). With nucleic acid, the alcohol gives a precipitate soluble in water.

After denaturation, i.e., rendering insoluble of proteins by alcohols, the linkages of the proteins appear to be altered. Because not only is its solubility affected, but also the combining capacity with both acids and bases is increased. The change of combining capacity makes for easier staining, but it is less marked with alcohol than with other fixatives. Tellyesniczky and Underhill (1932) described alcohol as one of the fastest penetrators of tissues. As alcohol diffuses into cells and becomes misable with their water content, the cytoplasm of each cell is precipitated and pushed to a side inside the cell. Absolute alcohol ranks as a fixing agent because it kills with such rapidity that structures have hardly time to get deformed in the process.

However, the Fixative I described here, i.e., a mixture of three parts of absolute alcohol and one part of normal hydrochloric acid offers an advantage over other fixatives. Very encouraging results are obtained when microfilariæ from the blood of vertebrates are fixed in this new Fixative I and stained with Delafield's and Ehrlick's hæmatoxylins, J.S.B. I and II, Giemsa as well as with Schiff's reagent followed by Fuelgen's technique. Structure of sheath in *W. malayi* and *W. bancrofti* from human blood as well as in microfilariæ from partridge and *calotes* are well brought out when stained with Delafield's and Ehrlick's hæmatoxylins, and fairly nicely with Giemsa.

The general appearance of body wall in all the microfilariæ from the blood of vertebrates seen so far, is good, and the nuclear structure is clear and distinct.

A comparative statement of results obtained by various methods of staining the human microfilariæ from blood smears, and also comparative description of structures in microfilariæ from *calotes* and partridge as well as from dog, are given in Tables I and II.

SUMMARY.

A method for fixing and staining microfilariæ from the blood of vertebrates involving a new and effective combination of absolute alcohol and normal hydrochloric acid, offering good results, has been described.

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SOME NEW RECORDS OF CULICINES* OF DEHRA DUN (UTTAR PRADESH) WITH A DESCRIPTION OF A NEW VARIETY.

BY

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[March 24, 1958.]

DURING the course of an entomological field trip to Dehra Dun†, a number of Arthropod specimens were collected. Amongst these were 25 mosquitoes, of which six species belonged to the tribe *Anophelini* and nineteen to the tribe *Culicini*. Eighteen of the culicines, which include a new variety, are new records for the valley.

The Dehra Dun District covers an area of 1,193 square miles between 77° 35′ north and 78° 20′ east longitude, and 29° 57′ and 31° 2′ north latitude, and comprises two distinct tracts, the Dun proper which is an open valley enclosed by the Siwalik hills and the outer scrap of the Himalayas, and the hill pargana of Jaunsar-Bawar, a wedge of mountain and gorge thrust between the Sirmur and Tehri (former States) and attached to the north-west corner of Dun. The valley has tributaries of Jamuna and Ganges flowing through it and is rich in forest wealth (Walton, 1911).

Mosquitoes reported in this paper were collected as larvæ and or adults near Mohand, Ganeshpur Village, Shespur, Kalsi, Lachhiwala range and Sahas Dhara (Map 1). All these places are within 40 miles perimeter from Dun town proper. The adult anophelines were collected resting indoors only.

Species-wise distribution of culicines, collected during the field trip, is given below. In case of one tree-hole breeder, the larvæ did not pupate. The specimens, according to the larval key of Knight and Mattingly (1950), is of *Orthopodomyia*

**Culicidae, Diptera.*
†The trip was made on September 22 to 25, 1957.

MAP 1.

SKETCH MAP OF DEHRA DUN
FOOTHILL AREAS
(Scale 1" = 8 miles)
N
CHAKRATA
KAILANA
KALSI
JUMNA R.
HERBERTPUR
PLACES VISITED FOR COLLECTION SHOWN UNDERLINED.
DEHRA DUN
SONG R.
LACHHIWALA RESERVED FOREST
GANESHPUR
TO SAHARANPUR
TO ROORKEE
R. GANGA

*andamensis** Barraud (1934). A larval description of this species has been included here for future confirmation†. The bred-out adults from larvæ of another tree-hole resembled those of *Aedes* (*Finlaya*) *greeni* (Theobald, 1903). However, the larvæ and male genitalia differed from the original description (Theobald, 1903) and the description given by Barraud (1924*a*). On the basis of these differences, it is considered that the present specimen is a new variety (var. *doonii*) and a description of it, along with the differences from the type form, is included in this paper.

LIST OF SPECIES.

(A) Anophelines.

Adults of *A. annularis*, *A. culicifacies*, *A. fluviatilis*, *A. hyrcanus*, *A. maculatus* and *A. subpictus* were collected resting inside human dwellings. In addition, larvæ of *A. fluviatilis* and *A. maculatus* were collected in the rivulet near Mohand culvert.

(B) Culicines.

1. *AEDES (FINLAYA) ALBOLATERALIS* THEOBALD, 1908.

Habitat.—Tree-holes and bamboo stumps. At Lungtch, adults were caught indoors.

Distribution.—It has been recorded from Assam (Golaghat, Sibsagar District, Halflong, Lungtch, Lushai Hills, Cachar Hills, Nongpoh, Khasi Hills District), North Bengal (Sukna, Kurseong, Darjeeling District, Meenglas, near Jalpaiguri, Duars), East Punjab (Simla, Solan, Kasauli, Koti near Kalka), North Kanara (Karwar) in India; and Rangamati, Chittagong Hill Tracts and Sylhet in East Pakistan (Barraud, 1934; Theobald, 1908). It has also been recorded from Malaya, Yunnan and Philippine in the same habitat (Horsfall, 1955).

In Dehra Dun, two females were caught in the forest around the rivulet near Mohand.

2. *AEDES (FINLAYA) ASSAMENSIS* (THEOBALD), 1908.

Habitat.—Tree-holes.

Distribution.—It has been recorded from Travancore, Assam (Nongpoh, Halflong, Dibrugarh) and North Bengal (Sukna) in India; and Sylhet, Rangamati, Chittagong Hill Tracts of East Pakistan (Barraud, 1923*b*:1934; Theobald, 1910; Senior White, 1923). It has also been recorded from Indo-China, Yunnan and Java (Horsfall, 1955).

In Dehra Dun, a single male was caught near the Song River in Lachhiwala Forest Range.

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*Barraud dealt it under *O. anopheloides* var. *andamensis* but is now recognised as a species distinct from *anopheloides* (Knight and Mattingly, 1950).

†Barraud did not describe the larva.

3. *AEDES (FINLAYA) AUREOSTRIATUS* *VAR *DOONII* (N. VAR).

Locality.—Kalsi, Dehra Dun. Type ♀, slide of ♂ terminalia and larva in collection of Malaria Institute of India.

Habitat.—Larvæ of this species, along with the larvæ of *Culex* (*Neoculex*) *brevipalpis* and *Orthopodomyia andamanensis*, were collected from a mango tree-hole in Kalsi. The bred-out adults resemble *A.* (*F.*) *aureostriatus* var. *greeni* (Theobald) in ornamentation but differ in the structure of male terminalia, and the structure of larvæ. The description of the male terminalia† and the larva‡ is given below:

Male terminalia (*Fig. 1*).—Lobes of ninth tergite (IX F-L) close together, each bearing five spines. Tenth sternite (X-S) sclerotized and curved apically. Phallosome (Ph) cylindrical and little longer than wide, claspette stem (Cl-S) about 1/3 length of basistyle, roundly expanded at the apex on the convex side

FIG. 1.

Male terminalia of *Aedes* (*F*) *aureostriatus* var *doonii* (n. var). A-L, Apical lobe. B-L, Basal lobe. Bs, Basistyle. CL-F, Claspette filament. CL-S, Claspette stem. Ds. Dististyle. Ds-C, Dististyle claw. IX L. Lobe of ninth tergite. Ph, Phallosome. X-S, Tenth sternite.

*Barraud (1934) dealt it as *A.* (*F.*) *greeni* (Theobald), 1908 in the text of his famous volume on culicidæ but in the appendix noted it to be a variety of *A.* (*F.*) *aureostriatus*. Subsequent work of Knight and Marks (1952) has discussed the position of this species in the *aureostriatus* group and the present nomenclature is followed after them.

†Terminology followed after Carpenter and LaCasse (1955).

‡Terminology followed after Belkin (1950).

(tubular in *greeni*) bearing three short spines, small setae confined to the inner aspect only; claspette filament (Cl-F) shorter than the stem, funnel shaped at the base and expanded apically; at the point of its origin (from claspette stem—CL-S) there is a narrow chitinized ring. Basistyle (BS) about four times as long as mid width, clothed with scales and long and short spines; basal lobe (B-L) distinct, bearing a row of flattened blades right from base to apex (confined to apex only in *greeni*), those at apex quite large; apical lobe (A-L) rudimentary represented by concentration of spines (absent in *greeni*). Dististyle (D-S) about 1/2 length of basistyle expanded basally and tapering apically; claw (Ds-C) represented by a short appendage.

Larva (*Fig.* 2).—Resembles *greeni* but can readily be distinguished from it by (*i*) antennal tuft which may be represented by a single (as in *greeni*) or a terminally bifid stout hair; (*ii*) pecten teeth which may be smooth, with one denticle or two denticles (one denticle in *greeni*); (*iii*) varying number of ridges of minute spicules present in anal saddle (absent in *greeni*); (*iv*) hairs of lower caudal tuft unbranched (branched in *greeni*); and (*v*) fringed scales (of comb) 55 in number (40 in *greeni*). A complete description of the fourth instar larva is given below:

Length: About 6 mm. long. Colour: Body slightly brownish, head and siphon blackish brown and slightly chitinized. Head (Fig. 2, '*A*') about as long as broad; antennae fairly long and slender nearly 15 times as long as wide, shaft with few spicules, tuft represented by a single stout barbed hair, which may sometimes split into two at the distal end, attached further from the base than the apex; terminal antennal hair well developed with two finger-like processes, antennal papillae about two-third length of the terminal hair. Mouth brush hair simple; preclypeal (1) spines short and slender; post-clypeal (4) small with multiple branches; upper frontal (5) three to four branches all barbed; lower frontal (6) single and exceptionally long, about twice the length of the head; pre-antennal (7) barbed and consists of 10-12 branches; sutural (8) simple dividing into two at the distal half; mentum (Fig. 2, '*B*') triangular with 10-11 teeth on each side, four teeth towards base large and widely spaced. Thorax: Tuft hairs of thorax normal. Abdomen (Fig. 2, '*C*'): well-developed hairs on 1st segment, II-VIII with small hairs; comb of eighth segment of about 55 fringed scales in a patch (Fig. 2, '*G*'). Siphon 3½ times the width at base; acus present; pecten of 26 small and pointed teeth, two most basal without any lateral denticles, next six with one denticle and the rest with two denticles (Fig. 2, '*D*', '*E*', '*F*'); siphonal tuft of 5-6 barbed branches at about 2/3 distance from base; anal segment short, 3/4 of dorsal surface covered with dark-brown saddle, posterior border with small teeth, whole saddle with ridges of minute spicules; lateral hair (LH) branching in the middle into 5-6 branches; upper caudal tuft (U.C.) of three branches, two long and one small; lower caudal tuft (LC) single, fairly long, and pointed. Anal gills pointed, dorsal of the size of the anal segment and ventral 1/2 of the dorsal. Ventral brush of moderate size.

4. *AEDES* (*MUCIDUS*) *SCATOPHAGOIDES* THEOBALD, 1901.

Habitat.—Open natural pools, chiefly during rainy season.

Distribution.—It has been recorded from Delhi, Rajputana (Nasirabad), East Punjab (Amritsar, Ferozepur, Karnal, Kasauli), Delhi, Uttar Pradesh

FIG. 2.

Larva of *Aedes* (*F*) *aurostriatus* var. *dosnil* (n. var.).
A. Head. Hairs : (1) Pr clypeal. (4) Post-clypeal. (5) Upper frontal. (6) Lower frontal. (7) Pro-antennal. (8) Sutural.
B. Mentum
C. Terminal segments. LC, Lower caudal hair. LH, Lateral hair: UC, Upper caudal hair.
D, E and F. Pecten teeth.
G. Comb scale.

(Saharanpur, Kanpur, Bareilly, Moradabad); Madhya Pradesh (Kamptee); Bihar (Pusa, Saran, Chatra and Kodarma, Hazaribagh, Purnea); Bengal (Bauria Damukdiaghat), Madras (Guindy, Anantpur, Trincomali) in India; and Sialkot and Jhelum in West Pakistan (Barraud, 1929*a*:1934; Senior White, 1923). Recently it has been recorded from Poona (Rao and Rajagopalan, 1957). It has also been recorded from Ceylon, Burma (Barraud, 1934), Sudan, West Africa, Uganda, Ethiopian region (Barraud, 1934; Senior White, 1934; Horsfall, 1955) and Chienquia in Northern Thailand (Bhatia, 1950).

In Dehra Dun, one female specimen was caught at Sahas Dhara in the village on bank of the Song River.

5. *AEDES* (*STEGOMYIA*) *ALBOPICTUS* (SKUSE), 1894.

Habitat.—Tree-holes, bamboos, leaf-axils and rarely in artificial receptacles or rock-pools.

Distribution.—It has been recorded from Andaman Islands; Assam (Gauhati, Shillong, Halflong, Dibrugarh); Bengal (Tirrihana and Marianbaree tea estates and Sukna); Orissa (Ululcuru, Keonjhar State); Bihar (Pusa); Punjab (Karnal, Kasauli); Delhi; Bombay (Bombay, Elephanta Island, Trombay, Hog Island, Belgaum, Nagargali); Coorg; Malabar Coast; Madras (Nilgiri Hills, Anamali Hills) in India; Chittagong Hill Tracts of East Pakistan and in Burma and Ceylon (Barraud, 1931; 1934). Recently, Rao and Rajagopalan (1957) recorded it from Poona. Outside Indian Region, it has been recorded from Philippines, Sumatra, Madagascar and Amboina (Senior White, 1923). Horsfall (1955) states that this species is found from Japan South and West to the continent, and eastward through Micronesia to the Hawaiian Island.

In Dehra Dun, specimens were caught biting in the forest areas near Mohand culvert, Lachhiwala Range and Kalsi.

6. *AEDES* (*S.*) *WALBUS* THEOBALD, 1905.

In India, it is known to be widely spread from Punjab to Bihar and North Bengal and through Peninsular India to Ceylon. Apparently absent from Assam and Burma (Barraud, 1934). It has been recorded from Narcondam Island in Bay of Bengal (Theobald, 1907); in Sukna, Darjeeling (Bengal) by Theobald (1910); in Matheran (Bombay State) and Pusa in Bihar (Senior White, 1923). Recently, Rao and Rajagopalan (1957) recorded it from Poona. It is also known from Siam, Formosa, Ceylon, Malaya, Hong Kong, Kuala Lumpur and S. China (Barraud, 1934; Horsfall, 1955; Mackie, 1954; Senior White, 1923).

In Dehra Dun, both males and females were caught from Mohand, Lachhiwala Range and Kalsi forests.

7. *AEDES* (*AEDIMORPHUS*) *PIPERSALATUS* (GILES), 1901.

Habitat.—Ground pools, water filled ditches and similar places.

Distribution.—It has been recorded in India from Bombay State (Kamptee, Nagpur District, Belgaum and Tavargatti); Bihar (Ranihat, Pusa); Orissa (Cuttack); Uttar Pradesh (Anwarganj, Kanpur District), and Madhupur in Bengal

(Barraud, 1928). Recently, Rao and Rajagopalan (1957) have recorded it from Poona.

In Dehra Dun, a single male was caught from forest at Kalsi.

8. ARMIGERES (ARMIGERES) OBTURBANS (WALKER), 1860.

Habitat.—Tree-holes, bamboos, domestic collections of foul water.

Distribution—In India, it has been recorded from Delhi; Punjab (Amritsar, Kasauli); Uttar Pradesh (Naini Tal, Meerut); Bombay State (Trombay, Belgaum, Nagargali); North Kanara (Karwar, Yellapur); Nilgiri Hills (Coonoor); Bengal (Calcutta); Eastern Himalaya Foot-Hills (Kurseong, Sukna); Assam (Lushai Hills, Nongpoh); Madras, Travancore, Bihar (Barraud, 1927). Common species of the genus, occurs from Punjab to Assam and Burma, and throughout Peninsular India to Ceylon (Barraud, 1934). In East Pakistan, it is known from Chittagong, Rangamati and Sylhet (Barraud, 1927). Recently, Rao and Rajagopalan (1957) recorded it from Poona (India). It is also known to occur in Malay Peninsula, China, Japan and Sumatra, Java, Formosa, Philippines, Amboina (Indonesia), Pupua Cerum, Celebes (Barraud, 1934; Horsfall, 1955 ; Senior White, 1923) and Bangkok in Thailand (Bhatia, 1951).

In Dehra Dun, a single female was caught in bamboo thickets near Kalsi.

9. CULEX (CULEX) BITAENIORHYNCHUS GILES, 1901. TYPE FORM.

Habitat.—Open weedy pools.

Distribution.—Oriental, Australian, African regions (Horsfall, 1955) and Japan (Barraud, 1934). In India, it has been recorded from Punjab (Amritsar); Uttar Pradesh (Shahjahanpur); Bombay State (Trombay, Parel, Belgaum, Tavargatti, Yellapur and Karwar); Madras (Salem, Nilgiri Hills); Bihar (Pusa, Singhbhum); Assam (Shillong, Nongpoh, Dinnapur, Dibrugarh and Halflong); Kerala (Travancore, Pellode, Kermaddi); Orissa (Balighai, Puri); Manipur, and Andaman Island and in East Pakistan from Chittagong, Rangamati and Sylhet (Barraud, 1924*c* ; Senior White, 1923). Recently, Rao and Rajagopalan (1957) have recorded this species from Poona.

In Dehra Dun, a single male and two females were caught in shrubs near Mohand culvert, Lachhiwala Range (near Song River) and Kalsi.

10. CULEX (CULEX) EPIDESMUS (THEOBALD), 1910.

Distribution.—"Widely spread from Punjab to Assam and southwards to Ceylon, but apparently never very abundant. Perhaps commonest in Bihar" (Barraud, 1934). It has been recorded from Simla (Punjab) ; Bhogaon, Pusa, Katihar (Bihar); Ranihat, Cuttack (Orissa), and Nongpoh (Assam) (Barraud 1924*c* ; Senior White, 1923). Recently it has been recorded from Poona (Rao and Rajagopalan, 1957).

In Dehra Dun, a single female was caught from Lachhiwala Forest Range near Song River.

11. CULEX (CULEX) FATIGANS WIEDMANS, 1828.

Habitat.—Domestic collections of water and in such places as flooded open cement drains, flooded latrines, overflow water from houses, kitchens, etc. Also in ground-pools, ditches, and shallow wells. Rarely in tree-holes or bamboos; not in jungle-pools, stream pools, etc., if far from human habitations (Barraud, 1934).

Distribution.—Abundant throughout India (Barraud, 1934). Encircles the world in the tropics and warmer temperate climates; covers the continents of Australia, Africa and South-America east of Andes (Horsfall, 1955).

In Dehra Dun, larvae were collected from rivulet* near Mohand and the adults were collected resting indoors at Mohand and in Dun town.

12. CULEX (CULEX) VISHNUI THEOBALD, 1901.

Habitat.—Ground pools of almost every description, ricefields, salt marshes (Barraud, 1934), streams (Chow, 1949).

Distribution.—Oriental Region (Horsfall, 1955). It is a common Indian species recorded from Punjab (Kalka, Madhopur, Amritsar); Uttar Pradesh; Bombay State (Trombay, Belgaum, Tavargatti, Hubli, Karwar); Madras (Ennore); Orissa (Chilka Lake); Bengal (Calcutta); Bihar (Singhbhum); Assam (Nalbari, Nongpoh, Dinnapur and Dibrugarh); Malabar Coast; and in East Pakistan from Chittagong, Rangamatti and Sylhet (Barraud, 1924*c*; Senior White, 1923). Recently it has been recorded from Poona by Rao and Rajagopalan (1957). It has also been recorded from Ceylon, Burma, Nepal, Sumatra, Hongkong, Celebes, Java and Philippines (Senior White, 1923.)

In Dehra Dun, both males and females were caught in shrubs at Mohand and Song River (Lachhiwala Range).

13. CULEX (CULEX) MIMETICUS NOE, 1899.

Habitat.—Streams, springs and pools (Horsfall, 1955).

Distribution.—This species has been recorded from Kashmir (Nar Nag); Punjab; Assam (Shillong, Lushai Hills and Cherrapunji,); Bihar (Pusa); Nilgiris (Coonoor); and Kanara. It is also known from Bakloh in West Pakistan, Nepal and from Ceylon (Barraud, 1934; Senior White, 1923). Recently, Rao and Rajagopalan (1957) recorded it from Poona. Outside Indian sub-continent, this mosquito has been found to occur in continental China and Formosa, Cyprus, Palestine, Syria and Lebanon (Barraud, 1934; Horsfall, 1955; Senior White, 1923).

In Dehra Dun, a single female specimen was caught resting underneath a rock in the shorebed of stream at Sahas Dhara.

14. CULEX (LUTZIA) RAPTOR EDWARDS, 1922.

Habitat.—Natural pools, shallow wells, domestic collections of water, etc. (Barraud, 1934).

Distribution.—It has been recorded from Punjab (Amritsar, Kalka) , Bombay State (Bombay, Parel, Trombay, Poona, Belgaum, Tavargatti, Hubli, and Kadra); Madras (Nilgiri Hills) (Barraud, 1924*b*).

*The flow of the rivulet was very slow and it was particularly dry in the third week of September.

In Dehra Dun, male and female specimens were hatched from the larvae collected from stagnant water at Ganeshpur Village.

15. CULEX (L) VORAX EDWARD, *1921*

Habitat.—As given for *raptor*.

Distribution.—Orient Region from Japan, China, Burma, to Himalayan Mountains (Horsfall, 1955). In India, it has been recorded from Kasauli Hills (Punjab); Bhowali, Mussooree (Uttar Pradesh); Kurseong and Mungpoo (Bengal); Shillong, Cherrapunji, Halflong, Cachar Hills, Mikir Hills (Assam); and Comibatore, Nilgiris in Madras State (Barraud, 1934).

In Dehra Dun, a single male specimen was caught from Kalsi.

16. CULEX (NEOCULEX) BREVIPALPIS (GILES), 1902.

Habitat.—Tree-holes, bamboos, once in open iron tank (Barraud, 1934).

Distribution.—Common over larger part of Indian Region east of a line drawn from the western boundary of Nepal to Bombay (Barraud, 1934). It has been recorded from Bombay State (Tavargatti, Kadra); Uttar Pradesh (Shahjahanpur); Madras (Madras, Ennore); Bihar (Pusa); Bengal (Sukna); Assam (Nongpoh, Halflong) and Tindharia in Eastern Himalayas; and from Chittagong and Rangamatti from East Pakistan (Barraud, 1924*d*: 1934; Senior White, 1923). Rao and Rajagopalan (1957) recorded it from Poona. It is also known from Malay, Siam and Cochin-China (Barraud, 1934).

In Dehra Dun, male and female specimens were hatched from larvae collected from a 5 feet high mango tree-hole in Kalsi along with the larvae of *Aedes* (*F.*) *aureostriatus doonii* (n. var) and *Orthopodomyia andamanensis* Barraud.

17. HEIZMANNIA HIMALAYENSIS EDWARDS, 1922.

Habitat.—Tree-holes and bamboos (Barraud, 1934).

Distribution.—It has been recorded from Kurseong and Sukna in Darjeeling District of Bengal and from Koti near Kalka (Barraud, 1934).

In Dehra Dun, two female specimens were caught one each from Lachhiwala Forest Range and Kalsi forests.

18. ORTHOPODOMYIA ANDAMANENSIS BARRAUD, 1934.

Habitat.—Rat-holes, bamboo stumps, and packing container (Knight and Mattingly, 1950).

Distribution.—Andaman Islands; Sukna (Darjeeling); Singapore (Barraud, 1934); S.W. Pacific (Knight and Mattingly, 1950).

In Dehra Dun, larvæ of this species were found breeding in a mango tree-hole in association with *Aedes* (*F.*) *aureostriatus doonii* and *Culex* (*N.*) *breviapalpis*. No adults could be hatched. A description of the larva is given below for future confirmation.

Larva (*Fig.* 3).—Readily distinguished from other culicine larvae by the presence of chitinized saddle on Segment VIII and absence of pecten teeth. Distinct

FIG. 3.

Larva of *Orthopodomyia andamanensis.*
A. Head. Hairs: (1) Preclypeal. (4) Post-clypeal. (5) Upper frontal. (6) Lower frontal. (7) Pre-antennal. (8) Sutural.
B. Mentum.
C. Terminal segments. LC, Lower caudal hair LH, Lateral hair. UC. Upper caudal hair.
D. Larger tooth of comb.
G. Small fringed tooth of comb.

from *anopheloides, maculata, maculipes* and *mcgregori* by the position of the siphonal tuft and from *anopheloides* by the saddle which is incompletely ringed posteriorly on the anal segment.

Length : About 6·5 mm. *Colour :* Body blackish-brown, head and siphon deep brown. *Head* (Fig. 3 'A') slightly wider than long; antennal shaft smooth, 0·41 mm. long (about 7 times as long as its greatest width), stout on basal half and tapering towards tip, tuft of 6-8 branches at one-third from base, two stout terminal spines with one finger-like process; anal papillae of the size of the latter. The hair in mouth brushes simple; pre-clypeal spines (1) fairly long and tapering, post-clypeal and lower frontal (4 and 6) with 6-8 branches, upper frontal (5) with 4 - 6 branches, pre-antennal hair (7) with 9 - 11 branches, sutural (8) long, divided into two distally; mentum (Fig. 3 'B') with 8 teeth on each side, middle tooth large and pointed, four basal teeth also large and widely spaced. *Thorax:* Lateral tufted hairs well developed, having some long hairs. *Abdomen:* First two segments with lateral tufted hairs, III-VII with fine hairs, some of which are simple; VII segment with a chitinized saddle covering rather more than dorsal half in side view, VIII nearly enclosing the segment. Comb with 7 to 9 larger teeth and about 26 small fringed teeth in definite rows. Larger teeth with 5 spines from expanded apex, central long and stout, the stem bulged basally (Fig. 3 'C', 'D', 'E'), siphon 1·1 mm. long (about 4½ times as long as diameter at base), siphonal tuft of 10 - 11 barbed hairs inserted at 0·40 length of the siphon. Pecten absent. Anal segment incompletely ringed posteriorly, a separate chitinized strip on each side at base, upper caudal tuft of dorsal brush (U.C.) splits into 9 branches, one much stout and longer than others, lower caudal tuft (L.C.) single and long, lateral hair (L.H.) with three branches, ventral about half the length of dorsal, ventral brush well developed, hairs arising from the brush well developed and each splitting into a number of branches some distance from base.

19. URANOTAENIA LUTEOLA BARRAUD, 1934.

Distribution.—It has been recorded from Andaman Islands and Malabar Coast.

In Dehra Dun, single specimen each of male and female were caught from a 'Sal' tree-hole at 7 feet height in Lachhiwala Forest Range. It is very likely that this species is a tree-hole breeder.

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STUDY OF THE RESIDUAL TOXICITY OF DIELDRIN IN HARDOI DISTRICT (UTTAR PRADESH).

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INTRODUCTION.

THE duration of residual toxicity of various insecticides varies in different parts of India. With the advent of dieldrin, a number of trials have been made with this insecticide and their results published. Pal *et al.* (1955) in their trial in Karnal area, Punjab, and Ganga Khadar, Uttar Pradesh, used dieldrin in different dosages, namely, 6·25, 12·5 and 25 mg./sq. ft. They concluded that the duration of residual effectiveness of dieldrin 12·5 mg./sq. ft. lasted for about seven to eight weeks, and dieldrin applied at the rate of 25 mg./sq. ft. did not show an increase in the duration of residual effectiveness proportionately. Dieldrin at a dosage of 6·25 mg./sq. ft. was ineffective as a residual spray.

Viswanathan *et al.* (1955) used dieldrin at dosages of 14 and 28 mg./sq. ft. in their experiments in Surat District, Bombay State, and came to the conclusion that a single application of 14 mg./sq. ft. of dieldrin, sprayed at the beginning of the season, would establish a high degree of successful malaria control and that the results with 28 mg./sq. ft. were practically the same as with a smaller dosage of dieldrin. Karani *et al.* (1956) found in Kharakwasla area that dieldrin, when sprayed at the dosage of 50 mg./sq. ft. in pucca buildings, still retained concentration of over 16 mg./sq. ft. up to 32 weeks. The incidence of anopheline and culicine mosquitoes showed a marked reduction initially and their densities were low for the

duration of the study (40 weeks). Dhir and Amrik Singh (1957) carried out the trial with 50 per cent dieldrin water dispersible powder at a dosage of 25 mg./sq. ft. in the rural areas of Delhi during 1956. They observed that there was significant rise in anopheline density after seven weeks.

The object of the present study was to determine the duration of residual toxicity of 12·5 mg./sq. ft. of dieldrin 50 per cent water dispersible powder in Hardoi District of Uttar Pradesh, assessed by entomological findings by comparing the density, survival rate, and the wing index of mosquitoes before and after insecticidal application. A limited quantity of dieldrin was received and spraying was carried out in the rural and urban areas of the district under the National Malaria Control Programme, during the first round of spraying in June-August, 1957. The spraying operations were completed in the study area within one week and observation carried out weekly afterwards. The toxic effects on human beings and animals were also observed, which have been presented separately.

AREA SELECTED FOR STUDY.

The area selected consisted of Hardoi and Bilgram municipalities and 147 villages between Hardoi-Bilgram and Hardoi-Sandi road and the Bilgram Extension Development Block (Map 1). The total population covered was 75,395.

CLIMATE AND METEOROLOGY.

The average annual rainfall in the area is 39 inches, while the rainfall during April to August is on an average 25 inches for the last 30 years. The hottest months are May and June. Table I shows the meteorological figures from May to August in 1955 and 1956.

TABLE I.

Months	Maximum temperature. Monthly mean.		Minimum temperature. Monthly mean.		Relative humidity		Rainfall (inches).	
	1955	1956	1955	1956	1955	1956	1955	1956
May	105·5	104·1	73·9	80·6	40	50	0·00	1·18
June	100·6	98·6	80·1	80·8	70	70	3·14	2·20
July	91·4	91·8	79·3	79·1	89	83	7·66	9·24
August	88·0	90·7	79·0	78·6	92	85	14·28	14·19

ANOPHELINE FAUNA.

The following species were identified from the area during the survey: *A. culicifacies*, *A. stephensi*, *A. annularis*, *A. pallidus*, *A. splendidus*, *A. subpictus*, *A. gigus*, *A. maculatus*, *A. hyrcanus*, *A. vagus* and *A. barbirostris*.

DETAILS OF EXPERIMENT.

The area had not been sprayed before, except Hardoi Municipality which had been sprayed only once with a dose of 100 mg./sq. ft. of DDT in November, 1956. The spraying with dieldrin was carried out in all human dwellings, mixed dwellings, and cattlesheds by the trained labour under strict supervision. The dose employed was 12·5 mg. dieldrin per square foot and stirrup pumps were

MAP I
N
S
SANDI
HARDOI
HARDOI (M.B)
CANAL
BILGRAM (M.B)
TEHSIL HARDOI
TEHSIL BILGRAM
SCALE
0 1 2 3 Miles
RIVER GANGES
DIST. FARRUKHABAD

used for spraying. The houses in all the villages had mud walls with thatched roof except in municipal and town areas where the houses are pucca with cement plastered walls.

COLLECTION OF DATA.

Five catching stations were fixed each in a different town/village within a radius of six miles from the Headquarters of the Unit. The catching stations consisted of human dwellings, cattlesheds and mixed dwellings in the area of trial. Three of the catching stations were with mud walls and two with pucca walls. The villages were so selected that they had all the important typical topographical features of the area like canals, lakes, seepage canals, waterlogging and storm-water drain, etc.

No comparison village was fixed, the pre-operational data for 12 weeks were available for comparison. Mosquito collections were made weekly from the catching stations for 1½ hours each and records were maintained for the following:

A. 1. Weekly density of all anopheline and culicine mosquitoes.
2. Identification of anopheline mosquitoes only.

B. Survival rate of both anopheline and culicine mosquitoes was determined. Random collections were made of mosquitoes from the catching stations every week and brought in cages to the laboratory for determination of survival rate. The cages were kept in mosquito hotels surrounded by *khas tatties** to lower the temperature, raise the humidity and provide semi-darkness. The survival rate was noted after 24 hours for both Anopheline and Culicine mosquitoes.

C. Enquiries were made of any ill effect on the spraymen, local inhabitants and animals.

OBSERVATIONS AND DISCUSSION.

1. Mosquito density.

The data collected are represented in Fig. 1 and Fig. 2. The analysis of the data shows that there was marked reduction in the anopheline density after

FIG. 1.

Weekly density (per man-hour) of total anopheline mosquitoes.

* Khas tatties are screens made of the roots of a particular type of plant.

FIG. 2.

Weekly density (per man-hour) of total culicine mosquitoes.

spraying but it never reached zero. The density started building up gradually and was higher than the pre-operational figure in the eighth week. The effect lasted up till seventh week in case of anopheline. The finding is almost similar to that of Pal *et al.* (1955) in their trial in Punjab and Uttar Pradesh that the effect of dieldrin 12·5 mg./sq. ft. lasts for 7 - 8 weeks. In the case of culicine mosquitoes, the density started building up in the fourth week and was higher than the pre-operational figure in the fifth week. The residual effect against culicine had waned off in the third week.

The density of Anopheles in catching stations, with mud walls and pucca walls with cement plaster, is given in Table II.

TABLE II.

Density of Anopheles per man-hour in different types of catching stations.

Week after spraying	IN CATCHING STATIONS WITH	
	Mud walls.	Pucca walls.
I	2·6	1
II	4·6	3·3
III	5·3	2·3
IV	8·2	3·3
V	8·2	2·6
VI	11·5	6·3
VII	8·4	6·6
VIII	16·4	7·6
IX	17·1	13
X	14·6	10·6

From the above figures, it appears that the density of Anopheles remained nearly double in the catching stations with mud walls as compared to that of pucca

walls. Probably the deterioration of the dieldrin on the mud walls, is at much quicker rate as compared to that on cement plastered walls.

2. Identifications of all species collected revealed that *A. culicifacies* and *A. stephensi* were not caught for eight weeks. In the ninth week, *A. culicifacies* appeared and *A. stephensi* was still not encountered. These species had been recorded from the catching stations in the pre-operational period. The species commonly recorded during the post-spray period were as follows in descending order: *A. subpictus, A. annularis* and *A. pallidus*. It is to be noted that the density of *A. subpictus* remained high throughout the post-spray period.

3. Survival rate data are represented in Table III and in Fig. 3 and 4.

TABLE III.

Survival rate of anopheline and culicine mosquitoes.

Week after spraying	Number of insects under observation		Number of insects surviving after 24 hours.		Survival rate (per cent)		REMARKS
	Anophelines	Culicines	Anophelines	Culicines	Anophelines	Culicines	
I	4	124	0	0	0	0	Density of anophelines remained very low for the first three weeks.
II	6	112	0	0	0	0	
III	15	56	0	5	0	9·0	
IV	35	112	11	70	31	62·0	
V	30	200	6	141	20	70·5	
VI	25	226	8	226	32	100·00	
VII	41	—	8	—	19·5	—	Collection of culicine mosquitoes was discontinued after sixth week.
VIII	49	—	10	—	21·4	—	
IX	66	—	15	—	22·6	—	
X	82	—	66	—	80·4	—	

In case of anopheline mosquitoes, the survival rate remained zero per cent for three weeks and then it started rising and crossed the 50 per cent limit in the tenth week. Accordingly the duration of residual toxicity was nine weeks for anopheline mosquitoes.

In the case of culicine mosquito, it remained zero for two weeks and suddenly was higher than the 50 per cent limit in the fourth week, the effect lasted for only three weeks.

TOXICITY.

Cases of dieldrin poisoning in human beings by ingestion (accidentally) and by constant inhalation were observed. The symptoms resembled that of major epilepsy (*petit mal.*).

FIG. 3.

Survival rate of anopheline mosquitoes.

SUMMARY.

1. A field trial of 50 per cent dieldrin water dispersible powder, with a dosage of 12·5 mg. dieldrin per square foot, was carried out in Hardoi District in Uttar Pradesh.

2. It was found that weekly density of all anophelines remained low for seven weeks, and of culicines for four weeks only.

3. Survival rate of mosquitoes, based on 50 per cent mortality, showed that the duration of the effect of dieldrin 12·5 mg. per square foot lasted for nine weeks in case of anopheline mosquitoes and three weeks in case of culicine mosquitoes.

4. *A. culicifacies* and *A. stephensi* remained absent for eight weeks while one specimen of *A. culicifacies* was caught.

5. *A. subpictus*, *A. annularis*, *A. pallidus* showed lower susceptibility to this insecticide as compared to the other species, as the densities of these remained high throughout the post-spray period.

6. The density of the anophelines remained nearly double in the mud-walled catching stations as compared to that of cement-plastered catching stations.

FIG. 4.

Survival rate of culicine mosquitoes.

7. Dieldrin poisoning produced symptoms exactly like major epilepsy in human beings.

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EFFECT OF ACTIVE METABOLITE OF BROMOGUANIDE (M.I.S. 11) ON THE SPOROGONY CYCLE OF *P. GALLINACEUM* (BRUMPT 1935).

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JASWANT SINGH *et al.* (1954) had observed that active metabolite of bromoguanide (Mal. Instt. Surv. No. 11) has a quinine equivalent of 512 against *P. gallinaceum* in chicks. Subsequently its quinine equivalent against *P. cynomolgi* was worked out to be 17 (Ray *et al.*, 1954). Further, during their studies to determine the antimalarial properties of this compound against *P. vivax*, Ray *et al.* (1956) recorded high degree of activity against the asexual erythrocytic forms though action against the gametocytes was found to be somewhat tardy.

The present paper records the findings on the effect of the drug on the developmental phases of *P. gallinaceum* in *Aedes aegypti*.

MATERIALS AND METHODS.

The experimental fowls (white leghorn) were inoculated intravenously with 50 million parasitized (*P. gallinaceum*) erythrocytes per kg. body weight. Blood smears from the infected fowls were stained with J.S.B. (Jaswant Singh and Bhattacharjee, 1944) and an Ehrlich's eye piece was used for gametocyte count.

Freshly hatched *Aedes aegypti* were obtained from a colony maintained in the laboratories of the Institute. These mosquitoes were starved overnight and then fed on experimental fowls at a time when patent infection was well established and fair number of gametocytes was detectable. The technique of feeding was similar to that adopted earlier by Jaswant Singh *et al.* (1952). Only the fully fed

mosquitoes were selected and kept in Barraud's cages at a temperature between 80° and 85° F., with relative humidity ranging from 80 to 85 per cent.

Batches of mosquitoes were fed on infected fowls for two consecutive days prior to drug administration referred to as (−2) and (−1) day (Table I) and these served as the comparison group. Subsequently, batches of mosquitoes were fed every four hours on each fowl on Day-0 (day of drug administration). Thereafter feeding was continued once a day for a period of six days.

TABLE I.

Result of dissection of Aedes *mosquitoes fed on fowls prior to and after drug administration.*

Time of feeding.	Series I. 250 mg. per 70 kg.			Series II. 500 mg. per 70 kg.			Series III. 250 mg. per 70 kg. a day for 2 consecutive days.		
	Total mosquitoes dissected.	Oöcysts rate, per cent.	Sporozoite rate, per cent.	Total mosquitoes dissected.	Oöcyst rate, per cent.	Sporozoite rate, per cent.	Total mosquitoes dissected.	Oöcyst rate, per cent.	Sporozoite rate, per cent.
−2 days	21	18·6	38·1	78	19·2	70·5	35	14·2	40
1 day ..	21	23·3	33·3	81	16·05	82·7	35	25·7	51·4
0 day, 4 hours	26	26·9	..	54	7·4	..	41	7·3	..
0 day, 8 hours	42	7·1	..	55	3·6	..	31	6·4	..
0 day, 12 hours	42	9·5	4·7	56	3·6	1·8	39	5·1	10·2
1 day	27	7·6	3·7	67	7·4	1·5	34	3·8	5·7
2 day	34	14·7	5·9	72	2·8	16·6	29	2·1	13·9
3 day	32	3·1	6·2	33	6·1	33·3	35	..	8·6

Sample dissections of three to five mosquitoes, from each batch, were commenced from the fifth day following the infective feed and continued for a period of 14 days to determine infection of gut and glands. Viability of sporozoite, when detectable, was determined by inoculation test in normal fowls at two mosquito equivalent dose.

Three dosage regimes of the compound were adopted and they were administered in terms of human equivalent doses of 250 mg. (Series I) and 500 mg. (Series II) single dosage schedule, as well a dose of 250 mg. (human equivalent) for two consecutive days (Series III), taking the average weight of an adult as 70 kilogram.

RESULTS.

Details of dissections of *Aedes aegypti*, fed on fowls receiving different human equivalent doses of the compound, are presented in Table I.

It would be evident from the data presented that in Series I, those batches (comparison group) in which the mosquitoes were fed on infected fowls prior to drug administration (−2 and −1 day), gut and gland infections were easily demonstrable. The oöcyst rate was found to vary between 18·6 and 23·3 per cent from the fifth to tenth day after the infective blood meal.

In the groups in which the mosquitoes were fed four hours after drug administration and dissected thereafter between five and ten days subsequent to the infected blood meal, the oöcyst rate was observed to be 26·9 per cent and in this respect there was hardly any significant difference between the comparison and the experimental groups. On the other hand, mosquitoes fed eight hours after drug administration and those fed subsequently up to three days, showed marked reduction in the oöcyst rates, the average for the five batches being 8·4.

The sporozoite rates were observed to be between 33·3 and 38·1 per cent in the comparison group as against nil in the two batches of mosquitoes which had received infected blood meals four and eight hours after the fowls were treated. In the subsequent batches, the sporozoite rates were found to be markedly lower than the comparison group, the average for the four batches being 5·1 per cent.

However, in the higher dosage schedule (human equivalent single dose of 500 mg.), the results were somewhat different in that the average oöcyst rate in mosquitoes, fed four hours after drug administration and thereafter up to three days, was 5·45 as against 19·2 to 16·05 per cent on Days −1 and −2 (Series II, Table I). The sporozoite rate in the comparison series was recorded to be 70·5 and 82·7 per cent against nil in batches fed four and eight hours after drug administration. The readings of the two subsequent batches were 1·8 and 1·5 per cent and this was indeed remarkable. However, the sporozoite rates in the batches fed thereafter increased steadily from 16·6 to 37·5 per cent up to Day 5.

The findings were somewhat similar in Series III, in which the oöcyst rates in the comparison series (−2 and −1 day) were 14·2 and 25·7 per cent as against 7·3, 6·4 and 5·1 per cent in batches fed 4, 8 and 12 hours respectively after drug administration. The subsequent rates for Day 1 and 2 were 3·8 and 2·1 per cent respectively. Again when the sporozoite rates were 40 and 51·4 per cent in the comparison series, the first two batches in the experimental group showed no sporozoites at all and the subsequent rates up to Day 3 ranged between 5·7 and 13·9 per cent.

Sporozoites, whenever detectable, were found to be viable as determined by the viability test.

DISCUSSION.

From the data presented, it would be evident that the pattern in all the series are somewhat similar inspite of individual variations, the most redeeming feature being the absence of sporozoites in all series in the batches fed four and eight hours after drug administration. Further, in the subsequent batches the sporozoite rates, as compared to the comparison series, were uniformly low up to Day 2. In this respect, it would appear that the lowest level was maintained in Series II.

As regards the oöcyst rate, the pattern was similar in Series II and III in that even after four hours subsequent to the drug administration the rates were significantly lower than in the comparison series. In Series I, the oöcyst rate appeared to be at the same level in the batch fed four hours after drug administration as in the comparison series. Nevertheless, the subsequent batches showed much lower rates though not up to the same level as in Series II and III.

Thus it is evident that development of the plasmodium in the mosquitoes was considerably hindered up to eight hours after drug administration and somewhat retarded up to two days after drug administration, the oöcyst and sporozoite rates being at significantly low levels as compared to the comparison series. Thus it may be concluded that the drug has got a transient inhibitory influence on the sporogony cycle of *P. gallinaceum* in *Aedes aegypti* similar to that reported earlier in respect of pyrimethamine (Jaswant Singh *et al.*, 1953). Similar studies with proguanil, undertaken earlier by Ramakrishnan *et al.* (1952), indicated that the effect of proguanil against the sporogony cycle was somewhat more prolonged.

From these observations, it may be concluded that all the three preparations, namely proguanil, pyrimethamine, the metabolite of bromoguanide, have inhibiting influence on the developmental cycle of *P. gallinaceum* in the invertebrate host. However, such effect is of transient nature only, and to be fully effective frequent drug administration would be necessary.

Obviously such investigations should be taken up on a large scale in respect of human plasmodia though, so far as proguanil is concerned, it has already been demonstrated by Ramakrishnan, Young *et al.* (1951) that the effect varied between two to six days against the earlier claims for seven to ten days by Mackerras and Ercole (1947) and Shute and Maryon (1948). Recently a few workers (Foy and Kondi, 1952; Shute, 1952; Jeffery *et al.*, 1956) have claimed that the effect of pyrimethamine lasted longer. Thus, from this, detailed investigations on large scale are indicated.

SUMMARY.

Active metabolite of bromoguanide, in three different dosage regimes (human equivalent), was tried to study the effect of the drug on the infectivity of the gametocyte of *P. gallinaceum*.

Sporozoites found in mosquitoes, fed on fowls with gametocytes, two days prior to drug administration and in those fed after one to three days of drug administration, were viable.

The inhibitory effects of the drug, on the growth of parasites in *Aedes* mosquitoes, were of transient nature.

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SCREENING OF COMPOUND 377C54* AGAINST AVIAN AND SIMIAN MALARIA PARASITES.

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FOLLOWING the observation that 1-cyclohexyl-aminomethyl-2-hydroxy naphthalene possesses some antimalarial action against avian parasite, the Wellcome Laboratories were able to synthesise a series of mono and di-hydroxy naphthalene compounds. One of these, having the following chemical structure and formula, is known as 377C54. The molecular weight of the compound is 455·22 and the base content is 84 per cent.

2:5 bis (cyclohexylaminomethyl) naphthalene-1:6 diol dihydrochloride.

*The drug was made available to this Institute by the Wellcome Laboratories through the Indian Council of Medical Research, for studies on its antimalarial properties.

Rollo (1956) and Bruce-Chwatt and Charles (1957) had studied some of the laboratory and clinical features of this compound. From their observations, it would appear that the drug possessed effective antimalarial activity against asexual forms of avian and simian malaria but there was little or no action against the tissue phase of avian parasite (*P. gallinaceum*). Further they had observed that tolerance to the drug was high.

The present paper records the findings of detailed investigations carried out in respect of this naphthalene derivative against *P. gallinaceum* in chicks and *P. knowlesi* in monkeys.

METHODS AND MATERIAL.

(*a*) *Plasmodia.*—(*i*) A strain of *P. gallinaceum*, maintained in fowls in the laboratories by serial blood passages for years, was utilized as the infective agent. The strain is highly virulent in chicks, the parasitic density reaching as high a level as 70 to 85 per cent. The infection almost always runs a fatal course.

(*ii*) *P. knowlesi.*—The newly isolated virulent strain (Nuri) (Jaswant Singh *et al.*, 1953; Edeson and Davey, 1953) was utilized for the studies. In uninterfered infection, the density attained is as high as 80 to 96 per cent in four to six days. The course is always fatal.

(*b*) *Host.*—(*i*) Laboratory hatched, 7-day old, white leghorn chicks were utilized for screening as well as for determination of the M.E.D. of the compound against *P. gallinaceum.*

(*ii*) Normal *M. mulatta mulatta* (commonly known as rhesus monkeys) were utilized. Prior to these investigations, the monkeys were subjected to tuberculin test (Nair and Ray, 1954) for any evidence of tuberculosis. To exclude any parasitic infection, blood was examined on three consecutive days.

(*c*) *Dosage schedules.*—For routine screening against *P. gallinaceum*, three dosage schedules were taken up to determine the range of activity. The dosages were calculated in terms of the M.E.D. of quinine (1·6 mg./50 gm. body weight) like 4Q,Q and 1/4Q. Subsequently the doses were graded to assess the Minimum Effective Dose (M.E.D.). The technique adopted was similar to that described by Jaswant Singh, Basu and Ray (1952).

For trials against *P. knowlesi* in monkeys, dosage schedules were calculated in mg. of base content/kg. body weight. Medication was commenced, when intensity of the infection in host attained a level of 0·01 to 0·02 per cent cell infection, and the class of activity (I, II, III of Shannon) was determined following the procedure adopted earlier by Jaswant Singh, Ray *et al.* (1952).

OBSERVATIONS AND RESULTS.

The dosage schedules, adopted against *P. gallinaceum* in chicks, were from 6·4 to 0·4 mg. per 50 gm. of the body weight of the chick, 1·6 mg. being equivalent to the M.E.D. of quinine. Table I indicates the dosage schedule as well as parasitæmia percentage ratio as compared to the comparison group. It would appear that, in doses ranging between 0·4 and 0·6 mg. the parasitæmia ratio, as compared

to the control group, varied from 76 to 32·8 per cent. With 0·65 mg., the percentage of parasitæmia was recorded to be 20·8 per cent. In the higher dosage schedules, the parasitæmia was still lower ranging from 0 to nine per cent. In view of the fact that 0·65 was the lowest dosage schedule to decelerate the course of parasitæmia to less than 25 per cent as compared to the comparison group (Class I effect of Shannon-Wiselogle, 1946), this dose was considered to be the minimum effective dose of the drug against *P. gallinaceum*.

TABLE I.

Screening of 377C54 *in chicks against* P. gallinaceum.

Schedule number	Dose in mg./50 gm. body weight.	Dose equivalent of quinine.	Number of chicks utilized.	Comparison with control group. (Percentage)	Remarks.
I.	6·4	4 Q	5	0·48	
II.	1·6	Q	5	0·0	
III.	0·8	1/2 Q	5	0·5	
IV.	0·7	7/16 Q	3	3·8	
V.	0·675	27/64 Q	3	9	
VI.	0·65	13/32 Q	3	20·8*	*M.E.D.
VII.	0·6	3/8 Q	7	32·8	
VIII.	0·5	5/16 Q	3	76	
IX.	0·4	1/4 Q	8	71·75	

It would thus appear that the drug is about two-and-a-half times as active as that of quinine.

Against *P. knowlesi* infection, the dosage schedules were varied between 12 mg. and 4·75 mg. per kg. of body weight of monkeys as shown in Table II. Up to a dosage schedule of 5·5 mg., Class II effect, i.e., the clearance of parasitæmia from the peripheral circulation by the day following the last dose of drug administration (Wiselogle 1946), was observed in all animals. The average time for parasite clearance was between 18 and 32 hours from the commencement of treatment. Since, in none of the animals any recrudescence of the blood-induced infection could be observed for a period varying from three to six months, it was presumed that sterilization of the blood-induced infection had occurred. Further, as none of them showed parasitæmia after splenectomy it was evident that, with the range of dosage schedule adopted, automatically Class III effect of Shannon, i.e., complete sterilization of blood-induced infection (Wiselogle, 1946 *loc. cit.*), was attained.

In the subsequent dosage schedules varying from 5·25 to 4·75 mg., it was observed that in Schedule VII (5·2 mg.) Class II effect was attained in four and Class I effect in one out of a total of five monkeys subjected to the investigation. The average time for parasite clearance in the three monkeys indicated above, was

TABLE II.

Determination of M.E.D. against P. knowlesi (*Nuri strain*).

Schedule number.	Dose in mg./kg.	Number of animals used.	Number under Class I effect.	Class II effect		Remarks.
				Number of animals.	Average time for parasite clearance after taking the drug. (Hours).	
I.	12	1	..	1	18	No relapse.
II.	8	1	..	1	24	
III.	6·25	3	..	3	25	
IV.	6	3	..	3	32	
V.	5·88	3	..	3	32	
VI.	5·5	4	..	4	54	
VII.	5·2	5	1*	4	124	*No clearance in one monkey.
VIII.	5	5	3*	2	96	*No clearance in three monkeys.
IX.	4·75	4	3*	1	96	*No clearance in three monkeys.

between 96 and 120 hours and in one about 162 hours (i.e., eight days). With a dosage schedule of 5 mg. (Schedule VIII), Class II effect was observed in two and Class I in three monkeys. In the next dosage schedule (Schedule IX), Class I effect was recorded in three and Class II in one. In those schedules (VIII and IX) in which Class II effect was recorded, the average parasite clearance was observed in 96 hours. Recrudescence occurred in the course of 7 to 40 days in all animals under the three schedules where there was initial clearance of parasitæmia. It would thus be observed that for Class II and III effects, the dosage schedule of 5·5 mg. should be considered as the minimum effective dose (M.E.D.).

DISCUSSION.

According to Rollo (1956), the compound 377C54 showed varying degrees of schizonticidal action against *P. gallinaceum*, *P. cathemerium*, *P. berghei* and *P. knowlesi*. From the results of the present series of investigations, it became evident that the minimum effective dose (M.E.D.) of the drug against *P. gallinaceum*, was 0·65 mg. per 50 gm. chick. Since the M.E.D. of quinine was observed to be 1·6 mg. (Jaswant Singh, Basu and Ray, 1952), the quinine equivalent of the new compound was about 2·5. Further, as compared to the activity of chloroquine diphosphate the M.E.D. of which was observed to be 0·0615 (Ray *et al.*, 1956), the drug seemed to be 10·5 times less active. In this respect, the findings would appear to be somewhat different to those observed earlier by Rollo (1956) who had

reported that 377C54 was half as active as chloroquine. This difference in activity in the same species of plasmodium, could be attributable to the possible difference in the strain, the host and/or the technique adopted.

Against *P. knowlesi*, the minimum effective doses of quinine and chloroquine were earlier established to be 30 and 1·8 mg. per kg. of the body weight of the monkeys, respectively (Nair *et al.*, 1953; Nair and Ray, 1953). As observed during the present studies, the M.E.D. for Class II effect in respect of 377C54 was established at 5·2 mg. per kg., under which dosage schedule there was complete clearance of parasites from the peripheral circulation. Thus while it appeared to be 5·8 times more active than quinine, it was three times so less than chloroquine.

From the above findings, it may be concluded that firstly the drug showed somewhat more powerful schizonticidal action against *P. knowlesi* than *P. gallinaceum* and that secondly such activity against both the plasmodia was less pronounced than that of chloroquine.

Bruce-Chwatt and Charles (1957) reported that during preliminary trials against human malaria, the schizonticidal action of 377C54, in 200 to 300 mg. single dosage schedule, was of a lower order than chloroquine and this was particularly so in *P. falciparum* infection. Recently Ray *et al.* (1958) also observed similar findings in respect of a dosage schedule of 600 mg., administered in three divided doses at intervals of 12 hours or a single dose of 400 mg. However, the results were well comparable to the action of chloroquine, when 377C54 was administered in a single dose of 600 mg., thus indicating that the drug had some potentialities.

It was also observed that, both during laboratory trials in birds and monkeys as well as on human subjects, 377C54 was well tolerated in the dosage schedules adopted.

SUMMARY.

A hydroxy naphthalene derivative, known as 377C54, was tested against *P. gallinaceum* in chicks and *P. knowlesi* in monkeys. The compound showed high degree of schizonticidal activity against both.

The drug was found to be 2·5 and 5·8 times more active than quinine against *P. gallinaceum* and *P. knowlesi* respectively.

As compared to chloroquine, it was about ten times less active against the former plasmodium and three times against the latter.

However, in view of its action against human plasmodia as observed recently, the drug seemed to hold some promise.

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FURTHER OBSERVATIONS ON THE EFFECTS OF A FEW HERBICIDES ON THE AQUATIC FLORA OF BOKARO RESERVOIR.

BY

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[February 17, 1958.]

INTRODUCTION.

THE aquatic vegetation in Bokaro Reservoir is mainly marginal and restricted to the fluctuation-level zones. An enumeration of these species was included in a previous investigation (Neogy, Kachroo and Biswas, 1957). The plants closely associated with anopheline breeding in this reservoir are emergent, floating and submerged types; the important problem-species are: *Polygonum, Scirpus, Jussiaea, Monochoria* and a few submerged plants not dealt with in this note.

Bokaro Reservoir, it may be recalled, is a constant pool-level pond located in Bihar (District Hazaribagh) at the confluence of Bokaro and Konar rivers; the sole purpose of this pond being to supply cooling water to the Bokaro Thermal Power House. Further, being a constant-level pond, it is expected to provide optimum conditions for a luxuriant marginal vegetative growth and was also feared to develop a high malariogenic significance.

Effects of a few herbicides, namely, dicotox, chloroxone and copper sulphate on the aquatic vegetation at the various stations in this reservoir were discussed in a previous paper (Neogy, Kachroo and Biswas, 1957) and the present study was undertaken to assess the effects of a few more herbicides on the same flora.

MATERIAL AND METHODS.

Fernoxone (80 per cent sodium salt of 2, 4-dichlorophenoxyacetic acid), agroxone (27·5 per cent (approximately) wt./vol. of sodium 2 methyl 4-chlorophenoxyacetate) and chloroxone (±same as agroxone) were used in this study.

Each area selected for spray was 42 square feet; since an assessment of the comparative effects of the three herbicides was intended, the areas thus selected were with pure communities of individual plants. For a normal dose, 2½ lbs. of herbicide in 22 gallons of water were sprayed per acre of land—a stirrup pump and a knapsack sprayer were used for spraying. It was found that agroxone mixed freely and readily with water, but fernoxone left a negligible residue and chloroxone a residue equalling 0·5 oz. per 2·5 lbs.

RESPONSE OF *POLYGONUM* TO FERNOXONE AND AGROXONE (AND CHLOROXONE).

The plot (Station 8-1*) selected for these tests contained a few weeds of other species: ***Mazus rugosus, Kyllinga monocephala, Eclipta alba,*** and grasses which consisted mainly of ***Cynodon dactylon.*** The soil appeared to be of relatively low fertility.

Results.—The normal concentration of both fernoxone and agroxone was quite effective, killing the entire plants within 1½ months (Table I). Since recovery responses were negligible, a second treatment was not considered necessary. The first effects, apparent in form of epinasty, were visible within 1-2 hours after treatment and the leaves started to become yellowish within 24 hours; later the gradual increase in yellowness continued till each plant was on verge of physiological death. At the end of 21 days-period, most of the leaves withered and fell. However, changes in the main curvature of the aerial stem did not undergo a marked deflection due to hardiness of ***Polygonum*** stems.

TABLE I.

Comparative effects of chloroxone, agroxone and fernoxone on a few weeds at Station 8-1 in the Bokaro Reservoir.

Plants	Period in which plants were killed		
	Chloroxone	Agroxone	Fernoxone
Polygonum tomentosum	30 days	36 days	34 days
Mazus rugosus	7 ,,	6 ,,	6 ,,
Eclipta alba	7 ,,	7 ,,	7 ,,
Kyllinga monocephala	120 hours	96 hours	96 hours
Cynodon dactylon	No	effects at	all.

It was interesting to note that in case of ***Polygonum*** (Table II), fernoxone showed more efficacy as compared to chloroxone and agroxone, whereas agroxone was comparatively more effective than chloroxone (even though these two herbicides are reported to have a similar constitution). On ***Eclipta alba,*** all the three herbicides behaved equally, but in ***Mazus*** and ***Kyllinga*** chloroxone was more effective.

*The stations referred to in this note are the same as those treated in Neogy *et al.*, 1957.

TABLE II.

Comparative effects of agroxone, fernoxone and chloroxone on Polygonum tomentosum.

Description	Chloroxone	Agroxone	Fernoxone
Time required for the first appearance of epinasty	1-2 hours	1-2 hours	1-2 hours
Percentage number of petioles showing epinasty after 24 hours ..	65 per cent	50 per cent	75 per cent
Percentage number of leaves showing no green after:			
24 hours ..	36 ,, ,,	35 ,, ,,	36·1(?) per cent
48 ,, ..	36 ,, ,,	45 ,, ,,	39·2 per cent
72 ,, ..	47 ,, ,,	70 ,, ,,	76 ,, ,,
96 ,, ..	53 ,, ,,	Not recorded	79 ,, ,,
144 ,, ..	60 ,, ,,	75 per cent	94 ,, ,,
30 days ..	Dead*	Dead*	Dead*
Mean curvature stem (°):			
Before treatment ..	85	85	85(-120)
After treatment: 48 hours ..	80	80	70
72 hours ..	45	72	72
144 hours-end	No further change till dead.		

*Average for well-sprayed individual plants.

RESPONSE OF *JUSSIAEA REPENS* TO AGROXONE AND FERNOXONE.

These experiments were carried at Station 1 which was formerly a paddy-field. The other weeds (Table III), in addition to the pure colonies of *Jussiaea repens*, were: *Sagittaria guayanensis*, *Alternanthera sessilis* and *Monochoria vaginalis*.

TABLE III.

Comparative effects of agroxone and fernoxone on a few weeds at Station 1, Bokaro Reservoir.

Plants	Agroxone	Fernoxone
Jussiaea repens	72 hours	48 hours
Sagittaria guayanensis	48 ,,	48 ,,
Alternanthera sessilis	96 ,,	96 ,,
Monochoria vaginalis	12 days	6 days

The effects of agroxone on *Jussiaea repens* were less marked as compared to fernoxone (Table IV), as shown by a study of percentage of leaves showing no green after treatment, and ultimate death of the plants; and the same was true of *Monochoria*. In case of *Sagittaria*, both these herbicides appeared to have a similar effect (Table V); and the same was the case with *Alternanthera*.

TABLE IV.

Comparative effects of agroxone and fernoxone on Jussiaea repens.

Description	Agroxone	Fernoxone
Mean deflection:		
Petiole —		
Before treatment	60°	70°
After treatment	30°	40°
Stem —		
Before treatment	45°	45°
After treatment	10°	10°
Percentage leaves showing no green after:		
25 hours	64	100
48 „	65	Dead
72 „	Dead	

TABLE V.

Comparative effects of agroxone and fernoxone on Sagittaria guayanensis.

Description	Agroxone	Fernoxone
Mean deflection petiole		
Before treatment	75°	70°
24 hours after treatment* ..	45°	30°
Percentage leaves yellowed after:		
24 hours	58°	80°
48 hours	Dead	Dead

*No further deflection was visible 24 hours after treatment.

CONCLUSIONS AND SUMMARY.

All the three herbicides, agroxone, chloroxone and fernoxone, discussed here have the capacity to be absorbed by the weeds through the roots or foliage but act more quickly and effectively through the latter—in the present study only the aerial portions of the plants were sprayed. The first effects visible were drooping of leaves, followed by twisting or contortion of foliage and stems; later the leaves blackened and ultimately withered.

From the experiments enumerated above, it appears that in overall effects fernoxone is more efficacious than agroxone (and chloroxone). However, a study of the initial deflection of the petioles, after treatment with the various herbicides (Table VI), showed that such gerneralization does not necessarily hold good for all the parts of a plant since such a behaviour of the petioles is controlled by their internal morphology.

TABLE VI.

Initial deflection in petioles of a few weeds 24 hours after treatment with chloroxone, agroxone and fernoxone.

Plants	Deflection of petioles 24 hours after treatment		
	Chloroxone	Agroxone	Fernoxone
Jussiaea repens	20·40	30	30
Sagittaria guayanensis	N.C.	45	30
Monochoria vaginalis	50	N.C.	25
Scirpus articulatus	10	0	0

N.C. = Not considered.

ACKNOWLEDGEMENTS.

The authors are grateful to Dr. B. P. Neogy for his keen interest in this work and encouragement. Thanks are also due to Dr. S. K. Das Gupta for a number of facilities at Bokaro and to Mr. K. Biswas for his assistance.

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NOTICE

HAFFKINE INSTITUTE DIAMOND JUBILEE CELEBRATIONS.

1899-1959

THE Haffkine Institute, Bombay-12, India, will be celebrating its Diamond Jubilee from the 10th to the 14th January, 1959, after the completion of 60 years of its service in medical research.

The Celebrations are expected to be inaugurated by the President of the Republic of India. The programmes will include lectures and scientific seminars on topics, such as Plague, Cholera, Rabies, Influenza, Poliomyelitis, Snakes, Venoms and Insect Resistance to Insecticides. These will be on international level for participation.

The Scientists from abroad, who are interested in the related scientific pursuits, are being invited. All Scientists, Scientific Organizations and Learned Societies are invited to attend or to send delegates so as to encourage free flow of research ideas and goodwill between Research Institutions in the world.

Indian Journal of Malariology, **12**, 4, *December 1958.*

SPECIAL NUMBER OF

INDIAN JOURNAL OF MALARIOLOGY

ON

RESISTANCE OF INSECTS TO INSECTICIDES.

INTRODUCTION.

DURING the past ten years, residual insecticides such as D.D.T., B.H.C. and dieldrin and more recently organophosphorus insecticides have been widely used in the control of many arthropod-borne diseases. Over this period, evidence has been accumulating that nearly forty species of insects of public health importance have developed resistance to these insecticides. This, as Prof. Brown* has stated, " has become a great concern to many countries in which the vigorous offensive now being pursued against arthropod-borne diseases such as malaria, typhus fever, and plague, risks a serious set back unless this new problem can be rapidly mastered ".

The World Health Organization, in collaboration with the Government of India, sponsored at New Delhi in 1958 the first international seminar of research workers on this subject. A total of 61 participants attended this meeting along with eight members of the World Health Organization staff as consultants. They came from 14 countries in the Eastern Mediterranean, South-East Asia and Western Pacific Regions, while the consultants came from Europe and North America. The papers presented at the seminar and a gist of the discussions have been distributed by the World Health Organization *vide* document *World Health Organization/Insecticides/76*.

The seminar stimulated the idea that further information on this problem may be obtained from the workers throughout the world. A request was, therefore, made to nearly 300 workers to contribute papers for the Special Number of the *Indian Journal of Malariology* on Resistance of Insects to Insecticides. Forty papers have been received on the various facets of the problem.

On some occasions, confusion has arisen with regard to insecticide resistance because of imperfect understanding of the precise meaning of certain terms. Drs. Busvine and Pal have contributed a note on terminology to clarify this matter by explaining the usage which has been followed by most of the contributors.

The papers have been grouped under various heads.

*Brown, A. W. A. (1958). *Insecticide resistance in arthropods.* World Health Organization Monograph No. 38, Geneva.

Under the first group, namely, characterization of resistance level, six papers have been included. Mr. Wright of the World Health Organization outlines the standard test methods for detecting and measuring the resistance in mosquitoes and some other insects, and also describes the kits which have been made available by the World Health Organization. Dr. Busvine has described some experiments with the original Busvine-Nash technique and the modified World Health Organization test kit. Dr. Fisk has critically examined the techniques for the measurement of insecticidal resistance in mosquitoes as they relate to the principles of bioassay. Dr. Ramachandra Rao has discussed the development of test methods for other insects of public health importance. Dr. Morrison and Drs. Oppenoorth and Dresden have discussed these methods from the standpoint of basic factors involved.

The second group of thirteen papers deals with the susceptibility of insects to insecticides in various parts of the world. Drs. Hawkins, Moore and Smith give the results of tests on the resistance of Anopheles larvæ in the region of Tennessee Valley Authority. Dr. Jose Blazquez and Dr. Pablo Cova Garcia have studied the susceptibility of larvæ and adults of mosquitoes respectively in Venezuela. Dieldrin resistance in *Culex pipiens* in North-Eastern Tanganyika has been discussed by Dr. Smith. Mr. Chow has presented the susceptibility levels of anopheline mosquitoes in Java. In India, Dr. Patel *et al.* have reported *A. culicifacies* resistant to dieldrin in Bombay. Mr. Bhatia *et al.* have determined the susceptibility of mosquitoes to insecticides in Bombay State and Drs. Ganguli and Sinha in West Bengal. Dr. Pal has reviewed the present status of susceptibility of some species of mosquitoes in India to D.D.T., B.H.C. and dieldrin and Dr. Sharma has reviewed the laboratory and field development of resistance in mosquitoes on global basis. The practical implications of studies on insecticide resistance in anopheline mosquitoes have been discussed by Dr. Davidson. Drs. LaBrecque and Wilson have discussed the resistance of houseflies to organophosphorus insecticides in the United States and Dr. Whitehead has reviewed resistance in the Blue tick *Boophilus decolaratus* in South Africa.

Susceptibility of resistant strains of insects to some newer insecticides has been discussed in the third group comprising six papers. Drs. Mathis and Schoof describe the effectiveness of malathion deposit against *Anopheles quadrimaculatus*. Dr. Pal and Kalra have evaluated the effectiveness of diphenyl methane derivatives and malathion against *Culex fatigans*. Dr. Bettini *et al* and Dr. Keiding have studied the effectiveness of cetyl and myristyl bromo and chloroacetates and cetyl bromoacetate respectively against houseflies. Dr. Nicoli discusses the halogenic intoxication of hexapodes and Dr. Carlo Jucci discusses the effect of pre-treatment with neurotopic drugs.

The fourth group of three papers deals with the genetics of resistance. Drs. Laven and Pal outline the procedures for the study of population genetics. Dr. Pal and Krishnamurthy give the results of crossability of mosquitoes *C. molestus* and *C. fatigans*. Inheritance of D.D.T. resistance in *Culex fatigans* has been discussed by Drs. Pal and Singh.

The fifth group of three papers deals with the biochemistry of resistance. Dr. Babers has reviewed the biochemistry and physiology of resistance by insects to insecticides; Dr. Pant has reviewed the mechanism of resistance involved with

regard to D.D.T. and Dr. Pal has discussed the biochemistry and resistance of organophosphorus compounds.

Other intrinsic factors concerned with resistance have been discussed in the sixth group comprising four papers. Two papers by Dr. Bradbury *et al.* and Dr. Neri *et al.* deal with the lipoid content in resistant strains. Dr. Spiller discusses the components of insecticide tolerance and Dr. Meltzer unspecific resistance mechanisms.

Effect of environmental factors on susceptibility forms the subject of two papers by Drs. Koshi and Ranganathan; and Dr. Ungureanu *et al.* Both papers discuss the effect of temperature and humidity conditions on the susceptibility of mosquitoes.

Three papers have been placed in the general category. Drs. Sautet, Aldighieri and Aldighieri point out that the resistance to insecticides is a danger in the struggle against diseases transmitted by insects and Dr. Ascher makes a plea that a common information source for all resistance problems in biology is urgently needed in view of the paucity of effective countermeasures to insecticide resistance.

Miss Sollers has prepared the bibliography of references on resistance of mosquitoes to insecticides from 1954 through September, 1958.

From the foregoing, it would appear that the various facets of the problem have been pretty well covered.

It is hoped that the Special Number will be of some utility in furtherance of the solution of this difficult problem facing the public health workers. To quote Prof. Brown* again "The rapid rise of resistant strains of flies (*insects*)† has thus focussed attention on the many sidedness of the problem of insect resistance in response to insecticides. On the physiological side it may be due to difference in habits, cuticle, enzymes or general vigour or to combination of any of these factors. On the genetical side, single, few or multiple genes may be involved. Moreover the same species may well respond to the same insecticide in different ways in different areas. The overall problem is challenging to the breadth and depth of entomologist's (*biochemist's*)† training and ability as an investigator.

—*Editor.*

*Brown, A. W. A. (1950) *Eightyfirst Annual Report of Entomological Society of Ontario* (Canada), pp. 34-35.
†Italics have been added by the Editor.

Indian Journal of Malariology, **12**, 4, *December 1958.*

RESISTANCE OF INSECTS TO INSECTICIDES.

A NOTE ON TERMINOLOGY.

BY

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AND

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[December 31, 1958.]

On some occasions, confusion has arisen in regard to insecticide resistance because of imperfect understanding of the precise meaning of certain terms. It seems therefore desirable to clarify this matter by explaining the usage which has been followed by most of the contributors.

The following definitions, which were formulated by the World Health Organization Expert Committee on Insecticides at its Seventh Meeting, have been adopted.

"Resistance to insecticides is the development of an ability in a strain of insects to tolerate doses of toxicants which would prove lethal to the majority of individuals in a normal population of the same species. The term 'behaviouristic resistance' describes the development of the ability to avoid a dose which would prove lethal."

Certain of these terms, however, need a little amplification.

IN REGARD TO INSECTICIDE RESISTANCE*.

It is generally believed that two types of insecticide resistance are possible: "true" physiological resistance and "vigour tolerance". *Physiological resistance*, which is specific to a particular type of poison, often gives a very high degree of protection approaching complete immunity. The resistance mechanism responsible is inherited, apparently by normal Mendelian process (and in several cases through single genes). Thus, it is comparatively easy to maintain resistance colonies for indefinite periods, without the selective action of insecticides.

*Some species of insects are not killed by insecticides even when these are used for the first time; a separate term such as "refractoriness" has been suggested for this phenomenon.

Although resistance of insects to insecticides has not resulted in a checkmate so far, yet it would seem that the serious threats to control of insects of public health importance are due probably to this type of resistance. It has been reported for over 30 species in the field.

Vigour tolerance is a term suggested by Hoskins and Gordon (1956) * to describe a generalised lowering of susceptibility to one or many types of poison, depending on non-specific defence mechanisms due to improved condition, greater weight etc. The consensus of opinion seems to be that this improved condition could be genetically controlled and pressure from the environment might select genotypes for this improved condition for which many genes might be responsible. However, improved vigour might be due to environmental effects alone such as improved nutrition and would therefore be reversible. Comparatively few instances of this have been described in the literature and nearly all of them refer to strains developed by artificial selection by insecticides on laboratory colonies. In such cases, the level of resistance attained is not very high and it is soon lost when the selection pressure is removed. Vigour tolerance does not appear to be of great practical importance.

IN REGARD TO BEHAVIOUR RESISTANCE.

The term *behaviouristic resistance* has been mainly employed in connection with mosquitoes. This phenomenon is not merely a change in the behaviour of the insects but a change due to selection following the use of insecticides. Thus, D.D.T. residues appear to have an irritant effect on several kinds of mosquitoes, which reduces their inclination to rest on treated surfaces. The term " protective avoidance " has also been used to describe this behaviour.

According to the World Health Organization definiton given above, the phrase "*behaviouristic resistance*" refers to the actual development (i.e. intensification) of this power of avoidance. Thus, *behaviouristic resistance* stands in the same relation to protective avoidance as physiological resistance does to normal susceptibility.

At the Seventh Meeting, the World Health Organization Expert Committee on Malaria suggested the term 'natural protective avoidance' and 'acquired

*Hoskins, W. M., and Gordon, H. F. (1956) *Annu. Rev. Ent.*, Vol. 1, pp. 89-122.

protective avoidance' for these two entities. However, we feel that the adjective 'acquired' is ambiguous, suggesting as it does a character acquired by individual mosquitoes. (For which reason it was rejected in connection with physiological resistance). Accordingly, we suggest that the term 'protective avoidance' is adequate to describe normal behaviour, while 'behaviouristic resistance' should refer to intensified behaviour developed by selective pressure.

The importance of behaviouristic resistance is difficult to assess in the absence of a universally accepted measuring behaviour change. The Expert Committee on Malaria at the Seventh Meeting has stated that natural protective avoidance is known to have prevented the interruption of transmission in the case of *A. cruzi cruzi*, *A. bellator*, *A. nunez-tovari*, *A. aquasalis* and *A. darlingi*. Only one case of behaviouristic resistance has, however, been definitely proved, though a few others may be suspected. The present ignorance and uncertainty with regard to this type of resistance has prevented a correct appraisal of the situation. It seems probable that this particular trouble is comparatively unimportant although there seems to be a growing belief that the problem of behaviouristic resistance is greater than is generally accepted.

COMBINATIONS OF RESISTANCE TYPES.

There seems to be no reason why vigour tolerance may not be superimposed on physiological resistance. Indeed, it is possible that such combinations may have been responsible for obscuring the clarity of certain laboratory investigations on toxicology and genetics. On the other hand, physiological resistance is likely to be combined with behaviouristic resistance, since the development of either is likely to blunt the selective action for the other. It is, however, worth noting that a combination of physiological resistance with protective avoidance may be of considerable practical importance.

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STANDARD TEST METHODS FOR DETECTING AND MEASURING THE RESISTANCE OF INSECTS TO INSECTICIDES.

BY

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[October 14, 1958.]

SINCE the emergence of resistance to insecticides in arthropods of medical importance, the World Health Organization has given considerable importance to the development of accurate and comparable test methods for measuring and detecting physiological resistance. Standard methods for adult and larval mosquitoes and for body lice have been established by the Expert Committee on Insecticides and test kits based on them have been distributed by the Organization in large numbers. Techniques for other insects of medical importance are also under study and it is planned that these will become available during 1959. This paper describes the principles on which existing test methods are based and the collaborative efforts which led to their development.

METHODS FOR ADULT MOSQUITOES.

In 1953, two procedures for detecting the resistance of adult mosquitoes were in general use; the first had been developed by Busvine and Nash (1954) in the London School of Hygiene and Tropical Medicine, London, U. K., and the other by Fay and his collaborators (1953) in the Communicable Disease Centre, Savannah, U. S. A. Both were based on the principle of exposing batches of mosquitoes to standard dosages of insecticides but differed on a number of points, among which the more important were the method of applying the toxicant to the test surface, the type and nature of the exposure chamber and the procedures involved in deriving the base line. As a consequence, the results obtained with the two techniques were not comparable; in addition, it soon became evident that both possessed disadvantages affecting their use in the field.

In 1956, the Expert Committee on Malaria of the World Health Organization proposed that the two methods be compared and the best features of both incorporated in a standard procedure that might be adopted in mosquito control programmes throughout the world. This suggestion was followed up by the Expert Committee on Insecticides in the same year and a Working Group drawn

from the Expert Advisory Panel on Insecticides consisting of Dr. J. R. Busvine of the London School of Hygiene and Tropical Medicine, Dr. R. W. Fay of the Communicable Disease Centre, U. S. Public Health Service, Savannah, Georgia, U. S. A. and Dr. C. Mofidi of the Institute of Malariology and Parasitology, Teheran, Iran, was established to study the problem.

By the end of 1956 the group had agreed upon the following principles upon which a modified test method might be based:

(*i*) Test exposure papers to be impregnated in accordance with the procedure originally devised by Busvine and Nash, by which filter paper was treated with different concentrations of insecticides dissolved in a clear, non-volatile mineral oil, such as Risella or Ondina 17.

(*ii*) Impregnation to be carried out at one central point under the control of the World Health Organization.

(*iii*) The type of exposure chamber developed by Fay to be adopted. This consisted of a plastic tube screened at one end and threaded at the other. Exposure tubes were to be lined with impregnated papers; tubes lined with clean paper were to be used as holding cages. The threaded ends could be attached to a slide, the manipulation of which permitted the insects to be transferred easily from one tube to the other.

However, before prototype kits could be assembled for field trial, the working group was required to solve the following problems:

1. IMPREGNATION OF PAPER AT ONE SOURCE.

To achieve the necessary degree of accuracy, a strict production control of the impregnated papers was established. This is based on the weight of impregnant applied to each paper, a tolerance of not more than 3 per cent above or below the required dosage being specified. Dye tests were used to confirm an even distribution of toxicant throughout the papers.

2. VALIDATION OF THE RESULTS OBTAINED WITH THE BUSVINE-NASH METHOD WITH THOSE OBTAINED WITH THE MODIFIED METHOD.

During the period 1953-1956, a considerable amount of data had been obtained from the use of the Busvine-Nash technique. It was important that the results obtained with the modified method would be consistent with these. This involved the following separate but related facts:

(*a*) *The impregnation rate of the commercially impregnated papers.*—Experiments performed by Busvine showed that an impregnation rate of 3·57 mg. of oil solution per sq. cm. of paper would give results congruent with those formerly obtained by the Busvine-Nash method. This figure was later adjusted to 3·6 mg./cm.^{2}.

(*b*) *The position of the tube during exposure.*—It was found that the difference in results obtained with the tube screen end up during the exposure, compared with the screen end down was insignificant. The former was, therefore, accepted on the basis of convenience.

(*c*) *The necessity of covering the two ends with treated paper.*—In the original Busvine-Nash method, the end of the cork inserted into the chamber during exposure had been covered with impregnated paper. The covering of the gauze end of the plastic tube in the same way was found to have little influence on the final results. End were, therefore, left uncovered.

(*d*) *Testing in darkness or light.*—It was found that testing in diffuse light gave results equivalent to those obtained with the Busvine-Nash technique.

3. INSECTICIDES AND CONCENTRATIONS.

It was decided to include only papers impregnated with D.D.T. or dieldrin in the first test kits, at the following dosage:

D.D.T.: 0·25 per cent, 0·5 per cent, 1·0 per cent, 2·0 per cent and 4·0 per cent.

Dieldrin: 0·1 per cent, 0·2 per cent, 0·4 per cent and 0·8 per cent. The para-para isomer of D.D.T. and H.E.O.D. were used. Papers impregnated with oil, but without insecticide, were provided as controls.

4. EXPOSURE TIME AND HOLDING PERIOD.

An exposure period of one hour was proposed, as had been used in the Busvine-Nash method, it being understood that adjustments to this might be required for highly resistant or susceptible strains. The holding period after exposure was fixed at 24 hours. No criterion for death was included in the first proposals.

During the middle of 1957, 36 kits based on the tentative method were issued to collaborators in different parts of the world for field and laboratory trial, including: Blazquez (Venezuela), Bruce-Chwatt (Nigeria), Busvine (United Kingdom), Davidson (United Kingdom), Fay (United States of America), Hadjinicolaou (Greece), Hamon (French Equatorial Africa), Mofidi (Iran), Mosna (Italy), Pletsch, McNeel and Fritz (Mexico), Ramachandra Rao (India), School (United States of America), Sharma (India), Wharton (Malaya) and de Zulueta (Geneva and Italy).

Comments received were generally of a favourable character but suggestions and advice were received on the following points:

(*i*) As far as the life of the impregnated papers was concerned, trials indicated that after seven months' storage in a constant temperature room at 27°C. and approximately 75 per cent R.H., no evidence of any decline in their efficiency was observed. The Communicable Disease Centre observed that after six months' storage, papers could be used 20 times for a period of at least three weeks after being removed from the sealed package in which they were sent. (On the basis of further work done subsequently by the Communicable Disease Centre and T. Ramachandra Rao, it was possible in 1958 to extend the life of the papers to one year from the date of impregnation).

(*ii*) A number of workers pointed out that "down" mosquitoes were inclined to stick to the metal slide, and suggested this be replaced with plastic.

(*iii*) It was also suggested that holding cages, other than the tubes provided, might in some circumstances be preferable or desirable, and that this point be left to the discretion of the worker.

(*iv*) The impregnated papers often became detached from the wall of the tubes during the transfer of the mosquitoes from one to the other. Spring clips or some other device were suggested to prevent this.

(*v*) The provision of a concentration of dieldrin lower than 0·1 per cent was considered to be essential for use against highly susceptible strains in some parts of the world. 0·05 per cent dieldrin papers were, therefore, added. (Papers at 0·025 per cent are also available from Geneva on request.)

(*vi*) There was a consensus of opinion that a clear definition of mortality was desirable. It was, therefore, decided that those mosquitoes unable to walk should be counted as dead.

(*vii*) It was agreed that the temperature at which the test was performed should be included in the report.

(*viii*) As far as the condition and number of mosquitoes were concerned, it was generally held that 20 insects per tube be exposed (if available) and that these be blood-fed females; if not, the proportion of the sexes and their nutritional state should be described.

(*ix*) After the preliminary test with the whole range of concentrations had been done, four concentrations should be chosen (the highest providing 100 per cent kill and the lowest less than 50 per cent). Two tests in duplicate should then be run with these on samples from the same population.

(*x*) No worker reported difficulty arising from contamination of the exposure or holding tubes. Decontamination material was, therefore, not provided in the kit.

These and other points were considered by the Expert Committee on Insect Resistance and Vector Control convened in November 1957. The Committee has described in its report (published as document *World Health Organization Technical Report Series No. 153*) a standard method and test kit. This report may be obtained on application to the World Health Organization. A limited number of test kits are now being distributed.

TEST FOR MOSQUITO LARVÆ.

In 1956, the Expert Committee on Insecticides was requested to recommend a method for determining the resistance of mosquito larvæ to insecticides. The Committee was guided in its work by a review of current techniques prepared by Dr. A. W. A. Brown, then of the World Health Organization. On the basis of this, the Committee proposed the adoption of a tentative procedure incorporating the best points in each for field testing on as wide a scale as possible. This was done with the expert advice of Dr. Busvine and Dr. Fay and in May 1957, 28 prototype test kits were distributed.

The kits were composed of the following:

(*i*) Five concentrations of each of D.D.T. (p, p'isomer), B.H.C. (gamma isomer) and dieldrin (H.E.O.D.) in the form of 50 ml. standard solutions

in ethanol, which when diluted at the rate of 1 ml. to 1 litre of water, gave concentrations of 0·004, 0·02, 0·10, 0·50 and 2·50 p.p.m.

(*ii*) Three 1-ml. pipettes.

(*iii*) One strainer.

(*iv*) Two stirring-rods.

(*v*) One 50-ml. bottle of detergent for decontaminating containers.

The user was expected to provide his own collecting and test vessels.

The tentative procedure involved exposing duplicate batches of 30 larvæ in the third or early fourth instar to each concentration in one litre of water. Enamel pans or basins were recommended as exposure containers. Exposure was for 24 hours, after which the larvæ were removed to clean water for a similar period.

Among those who placed the tentative method under test were: Armstrong and Williams (Arusha, Tanganyika), Blazquez (Maracay, Venezuela), Bunn (Washington, D. C.), Busvine (London, U. K.), Elliott (Lagos, Nigeria), Fay (Savannah, Georgia), Floch and Fauran (Cayenne, French Guiana), Gad (Fassayil, Jordan), Gjullin (Corvallis, Oregon), Grammiccia (Basrah, Iraq), Hawkins (Wilson Dam, Alabama), Lewallen (Berkeley, California) and Smith and Ford (Orlando, Florida).

In general, comments were favourable, although a number of proposals on detail were submitted. The majority of these were incorporated into a modified procedure.

The World Health Organization is particularly grateful to Dr. J. Colvard Jones of the National Institutes of Health, Bethesda, Maryland, for the results of a valuable series of trials and the numerous suggestions that he provided.

All comments received were again reviewed by the Expert Committee on Insecticides in 1957 when the following modifications were made to the original method:

1. The volume of water for exposure was reduced to 250 c.c. and conventional glass containers were substituted for the enamel vessels.

2. The second 24-hour period, during which observation was originally carried out, has been omitted. Dead, living and moribund larvæ are counted after the 24-hour exposure period. Dead larvæ are those that cannot be induced to move when probed with a needle in the siphon or cervical region. Moribund larvæ are those incapable of rising to the surface within a reasonable time or of showing the characteristic diving reaction when the water is disturbed.

3. Two eye-droppers and one metal strainer have been included; the stirring rods have been omitted since stirring is performed by the pipette.

The description of the kit and the instructions for its use are to be found in the Report of the Expert Committee on Insect Resistance and Vector Control (*World Health Organization/Technical Report Series No. 153*). A limited number of kits are available for distribution by the World Health Organization.

TEST KIT FOR BODY LICE.

In 1953, a world-wide survey of the susceptibility of body lice to insecticides was initiated by the World Health Organization. Fifty-one national health administrations agreed to participate and to date results have been received from over 40 of these. The data available up to June 1956 have been analysed by Wright and Brown (1957) and a second survey, which it is anticipated will be more comprehensive than the first, has been started.

The test method used in the first series of observations was based on suggestions made by the U. S. Department of Agriculture Laboratory in Orlando, Florida, to the Associate Committee on Insect and Rodent Control of the U. S. Armed Forces, the latter being good enough to provide the World Health Organization with the necessary data and a specimen test kit. This consisted essentially of treating a square of cloth with dusting powder, placing a number of lice upon it under a suitable container (usually a Petri dish or equivalent) and assessing the percentage mortality 24 hours later. The following concentrations of insecticides were provided with each kit:

D.D.T. (technical grade) 0·1 per cent, 0·5 per cent, 1·0 per cent, 5·0 per cent.

B.H.C. (gamma isomer) 0·25 per cent, 0·5 per cent.

Pyrethrins (synergized) 0·02 per cent, 0·04 per cent.

Two samples of 10-20 lice were required to be tested on three successive days, making a total of six replicates, at each concentration. The tests were to be performed at approximately 25°C. in darkness. Lice were to be collected shortly before being exposed. The exposure cloths were approximately 90 cm.^{2} in area, and 0·5 g. of insecticide were to be applied to each square to give a deposit of 5·5 mg. of powder per sq. cm. Untreated cloths were used as controls. After 24 hours' exposure, the lice were counted into three categories: normal, affected (incapable of co-ordinated movement) and dead (no movement discernible). Checks were made during the course of the survey regarding the quality of the powders. Chemical analyses of the D.D.T. and B.H.C. were carried out in 1956, three years after packaging. The D.D.T. was unchanged; the effectiveness of the B.H.C. had fallen off slightly but not sufficiently to be of biological significance. Since M.Y.L. powder was known to deteriorate only slightly in storage no change was anticipated; this was confirmed.

The following difficulties in the use of the kit were reported:

(*i*) It was unfortunate that the concentrations of insecticides provided were not suitable for the calculation of the LC_{50}. Therefore, in order to obtain a single figure for comparative purposes the average of the mortalities at all the concentrations was calculated. It was considered important to change the range of concentrations to a geometrical progression.

(*ii*) Difficulty was experienced in obtaining sufficiently large number of lice every day for testing on each of the three days specified. The temptation of gathering sufficient lice at any one time for the whole test was frequently too great to resist, with the inevitable results.

(*iii*) The amount of insecticide in each package varied.

(*iv*) The cloths were too small.

The test kit has, therefore, been amended as follows:

(*a*) Insecticides are provided at logarithmically spaced concentrations, as follows:

D.D.T.: 0·04 per cent, 0·2 per cent, 1·0 per cent, 5·0 per cent.

B.H.C.: 0·02 per cent, 0·1 per cent, 0·5 per cent, 2·5 per cent.

Pyrethrins: 0·0016 per cent, 0·008 per cent, 0·04 per cent, 0·2 per cent.

(*b*) Tests to be done in triplicate and on one day only.

(*c*) The amount of insecticide in each package is now within close limits of tolerance.

(*d*) The test cloths have been increased to 156 cm.[2]

Test kits may be obtained from the World Health Organization on application.

OTHER INSECTS OF MEDICAL IMPORTANCE.

There is an urgent need for test methods for other insects of medical importance in addition to those already referred to, including fleas, bed-bugs, houseflies, sand-flies and simulium. This was considered by the Expert Committee on Insect Resistance and Vector Control in 1957, when two general principles were established: firstly, it seems desirable that, as far as possible, presently available equipment be used for all methods, and secondly insects be grouped as follows:

(*a*) Adult flies, including *Musca*, *Stomoxys*, *Phlebotomus*, and *Glossina*.

(*b*) Crawling arthropods such as *Cimex*, *Triatoma*, certain ticks and possibly cockroaches.

(*c*) Fleas and simulium larvæ would probably require separate and special attention.

Since this meeting, the World Health Organization has taken the following action to meet the Committee's recommendations.

FLIES.

Whereas a considerable number of laboratories use methods of different types for determining the susceptibility of houseflies to insecticides, none of these have to date been successfully adapted for field use. With the view of extending the adult mosquito method to this insect, studies are being carried out in a number of institutes; one of these investigators, Dr. F. J. Oppenoorth of the Laboratory for Research on Insecticides at Utrecht, is being assisted materially by the Organization.

Trials with *Stomoxys* and *Tabanidæ*, along the same principles, are also being conducted by Mr. H. C. M. Parr of the Colonial Insecticides Research Unit at Entebbe, Uganda, and Dr. Norman Gratz of the World Health Organization is trying all described methods in a field project in Liberia.

SAND-FLIES.

A test method for sand-flies has been developed by the World Health Organization in collaboration with Dr. Hadjinicolaou of the Department of Malariology and Tropical Diseases, Athens School of Hygiene, Greece (now of

the World Health Organization). A prototype kit is at present under trial by him and it is anticipated that a tentative procedure for more general use will be ready by early 1959.

BED-BUGS.

Tests using the impregnated papers as a means of detecting and measuring the resistance of bed-bugs to insecticides has been recently described by Busvine in an unpublished World Health Organization document. Glass tubes were lined with the paper and the bugs were exposed to them five days after feeding. Batches of 10-12 were used, with an exposure period of up to five days, followed by a two-day observation period.

The promising results so obtained have prompted T. Ramachandra Rao of Poona (India) and C. Mofidi of Teheran (Iran) to develop the method further.

FLEAS.

Investigations towards developing a test method for fleas are being investigated by S. Asahina of the Division of Medical Entomology, National Institutes of Health, Tokyo, and M. Baltazard of the Pasteur Institute, Teheran, both with assistance from the World Health Organization.

It is anticipated that a tentative method will shortly become available for distribution and field testing.

TICKS.

A method for determining the resistance of ticks to insecticides, using oil impregnated papers, was described by Busvine in 1953. In 1956, Mofidi reported to the Expert Committee on Insecticides the results of a series of tests carried out with filter papers impregnated with insecticides dissolved in xylene. Both methods are still under study.

SIMULIUM LARVÆ

A procedure for "establishing a base line which can be used to indicate any distinct increase in tolerance to normal field dosages of insecticides" for use in connection with simulium larvæ, developed by Dr. Muirhead-Thomson, was considered by the Expert Committee on Insect Resistance and Vector Control in 1957. This had been reviewed by Dr. Barnley of Kampala, Uganda; Dr. Herbert T. Dalmat of the National Institute of Health, U. S. A.; Dr. Arden O. Lea, Jr., of Ohio State University and Dr. McMahon of Uganda. It is proposed to circulate a revised method for trial shortly.

It is anticipated that all work on these test methods will have been completed by 1959 when a further meeting of the Expert Committee is planned.

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EXPERIMENTS CONCERNED WITH THE DEVELOPMENT OF THE WORLD HEALTH ORGANIZATION TEST FOR RESISTANCE IN ADULT MOSQUITOES.

BY

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THANKS to the extensive connections of the World Health Organization, the tests for resistance advocated by its Expert Committees are perhaps the most widely used techniques concerned with insecticides. This is especially true of the test for resistance in adult mosquitoes, by means of which an impressive body of data is accumulating on susceptibility levels in normal and resistant strains, which should be very helpful for reference purposes in many countries.

In the preceding article, Mr. J. W. Wright outlines the development of the present test method; in this paper I wish to describe some of the experimental work supporting it. As Mr. Wright says, the Expert Committee on Insecticides adopted the test exposure papers impregnated with oil solution of insecticide, as originally devised by Busvine and Nash (1953). This was retained because it (*i*) ensures a uniform impregnation, (*ii*) by keeping the amount of oil constant, the degree of contamination of the exposed insects is standardized, and (*iii*) by using papers impregnated with different concentrations of insecticide, a dose/kill curve can be obtained. For most normal strains of mosquitoes, this curve is steep, indicating a sensitive correlation between dose and effect.

The use of several concentrations, to provide a dose/kill regression line, permits actual measurement of the degree of resistance present in homogenous strains. Where, however, mixtures of resistant and non-resistant insects are concerned, the shape of the curve can give information on the composition of the population. These refinements may not always be feasible under field conditions, where the essential requirement is primarily the detection of resistance. However, when resistance is detected, it is obviously convenient to use the same method under more standard conditions for extended observations on the degree and type of resistance. This indeed has been possible with the Busvine/Nash test which, apart from field use, has been extensively used in the laboratory for genetical investigations etc. (Davidson, 1958; Coker, 1959).

A disadvantage of the original Busvine/Nash technique was the difficulty of preparation of test papers by individual workers and the possible source of

variation on this account. Fortunately the World Health Organization solved this problem by undertaking to impregnate papers centrally and distribute them throughout the world.

Another test for resistance considered by the Insecticides Committee was that described by Fay *et al.* (1953). The outstanding advantage of this test was the ingenious idea of using a simple cylinder of paper, similar to the exposure chamber, as a holding cage. Mosquitoes could be simply transferred from one to the other, by opposing the ends and gently blowing. In the summer of 1956, this idea was further developed at the Communicable Diseases Centre, Savannah. In the improved version, the papers are supported in plastic tubes with a wire mesh screen at one end; the other end is threaded to allow attachment of a slotted holder carrying a closure slide. This simplifies the following: (*i*) as the mosquitoes are caught, they are stored in batches in the (clean) holding tubes prior to testing. This allows detection and removal of any damaged specimens, (*ii*) transfer to exposure chamber and (*iii*) return to holding cage for mortality counts on the following day. This apparatus and procedure were grafted on to the Busvine/Nash test.

PROBLEMS RAISED BY THE IMPROVED TEST METHOD.

Following agreement in principle about the new method, it was necessary to investigate its feasibility. This involved certain experimental work, which was done in 1957 by Dr. R. W. Fay and myself, in Savannah and London respectively. My own contributions are described in the following sections:

1. ADJUSTMENT TO THE BUSVINE/NASH TEST.

The Busvine/Nash method had been advocated as an interim test for resistance by the World Health Organization Malaria Committee in 1954. Consequently, a considerable amount of data had been accumulated between 1953 and 1956 and it seemed reasonable to adjust the new test to give results consistent with these, so that the value of these observations be not lost.

(*a*) *Rate of impregnation.*—The first papers impregnated in Switzerland, at a rate believed to be equivalent to the Busvine/Nash test, gave kills far too low. Two higher impregnation rates were then tried and the papers tested in London, with the results shown in Table I. The mosquitoes used in these tests were not blood-fed but were given syrup.

These experiments suggested that papers impregnated at 3·6 mg./cm.^{3} by the Swiss method would give results similar to the Busvine/Nash test. Actually, an estimate based on these data gave 3·57 mg./cm.^{2} as the best approximation and this was adopted for subsequent impregnation.

(*b*) *Use of a treated paper or a wire-gauze end on the exposure tubes.*—In the Busvine/Nash method, the cork at the top of the exposure tube is lined with treated paper, so that the mosquitoes cannot rest anywhere on an untreated surface, except on the glass bottom. In contrast, the top end of the exposure tube devised by Fay consisted of wire gauze, which suggested that some mosquitoes might escape the toxic surface at least part of the time. However, some preliminary tests at Savannah

did not show reduced kills with the gauze ends; indeed the mortality was slightly higher. More extensive experiments were conducted in London, with the results shown in Tables II, III and IV. These tests were done in the new type plastic paper holders with papers impregnated in Switzerland. Three types of comparison, with or without treated ends, were made, as indicated in the Table captions. In all cases the "treated ends" were circles of impregnated papers and the "untreated ends" were clean paper discs.

TABLE I

Comparison of test papers impregnated in Switzerland with the Busvine/Nash method.

Mosquito	Papers	Per cent D.D.T.	Kills				Average kill, Per cent
			Males	Per cent	Females	Per cent	
Anopheles gambiae	Busvine/Nash	1·0	22/22	100	13/13	100	100
	WH 3·6 mg./cm.²	,,	16/16	100	11/11	100	100
	WH 3·3 ,,	,,	14/19	74	7/8	87	80
Anopheles gambiae	Busvine/Nash	0·5	40/59	68	32/48	67	67
	WH 3·6 mg./cm.²	,,	29/39	74	17/28	61	68
	WH 3·3 ,,	,,	12/29	42	18/29	62	52
Aedes aegypti	Busvine/Nash	2·0	13/13	100	24/34	71	85
	WH 3·6 mg./cm.²	,,	14/14	100	32/43	74	87
	WH 3·3 ,,	,,	9/10	90	25/48	52	71
Aedes aegypti	Busvine/Nash	1·0	19/29	66	8/46	17	41
	WH 3·6 mg./cm.²	,,	20/27	74	7/43	16	45
	WH 3·3 ,,	,,	4/16	25	0/39	0	12
Aedes aegypti	Busvine/Nash	0·5	2/34	6	0/3	0	3
	WH 3·6 mg./cm.²	,,	3/19	16	0/0	..	(8)
	WH 3·3 ,,	,,	2/29	7	0/0	..	(3)

In these tests, the use of a treated end has much less effect than might be expected. There seemed to be slightly lower kills with a gauze top instead of a treated top (Table IV); but the difference was small compared to the large variation in susceptibility associated with age (*see* Table I).

(*c*) *Effects of illumination.*—Some unpublished work of Dr. C. W. Kearns in 1957 had shown that house-flies exposed to residual deposits picked up larger doses in light than in darkness. It seemed likely that this might be true with mosquitoes and accordingly some tests were made with the new method in comparison with the Busvine/Nash test, which should be run in darkness. The tests were done with unfed *Aedes aegypti* mosquitoes and papers impregnated with different concentrations of dieldrin (Table V). From these results it is apparent that tests in diffuse light give higher kills than tests in darkness, probably because of the increased activity of the mosquitoes. Incidentally this may explain why gauze-ended tubes are almost as effective as tubes with treated ends, since the gauze lets in more light and this makes the mosquitoes more active.

TABLE II.

Treated or untreated end upwards, gauze end downwards.

(Figures in the body of the Table are percentage kills, with number used in brackets.)

	Adult age	Per cent D.D.T.	Treated end up			Untreated end up		
			Males	Females	Average	Males	Females	Average
Aedes aegypti	1 day	2	86 (21)	75 (24)	80	100 (24)	84 (25)	92
		1	17 (24)	3 (30)	10	22 (23)	10 (38)	16
	2 days	2	100 (35)	93 (27)	96	100 (35)	85 (34)	92
		1	56 (34)	17 (24)	36	42 (43)	5 (22)	23
	3 days	2	100 (44)	100 (42)	100	100 (36)	97 (32)	98
		1	83 (18)	58 (31)	70	84 (19)	50 (24)	67
	All ages	2	97 (100)	91 (93)	94	100 (95)	89 (91)	94
		1	50 (76)	27 (85)	38	46 (85)	20 (34)	33
Anopheles gambiae	1 day	1	50 (16)	44 (7)	47	73 (12)	44 (9)	58
		0·5	25 (16)	13 (15)	19	29 (21)	36 (14)	32
	2 days	1	88 (31)	97 (31)	92	91 (23)	100 (26)	95
		0·5	59 (27)	69 (35)	64	57 (23)	41 (17)	49
	3 days	1	100 (10)	100 (10)	100	100 (10)	100 (10)	100
		0·5	92 (26)	87 (8)	91	95 (20)	80 (5)	87
	All ages	1	81 (57)	88 (50)	84	85 (55)	89 (45)	87
		0·5	64 (69)	57 (58)	60	59 (64)	44 (36)	51

TABLE III.

Treated or untreated end downwards, gauze ends upwards. Tests with adults of mixed ages.
(The figures in the body of the Table are percentage kills, with numbers used in brackets.)

	Per cent D.D.T.	Treated end up			Untreated end down		
		Males	Females	Average	Males	Females	Average
A. aegypti	2	100 (66)	91 (47)	95	100 (40)	89 (54)	94
	1	36 (66)	8 (46)	22	15 (67)	10 (50)	12
A. gambiae	1	87 (23)	86 (22)	86	(16)	72 (18)	73
	0·5	45 (22)	32 (22)	38	36 (11)	25 (28)	30

TABLE IV.

Treated end up plus gauze end down versus gauze end up plus untreated end down.
Tests with adults of mixed ages.
(Figures in the body of the Table are percentage kills, with numbers used in brackets.)

	Per cent D.D.T.	Treated end up			Gauze end up		
		Males	Females	Average	Males	Females	Average
A. aegypti	2	97 (29)	90 (68)	93	97 (35)	80 (59)	88
	1	63 (35)	37 (86)	50	41 (22)	37 (89)	39

TABLE V.

Tests in diffuse daylight or darkness.

Paper	Light or dark	Per cent dieldrin	Kills				Average kill, per cent
			Males	Per cent	Females	Per cent	
WHO 3·57 mg./cm.²	Dark	0·8	41/41	100	30/30	100	100
,,	,,	0·4	88/90	98	59/88	67	82
,,	,,	0·2	48/109	43	13/91	14	26
,,	,,	0·1	1/52	2	0/15	0	1
WHO 3·57 mg./cm.²	Light	0·8	52/52	100	36/36	100	100
,,	,,	0·4	79/82	96	110/120	92	94
,,	,,	0·2	40/45	89	16/109	15	52
,,	,,	0·1	3/25	12	1/25	4	8
Busvine/Nash	Dark	0·8	34/34	100	32/32	100	100
,,	,,	0·4	87/87	100	31/35	88	94
,,	,,	0·2	48/72	67	17/51	34	50
,,	,,	0·1	8/46	17	4/28	14	15

The results in Table V suggest that the Busvine/Nash test gives higher kills than the new test in darkness, but about the same results if the World Health Organization test is done in diffuse light. In the past year, further comparisons have been made of the two methods under similar conditions. In most cases they were equivalent, but in a few instances the World Health Organization test gave *higher* kills than the Busvine/Nash tubes (Zulueta, 1958).

2. QUESTIONS OF CONCENTRATION AND EXPOSURE TIME.

Papers impregnated at the following rates (pure *pp*'D.D.T. or HEOD) are provided with the test kit prepared by the World Health Organization.

D.D.T.: 0·25 per cent, 0·5 per cent, 1·0 per cent, 2·0 per cent and 4·0 per cent.

Dieldrin: 0·1 per cent, 0·2 per cent, 0·4 per cent and 0·8 per cent.

Using the standard one hour exposure time, these concentrations are adequate to provide satisfactory data for nearly all susceptible mosquitoes and several resistant strains. In some cases, however, there may be negligible mortality at the highest doses (resistant strains or species of normal low susceptibility such as *Culex fatigans*); and sometimes a complete kill results from the lowest dose used. So far as dieldrin is concerned, this difficulty is met by additional concentrations (0·025 per cent, 0·05 per cent and 1·6 per cent, 4·0 per cent) supplied on special request. With D.D.T. the main difficulty is that the concentration cannot be raised above four per cent in Risella 17, which represents saturation. Some workers have attempted to solve this problem by adding other solvents or by increasing the rate of deposit. Both practices are inadvisable since the data attained from such special preparations cannot be plotted on the same graph as those from standard papers; this defeats the object of the special impregnation.

A very simple solution is available; that is to alter the exposure time. In every case, the normal time should be used first to provide basic data. If it does not provide enough suitable mortalities, tests with longer or shorter exposures may then be made. It is suggested that the alternative exposures should be simple

TABLE VI.

Relation between equitoxic concentration and exposure time.

Per cent dieldrin	Exposure (hours)	Kills				Average kill, per cent	LC_{50}
		Males	Per cent	Females	Per cent		
0·8	1/4	48/59	80	57/66	85	82	0·62
0·4		3/41	7	4/43	9	8	
0·4	1/2	36/46	78	58/70	84	81	0·25
0·2		15/35	43	18/50	36	39	
0·4	1	29/29	100	42/43	98	99	0·14
0·2		27/28	96	37/59	64	80	
0·1		9/36	25	7/58	12	18	
0·2	2	81/81	100	64/67	96	98	0·085
0·1		51/80	65	62/84	74	69	
0·05		2/15	13	1/12	8	10	
0·1	4	48/48	100	78/81	96	98	0·041
0·05		11/12	92	18/39	46	69	

geometric multiples of the normal. Some tests undertaken to investigate the effects of time on LC_{50} are summarized in Table VI. It will be found that, over this restricted range, the concentrations giving equal effect are inversely proportional to the exposure period. In other words $LC_{50} \times$ time = constant. The actual products, obtained from the results in Table VI, are:

0·155, 0·125, 0·140, 0·170, 0·164.

3. EFFECTIVE LIFE OF IMPREGNATED PAPER.

In 1957, both Dr. Fay and I made some preliminary evaluation of the lasting powers of treated papers, before and after removal from their sealed boxes. We found that, even after removal and insertion in the exposure tubes, the papers were effective for some six weeks; and they could be used for a dozen times or more. Subsequently, additional tests have been made, at Savannah, by Dr. T. Ramachandra Rao in India and by Mr. R. J. Wood in London. These trials showed no evidence of deterioration after storage for one year, before use.

DISCUSSION.

I have thought it worthwhile putting the foregoing data on record to show that the present World Health Organization test for resistance in adult mosquitoes has been the subject of considerable laboratory investigation. (My own experiments comprise only a part of this). However, it must be realized that any test of this type cannot be satisfactory in every respect, since it is the outcome of a series of compromises. Thus: (*i*) The use of a paper test surface impregnated with oil film is a little unsatisfactory in that it is quite unrelated to the type of insecticide deposit which the insect encounters in the field. Nevertheless, it was adopted because it permits a precision and repeatability not possible with crystalline residues.

(*ii*) Having agreed to use an artificial test for this particular purpose, one would prefer to avoid dosing the mosquitoes by merely exposing them to treated surfaces. Any method of this kind involves several undesirable variables, since the true dose picked up by the insect depends on the structure of its tarsi and on its activity (which in turn is affected by temperature, illumination, etc.).

However, the alternative method of topical application of individual mosquitoes, seems too difficult to employ widely in the field.

(*iii*) The slide-holding attachment screwed on to the new type exposure chambers very greatly facilitates introduction of the mosquitoes as compared to the Busvine/Nash test. However, it must be admitted that this simplicity is purchased at the expense of a certain number of mosquitoes being crushed if they happen to stick to the slide during exposure.

(*iv*) The use of centrally impregnated paper is a great advance in the direction of standardization. But, once again, one must observe a slight loss of flexibility since one cannot prepare papers at particular rates for special purposes.

All these questions have been carefully considered by the members of the Expert Committee on Insecticides, after considerable field use and the receipt of comments from various users. Decisions were made which may not satisfy everybody. However, the point should be emphasized that it is far better for everyone to use exactly the same technique, than for individuals to introduce their own

modifications. That will merely tend to isolate their results by preventing direct comparison with those of other workers.

USE OF IMPREGNATED PAPERS IN OTHER TESTS.

The central impregnation of papers by the World Health Organization provides a valuable asset in devising tests for resistance in other insects. Already these papers have been found to be satisfactory for detecting and measuring resistance in bed bugs, sand-flies, triatomid bugs, fleas and, perhaps, ticks. The actual techniques differ somewhat from those suitable for mosquitoes, of course, in most cases they are considerably simpler.

Yet another use for these papers has been to measure "protective avoidance" in mosquitoes (induced by the irritant action of D.D.T.). From such tests, it is possible to detect strains with abnormally-developed sensitivity, which therefore display "behaviouristic resistance" (Brown, 1958; de Zulueta, 1958). The development of a reliable test for this phenomenon will be a welcome means of dispelling some of the confusion and ignorance on the subject.

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TECHNIQUES FOR THE MEASUREMENT OF INSECTICIDE RESISTANCE IN MOSQUITOES AS THEY RELATE TO THE PRINCIPLES OF BIOASSAY.

BY

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INTRODUCTION.

WITH the advent of D.D.T., B.H.C., chlordane, and related chemicals at the close of World War II, we entered into a Utopian period where the eventual destruction of all insect pests seemed only to wait upon the ability of man to produce and distribute enough of the new potent insecticides to cover the earth wherever pest insects were to be found. But the mind of man reckoned without a proper recognition of the potentialities of his six-legged adversary. Scattered reports of insecticide resistance among insects, at first discredited, became so numerous and so alarming that they could no longer be ignored or refuted. From that time onward, relatively enormous efforts have gone into the investigation of insecticide resistance from several approaches. Though few of us would share the pessimism of some fly control entomologists who favour a return to the fly swatter, yet we are all concerned over the growing complexity of the problem. At this writing, in only three genera of medically important insects, namely *Simulium*, *Phlebotomus* and *Glossina*, has resistance not yet been reported (Brown, 1958*a*). In all other genera of medically important insects, as well as a host of agricultural pests, there are one or more instances of increased tolerance to insecticides.

The World Health Organization, through its Division of Environmental Sanitation and its Expert Committees on Insecticides and on Insect Resistance and Vector Control, has taken cognizance of the growing importance of insecticide resistance and during the last few years has promoted an extensive programme towards stimulating, coordinating, and publicizing effective research on this problem. The detection and measurement of insecticide resistance in field populations of insects is one small, though important, phase of the general problem. Tests have been developed under World Health Organization sponsorship for adult mosquitoes, human body lice, and mosquito larvæ. Other agencies have developed standardized tests for insecticide resistance in house flies, cockroaches, and *Drosophila* flies to name a few.

PRINCIPLES OF BIOASSAY.

Let us first consider the principles of bioassay procedure and then examine the approved tests for insecticide resistance in mosquitoes in the light of these principles. Bioassay may be defined as any quantitative method whereby the potency or amount of a drug (or insecticide) is determined by the use of biological organisms. The organisms involved range in size from bacteria to dogs, but so long as the amount or potency of the chemical is assayed by means of the response (it may be death) of some living organism, we have bioassay. Bliss (1957) has listed the principles of bioassay as follows: (*1*) Potency is a property of the drug (insecticide), not of the response. (*2*) Potency is relative, not absolute. The potency of one preparation (the " Unknown ") can be measured only in relation to that of a second preparation (the " Standard ") which elicits a similar biological response. (*3*) The assayed potency (quantity) of an " Unknown " resulting from a given test is only an estimate of its true value. (*4*) Both the reliability and efficiency of a bioassay are linked inseparably with its design.

Bliss (1957 *loc. cit.*) interprets the first principle to mean that the response of the organism must be suitable for determining the amount of poison. " The preferred relation is that where the response, however defined, plots linearly against the log-dose of a drug." " The interpretation of the results of an assay is essentially a comparison of parallel straight lines. . . ." Principle two is very important. In order to properly assay an unknown, we must have a standard containing the same chemical (or one eliciting a similar response). So long as we have a known quantity of a standard insecticide to compare with the unknown tested at the same time, then minor variations *between tests* of test animals, environmental conditions, or details of operation will not shift the apparent potency of the unknown nor destroy the validity of the tests. The relative effectiveness of " Standard " versus " Unknown " on the insect is sometimes referred to as Toxicity Index (Sun, 1950; 1957).

Principle three refers to the fact that, just as the actual insects tested are but a sample of the population from which they are drawn and the chemicals used are just samples or extracts from a given source or sources, so the results from a single assay are only an estimate of the true value that would be obtained from testing the whole population of insects against an infinite number of samples under ideal conditions. Bliss (1957) states: "A suitable measure of the precision of each estimate must be determined from the internal evidence of the assay," e.g. the statistical reliability of the assay must be capable of being determined from the data of that particular test. The statistical treatment may be simplified or involved depending upon the design of the experiment, and, as always, upon the limits of reliability (variation) which will be acceptable to the experimenter or statistician.

Principle four simply emphasizes that: " A sound experimental design is thus the last but not the least of our requirements ".

How does the testing of insect populations for insecticide resistance relate to bioassay? Here the assumed variable or " Unknown " is not the chemical but the insect. Since the chemical is no longer a variable quantity it is most important that it be standard as to source, quantity, formulation, and exact method of

application. Since the insect population now becomes the " Unknown ", there must be a " Standard " or " normal susceptible " population with which it can be compared and the ratio " Unknown " to " Standard " strain establishes a Resistance Index comparable to the Toxicity Index of Sun (1950).

In order to conform to the principles of bioassay, a successful Insecticide Resistance Test should include: (*a*) sound experimental design; (*b*) a highly standardized source of formulation of insecticide, and (*c*) a comparison of the " Unknown " (suspected resistant) insect with a " Standard " (susceptible) strain of the same species. Unfortunately the field entomologist may not have access to the " Standard " (susceptible) strain or may not have the facilities to rear it alongside the " Unknown " strain so as to have samples of each available on the day of test. Where the original susceptible strain is not available it would seem advantageous to use the best available " Standard " strain so that the principle of comparing two or more populations on the same day by the same operator can be carried out. If the populations prove to be different in susceptibility and a Resistance Index of any one of them has already been established, then Resistance Indexes for the others can be estimated.

TEST METHODS FOR MOSQUITOES.

The World Health Organization official method for assessing susceptibility of adult mosquitoes to insecticides (World Health Organization, 1954 and 1957) is a modification of the Busvine and Nash (1953) test and in its most recent form is also described by Busvine (1957). A condensed description follows:

(*1*) *Test insects.*—Adult female mosquitoes, ranging in age from five to ten days after emergence and from " a few " to 24 hours after a blood meal, are preferred. This means they should be reared from larvæ or pupæ so that their age may be known. Unreliable results and high control mortalities may be expected if field-collected adults are used. The mouth-controlled collecting tube is suitable for transferring many species, but some, such as *Anopheles gambiae*, will be damaged unless great care is taken.

(*2*) *Test containers.*—The containers now used are two sets of plastic tubes each 5 × 1-3/4 inches, which can be kept separate or can be connected with a sliding partition between them. One set are holding tubes which are lined with clean paper and used for accumulating and holding the mosquitoes before test and later serve as recovery tubes following the exposure period. The second are exposure tubes which are lined just before test with the proper impregnated papers. The test insects are transferred to the exposure tubes for the exposure period, then carefully returned to the holding tubes.

(*3*) *Insecticide papers.*—These are sheets of filter paper especially impregnated with minute, known amounts of D.D.T. or dieldrin dissolved in a non-volatile white mineral oil (Shell "Risella" oil*) plus a suitable volatile solvent. Evaporation of the volatile solvent leaves a dissolved residue of insecticide adhering firmly

* Other products might be used. A low viscosity, non-toxic silicone oil (Dow-Corning 200 fluid) is suggested.

to the paper. These papers, covering a range of six concentrations for each insecticide are prepared by the World Health Organization in Geneva and shipped out to experimenters in sealed containers along with the rest of the Test Kit.

(*4*) The standard exposure time is one hour, in moderate diffuse illumination at a suggested temperature of 27°C. followed by a 24 hour recovery period during which the holding tube is supplied with a moist cotton plug and held at 27°C.

(*5*) Mortality counts at the end of the 24 hour period may be made without much difficulty, since the adult mosquitoes will either be dead or quite active (able to walk or fly) at this time. Control experiments, using exposure tubes lined with oil-treated papers will be necessary. If mortalities over five per cent are found in the control groups the mortalities should be corrected by Abbott's formula. Tests at critical concentrations (i.e., those with partial mortality from which dose/kill graphs can be drawn) should be replicated five times if possible. Log-concentration/probit regression lines are drawn using the average mortalities and the median lethal concentrations (LC_{50}'s) can be estimated graphically. If two or more populations of a given species are being tested the LC_{50}'s may be then converted into Resistance Indexes.

A tentative official test for determining insecticide resistance in mosquito larvae was published by Brown (1957) and given a practical trial by several key workers. As a result of their experience and comments, the Expert Committee on Insect Resistance and Vector Control set up the current official test method (World Health Organization, 1957) which is described fully by Brown (1958*b*). Its principal points are as follows:—

(*1*) *Importance of early tests.*—In order to detect the development of resistance in a given area it is important that the normal susceptibility levels of the target mosquito species be determined before treatments start (at least eight tests) and then continued at regular intervals as the programme progresses. Indications are that resistance should be suspected when the LC_{50} of D.D.T. is in excess of 0·1 p.p.m. for *Aedes* or *Anopheles* or in excess of 1 p.p.m. for *Culex*.

(*2*) *Composition of Test Kit.*—The kit as shipped out from Geneva includes:—

(*a*) five different concentrations (in ethyl alcohol) of p, p′ D.D.T., gamma-B.H.C. or lindane, and dieldrin.

(*b*) three 1-ml. pipettes, one for each insecticide.

(*c*) length of rubber tubing to act as mouthpiece for the pipettes.

(*d*) two eye-droppers.

(*e*) metal strainer for transfer of larvæ.

(*3*) *Test insects.*—Lots of 20 to 25 third or early fourth stage larvæ are counted out into 12 small beakers each containing 25 ml. water. Younger larvæ are difficult to count and handle and will vary in susceptibility (generally more sensitive); older larvæ may pupate during the experiment and then cannot be counted in the test. The sex of the larvæ does not alter their susceptibility and so may be ignored (Jones, 1957).

(*4*) *Test containers.*—Glass vessels, approximately three to four inches in diameter, are specified. They may be jars, bowls or 500 ml. beakers. In the

author's experience, paper cups have proved very useful in large scale bioassay procedures with *Aedes aegypti*. Used cups are destroyed and the fresh cups need no washing or decontamination before use. However, Hawkins and Kearns (1956) and others have severely criticized the use of paper containers because the ionic activity of the paper tends to flocculate the colloidal insecticide suspensions, rendering them about half as stable in paper as in enamel or glass containers.

(5) *Insecticide.*—The kit comes with a range of concentrations of D.D.T., lindane and dieldrin. Additional concentrations may be prepared by dilution with ethyl alcohol. (If higher concentrations are required they may be obtained from the World Health Organization upon request.) Insecticides, other than the three just mentioned, must be procured by the experimenter. In preparing the test mixtures exactly 1 ml. of the appropriate standard insecticide is delivered beneath the surface of 225 ml. of water in the test container and stirred vigorously for 30 seconds with the pipette. Beside the duplicate control (no poison), two replicates of each concentration, beginning with the most dilute, are prepared. The water used may be distilled, rain, stream or tap-water, as free as possible of chlorine or other contaminants and from solid detritus. It should be within the range 20° to 30°C., preferably close to 25°C.

(6) *Exposure time.*—Within 30 minutes of the preparation of the test dispersions, the beakers containing the larvæ are tipped into the larger vessels, making a total volume of 251 ml. in each. This begins the exposure period which lasts 24 hours. At that time the number of dead, living, and moribund larvæ are counted and recorded on the form provided. Except with quick-acting poisons the assessing of mortality at this time is not a simple task. Poisoned larvæ at first appear normal except for occasional erratic movements. The erratic movements increase but at the same time become increasingly sluggish until death finally ensues. The designated criterion of death is that the larvæ shall not move even when probed with a needle. Moribund larvæ are defined as those which fail to rise to the surface or, if on the surface, fail to dive normally when the water is disturbed. Any pupae present are eliminated from the counts and if more than ten per cent of the control larvæ pupate, the test is thrown out. Log concentration/probit regression lines are drawn and from these the LC_{50} or LC_{90} values are read.

In view of the difficulty of readily assessing mortality in the official method, some alternate techniques may be mentioned. Jones (1957) uses much higher concentrations of test insecticides and a series of shorter exposure times, none over 75 minutes. However, the mortality counts continue over a several day period and do not offer any advantage in speed or simplicity. Jones (1957 *loc. cit.*) expresses results in terms of time-mortality, e.g., M.L.T.'s (LT_{50}'s). Earlier Burchfield, Hilchey and Storrs (1952) proposed a photomigration technique. The larvæ are put in the test suspension held in a glass trough set before a controlled light source. Removable barriers separate the trough into three compartments and the larvæ, initially added closest to the light source, are allowed to migrate away from the light as it is turned on. After a minute, the light is turned off and the barriers lowered and the positions of the larvæ noted. The healthy mosquitoes will be in the far compartment, while the morbid and dead will get no further than the centre and near compartments. The procedure is repeated at intervals until the

time at which less than 50 per cent of the larvæ reach the far compartment is noted, e.g., Inactivation Times (TI_{50}'s).

This procedure has been greatly simplified in the author's laboratory by holding the larvæ in the test dispersions undisturbed for 18 hours and then employing the photomigration trough just once for each test group to determine per cent mortality. Larvæ reaching the far half of the trough were counted alive, those remaining in the near half were counted as dead.

DISCUSSION.

If, for the purposes of this paper, the World Health Organization methods for determining insecticide resistance in adult and larval mosquitoes are considered as examples of modified bioassay techniques, they appear to carry out effectively the principles of bioassay*. They are sufficiently simplified in design to be carried out without expensive facilities, yet they are standardized in sufficient detail as to yield comparable results no matter where they are carried out, so long as the instructions are followed. Further their design is statistically sound so that valid quantitative data may be secured from them (assuming that the tests themselves appear valid, i.e., reasonable agreement of replicates, low control mortality, log concentration/probit regression lines of expected slope and nearly parallel). Potency or effectiveness of the chemical is based on a clear-cut response of the organism (death), though in the larva test this may be tedious to assess. With respect to the standardization of the chemical (insecticide), this is handled very well by the use of test kits prepared at a central point, Geneva, and distributed worldwide from there, thus assuring all who use the kits that they have the same source of standard chemicals. However, some experimenters will wish to test insecticides other than the three available in the kits or they may extend and modify the standard test methods to investigate a particular problem (Shidrawi, 1957). Such investigators are faced with the problem of standardization of the insecticide.

How may this problem be solved? One may use only insecticides meeting World Health Organization specifications. By the use of the manual (World Health Organization, 1956) these specifications may be checked by the appropriate chemical and physical tests described therein. It is likely that batches of a given insecticide from different suppliers, though conforming to the minimum World Health Organization physicochemical specifications, might differ very definitely in biological activity. Another source of standard insecticides now available are those sponsored by the Committee on Insecticide Reference Standards of the Entomological Society of America. There are now stocked† eighteen standard insecticides as follows: technical aldrin, technical allethrin, technical aramite, reference grade chlordane, technical D.D.T., pure p, p′ D.D.T., technical dieldrin, technical endrin, technical heptachlor, lindane, technical methoxychlor, technical methyl parathion, technical ovex, technical parathion, technical perthane, technical phosdrin, technical strobane and technical toxaphene. Although most of these materials are listed as technical grade, they are the most highly concentrated form

*Actually these tests were designed solely to detect and measure resistance to insecticides and were never intended to be considered in relation to bioassay.

†May be purchased from Nutritional Biochemicals, Inc., Cleveland 28, Ohio, U.S.A., at $1·25 each or $20·75 per set of 18.

supplied by the basic manufacturer to formulators and suppliers. Net weight of the samples range from 50 to 100 grams each, depending on the material (except allethrin which is 10 grams). With each shipment of standards is supplied a folder giving physicochemical specifications for all of the standards. Each user of these standards has the assurance that he is using essentially the same batch of insecticide as are other experimenters employing the E.S.A. Reference Standards. While the standards available have been selected in part for stability and are reasonably stable as packed the investigator should realize that, like the World Health Organization Test Kits, they will not last indefinitely on the shelf, especially if they are stored under tropical conditions of high temperatures and humidities.

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DEVELOPMENT OF TEST METHODS FOR OTHER INSECTS OF PUBLIC HEALTH IMPORTANCE*.

BY

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ENTOMOLOGISTS have been for a very long time using test methods of several kinds for assessing the effectiveness of insecticides. One cannot scan through the old volumes of the *Journal of Economic Entomology*, or the *Bulletin of Entomological Research* without being bewildered by the vast variety of test methods and equipment employed. It would be impossible to review in a brief paper such as this the entire course of the evolution of the methods. Nor would it be necessary to do so as we are primarily interested at the present moment with test methods to be utilized in connection with only insects of public health importance.

Busvine (1957) in his recent book dealing critically with the techniques for testing insecticides has rightly indicated that the two main interests of such tests lie in determining (1) the toxicity of insecticides and (2) the susceptibility of the insect. The group of workers assembled here are probably more immediately concerned with the determination of susceptibility of the insect rather than with the toxicity as such of insecticides.

Those in charge of vector control programmes are at present deeply and urgently interested in the development of standard test methods which would be (1) as simple as possible, consistent with scientific accuracy, so that even field workers of average ability could carry out the tests and (2) as uniform as possible so that the results obtained in one place or by one person could be compared with those obtained by others at other places. Never before has the need for such methods been as keenly felt as at the present time when modern synthetic insecticides are being used in such a large scale all over the globe and vector control programmes have practically to run a race against time to keep ahead of the challenge which insecticide resistance offers.

The standardization of any scientific experiment involving the use of simple equipment and procedures will undoubtedly result in a certain decrease in the

*Paper read before the World Health Organization Seminar on Insecticide Resistance held in Panama, Republic of Panama, from 21 to 27 June, 1958, at which the author participated as a World Health Organization Consultant.

sensitivity of the tests. They can never, and should never be intended to, replace refined tests to be carried out in advanced laboratories in connection with specialized investigations. But the standard methods make up for the slight lowering of the sensitivity by facilitating accumulation of a mass of comparable data from a wide variety of regions. The experience with the World Health Organization test methods for mosquitoes and body-lice gained during the last two years has amply demonstrated the utility of standardization and the desirability of developing similar tests for other insects of public health importance.

Any test method has necessarily to take into account the habits of the insect dealt with and the equipment and procedures have to be adopted to suit the habits. But most practical field workers in vector control will agree and demand that the equipment and procedures used should, consistent with basic technical soundness, be as uniform as possible for several groups of insects. It is therefore necessary to explore the possibility of using a common equipment, the whole or parts of which could be used with a variety of insects. It is, however, admitted that there will be some groups of insects which will not lend themselves to be tested by any standard kit.

A considerable amount of work has been done in several laboratories in the world towards the advancement of this objective and later during the meetings, the details of the test procedures in respect of each one of the major groups of workers will be dealt with by several workers competent to deal with them. It has occurred to me that it would be very useful in an introductory paper such as mine broadly to deal with the basic principles of test procedures and indicate in a general way the lines on which standardization could be attempted. Such a review has of necessity to be short and limit itself to the major factors which are likely to play a part in the devising and use of the test method. No attempt will be made to secure any degree of completeness in giving references which will be chosen more for purposes of illustration than for exhaustive presentation.

The essential steps in any test dealing with insects are:

1. Selection of test insects
2. Exposure to insecticides
3. Post-exposure holding
4. Measurement of effect, and
5. Interpretation of data.

There should be a measure of agreement among workers on the standard procedures in respect of each one of these steps. We shall now deal with each one of these steps to see how far standardization is possible.

SELECTION OF TEST INSECTS.

Sex, age, size, physiological condition and the stage in the life cycle of the insect are all now known to have a profound influence in determining the degree of susceptibility to insecticides. Agreement is necessary on (1) the best developmental stage to use, (2) the physiological condition, particularly in relation to feeding,

and wherever possible, size, (3) the sex and (4) whether wild-caught or laboratory-bred specimens should be used.

The developmental stage and the physiological condition of the test insects to be used are determined by biological characteristics of the insects themselves. For insects such as *Siphonoptera*, one would naturally expect only the imagos to be used but for *Blattidae* and *Cimecidae* some late developmental stages could be employed in addition to the imagos. For many Diptera, it is obvious that both larval and imaginal stages could be used, though in different types of tests.

The effect of feeding on the degree of susceptibility is now well established. Both the quantity and quality of feeding may have a profound influence on susceptibility. For instance Kerr (1948) showed several years ago that domestic flies had more resistance if they were deprived of food, mainly as a result of the reduced flying activity which led to a lower pick up of the insecticide. The work of Hadaway and Barlow (1956) has demonstrated that in the case of mosquitoes there is a regular cycle of susceptibility corresponding to the gonotrophic cycle. Barnes (1946) and Busvine and Harrison (1953) have shown that freshly-fed bedbugs were more resistant to D.D.T. than during a few days later. Published literature abounds in references such as this of the state of feeding having an influence on susceptibility. A similar phenomenon occurs in relation to mosquitoes in the hibernation phase, which are less susceptible. A general agreement on the stage and physiological condition of the test insect is therefore an important necessity.

Similarly, whether a wild-caught or a laboratory-bred specimen could be used preferentially is a moot question. As the tests now under consideration are mainly for the purpose of determining the degree of susceptibility in the field, it seems that the best insect to use would be the one actually collected in the field. There may, however, be situations in which field collected material may not be numerically adequate and therefore recourse would have to be taken to testing the progeny reared in the laboratory. The hazards of unconscious selection in the course of breeding the next progeny have to be borne in mind.

EXPOSURE TO INSECTICIDES.

The choice of the method used in this, perhaps the most important, step in the test procedures, has often been arbitrary, though we would expect that the method of exposure used should more or less simulate the conditions of exposure to which the insects are subjected under natural conditions. Some of the important methods which have been used and their merits and demerits are briefly discussed below.

1. *Microinjection.*—Introduction of accurately determined quantities of the insecticide into the body of insects, as was done by Van Asperen (1956) in the case of cockroaches. Many mechanical devices have been developed for the purpose. But it is doubtful whether this method can even be used for standardization in field tests, though undoubtedly it is of great importance in basic toxicological studies. Most small insects would not lend themselves readily for microinjection. Apart from the fact that the mode of entry is quite unnatural, the method would

fail to bring out cases in which the degree of susceptibility may depend on the facility of penetration of the insecticide through the insect cuticle.

2. *Topical application.*—A carefully measured quantity of the insecticide in a solution is placed on the body of the insect with mechanical aids such as microsyringe or microloop. Busvine and Harrison (1953), Kochar and Ascher (1954) and others have used this method for house-flies. In view of the accuracy with which the dose can be regulated, it would at first sight appear to be the method of choice. But there are several factors which mitigate against its adoption for standard field tests. There is no guarantee that the dose placed on the insect does all adhere to the body, and the vehicle used as a solvent may considerably influence the penetration into the cuticle. Moreover, the mode of entry is not entirely natural, except in the case of insects which are directly sprayed upon in vector control programmes. The insects have to be immobilized and individually treated, a procedure which is time-consuming.

3. *Exposure to droplets, aerosols, etc.*—Fine droplets are applied directly on to the body of insects either in a Potter's Tower or on a turn-table, or the droplets are picked up by the insects during flight as in the Peet-Grady chamber. Though extensively used on flying insects such as flies and mosquitoes, and sometimes bedbugs, the method is not suitable for field use because of the bulky equipment required.

4. *Exposure to dusts.*—Powders containing known concentrations of insecticides are placed on paper, glass, wood, or cloth surfaces, and insects are exposed to them, a method which is highly suitable for use with crawling insects such as body-lice (Wright and Brown, 1957) and bed-bugs (Ramachandra Rao and Halgeri, 1956). The chief disadvantages are that the exact quantity picked up and *retained* depends upon several factors such as the type of surface, the size of the particles of dust, etc. The abrasive action of dusts on cuticles leading to higher mortalities is an unknown factor. But the method has much to commend itself for adoption with crawling insects if other methods cannot be used.

5. *Exposure to impregnated material.*—Paper, cloth, or any other absorbent material is impregnated with known concentrations of the insecticides from solutions and the insects are exposed to them in suitably designed containers. The very-extensive use of the impregnated paper technique in the World Health Organization test kits for mosquitoes has demonstrated the soundness and utility of this method. Recent work in Poona in our laboratory has shown that for susceptible bed-bugs (*Cimex hemipterus*) also the method works very satisfactorily. Exposures for one hour and counting mortality 24 hours later showed an MLC of 0·7 per cent D.D.T., using the World Health Organization D.D.T.-impregnated papers. The resistant strains from Poona failed to be killed even with 8 per cent papers. The method has been used by Mofidi and colleagues (Mofidi, 1948) in Iran with results showing that their normal strains of *C. lectularius* gave low kills with the impregnated paper. Ansari *et al.* (1956) have also used the impregnated papers for testing the soft tick *Ornithodorus tholozani* and *O. lahorensis*. Eddy *et al.* (1954) used this method several years ago with body-lice and recent work in Orlando with a view to replace dusted cloth by impregnated papers for this insect have met with some success.

Hadjinicolaou (1958) has very successfully used the World Health Organization impregnated paper for tests with *Phlebotomus* in Greece. Shawarby (1953)

used impregnated papers several years ago for tests with fleas and recently Ashahina has been testing the susceptibility of dog and cat fleas in Japan by using World Health Organization papers. While one cannot yet state whether the impregnated papers will finally offer the best method of exposing all the above mentioned groups and several others to insecticides, it holds out the best promise among all methods and the equipment required for exposure is simple. The previous preparation of the papers at a central source entirely eliminates the variabilities associated with such preparations of test surfaces by individual workers. One serious drawback is that when dealing with insects which have already developed resistance, even the papers at the highest concentrations may not give any kill. Nevertheless when one realizes that the test methods now required are mainly to determine susceptibility the technique may be useful even in such cases by directly showing whether the insect populations still retain their susceptibility.

Schoof (1957) has described a method for testing house-flies based on more or less the same principles. He uses a plastic screen cylinder which was previously immersed in the insecticide. The cylinder is placed inside a cardboard carton and the transfer of flies to and from the cylinder is more or less on the same lines as in the case of mosquitoes in the new World Health Organization standard kit.

A closely allied method, if not exactly similar, is the use of glass containers the interior surfaces of which are coated with a deposit of the insecticide. As far back as 1951, Pal and Sharma used glass panels for testing house-flies. Hopkins and Hoffman (1955) used glass jars coated with Dilan. The U.S. Army now has a method of exposing flies to insecticide-coated glass jars for testing house-flies. Intense work in this direction is going on in several laboratories and it may be possible that glass jars or vials may prove themselves to be very good substitutes for impregnated paper where heavy deposits of insecticides are required.

The optimum duration of exposure, the optimum number of insects to be used per test, the concentration of the insecticides to be used and the number of replications required are matters which require careful consideration and standardization in respect of each group of insects.

POST-TREATMENT HOLDING.

Except in cases where the final results are read immediately after the predetermined period of exposure, insects have to be held for further observation in suitable containers. Uniform conditions of temperature and humidity during this stage of the tests are very important. They can be obtained in any well-equipped laboratory but it is rarely possible in field conditions. However, the excellent results obtained with mosquito test-kits used in a wide variety of places and climates justify the conclusion that even with simple precautions it would be possible to obtain satisfactory post-exposure holding conditions.

MEASUREMENT OF RESULTS.

The criteria commonly used for the end results are mainly (1) death and (2) knockdown. In certain experiments with aquatic stages photo-sensitivity has been used as a measure of the intoxication. Death is perhaps the most satisfactory criterion to be used. Knockdown has been employed quite often but in most cases

with the modern insecticides knockdown is followed by death. Its main advantage is in that it provides an intermediate reading which helps in measuring the speed of intoxication.

The tests with insects are almost always an indirect assay of the effectiveness of insecticides, depending upon exposure of batches of individuals to standard doses and recording their responses (Finney, 1952). There are two kinds of responses, a quantitative and a quantal response. In quantitative response, some property of the subject is measured, as for instance the time taken for knockdown. In a quantal response, an all or nothing type of response, death is the criterion most often used. The relative advantages of the two are to be carefully considered. The end results may be determined either by

(1) exposing test insects to a multiple range of concentrations and determining the LD_{50} or LC_{50} at a given period of time after exposure; or

(2) exposing insects to only one concentration and determining the susceptibility by the speed of knockdown or death.

Limitations of time and equipment, apart from the inadvisability of leaving the measurement of end points to the subjective experience of the field investigators, make quantitative responses less suitable than quantal responses for field tests.

INTERPRETATION OF DATA.

Statistical procedures are now available to all field workers and there is no longer any excuse for not using them. The use of probit/log concentration transformations has now been widely accepted and the method of Finney (1952) and Lichtfield and Wilcoxon (1949) are both acceptable. Swaroop (1957) has now provided non-mathematical workers with a very useful guide to probit analyses. The most common method of expressing the results are as median lethal dose, median lethal concentration, or median effective dose. When speed of knockdown is measured the result can be expressed as median knockdown time. Some workers also prefer to use the LD or LC_{90} before expressing an opinion regarding the degree of susceptibility discovered.

DISCUSSION.

The purpose of this review was mainly to focus attention on the problems connected with the development of standard test procedures. While no method can be ideally suited for all insects, the use of impregnated paper or previously treated surfaces offers the most promise for standardization. It is now necessary for groups of workers to carry out trials in a coordinated manner to determine the exact details. The experience gained with the development of mosquito test kits should be utilized and similar methods adopted for other insects.

No mention has been made in this note regarding the tests with aquatic insects, whose problems are of a special nature. The mosquito larvae testing kits point the way in which test kits can be developed for other aquatic insects. Muirhead Thomson's work with *Simuliidae*, however, shows that there will be groups of aquatic insects each requiring a specific method of its own.

The disturbingly rapid increase in the number of insect species which have been reported to have developed resistance during the last two years, makes it very

necessary that urgent steps are taken regarding the development of standard test methods for as many insect groups as possible and placing them in the hands of field workers. There will undoubtedly be many situations in the world where vector control or disease control fails to reach the perfection desired. Quite often they may be due to purely administrative reasons and lack of thoroughness of work. Sometimes they will arise because of some behaviour characteristic of the vector species which had gone unnoticed formerly, not necessarily a change in behaviour, and which leads to a part of the vector population not getting adequate contact with the insecticide. In such situations susceptibility tests will often clinch the issue and direct attention to the proper cause of the failure. Resistance is a reality today, though experience in my country has shown that with two of our most important and widespread vectors of malaria, D.D.T. spraying for over 10 years has not produced resistance. One need not see the spectre of insecticide resistance in every place and become unduly alarmed. At the same time, one cannot altogether ignore the reality of resistance which has in some situations caused havoc with vector control. A calm and deliberate way is to carry out periodic tests in as many places as possible and take such steps as may be necessary to combat resistance if it appears. Development of standard tests are a *sine qua non* for such a vigilance.

In this presentation I have dealt with the matter in an extremely general manner. I could not have done otherwise because the number of insects to be dealt with is considerable and because the details of the methods in respect of each group of insects will be discussed separately by several participants tomorrow.

The development of standard methods is still in a very preliminary stage. I have tried to highlight features of such steps in the test procedures on which we still need more information and discussion. I hope that, very elementary as my presentation has been, it will form the basis of discussion by the distinguished groups of workers assembled here.

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THE INFLUENCE OF THE TEST METHOD ON THE DEGREE OF INSECTICIDAL RESISTANCE REPORTED.

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[October 27, 1958.]

A LARGE body of literature on the laboratory testing of insecticides has accumulated over the years (Busvine, 1957). In it, the fact that numerical expressions of relative toxicity, based on experimental results, are always as much functions of the test method used as of the materials under test, has been emphasized (Morrison, 1943) and has formed the strongest argument for the standardizing of comparative tests. The problem is much the same whether we wish to compare chemicals or the relative susceptibilities of species, strains or stages of insects, to the same chemical.

The evolution of insect strains, which were not as easily or not at all controlled by measures previously effective, was suspected when the resultant poor control was shown as not due to either techniques or materials. Final proof came from comparative laboratory tests on different strains. It then became desirable to measure the differences quantitatively. The measured difference has been called resistance by most workers though some prefer the opposite side of the coin and measure susceptibility, while others subdivide resistance according to its real or suspected causes. Low degrees of resistance are termed "tolerance" by Brown (1958). The World Health Organization Expert Committee on Insecticides (1957) distinguished between "behaviouristic resistance" and "physiological resistance", reserving the latter term for resistance that cannot be explained on a basis of changes in insect behaviour such as increases in the avoidance reactions.

Practically it becomes important to be able to measure the level of resistance for at least two reasons; (1) to establish when a change in control method is warranted, (2) to carry out studies on the inheritance of resistance. Decker and Bruce (1952) considered that levels of resistance, measuring five to ten times that of the normal strain of house-fly, constitute resistance. Knipling (1950) and Keiding (1956) state that a resistance of ten times makes house-fly control unsatisfactory. Brown (1958), as previously pointed out, uses 'tolerance' for levels of less than five times.

For purposes of establishing the need for a change in control it is sufficient to show that the old methods applied efficiently are no longer effective, but in

order to study the physiological basis and inheritance of resistance it must be measured quantitatively with enough accuracy that different degrees of resistance, can be identified. The present paper suggests that we need to be sure of what we are measuring or at least that we are not measuring different things at different times and comparing the incomparable. If test results differ, because the tests differ in sensitivity, the adjustment of results for comparison is possible. Actually the greater the sensitivity the more readily differences will be distinguishable from random variations. But if test results differ because they measure something different, confusion is bound to result.

One's suspicions are aroused when differences of 100 to several thousand times in resistance levels are reported (March, 1952). Winteringham (1952) put his finger on one aspect of the problem when he reported, " the higher the dose applied topically the smaller was the proportion absorbed by the adult house-fly..... when these facts are taken into account it is believed that the spectacularly high levels of resistance observed become very much reduced when the " dose " relates to the actual weight of insecticide which is absorbed by the insects and provided that the insect can cope with the insecticide absorbed, " infinite " levels of resistance could be recorded but the biochemical level of resistance would not be necessarily alarming ".

We can set up a model of this effect if we assume that a small child is so constituted in his desires, stomach size etc., that he can and will, if given the opportunity, consume five apples in a given length of time but that children vary in their physiological response to this dosage of apples, some being rendered ill by as few as one apple, others being unaffected by five. Now take six such children so selected that they can individually be made ill by one, two, three, four, five and six apples respectively. Blindfold each child and place him for a given length of time in an enclosure in which there are a fixed number of apples. He may eat each apple that he finds and picks up. When only one apple is supplied we will suppose that no child finds it. When three are supplied, each child finds one and one child becomes ill. When six are supplied, each child finds three and three children are affected. When ten are supplied, each finds four and when fifteen are supplied each finds five. Thus a dose of fifteen apples renders five children ill but no dosage will render the sixth child ill since he is so constituted that he cannot eat the necessary " lethal " dose. Though the figures given are pure conjecture there is good reason to believe that there would not be a straight line relationship between the number of apples supplied and the number found.

If we were to repeat the test with six other children so constituted that each could consume ten apples and required two, four, six, eight, ten and twelve apples respectively to render them ill, dosages up to fifteen apples might well result in five being eaten by each child and only two children becoming ill. It might require dosages of up to fifty apples, depending on the size of the enclosure and nature of the terrain, inquisitiveness of the child etc., to render five of the six children ill. Thus the relative susceptibility of the children would not be measured by the relative size of the dosages which would have to be supplied to cause equal numbers of reactions.

The proportion of ' apples found ' to ' apples supplied ' might be increased by doing something to prevent apples from rolling out of reach when touched or

bumped into. This should improve the accuracy of the estimate of "physiological susceptibility" based on observed symptoms. The proportion found might be similarly decreased by increasing the places where apples could become inaccessible or by greasing the children's hands, etc.

The logical conclusion is that test methods must be so devised that: (1) there is as exact a parallel as possible between dosage applied and effective dosage, and (2) the effects of as small differences in dosage as possible are measurable. To do this, we must study the mode of action of the insecticide. This is where the great gap in our knowledge exists. We know very little about the physiological action of most insecticides and in general toxicological tests have failed to take cognizance of what we do know.

Hockenyos and Lilly (1932) reported that nicotine was more effective on *Celerio lineata* larvæ the closer the point of application was to the head. Fisher (1952) spotted small amounts of D.D.T. on different parts of the adult house-fly. He found that equal dosages produced lower mortalities as the locus of application moved distally along the legs and posteriorly from head to abdomen. The only exceptions occurred on membranes at the bases of the wings and in the tibio-femoral area, where the membranes lay directly over pulsatile organs. The same author found a correlation between the area of integumental contact with D.D.T. and toxicity. He reported also great sensitivity to D.D.T. applied to the labella. The last result was verified by Morrison and LeRoux (1954) who reported that the same dosage on the labella and on the leg of house-fly adults produced 83 per cent and 65 per cent mortality respectively in twenty-four hours.

LeRoux and Morrison (1954) observed greater mortality when D.D.T. solution was allowed to spread over integumental areas of the house-fly's leg than when it was confined. They also found that application to the labella or gena were highly effective due to the rapid accumulation of D.D.T. within the head from such applications. One and one half micrograms of D.D.T. applied to the labella of susceptible flies and left in contact for 30 minutes caused greater mortality after 24 hours than a similar amount allowed to remain on the leg for four hours D.D.T. resistant flies, treated on the leg with the same amount (1½ micrograms), showed 25 per cent mortality in the twenty-four hours while those treated on the labella showed 60 per cent mortality. Both strains suffered 100 per cent mortality when three-tenths micrograms (approximately 20 per cent of the dosage used on the leg to produce 25 per cent mortality in the resistant and 65 per cent mortality in the susceptible fly strains) of D.D.T. were administered directly within the head. The material was applied as a benzol solution. [Fisher (1952) had been unable to affect flies by placing crystals of D.D.T. directly under the thoracic integument]. A dosage of 0·05 micrograms, applied directly within the head, resulted in 84 per cent mortality of susceptible and 66 per cent mortality of resistant flies. We thus see that using a single discriminating dosage, application of D.D.T. to the legs of the two strains of house-flies gave a resistance ratio of 68/25 or 2·7; application to the labella gave a ratio of 87/60 or 1·5; while application to the brain tissue yielded a ratio of 84/66 or 1·37. Had the method of exposure to a residual film been used, the ratio would undoubtedly have been much higher than any of those reported here since the resistant strain was being reared by Dr. R. W. Fisher in the

Dominion Science Service Laboratory at London Ontario in the constant presence of residual D.D.T.

Low ratios of measured resistance in two strains should be most accurate if they can be proved to be reasonably constant over a range of dosages and providing they are not low because the technique bypasses some physiological mechanism acting under ordinary field exposure conditions.

The effect of technique may be suspected for reasons other than high ratios. If the differences are disproportionate for different dosage levels it may be possible to correct for this by statistical manipulations. Thus the sigmoid toxicity curve (Bliss, 1935) is usually made symmetrical by expressing the dosage on a logarithmic scale on the assumption that absorption is logarithmic. But much work on the relation of technique to results has yet to be done.

Can we expect comparable results from different criteria? Harrison (1951; 1954) concluded that in the resistant strain he worked with, resistance to knock-down was differently inherited from resistance as measured by mortality. Milani (1957), reviewing the work done on inheritance of resistance, divides the reported experimental work into two categories depending on whether resistance was measured by differences in response to a single 'discriminating dose' or on comparison of probit log-dosage regression lines or by similar scalar methods. There was a marked correlation between the interpretation of results as due to mono-factorial or multifactorial inheritance and the test technique used. Milani (1957) expressed some concern over the possible effects of normalizing transformations on the results and pointed out that "the use of scalar doses in genetic research presupposes the existence of genotypes capable of producing discontinuities of such a size in distributions that they cannot be confused with random variations....".

The writer contends that the test method so influences the results in testing comparative insect resistance to insecticides that all investigators should make every effort to determine that their test method; (1) actually measures the physiological resistance of the insect and is not influenced by insect behaviour, uncontrolled physical factors etc., and (2) is so designed that the applied dosage has a close, constant, and if possible, known relationship to the dosage which acts on the insect. These ends can be best attained by more intensive studies on the physiology of toxic action.

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DISCUSSION OF SOME METHODS FOR THE MEASUREMENT OF DEGREES OF RESISTANCE.

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INTRODUCTION.

SINCE the development of insect populations resistant to insecticides, methods have been needed to estimate the resistance quantitatively. It is necessary to dispose of such methods when countermeasures in the field are considered and for the comparison of different strains under investigation in the laboratory. Moreover, the different data revealed by various methods are helpful in a physiological analysis of the resistance, provided that they are carefully interpreted.

Nearly every method used for the estimation of the susceptibility of the original population has also been used for the resistant ones. However, in many cases difficulties arise where very high doses are needed for the resistant strains, and it appears that the degree of resistance found depends largely on the methods used. Since there is no generally accepted standard method, the data obtained by different authors cannot always be compared. This has led to the present situation when data on the degree of resistance are often no longer supplied in studies of the physiology of resistant strains, which causes great difficulty in the interpretation of results.

This paper deals with the advantages and disadvantages of some of the methods. The physiological properties responsible for the resistance will manifest themselves to a larger or smaller extent, depending on the method used for the estimation of this resistance. It follows that it is impossible to define "true" resistance, so that the significance of the results obtained with any method will be only relative. Nevertheless it is important that a standard method will be adopted. In this respect special attention has been paid to a possible application of risella oil in resistance studies, because it has been widely used as a solvent of insecticides in the method of Busvine and Nash for mosquitoes, and seems to be promising in a more general method for many species of insects.

ESTIMATION OF DOSE-MORTALITY LINES.

In order to estimate dose-mortality lines for susceptible and resistant strains it is, of course, essential to have a method by which the flies—also the resistant ones—can be killed. This is not always a simple matter, because there are strains which cannot be killed by any practicable method known. Resistance is often expressed by a resistance factor, which is generally defined as the LD_{50} for the resistant strain divided by that for a susceptible strain.

It is evident that this factor is dependent on the susceptibility of the strain with which the comparison was made, while the meaning of a factor can only be clear if more or less homogeneous populations with similar slopes of the dose-mortality lines are involved.

The insecticides can be administered in many ways, of which the most important ones will be discussed below.

CONTACT AND TOPICAL APPLICATION.

In cases where pick up of the insecticides in the field is by contact, measurement of the resistance by a contact method has certain advantages as compared with topical application. First, with this method the activity of the insects—locomotion, cleaning movements—influences mortality, and possible differences between susceptible and resistant strains that are due to this factor may thus become apparent. Second, the site where the poison enters the insect body is more "natural" and it has been shown that the site of entrance greatly influences the mortality (Bigelow and LeRoux, 1954). Third, in a contact method absorption by the legs may be considerable, and it has been found by Wiesmann (1957) that differences between the legs of susceptible and resistant strains do occur.

A disadvantage of the method is that the large spread of the activity commonly found in the insects results in a corresponding spread in mortality. This may be largely overcome, however, by the addition of a non-volatile solvent to the insecticide (Hoskins *et al.*, 1952; Busvine and Nash, 1953).

In many cases where resistance has been measured by applying the insecticides in acetone or other volatile solvents to the contact surfaces or to the insects themselves, very large amounts have been needed to kill the resistant strains, if they were killed at all. This results in very high resistance factors, as have been found for instance in many strains of houseflies resistant to D.D.T. However, Busvine (1951) has pointed out that these high values have little meaning, since with the higher doses an absorption limit is probably reached. He found that the use of risella oil, as a solvent for the insecticide, greatly reduces the factor of resistance. To explain this difference, Busvine assumes that the oil enhances the absorption of D.D.T. as compared with the absorption after application in acetone. Barker and Rawhy (1957) compared the absorption of 0·8 μg. of D.D.T. applied with the two methods to a resistant strain. This dose is rather low, since the LD_{50} for their susceptible strain with risella oil was 0·57 μg., while those for the susceptible and resistant strains of Busvine were 0·4 and 7·2 μg. respectively. In 24 hours, 74 per cent was absorbed after application in acetone, 41 per cent after application in risella oil. It is possible that at higher doses the opposite situation may be

found, but it is interesting to note that the dose used gave a mortality of 2 per cent with acetone and 21 per cent with risella oil. This indicates the importance of other factors besides absorption when risella oil is used as a solvent.

Hewlett (1958) gives a theoretical discussion of the fact that in many cases the dose-mortality lines of resistant strains have lower slopes than those of susceptible strains (at least with D.D.T. applied in acetone to the housefly). He considers whether this could be caused by the relation between the dose applied and the amount absorbed. It is concluded that this will indeed be the case if the relation between the log dose and the log amount absorbed is not linear, but bends over and becomes less steep as log dose increases.

The available data in the literature do not clearly show this effect (Perry *et al.*, 1953; data collected by Busvine, 1953). However, Hewlett draws attention to the fact that these data have been obtained after a 24 hours' period. It may be more correct to consider only the time during which absorption really contributes to the mortality. This period, which he calls the "relevant time" is, of course, not known. But if it were possible to make estimations after this period, it might appear that the slope of the line becomes less steep as log dose increases.

Hewlett has limited his considerations to an explanation of the slopes of the dose-mortality lines. They may be adduced for the explanation of the differences of resistance factors as found with different methods. If the relation between dose and amount absorbed is more closely linear with the use of risella oil than with acetone, this would result in a lower resistance factor. The amount absorbed should again be measured over the " relevant period ", and it is of course conceivable that not only the absorption rates differ in the two methods, but also the " relevant times ". This may have a large influence on the resistance factor found. In the discussion of injection methods it will also be shown that the time, after which the intoxication is completed, may play an important part.

Besides the influence of the rate of absorption and the rate of intoxication there are some other factors that might be responsible for the lower degree of resistance found with risella oil as compared with acetone. In the first place, certain differences in the integument of the strains may be overlooked when using risella oil. Hadaway and Barlow (1958) showed that beeswax is dispersed in the oil, and in certain resistant strains it was found that it is probably an altered waxy layer which is responsible for the slower absorption of the insecticides (Oppenoorth, 1956). In the second place, there are indications that the solvents themselves have an influence on the insect, which might indirectly affect mortality. Topical application of 1 μl. of risella oil to the housefly causes a visible abnormality in behaviour, but no mortality. The flies are nearly unable to fly and keep their legs in a characteristic position. Application of n-hexadecane results in a similar behaviour, but it causes also a considerable mortality. It should be borne in mind that these harmful effects, even if they do not cause mortality themselves, may influence the mortality resulting from insecticidal action.

INJECTION.

Although injection is too laborious for routine measurements of resistance, the method can be useful in special cases. The difficulty, sometimes encountered

in other methods, to give enough insecticide to kill the highly resistant strains, does not apply here. The amounts of insecticide that can be easily injected, are large enough to be lethal even for these strains.

In injection methods, the solvent is of course very important. Water-soluble insecticides can be injected dissolved in physiological salines. For the more common lipid-soluble insecticides, solutions in acetone have been used (Winteringham, 1951; Babers and Pratt, 1953) besides oily solutions (Busvine, 1951) and emulsions (Oppenoorth, 1956).

Babers and Pratt (1953) injected D.D.T. into houseflies. The curious result was obtained that increasing doses did not cause an increasing mortality, whereas the amount of D.D.T. converted into D.D.E. decreased. A possible explanation would be, that upon injection of the more concentrated solutions (up to 28 per cent) the D.D.T. crystallized at the site of injection instead of being transported throughout the body. The distribution in the body will be much better if oil or an oily emulsion is injected. Especially the latter will be rapidly distributed. Other advantages of emulsions are their low viscosity and hydrophilic properties which facilitate the injection and prevent the liquid from spreading on the lipophilic cuticle.

There are two important differences between injection and the other methods of application. First, resistance factors depending on properties of the integument do not play a part, because the integument is by-passed. Second, the whole dose of the insecticide is suddenly introduced into the body. Injection has often been used as a means to study the possible influence of the integument. However, if no difference between a susceptible and a resistant strain is found after injection, this need not mean that the integument is the site of the differences between the two strains. Resistance factors may also be present that effectively protect the insect from intoxication only if the insecticide is slowly entering the body. The experimental facts presented in Chart 1 may serve as an example of this possibility.

The susceptibility of three strains of houseflies to parathion was measured with a contact method and an injection method. As it is known that parathion, in order to exert its toxic action, has to be converted in the body into paraoxon, the susceptibility for this compound has also been measured. The strains were found to have different susceptibilities for injected parathion. This indicates the presence of internal factors for resistance. Upon injection of paraoxon, however, there is only a slight difference apparent, which could mean that the resistance to parathion depends on a decreased conversion to paraoxon. This explanation, however, would make it difficult to understand the differences found with paraoxon in the contact experiments. A solution of this problem suggests itself if the time course of the intoxication is taken into account. Upon injection of parathion several hours elapse before symptoms develop; with paraoxon they set off immediately, and the ultimate mortality is reached within a short period of time. It seems probable, therefore, that as a result of this rapid action the "protective mechanisms" of the resistant strains (for instance, a detoxication process) cannot play an important part. This example stresses the importance of observations on the speed of development of the symptoms.

CHART I.

Dose-mortality lines for three strains of houseflies.

ESTIMATION OF TIME-KNOCKDOWN CURVES.

For the estimation of time-knockdown curves a group of insects is treated with the insecticide and for every insect the moment of knockdown is noted. In principle, the insecticide can be administered in each of the ways mentioned above.

The advantage is that the resistance of individual insects can be estimated. This is of special importance in genetical work (Harrison, 1953; Barbesgaard and Keiding, 1955). It has been pointed out by Busvine (1951) that resistance to knockdown and resistance to kill need not be the same. He found an interesting case in which a difference between the two was apparent in D.D.T. resistant houseflies. In general, however, the two criteria seem to be closely correlated.

It is clear that resistance, as measured by the knockdown-time method, is not quantitatively comparable with that measured by dose-mortality estimations, but a good qualitative agreement is generally found. It is important to note, however, that exceptions may be found. This can be illustrated by the following

example, where the dose-mortality lines and the knockdown-time curves for three strains are presented in Chart 2.

CHART 2.

Dose-mortality and time-knockdown curves for three strains of housellies.

The strains used in this experiment were: S, susceptible; U_2 a gamma-B.H.C. selected strain, highly resistant to this insecticide; D, selected with parathion and resistant to many organophosphorus insecticides. The left half of the chart shows dose-mortality lines in which the mortality has been counted after a 24 hours' exposure to diazinone, in a contact method. The right half shows time-knockdown curves, obtained with a high dose of diazinone, administered in the same way. It can be seen that in this example the two methods give quite different results. Strain U_2 is known to absorb gamma-B.H.C. at a lower rate than strain S (Oppenoorth, 1956). The same has been found for the absorption of aldrin and dieldrin, and it seems likely that it holds also for other lipophilic substances. This will probably have more effect on the time of knockdown than on the mortality after 24 hours.

SUMMARY AND CONCLUSIONS.

Different methods for the measurement of insect resistance often give very diverging results. Possible causes of this fact are discussed. For the explanation of the differences in resistance found upon application in acetone and in risella oil, four possibilities are considered:

1. The effect of the solvent on the rate of absorption, especially at high doses.

2. The effect of the solvent on the rate of intoxication.

3. The possibility of by-passing of resistance factors located in the cuticle.

4. The direct influence of the oil on the physiology of the insect, and thus on the mortality.

In view of the possible importance of risella oil in a standard method for the estimation of resistance in other species than mosquitoes, more insight into its influence on the intoxication is needed.

Two important points in which the injection method differs from other methods are discussed. One is the by-passing of the integument, the other the more rapid intoxication.

The importance of information on the time course of intoxication processes is stressed.

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TESTS ON THE RESISTANCE OF *ANOPHELES* LARVÆ IN THE REGION OF THE TENNESSEE VALLEY AUTHORITY.

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[September 22, 1958.]

After thirteen years of use, D.D.T. is still the insecticide of choice to supplement the physical and cultural measures for controlling *Anopheles quadrimaculatus* in the impoundages of the Tennessee Valley Authority. D.D.T. has in the main been applied as a larvicide, and, though during the early days a limited amount of adulticiding was carried out, the Tennessee Valley Authority is primarily concerned with the response of the *Anopheles* larvæ to D.D.T. Studies were initiated in 1950, and have continued to the present, to learn if *A. quadrimaculatus* in the Tennessee Valley Authority reservoirs was becoming resistant to D.D.T.

Larval bioassay was one of the methods chosen to measure the response of *A. quadrimaculatus* to D.D.T. However, it soon became apparent that discrepancies in the results must be due to unsuspected effects, and an investigation of factors affecting larval response to D.D.T. was undertaken. Of those factors subsequently defined, one of the more important was found to be the flocculation of the D.D.T. in the bioassay suspensions. This had been demonstrated to us when larvæ were placed at about one-half hour intervals in 0·1 p.p.m. D.D.T. suspensions which had been prepared at one time (Kruse' *et al.*, 1952). Of those larvæ introduced into the suspension during the first half hour, 68 per cent were dead after 24 hours; of those introduced during the second half hour, 24 per cent died; and of those introduced during the third half hour, 16 per cent died. When larvæ were introduced into the suspensions at equal times after preparation, the 24-hour kills were recorded as 67 per cent, 62 per cent, and 83 per cent for the respective groups.

Subsequently measurements were made of the rate of flocculation of D.D.T. from an ethanol-water suspension and xylene-Trex emulsion. After 24 hours

about 40 per cent of the D.D.T. had disappeared from the suspensions in glass and enamelled dishes, while about 75 per cent of the suspended D.D.T. had flocculated in paper containers. The emulsions of D.D.T. were quite stable in both glass and enamelled dishes and no measurable amount of D.D.T. was lost. But in paper containers the strength of the ionic environment was evidenced as 20 per cent of the emulsified D.D.T. flocculated on standing 24 hours (Kruse' *et al.*, 1952).

Because in general the stability of a sol is directly related to the amount of charge borne by the colloidal particles, which in turn is related to the ionic environment, an electrophoretic study of the D.D.T. sols was made (Hawkins and Kearns, 1956). It was found that D.D.T. suspensions in glass or enamelled dishes possess a zeta potential of about −25 millivolts while those suspensions placed in paper containers always possessed a zeta potential of zero. When increasing amounts of salts having mono- and divalent ions were added to the D.D.T. suspensions prepared in glass, the zeta potential was depressed until it reached zero. In the presence of tri- and tetravalent ions the zeta potential of the D.D.T. decreased from about −25 to zero, then the sign of the charge reversed to become positive and reached a maximum of about +25 mv., only to return to zero with further additions of the two ions. Spectrographic analyses showed the paper cartons to be a source of ions of more than the quantity needed to depress the zeta potential to zero. Undesirable amounts of ions were also found to be associated with the recrystallized sample of D.D.T. and could contribute to the contamination.

Evidence has been found that another factor influences kill in larval bioassays. This is the concentration of D.D.T. solvent in the test suspensions (Hawkins, 1956*a*). In general, kill increases as solvent content increases. It was found that when the ethanol concentration was reduced from 1·0 per cent to 0·1 per cent and further to 0·01 per cent, the LD_{50} of the larvæ doubled with each reduction. There was no difference in the LD_{90} at 1·0 per cent and 0·1 per cent ethanol concentrations, but the LD_{90} doubled when the ethanol concentration was lowered to 0·01 per cent. Larvæ conditioned for four hours in a two per cent ethanol solution, prior to being introduced into the D.D.T. suspensions, were more readily killed than unconditioned larvæ.

Tests have failed to reveal any significant degree of resistance to D.D.T. of the larvæ or adults of *A. quadrimaculatus* from reservoir areas treated with D.D.T. larvicides (Ludvik *et al.*, 1951; Hawkins and Hall, 1954). That resistance could develop, was considered to be a very real possibility. In view of this considered opinion, studies of the response of both *A. quadrimaculatus* adults and larvæ to selective exposures of D.D.T. were made. Virgin adults were selected by the topical application of measured amounts of an ethanolic D.D.T. solution. The survivors were allowed to mate and the progeny reared and treated. There was appreciable variation in the kill so that the selection pressure varied between 50 per cent and 90 per cent. The response to D.D.T. of the adults of this colony, following selection for eighteen generations, did not differ from that of the routine insectary colony adults (Gartrell and Ludvik, 1954).

Following these efforts to produce a D.D.T. resistant colony by treatment of the adult mosquitoes, selective exposures of the larvæ were begun. Early fourth-stage larvæ were exposed to suspensions of D.D.T. for 24 hours, at the end of which time the survivors were removed to fresh tap water, fed, and allowed to

develop. This colony has reached the 24th filial generation with dosage mortality data having been collected on the larvæ of each generation. Dosage mortality data showed that due to selection the larvæ of the F_5 generation were some two to three times more tolerant of D.D.T. at both the LD_{50} and LD_{90} levels than were larvæ of the parent insectary colony (Hawkins, 1956*b*). This two- to three-fold increase in tolerance has remained fairly constant, but has not increased with selection of the eighteen succeeding generations (Graph 1).

GRAPH 1.

Dosage response of 4th instar larvæ of various generations of BD and BDR colonies. (Conditions of testing: 25 larvæ per replicate, 23-26°C., 100 ml. of 1 per cent ethanolic distilled water D.D.T. suspension per test. 90 mm. "pyrex" glass crystallizing dishes).

The larvæ of the eleventh filial generation were divided into two equal groups—one group being subjected to selection and the other being allowed to develop without selection. After five generations the D.D.T. tolerance of that portion of the colony, receiving no further exposure to D.D.T., had returned to the lower levels of the insectary colony. The readiness in returning to the lower

tolerance would seem to strengthen the suggestion made earlier (Hawkins, 1956*b*) that probably only a vigour tolerance has been produced by selection.

Dosage response data (World Health Organization adult test kit method) for adults of the F_{24} generation of the selected (BDR) colony and for the insectary (BD) colony indicate that the tolerance levels of the BDR adults are greater than those of the unselected BD colony and of about the same magnitude as those for the larvæ (Graphs 2 and 3). Of some possible significance may be the divergence of the response curves in the regions of higher kills since the larval response curves tend to diverge in the regions of lower kills. However, this apparent reversal may be artifactitious and due simply to the different techniques of applying the D.D.T. to the two stages.

GRAPH 2.

Dosage response of 2-3 day old males to WHO *Risella* oil-D.D.T. impregnated papers. (Insects held at 23-26°C. throughout test period, sugar-water fed, exposed to D.D.T. papers 1 hour).

Tennessee Valley Authority's concern with resistance of *A. quadrimaculatus* was prompted by the occurrence of resistance among other insects of public health importance, and the assumption that *A. quadrimaculatus* possesses the potentiality for resistance to D.D.T. The failure of our field populations to show any measurable resistance would indicate that those selective conditions, necessary for

GRAPH 3.

Dosage response of 2-3 day old females to WHO *Rissla* oil-D.D.T. impregnated papers. (Insects held at 23-26°C. throughout test period, sugar-water fed, exposed to D.D.T. papers 1 hour).

developing resistance, have not existed for *A. quadrimaculatus*. The relative importance of the factors that have contributed to preventing resistance cannot be determined, but no doubt the principal reliance on physical and cultural methods for controlling mosquitoes is the major factor involved. It must also be pointed out that only once or twice during the past thirteen years has as much as 25 per cent of the potential breeding acreage been larvicided during the summer, the percentage usually being much less. Should a selection for D.D.T. tolerance occur among the mosquitoes in a larvicided area during the spraying season, there is the possibility that the infiltration of mosquitoes from adjacent untreated areas would tend to dilute the selected strain. Since each year the spraying programme is begun only when the numbers of *A. quadrimaculatus* have reached some critical value and is stopped when production declines in the fall, a few generations are never exposed to D.D.T. If it is valid to project our laboratory findings on the response of an *A. quadrimaculatus* colony released from selection with the response of field colonies, it would suggest that any tolerance developed during one spraying season could be lost before the following season.

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TESTS WITH LARVÆ OF *AEDES*, *CULEX* AND *ANOPHELES* IN VENEZUELA.*

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THE Department of Malariology of Venezuela, with the help of the Department of Toxycology of Insecticides, have carried out the following work for testing the susceptibility of the larvæ of mosquitoes to various insecticides. These investigations determined in general by the apprehension of some problems observed in the field, are part of those that are being studied at present and represent only a partial vision of the present state of physiological resistance of mosquitoes to the synthetic insecticides. There is evidence to show that at the end of the investigations the panorama will look even more cloudy than what these data indicate.

The tests summed up here have been done in five different places of the country. In some of them, investigations on the susceptibility of the larvæ of *Culex* or *Aedes* to the chlorinated hydrocarbon insecticides were carried out. The objective of this work was to clarify the cause of the failure of these insecticides in the control of these insects. In others, the susceptibility of larvæ of *Anopheles* was studied as a measure of preliminary work.

The treatments with insecticides carried out in the places studied and the causes that motivated the tests are given below:

(*1*) In Carupano, the important coastal region of North East of the country, while studying the *Aedes* index at the end of 1955, it was found that there was a high percentage of positivity, with captures even in the houses recently disinfected. This region was subjected at the time of the examination to 17 partial and residual coverages with D.D.T., with an anti-malarial end in view (1947-1955). A focal *Aedes*-control treatment was also carried out with the same insecticide in 1955. The studies of various samples of scrapings of the walls of the house in this zone demonstrated in biological and chemical determinations that the quantities of D.D.T. varied between 0·8 and 10 gm. per sq. metre.

(*2*) In Caracas, at 922 metres (approximately 3,000 feet) of altitude in the northern central region of the country, there were no systematic campaigns with insecticides up to October, 1954, at which time an intensive campaign of residual

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disinfection with D.D.T. was initiated. Simultaneously anti-larval treatment with the same insecticide was carried out. The total coverage of the locality was completed in April, 1955, which was followed by partial coverages with D.D.T. and Dieldrin. Simultaneously, four perifocal treatments were carried out. In May 1956, the tests of susceptibility, which are being described, were done with the larvæ of *Aedes aegypti* and *Culex fatigans* covering from natural breeding places found in zones under mixed treatment (larvicide and residual).

(*3*) In Puerto Cabello, a city at the centre of the northern coast, tests of susceptibility of larvæ of *Culex fatigans* were done when adults of these species were found repeatedly in houses recently disinfected. The locality, from 1946 to 1953, had 15 *Aedes*-control partial coverages with residual disinfections with D.D.T. Between 1954 and 1955, there was total coverage of D.D.T. and residual Dieldrin with anti-malarial objectives and in 1956 partial anti-malarial coverage with D.D.T. At the same time between 1951 and 1957, seven larvicide treatments in the whole locality and 51 in the sector were carried out, all with D.D.T.

(*4*) In Barquisimeto, tests with larvæ of *Aedes aegypti* were conducted as there was suspicion of an increase of tolerance to D.D.T. detected from the findings in the control work. The locality received five partial residual treatments with D.D.T. from 1948 to 1950 with anti-malarial objective and residual disinfection of total coverage in 1951. At the same time, from 1949 to 1956, ten specific larvaecide treatments with D.D.T. were done.

(*5*) The tests of susceptibility with larvæ of *Anopheles*, effected as a measure of study and preliminary work of orientation, were done with larvæ (90 per cent *A. albimanus*) caught in Taiguaiguai, a place near Maracay, after having received from 1948 to 1952, eight residual disinfections with D.D.T. and in 1954 one with Dieldrin.

The tests were performed in the same localities beginning the exposure in the 24 hours following the collection of larvæ in their natural breeding places.

The collection of these was effected with small ladles and taking them to the laboratory in recipients of glass, with coolers of wood fitted with gauze and cotton.

In these tests, the larvæ of 3rd and 4th instar, brought from natural breeding places were utilized. The colonies maintained in the laboratories since 1947 (without insecticidal pression) were also utilized.

For each test, 20 larvæ were placed in vessels of cardboards with 99 c.c. of water and 1 c.c. of alcoholic solution of the insecticide or 30 to 50 larvæ in enamelled trays with 1 litre of water to which 1 cm. cubic of corresponding alcoholic solution of the insecticide was added. In almost all the cases, 2 to 6 tests were done for each concentration and readings were taken after 24 hours, calculating as dead moribund larvæ.

The details of the technique were as given by the Committee of Experts of Insecticides of the World Health Organization though some small modifications were necessary regarding the handling of the larvæ. We have devised for the handling of the larvæ a small oval racket made of metallic meshes (Figure 1).

The data obtained in the tests are summarized in Tables I to V. The percentages of mortality have been corrected by the Abbott's formula when the

mortality was more than five per cent and values of LC_{50} have been calculated by graphic method.

FIG. I

RACKET FOR HANDLING OF LARVAE

CONSTRUCTION OF GALVANIZED COPPER WIRE AND METALIC CLOTH OF 8 THREADS PER CENTIMETRE

In Table I, it is demonstrated that LC_{50} of D.D.T. to the larvæ of *Culex fatigans* collected in Caracas was 1·07 ppm. and to the sample from Puerto Cabello 5 ppm. These figures, compared with the corresponding LC_{50} of the strain of the laboratory, indicate the existence of considerable degree of D.D.T. resistance in the sample of the larvæ from Caracas and a high degree of D.D.T. resistance in the sample from Puerto Cabello.

TABLE I.

Mortalities and LC_{50} of Culex fatigans *larvæ collected from natural breeding places and laboratory strain. The larvæ were exposed for 24 hours to concentrations of pp' D.D.T. in water as indicated.*

D.D.T.	Caracas			Puerto Cabello			Laboratory strain					
	May, 1956			April, 1956			April, 1956			November, 1956		
p.p.m.	A	D	Per-cent.*	A	D	Per-cent.	A	D	Per-cent.	A	D	Per-cent.
10	0	20	100	12	78	86·6	0	156	100	..	..	..
5	1	19	94·2	45	46	50·5	0	170	100	..	..	..
1	9	11	47·5	75	13	14·8	0	176	100	0	60	100
0·5.. ..	11	10	38·9	68	18	20·9	0	177	100	13	527	97·6
0·1.. ..	18	4	4·6	84	5	5·6	15	161	91·5	133	370	73·5
0·05 ..	16	6	15·1	83	3	3·5	50	116	69·5	..	..	..
0·02 ..	..	..	..	..	..	..	..	..	..	420	60	12·5
0·01 .	18	5	8·6	84	4	4·5	167	10	5·6	..	..	..
0·004 ..	..	..	..	..	..	..	..	..	..	429	256	5·7
Control ..	18	3	14·3	65	3	4·5	87	1	1·1	551	19	3·3
LC_{50} ..	1·07 p.p.m.			5·0 p.p.m.			0·034 p.p.m.			0·053 p.p.m.		

p.p.m. = parts per million. A = alive. D = dead.
* Percentage mortality corrected by Abbott's formula.

In Table II, one can compare the mortality of the larvæ of *Culex fatigans* of Caracas with those of the laboratory in different concentrations of Dieldrin. It is observed that tolerance in the sample from Caracas is about five times as compared to the values obtained with larvæ of our laboratory.

The results of the tests performed with the larvæ of *Aedes aegypti* are summed up in Tables III and IV.

The values of LC_{50} for D.D.T. as demonstrated in the three places, where investigations were carried out, show that the tolerance developed has been significant. If we compare with the normal strain which fluctuates between 0·004 ppm. and 0·05 ppm. to the susceptible races of the laboratories, we see that the sample of Caracas was that which showed the maximum tolerance (LC_{50} equal to 1·95 ppm) while the larvæ collected in Carupano gave LC_{50} equal to 0·34 ppm. which demonstrates an appreciable tolerance to D.D.T. The larvæ of samples from Barquisimeto demonstrate different levels of tolerance. This is explained by the fact that the sample of the Sector A comes from the Foci situated in places

TABLE II.

Mortalities and LC_{50} of Culex fatigans *larvæ collected from natural breeding places and laboratory strain. The larvæ were exposed for 24 hours to varying concentrations of technical dieldrin in water.*

Dieldrin	Caracas			Laboratory strain					
	May, 1956			November, 1957			April, 1958		
p.p.m.	A	D	Per cent.*	A	D	Per cent.*	A	D	Per cent.
10	2	40	94·7	..	..	..	..	..	..
5	1	42	97·3	..	..	..	..	..	..
1	4	38	89·5	..	..	..	0	200	100
0·5	29	12	21·6	9	425	97·7	0	200	100
0·25	..	..	..	..	..	..	53	234	81·5
0·1	22	18	39·1	200	230	50·2	98	113	57·0
0·05	32	8	11·4	..	..	..	163	47	22·4
0·02	..	..	..	340	99	16·9	..	..	..
0·01	39	7	6·1	..	..	..	554	36	6·1
0·004	..	..	..	368	53	6·3	..	..	..
Testigo	37	4	9·7	401	29	6·7	1102	2	0·2
LC_{50}	0·35 p.p.m.			0·098 p.p.m.			0·088 p.p.m.		

p.p.m. = parts per million. A = alive. D = dead.
* Percentage mortality corrected by Abbott's formula.

TABLE III.

Mortalities and LC_{50} of Aedes aegypti *larvæ collected from natural breeding places. The larvæ were exposed for 24 hours to varying concentrations of pp' D.D.T. in water.*

D.D.T.	Caracas May, 1956			Carupano April, 1956			Barquisimeto May, 1958					
							Sector A			Sector B		
p.p.m.	A	D	Per cent.	A	D	Per cent.	A	D	Per cent.	A	D	Per cent.*
10	0	21	100	3	111	97·4	..	..	..	..	..	..
5	3	18	85·6	9	117	92·8	..	..	..	..	..	..
2·5	..	..	..	..	..	..	74	97	56·7	30	180	84·5
1	17	5	22·4	33	103	75·6	..	..	..	..	..	..
0·5	16	4	17·4	24	90	78·6	..	..	..	..	..	..
0·1	13	11	45·8	122	10	7·6	104	66	38·8	109	114	47·1
0·05	19	2	9·5	113	10	8·1	..	..	..	..	..	..
0·02	..	..	..	..	..	..	149	38	20·3	200	48	12·9
0·01	21	1	4·5	114	6	5	..	..	..	..	..	..
0·004	..	..	..	..	..	..	36	4	10·0	..	..	..
Testigo	20	1	4·8	90	4	4·3	158	6	3·6	185	15	7·5
LC_{50}	1·95 p.p.m.			0·34 p.p.m.			0·8 p.p.m.			0·12 p.p.m.		

p.p.m. = parts per million. A = alive. D = dead.
* Percentage mortality corrected by Abbott's formula.

TABLE IV.

Mortalities and LC_{50} of Aedes aegypti *larvæ collected from natural breeding places and laboratory strain. The larvæ were exposed for 24 hours to varying concentrations of technical dieldrin in water.*

Dieldrin	Caracas May, 1956			Carupano April, 1956			Barquisimeto May, 1958			Laboratory Strain March, 1952		
p.p.m.	A	D	Per cent.*	A	D	Per cent.	A	D	Per cent.*	A	D	Per cent.
10	0	41	100	0	76	100	..	..	..	..	..	..
5	0	40	100	..	78	100	..	..	..	..	..	..
1	0	39	100	3	97	97·0	..	..	..	..	..	..
0·5	0	40	100	5	89	95·6	0	200	100	..	..	..
0·1	0	41	100	11	84	88·3	9	144	93·7	0	138	100
0·05	1	41	96·9	23	74	76·3	..	..	..	..	..	..
0·02	..	..	..	..	..	..	42	307	87·1	..	..	..
0·01	0	43	100	84	4	4·8	116	168	56·6	11	125	91·9
0·005.. ..	..	..	..	..	..	..	..	..	..	51	100	66·2
0·004.. ..	..	..	..	..	..	..	113	96	42·6	..	..	..
0·0025 ..	..	..	..	..	..	..	..	..	..	30	45	60·0
0·00125 ..	..	..	..	..	..	..	..	..	..	76	40	34·0
Testigo ..	34	9	20·9	81	2	2·4	343	21	5·8	116	5	4·1
LC_{50}	0·01 p.p.m.			0·033 p.p.m.			0·0062 p.p.m.			0·002 p.p.m.		

p.p.m. = parts per million. A = alive. D = dead.
* Percentage mortality corrected by Abbott's formula.

subjected to systematic adulticide and larvicide treatment simultaneously; while the larvæ of the Sector *B* were collected in places treated solely with sporadic residual disinfections with D.D.T. In the Sample *A* we obtained a value LC_{50} equal to 0·8 ppm. and with the sample *B*, 0·12 ppm. Both the values, as one can see, are above the normal susceptibility to D.D.T. The mortality of the larvæ of *Aedes aegypti* captured in Carupano and Barquisimeto in the tests with different concentrations of Dieldrin (Table IV), has not demonstrated significant increase of tolerance though there is a slight indication towards this. The sample from Caracas indicates a susceptibility to Dieldrin at the end of the test, similar to that obtained in 1952 in the strain which we had maintained in our laboratory. The tests with anophelines (90 per cent *Anopheles albimanus*) are summed up in Table V and demonstrate that the samples that have been studied show a normal susceptibility to the three tested insecticides giving the values LC_{50} of 0·0011 ppm. for DDT, 0·0029 ppm for Dieldrin, 0·007 ppm. for gamma B.H.C.

TABLE V.

Mortalities and LC_{50} of anopheline larvæ (90 per cent anopheline albimanus) collected from natural breeding places. The larvæ were exposed for 24 hours to varying concentrations of pp' D.D.T., technical dieldrin and gamma isomer of B.H.C. in water.

p.p.m.	D.D.T.			Dieldrin			B.H.C.		
	A	D	Per cent.	A	D	Per cent.*	A	D	Per cent.*
2·5	0	30	100	..	..	..	0	25	100
0·5	0	58	100	0	60	100	..	..	..
0·25	0	120	100	..	..	..	..	..	..
0·1	0	144	100	0	61	100	4	80	94·8
0·02	30	213	87·6	0	62	100	44	56	52·3
0·004	67	155	69·8	8	41	75·4	64	67	47·7
0·0008	13	36	45·5	..	..	..	..	..	..
Testigo Control	132	6	4·3	34	17	33·3	85	7	7·6
LC_{50}	0·0011 p.p.m.			0·0029 p.p.m.			0·007 p.p.m.		

p.p.m. = parts per million. A = alive. D = dead.
* Percentage mortality corrected by Abbott's formula.

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RESISTANCE MANIFESTATIONS IN THE BEHAVIOUR OF THE VECTORS IN VENEZUELA.*

BY

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THE history of malaria in Venezuela can be divided in two big stages: the first ending in 1945 is characterised by the reduction of vectors due to the fight against their larvæ with drainage and use of mineral oils; this was used only in big populated centres due to economic reasons. In the second stage, the fight is directed specifically against the adults and can be extended easily to the rural areas.

During the first stage, a detailed study of the disease and its vectors was made. It was shown that among these there were some that, in a high percentage, feed and rest inside the houses; that others bite and stay outside the houses and that lastly there are some that possess both the phases, i.e., they can indifferently bite and stay inside or bite and stay outside the houses.

A. darlingi belongs to the first group. This mosquito is in close contact with man and is the most virulent transmitter of the disease. The remaining mosquitoes belong to the second and third groups. Their participation in the transmission of the disease is of little or no relative importance.

Based on these facts, and possessing new insecticides of residual action, the second stage with intradomiciliary spray (indoor residual spray) was initiated; and as it was thought, malaria decreased in such a manner in a large zone of the country that it led to believe that its total elimination was already an accomplished fact.

In four years, i.e., from 1945 to 1949, of intradomiciliary use of D.D.T., this insecticide had acted in two ways: on the one hand it had eliminated in a large area the existence of *A. darlingi* and on the other hand the other species, chiefly *A. albimanus* and *A. aquasalis*, were left unaffected. This demonstrates: (*a*) that the species really responsive for the transmission was *A. darlingi* and that for this reason its eradication brought about the eradication of the disease; (b) that the other species, *A. albimanus* and *A. aquasalis*, played a secondary role and where they were vectors the effect of the insecticide could be considered as a result of interception and rest as a production of their reduction.

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Posed the problem in this way, it was necessary to solve the following question: Why is it that in this area the eradication of *A. darlingi* was easy and that of *A. albimanus* and *A. aquasalis* was completely inexistent?

It could be easy to say that the eradication of *A. darlingi* was due to the fact that this species is eminently domestic and that *A. albimanus* and *A. aquasalis* do not possess this quality in identical proportions; but it happens that in other areas of the country where the same insecticides were used, *A. darlingi* remains in conditions more or less similar to those shown by *A. albimanus* and *A. aquasalis* in the areas of eradication. This led to think that the species is divided in two races: one anthropophilic which penetrates into the houses and disappears with the use of insecticides of residual action, and the other zoophilic which feeds on animals and which is resistant.

This theory which interprets the current notion and states that " In its centres of dispersion *A. darlingi* is chiefly zoophilic and as it runs away from them is becoming anthropophilic so that its predominant characteristic is zoophilic and the anthropophilic is only a quality acquired by some races ". Though very interesting, this theory does not correspond to the reality, as all the other anopheline species chiefly *A. albimanus* and *A. aquasalis* would fall in the same category and this case we could state that, due to the use of insecticides the anthropophilic races died and only the zoophilic races survived. This is, however, not true as these species bite men and animals in the same proportion as they were doing before the use of D.D.T.

If it were possible to transplant the species *A. albimanus* and *A. aquasalis* to the regions where the insecticide does not prevent the life of *A. darlingi* and if they were able to adapt after thousand difficulties to the conditions of the natural habitat of this latter, there is no doubt that D.D.T. would have the same effect on them as on *A. darlingi*, not because new races were formed but because these races would live in a precarious condition and in an environment which is totally hostile. In these circumstances, any perturbing element could bring about a disequilibrium which, without any doubt, would result in their total elimination. Moreover, *A. albimanus* and *A. aquasalis* have as their natural habitat the coasts of the country and in the remaining part of our territory, with the exception of the Lake Tacarigua, they are rarely found, as the very ecologic elements where they have reached as the invading agents, have undertaken to prevent their survival.

It is observed that while in some places these species disappear due to the action of the environment, in other places as in the Lake Tacarigua, they live in perfect conditions of life notwithstanding an intense use of D.D.T., this happens because they reach an environment which offers them exactly the same ecologic conditions as from where they have come.

In Venezuela, *A. darlingi* is an invading mosquito, a complete stranger to the environment where its behaviour during the period of rains is different from that of the dry season, while during the rains it penetrates all the places, during the dry season it takes refuge in humid places where it bites men and animals indiscriminately as is shown in Table I.

This fact can also be observed in the studies carried out by Dr. Guzman Gacia Martin at the South of the Lake Maracaibo, where in captures, effected in the year

1955, it was found that *A. darlingi* was equally distributed in men and animals (*See* Table II).

TABLE I.

Places where A. darlingi *was captured.*

Area	Positive captures in human dwellings		Positive captures in traps		1936-1945 Total captured positive
	Number	Per cent.	Number	Per cent.	
Humid weather conditions					
Calabozo	73	81·1	17	18·8	90
Maturin	224	99·5	1	0·4	225
Dry conditions					
Calabozo	9	60	6	40	15
Maturin	20	51·2	19	48·8	39

TABLE II.

Captures of A. darlingi *at the South of Lake Maracaibo.*

	Captures for night	Percentage
Fed on donkeys	8	12·9
,, ,, pigs	15	24·2
,, ,, cattle	13	20·9
,, ,, humans	26	42·0

The adaptability of *A. darlingi* to all the environments, where there is notable influence of rains, can be temporal in some regions of the country and permanent in others. It is permanent when the environment and its ecologic conditions are similar to the natural habitating margins of the great masses of water and thick jungles, south of the Maracaibo Lake, course of the River Orinoco, Large Morichales of Monagas State and some other places with more or less similar conditions; it is temporary when the ecologic links are weak, *i.e.*, where the elements that unite it to the environment almost depend on the rains, temperature and humidity.

In this way it can be explained that the arrival of D.D.T. eradicated it from these regions and that it remained even today, after almost 12 years of saturation of the country with insecticides of residual action, in the areas where it could find conditions similar to its place of origin where neither D.D.T. nor any other insecticide will be capable of its extermination.

In Table 1 where the comparative data of the cities Calabozo and Maturin during the dry and rainy season are given, this observation is clear, as while in Calabozo where the species disappears just as in the whole Guarico State with the exception of the Southern limits with Orinoco, with the use of D.D.T. in Maturin and in general almost all over the State of Monagas, the species is in the same conditions as it was before the use of D.D.T.

Thus there is no formation of zoophilic and antropophilic races and their selection by the use of D.D.T. *A. darlingi* bites equally men and animals not only in its natural habitat but also when it invades inhabited centres; that the proportion in which this happens is different, means nothing; potentially the species is capable of feeding on any of them, depending only on the place where it is found. In an inhabited centre it will bite human beings more than animals, and in jungles and margins of great masses of water it will bite men less than animals. The saying " *A. darlingi* either bites men, or commits suicide " could be created when our attention was concentrated in the houses, forgetting that there were other places where its existence was secure. After 12 years of attack against this species, we have succeeded in finding its natural centres of life, which could be called secondary centres of dispersion, and there we find it in constant " acecho " ("lying in ambush") waiting for the opportune moment to begin a new invasion.

If we think that we have eliminated, by the action of D.D.T., the domestic races, and that only the zoophilic race remains,then the problem is solved, because this race could never constitute a danger to the country; but if, on the contrary, we think that there are no types of race, but that the species is the same and that for unforeseen reasons it had to stay in places which offered better security, then the situation would change completely; because if it is true that in the first case we could continue the spray with residual insecticides without any danger, in the second case we could not do it because we would run the risk of forming artificially a resistant race (and here the word race can be used) which would put the country in difficult situation and the future generations would have not thanked us in any way.

If it is true that it is thought today that " the insecticides are used primarily to interupt the transmission of diseases and not to control their vectors, in such a manner that once this objective is achieved, the degree of control of their vector is not important ", it is not less true also: (*a*) that in places where the vector has been eradicated, the certainty that the disease will not revive, is more firm than in places where it continues to exist, and (*b*) that, if those that remain due to some reason or other, could get selected for resistance, the situation would be disagreeable and the nation would be exposed to new attacks of a disease which was believed to have been conquered, with the aggravating circumstance that it would lack the effective means of combat and perhaps would have come back to the procedures that were abandoned in 1945.

There is no doubt that against this one can say that, in the future, other insecticides will be available and that we should not pre-occupy ourselves with this problem; but this is problematic. What is certain is that we possess today certain means which we should keep with maximum care and use with prudence. In Venezuela, as in any other region where the insecticides have carried out their mission, they should not be used but kept only for the emergency cases. To

saturate the country with insecticides, not only in the field of agriculture, but also in sanitation field, is to expose ourselves to grave contingencies. The resistance in the insects is not a mystery and our duty, once we cannot exterminate them, is to let the procedures and elements which we have used, be used by future generations with the same effectiveness as in our times.

It is not at all new that in various parts of the world, the flies, and some species of *Aedes* and *Culex* as also of *Anopheles*, have developed resistance and there is but one anxiety, observed in recent International Conferences while recommending the necessity of transforming the present schemes of the fight against malaria into programmes of eradication of malaria so that it may be possible to suspend the insecticide campaigns before the *Anopheles* develop resistance.

It is possible that we may have a chance of suspending the sprays, although there are clear indications that the *Anopheles* have begun to show some resistance as is seen in *A. nunez-Tovari* where a slight resistance is noted and is quite high in *A. darlingi*, being maximum in *A. albimanus*. It is this, and not any other thing, that is concluded from the work which we are trying to analyse, carried out by Dr. Guzman Garcia Martin on the principal vectors of malaria in Zulia in 1955 and his monthly letter of October, 1956. In this respect, it is good to remember that in the survey carried out by the Division of Malariology in the beginning of 1951 on the malarial conditions of the country, it was found that this disease, notwithstanding the constant use of D.D.T., persisted in Guanta and Pertigalete (Anzoategui); Santa Fé and Ayacucho (Sucre), Municipios Miranda and La Ceiba (Trujillo), Municipios Sur e Oeste (Tachira) e Costa Sur, bag of Maracaibo (Zulia). This posed the problem of knowing the cause of this persistence of the disease notwithstanding the insecticide treatments since 1947. It was thought that the insecticide did not give result when applied to the surfaces already sprayed or that it was due to the building of new houses. This led to the decrease of the interval between treatments which from six months came down to three months. With the use of this method an attenuation of the disease was obtained but not complete eradication. As a result, Dr. Guzman Garcia Martin began a detailed study of anophelines at south of the lake and found that in 38,643 captures from 1953-1955, there were 1,289 adults of *A. darlingi*, 898 of *A. albimanus* and 2,484 of *A. nunez-tovari*. With these data, by comparative studies, the degree of resistance in the three species was established, taking as a base the ratio between live and dead adults found in disinfected houses.

Table III, which summarises this work, demonstrates that the first place is occupied by *A. albimanus*, second by *A. nunez-Tovari* and the third by *A. darlingi*. Thus in 100 positive captures for every one of the three species 45·3 per cent correspond to *A. albimanus* found dead and 54·7 per cent to those found alive; in the case of *A. nunez-tovari* the percentage of the dead was 52·9 per cent and of the alive 47·1 per cent; in the case of *A. darlingi* the percentages were 56·5 per cent and 43·5 per cent respectively; this means that the most resistant mosquito to the action of D.D.T. is *A. albimanus*. Moreover in 17,737 captures in the year 1956, 830 live mosquitoes were found after treatment with D.D.T. against 265 specimens in 1953-55, this phenomenon was not accompanied by any increase in the number of adults found dead. The opposition of marked physiological resistance in *A. albimanus* is, therefore, evident.

TABLE III.

Number of anopheline vectors per 100 positive captures in a day in houses sprayed with D.D.T.

Species	Number alive	Per cent.	Number dead	Per cent.	Total
A. albimanus	265	54·7	220	45·3	485
A. nunez-Tovari ..	275	47·1	310	52·9	585
A. darlingi	163	43·5	212	56·5	375

On the other hand, *A. albimanus* is a mosquito which seeks shelter in houses as was seen in the test carried out in San Rafael. 95·5 per cent of the mosquitoes that penetrated and fed inside the houses, remained there (in the houses). This test consisted in allowing the mosquitoes to bite under human legs until they were full with blood. At the same time, an inspector was identifying them and counting the number of those that ran away after the feeding; another inspector captured the mosquitoes found in the walls and furniture. The house had one door and two small windows, all of them open. This experiment was also carried out in Minicipios Urribarri and Haras. In Urribarri, 11 per cent of *A. darlingi* were found inside the house and 89 per cent outside. In Haras, only 15·5 per cent of *A. nunez-Tovari* was inside and the remaining outside. Thus *A. albimanus* occupies, undoubtedly, the first place among the vectors which stay inside the houses, followed in very small percentages by *A. nunez-Tovari* and *A. darlingi*.

In the same Table III, one observes also that the mosquito which dies more frequently after *A. darlingi* is *A. nunez-Tovari* which, it appears, does not distinguish like *A. albimanus*, between surfaces with and without D.D.T. On this point, it is better to clarify that this mosquito is less likely to penetrate in the houses as was seen in the captures carried out simultaneously inside and outside the houses during the three-year period 1953-1955; 29 per cent were captured inside and 71 per cent outside. In 1956, this last percentage increased to 75·5 per cent. In their quality of avoiding surfaces with D.D.T., there was also some increase, as in 1955 the difference between those that were found on surfaces with or without D.D.T. was 55, while in 1956 it was 58. In the same way, the number of mosquitoes found dead in 1953-1955 was 310 while in 1956 this number was only 226. Thus in *A. nunez-Tovari*, undoubtedly, there is an increase in their quality of living outside the houses and also in their ability to run away from D.D.T.

Regarding *A. darlingi*, Table IV shows that this mosquito is found less frequently on the walls with D.D.T. (34·5 per cent), discriminates between the places with and without D.D.T., giving in this way signs of ecological resistance which was confirmed by the experimental tests carried out in Caserio Santa Rosa (Municipio Urribarri); here 69 per cent of captures corresponded to surfaces without D.D.T. and 31 per cent with the insecticide, although 95 per cent of the surface of houses was sprayed with D.D.T. In other tests, as in the case of placing simultaneously two workers, one inside and the other outside the house, the same results were

TABLE IV.

Number of anopheline vectors per 100 positive captures during day in houses sprayed with D.D.T. and without D.D.T.

Species	Number captured without D.D.T. spraying	Per cent.	Number captured with D.D.T. spraying	Per cent.	Total
A. albimanus	310	53·9	265	46·1	575
A. nuñez-Tovari ..	330	54·5	275	45·5	605
A. darlingi	310	65·5	163	34·5	473

obtained, as is seen by the number of *A. darlingi* captures inside the house which was 22 per cent within three months of D.D.T. spraying while this percentage increased to 56·5 per cent after this period. In order to make the idea even clearer, two persons with uncovered legs were kept in a house which was D.D.T.-sprayed seven days before. At the same time, three buffaloes were kept outside the house. It was observed that notwithstanding the known liking of this species for human blood, 78 per cent were captured while biting the buffaloes which demonstrates that *A. darlingi*, in these circumstances, prefers to feed on animal blood rather than to accept the risks of the action of D.D.T. By 1956, the results were even more patent, as no live mosquitoes were found on the surfaces sprayed with D.D.T. while in 1955 their number was 163. The mortality inside the houses also came down from 212 to 200.

In the East, the situation is more or less similar regarding *A. aquasalis*. It must be made clear that according to our studies in Puerto Cabello there are two types, one with the eggs, very similar to those of Panama and the other totally different (Fig. 1), this last being the type encountered in Maracay (Aragua), Santa Fé (Sucre) and Guanta (Anzoategui). It is also worthy of note that the opposition of *A. aquasalis* in the banks of the Lake Valencia is of recent origin, as the first samples were captured in July 1953.

Leaving aside these entomological considerations and coming back to our thesis, it must be stated that in Venezuela there is the presumption that in the States where still there is malaria, the transmission is largely taking place inside the houses. This presumption seems to be based on the fact that the majority of the patients are children of tender age. In Santa Fé (Sucre) for example, 43·8 per cent are children in the age-group 1 - 4, 17·8 per cent in the age-group 5 to 9 and 38·4 per cent in the age-group 10 or more. Not that the insecticide does not act on transmitting species because these do not enter the houses and transmit the diseases outside the houses, but chiefly because the insecticide has no action on the above mentioned species or because they enter the houses, bite and then get out, or because they enter, bite and rest on the walls of the houses; in any case the resistance does not admit any discussion. In the first case, we will have an advanced resistance and in the second, a resistance very near to the physiological resistance, as we have already said it is happening in *A. albimanus*.

FIG. 1.

Eggs of *A. Aquasalis*

Regarding the laboratory tests with anophelines captured in States near San Carlos (Cojedes), in 1955 and 1957, following the Busvine and Nash method modified by Blázquez, we found as seen in Table V, that in 1955 the concentration of D.D.T. necessary to bring about 50 per cent of mortality was 0·87 per cent while in 1957 it was 1·1 per cent. This indicates that in the period of two years, notwithstanding that the captures were made in the countryside, more quantity of D.D.T. was needed to kill the same anopheline species. This leads to suppose that in *zones* covered constantly with D.D.T., the mosquitoes, in general, have created some resistance towards the insecticide.

TABLE V.

Mortalities of anophelines captured in traps fixed in stables in San Carlos.

D.D.T. Per cent.	Mortalities after 24 hours					
	November, 1955			December, 1957		
	Alive	Dead	Per cent.*	Alive	Dead	Per cent.*
0·5 ..	..	..	..	38·0	14	20·9
1·0 ..	36	66	58·8	22	21	42·9
2·0 ..	18	242	91·9	5	51	90·1
Control ..	84	14	..	52	5	..
LC_{50} ..	0·87 per cent			1·1 per cent		

* Mortalities corrected with Abbott's formula.

These facts and not any other motives have led to think that to continue with the use of residual insecticides in the belief that other health benefits may be obtained, is dangerous and not at all effective; regarding Chagas' disease it is known that with the intensive and regular use of dieldrin it is possible to get a considerable decrease in the density of *R. prolixus*, but never its total eradication. We know by laboratory experiments that *R. prolixus* begins to become resistant by ingestion in the second generation which leads us to think that equal results can be obtained in natural environment by contact. Regarding *Aedes aegypti*, it must be stated that there is no justification for a continuous disinfection; there is no guarantee that it will not develop resistance as this has been found in disinfected places.

This indiscriminate and uncontrolled use of the insecticides has changed these from the elements of high effectivity into substances of little or no value in the control of metaxenic diseases. Perhaps this idea and the presence of resistance in the insects have given to the sanitarians the impression that the period of the use of insecticides in the fight against the vectors, is ending. We have a duty to avoid or at least to prolong this end as much as we can.

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DIELDRIN RESISTANCE IN *CULEX PIPIENS FATIGANS* IN NORTH EASTERN TANGANYIKA.

BY

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[November 5, 1958].

DIELDRIN resistance in *C. p. fatigans* has been observed in several countries. Wharton (1957) reported adult resistance at Ubai in Malaya, and Ramsdale (1957) in Liberia, where residual treatment of huts was carried out. Wharton (1957) also found that resistance extended to the larvæ. Resistance to dieldrin has also developed, linked with B.H.C. resistance, in South India (Rajagopalan *et al.*, 1955) and in Malaya (Reid, 1955) where B.H.C. was used as a larvicide. Charles (1954) has shown, however, that *C. p. fatigans* can be virtually eradicated by combined spraying and larviciding with dieldrin. The findings described in the present paper are from the Pare area of north eastern Tanganyika, where an experiment is in progress to find whether or not malaria can be controlled by residual treatment of huts with dieldrin. A water dispersible powder was used at an approximate wall dosage of 80 mgs. per square foot of dieldrin during the first cycle, completed in November 1955. Subsequent treatments were done, and continue, at intervals of eight months, using half the above dosage.

FIELD OBSERVATIONS.

(a) SPRAY CATCHES IN HUTS.

Before residual spraying, substantial hut infestations by *C. p. fatigans* occurred in the large villages situated along the main road. The average hut density, as measured by spray catches, was 5·6 for the year before dieldrin treatment, and 1·3 the first year, 4·2 the second year and 9·0 the third year after treatment. These results show that a reduction in hut density by 77 per cent occurred during the first year and by 25 per cent in the second year after residual spraying; but that densities were higher in the third year than before treatment. The progressive increase in infestation of huts by *C. p. fatigans*, after residual treatment, is shown in the graph.

(b) WALL TESTS.

C. p. fatigans from mosquito nets and *A. gambiæ* caught in box shelters round cattle pens (Draper and Smith, 1957) in November 1957, were put into

GRAPH.

Monthly spray catches of C. fatigans in huts, before and after dieldrin treatment.

small cages (5″ × 3″ × 3″) of mosquito netting, and exposed to the walls of a treated hut for three hours. They were then removed to an untreated building, and their mortality twenty four hours later recorded. In five tests, only 1 died of 161 *fatigans*, and all 151 *gambiæ* died, showing that *fatigans* survived although the wall was fully toxic to *gambiæ*. Control mortalities were two per cent for 174 *fatigans*, and 17 per cent for 150 *gambiæ*.

LABORATORY TESTS.

Mosquitoes from huts in the dieldrin-treated Pare area, and from huts in an untreated area about 70 miles away, were then tested for their susceptibility to dieldrin and to D.D.T., by means of World Health Organization test-kits. Tests were made on wild-caught adults, and on 3rd and early 4th stage larvæ from numerous egg batches laid by wild caught adults. Median lethal concentrations obtained from regression lines of the results, are shown in Table I and are expressed as percentages for the adults and as parts per million for the larvæ. Adult mosquitoes were exposed for 24 hours to dieldrin, and for 1 hour to D.D.T. treated papers.

TABLE I.

Median lethal concentrations of dieldrin and of D.D.T. to adults and larvæ of C. p. fatigans.

	Dieldrin		D. D. T.	
	Pare (treated)	Machemba (untreated)	Pare (treated)	Machemba (untreated)
Adults	1·8	0·17	3·7	1·9
Larvæ	0·92	0·09	0·035	0·017

The results show that adults and larvæ from the treated area were ten times as resistant to dieldrin, and twice as resistant to D.D.T., as those from the untreated area.

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INSECTICIDE SUSCEPTIBILITY AND RESISTANCE IN ANOPHELINE MOSQUITOES IN JAVA.

BY

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Part I. Comparison of the Busvine and Nash Method and the new W.H.O. test kit on D.D.T.-susceptibility test.

SINCE August 1957 when the new World Health Organisation test kits were received, a comparison was made between the Busvine and Nash method [recommended by World Health Organization Expert Committee on Malaria (*World Health Org. Tech. Rept. Ser. No.* 80, 1954)] and field tests using the new kits. Wild-caught anopheline mosquitoes from an unsprayed area (Malang, East Java) were used for the comparative tests by the same workers at the same laboratory and on the same day. A series of tests was carried out during five to ten consecutive days in each of most of the months during the period November 1957-May 1958. Table I gives

TABLE I.

D.D.T.-susceptibility tests of A. aconitus *and* A. annularis *in Malang City, East Java, at different periods, and by comparison of (A) Busvine and Nash method, (B) new World Health Organization test kit received in August* 1957 *and (C) new World Health Organization test kit received in May* 1958.

Insecticides	Method	Date	*aconitus*			*annularis*		
			Number tested	Per cent dead	LC_{50} D.D.T. per cent	Number tested	Per cent dead	LC_{50} D.D.T. per cent
Check:—	(A)	Sep.-Nov. 1957	137	3				
D.D.T. per cent:								
0·25			196	22				
0·5			243	37				
1·0			188	69	0·64			

(*Contd.*)

*Now at the World Health Organization Regional Office for South East Asia, New Delhi.

TABLE I—(*Concld.*).

Insecticides	Method	Date	*aconitus*			*annularis*		
			Number tested	Per cent dead	LC_{50} D.D.T. per cent	Number tested	Per cent dead	LC_{50} D.D.T. per cent
Check:—	(A)	Nov. 1957 to Mar. 1958	200	4		80	1	
D.D.T. per cent:								
0·25			159	13		42	12	
0·5			333	30		121	13	
1·0			237	60		202	18	
2·0			49	76		130	60	
4·0			..	..	0·8	39	92	1·6
Check:—	(B)	Same date	126	14		118	0	
D.D.T. per cent:—								
0·25			91	2*		27	1	
0·5			280	42*		78	23	
1·0			162	79*		152	28	
2·0			28	96*		167	72	
4·0			..	..	0·55	72	100	1·4
Check:—	(A)	Apr. 1958	189	1		31	0	
D.D.T. per cent:								
0·5			282	35		84	12	
1·0			287	79		72	40	
2·0			248	92	0·6	68	65	1·3
Check:—	(B)	Same date	167	1		35	3	
D.D.T. per cent:								
0·5			151	68		76	38	
1·0			189	94		40	65	
2·0			168	100	0·38	44	98	0·7
Check:—	(C)	May 1958	110	3		6	0	
D.D.T. per cent:								
0·5			177	49		11	10	
1·0			161	92		10	40	
2·0			83	100	0·5	23	74	1·25
Check:—	**Grand total**		929	4		270	1	
D.D.T. per cent:								
0·25			446	17		69	10	
0·5			1466	41		370	20	
1·0			1224	78		476	29	
2·0			576	90		432	70	
4·0			..	..	0·6	111	97	1·4

* Corrected mortality.

the results of tests with *Anopheles aconitus* and *A. annularis*, of which sufficient numbers were available at the time. No comparison was made on the dieldrin susceptibility test because the lowest available dosage of dieldrin pre-impregnated papers received before May 1958 was 0·1 percent, which was too high for the local anophelines.

It would appear that the new kit gave higher kills than the Busvine and Nash method. The LC_{50} (median lethal concentration, expressed in percentage of insecticide in Risella oil) obtained by the new kit was lower than that obtained by the Busvine and Nash method. The difference in LC_{50} was not much in the first series of comparative tests made during the period of November 1957-March 1958, as shown by 0·55 against 0·8 in *aconitus* and 1·4 against 1·6 in *annularis*, but it was rather large in the second series of tests made in April 1958, as shown by 0·38 against 0·6 for the former species and 0·7 against 1·3 for the latter one.

Part II. Insecticide-susceptibility tests with common anophelines in unsprayed areas.

It is well recognised that normal levels of LC_{50} of local, important species of anophelines to insecticides should be obtained for future comparison after spraying. The tests were made in the field, mainly using the Busvine and Nash method. In the case of *A. aconitus* and *A. annularis* for which both that method and the new kits were used, as mentioned above, an average of the results obtained is also furnished within parenthesis in Table II. For dieldrin tests, only the Busvine and Nash method was employed, with the insecticide solution prepared with technical dieldrin obtained from the local Shell Co. and Risella oil from World Health Organization Headquarters. This locally prepared dieldrin oil solution was checked for biological efficacy from time to time by exposing laboratory-bred susceptible *Aedes aegypti* and anophelines collected from unsprayed areas, and found good. In all cases, 100 per cent mortality was obtained with one hour exposure at the concentration of four per cent dieldrin. The insecticide-treated papers were used only five times in a period of three days. Mosquitoes were exposed to the insecticides for one hour, and the percentage mortality was recorded after twenty-four hours, and corrected by Abott's formula if the check mortality was above five per cent. Occasionally, when the check mortality was too high, the whole series of tests was discarded. The prevailing temperature and humidity conditions could not be recorded as no such facilities were available in the field. The temperature and humidity conditions in this part of Indonesia, however, do not vary appreciably throughout the year, the average temperature being 88°-90°F., and humidity 60–80 per cent.

Wild-caught, mostly fed, females were used. The tests were carried out mainly in Malang City, East Java. This city and its surrounding areas for some distance have never been sprayed with insecticide either as a public health measure or for agricultural pest control. Certain species which were not available for tests in this city were tested in other unsprayed localities, such as *A. maculatus* in West Java, and *A. sundaicus* and *A. subpictus* in Middle Java.

TABLE II.

Insecticide-susceptibility tests of anopheline mosquitoes in unsprayed areas in Java.

Insecticides	*aconitus*		*annularis*		*barbirostris*		*kochi*		*maculatus* (1)		*subpictus malayensis*		*subpictus subpictus* (2)		*sundaicus* (3)		*vagus*		*vagus* (4)	
	Number tested	Per cent Mortality	Number tested	Per cent Mortality	Number tested	Per cent Mortality	Number tested	Per cent Mortality	Number tested	Per cent Mortality	Number tested	Per cent Mortality	Number tested	Per cent Mortality	Number tested	Per cent Mortality	Number tested	Per cent Mortality	Number tested	Per cent Mortality
Check:—D.D.T.	526	2	111	1	81	0	152	3	71	4	17	0	33	24	117	5	228	3	47	0
0·2	..		..		..		..		..		..		..		69	37*	..		..	
0·25	355	18	42	12	45	9	..		..		56	27	15	12*	..		242	22	44	32
0·3	..		..		..		..		127	24	..		..		..		..		..	
0·5	858	33	205	12	111	19	253	21	..		94	44	22	41*	111	55*	429	35	40	50
0·6	..		..		..		..		112	46	..		..		..		..		..	
0·75	..		..		..		..		..		..		22	64*	..		..		..	
1·0	712	69	274	24	148	25	251	53	131	60	50	90	..		36	100	328	60	44	82
2·0	297	90	198	61	146	50	139	83	..		17	100	..		58	100	65	98	..	
4·0	..		39	90	48	85	..		..		..		..		..		..		..	
Check:—Dieldrin	115	6	241	0	81	0	152	3	71	4	17	0	33	24	61	3	228	3	47	0
0·02	99	13*	87	3	83	10	12	9	..		38	45	..		..		70	21	..	
0·025	..		..		..		..		104	22	..		13	9*	16	0	..		55	47
0·04	140	40*	160	12	83	18	27	15	..		57	74	..		..		132	63	..	
0·05	..		..		..		..		113	28	..		..		77	23	..		51	53
0·075	..		..		..		..		120	77	..		34	80*	81	70	..		..	
0·08	..		..		78	58	..		..		30	97	..		..		57	84	..	
0·10	88	88*	247	42	20	60	24	92	..		6	100	22	93*	48	90	28	96	57	70
0·20	13	92*	163	89	59	98	6	100	..		..		..		..		7	100	..	
0·40	14	100	22	100	..		..		..		..		..		..		..		..	
LC_{50}: D.D.T. per cent	0·7 (0·6)x		1·6 (1·4)x		2·0		0·9		0·7		0·47		0·6		0·4		0·75		0·5	
Dieldrin per cent	0·045		0·11		0·07		0·06		0·068		0·024		0·05		0·065		0·034		0·035	

Remarks :

* Corrected mortality by Abott's formula

(x) The figures in parenthesis show the average LC_{50} for *A. aconitus* and *annularis* using the new World Health Organization test kits and Nash and Busvine method.

All of the above mosquitoes tested were collected from Malang City, East Java in 1957-58 except:

(1) *A. maculatus* from Tjikeris, Purwakarta, West Java in 1956;

(2) *A. subpictus subpictus* from Karanganjar, south coast of Middle Java in 1955, tested by G. Davidson, S. Sundararaman and the author;

(3) *A. sundaicus* from Jepara, north coast of Middle Java in 1955 for D.D.T. tests, by the author; and from Karanganjar, south coast of Middle Java in 1955 for dieldrin tests by Davidson, Sundararaman and the author;

(4) *A. vagus* from Menongo, Surodadi, Lamongan, East Java in 1958.

As shown in Table II, LC_{50} for common anophelines in Java are as follows :

		LC_{50}	
		D.D.T. (Per cent)	Dieldrin (Per cent)
1.	*A. aconitus*	0·7	0·045
2.	*A. annularis*	1·6	0·11
3.	*A. barbirostris*	2·0	0·07
4.	*A. kochi*	0·9	0·06
5.	*A. maculatus*	0·7	0·068
6.	*A. subpictus malayensis* ..	0·47	0·024
7.	*A. subpictus subpictus*	0·6	0·05
8.	*A. sundaicus*	0·4	0·065
9.	*A. vagus*	0·5-0·75	0·035

Part III. Dieldrin resistance in *A. subpictus subpictus* Grassi, 1899; *A. subpictus malayensis* Hacker, 1921; *A. vagus* Donitz, 1902; *A. barbirostris* van der Wulp, 1884 and *A. annularis* van der Wulp, 1884.

Along with *Anopheles sundaicus* found to persist in Semarang City in June 1955 and in Surabaja City in June 1956 after some years of D.D.T spray, the presence of some *A. subpictus subpictus* was also noticed (Chow and Soeparmo, 1956). At that time, all attention was focussed on the status of resistance to D.D.T. of only the former species, and no attempt was made to test the latter species because of its small number. After the change-over from D.D.T. to dieldrin for controlling D.D.T.-resistant *sundaicus*, both *sundaicus* and *subpictus* disappeared. In Surabaja City, the capital of East Java Province, few adults of *subpictus subpictus* were discovered in routine collections immediately after the second round of dieldrin sprayed in July 1957; a considerably larger number was noticed from September-October 1957. During this period a few *A. vagus* specimens were also collected. Tests made in November, 1957 of the susceptibility of these two species to insecticides showed that both of them had become highly resistant to dieldrin. At the same time, resistance of *subpictus subpictus* to dieldrin was also reported in West Java (Davidson, 1957*a*) and Middle Java (Ronnefeldt, 1957). Later on, in Surabaja City a few *A. subpictus malayensis* were collected in December 1957, and a considerable number of *A. barbirostris* in March 1958; both of them showed high resistance to dieldrin.

In order to see whether there was any relationship between the development of insecticide resistance in the mosquitoes and the number of spraying cycles, etc., tests were made in different localities of East Java.

The mosquitoes (only females were used for tests) were caught mostly in cattle-sheds and their surroundings during the early night, and were tested the following morning, along with a small number collected in the day-time in houses and stables and tested the same morning. In Surabaja City, some female mosquitoes hatched out from larvae were also tested as indicated below. For the

D.D.T.-susceptibility test, the new World Health Organization test kit was used, because the insecticide pre-impregnated papers with D.D.T. dosages of 0·25 per cent were available. But for the dieldrin susceptibility tests, the same kit was used, only with the available papers pre-impregnated with dieldrin dosages of 0·1 - 0·8 per cent. As for the dosage below 0·1 per cent and above 0·8 per cent dieldrin, the Busvine and Nash method was employed, as mentioned in Part II. All the tests were made by the same workers, who also carried out the tests mentioned in Part II, and in an unsprayed field laboratory or house close to the spot where the mosquitoes were collected. Mosquitoes were exposed to the insecticides for one hour unless otherwise indicated.

RESULTS.

(1) IN AREAS SPRAYED FIVE TIMES WITH D.D.T. AND TWICE WITH DIELDRIN (SURABAJA CITY).

Surabaja City was sprayed for malaria control with five rounds of D.D.T. at a dosage of 2 gm./sq. m., once a year, from 1952 up to the middle of 1956, and then with two rounds of dieldrin at 0·5 gm./sq. m., in July 1956 and July 1957. The per man-hour per station density of anopheline mosquitoes (except for *sundaicus*, which occurred in great numbers after four to five rounds of D.D.T. but disappeared after the first round of dieldrin) in routine daytime collections from sprayed houses is as follows:

	subpictus subpictus	*vagus*
1956		
January (Fifth round D.D.T. starting) ..	3	0
February	5	0
March	1	0
April	0	0
May	9	0
June	12	0
July (First round dieldrin starting) .	15	0
August-September	..	..
October-December	0	0
1957		
January-May	..	..
June	2	0
July (Second round dieldrin starting) ..	1	0
August	3	0
September	14	1
October	16	14

Table III gives the results of tests with dieldrin. In the beginning, a few mosquitoes were used for check and all found living. Later on, in order to have more material available for test and in view of the knowledge of a high survival of mosquitoes even after having been exposed to four per cent dieldrin, no check mosquitoes were used.

TABLE III.

Susceptibility tests at various concentrations of dieldrin in Risella oil with anophelines *caught from Surabaja City in different periods.*

Dieldrin per cent	*barbirostris*	*vagus*			*subpictus subpictus*			*subpictus malayensis*		
	(2)	(1)	(2)	Total	(1)	(2)	Total	(1)	(2)	Total
0·8:—										
Number tested	25	26	76	102	5	68	73	..	7	7
Number dead	1	7	10	17	0	10	10	..	0	0
Percentage mortality	4	27	13	17	0	15	14	..	0	0
2·0:—										
Number tested	..	..	..	..	..	..	..	..	16	16
Number dead	..	..	..	..	..	..	..	..	1	1
Percentage mortality	..	..	..	..	..	..	..	..	6	6
4·0:—										
Number tested	158	128	769	897	156	604	760	19	27	46
Number dead	29	70	214	284	59	189	248	9	7	16
Percentage mortality	18	55	28	32	38	31	33	47	26	35

REMARKS.— (1) indicates—Tested in November-December 1957
(2) indicates—Tested in March-May 1958

In addition, 66 *subpictus subpictus* hatched out from larvæ were tested with four per cent dieldrin, with 18 per cent mortality (12 dead).

The above results show that *barbirostris, vagus, subpictus subpictus* and *subpictus malayensis* all have become highly resistant to dieldrin, the degree of resistance being roughly proportionate to the number of exposures to the insecticide. As will be seen from Table XI, most of these species have increased their tolerance to dieldrin over a hundred-fold.

In order to see how many hours of exposure to insecticides would yield a hundred per cent kill, some mosquitoes were exposed for different lengths of time to one concentration of insecticides after another. A small number of mosquitoes were tested as follows:

For *subpictus subpictus:*

(1) Six exposed first to four per cent dieldrin for one hour as usual, none dead after 24 hours' observation; then again to 0·8 per cent dieldrin for *twentyfour* hours, also none dead.

(2) Thirtyone exposed to four per cent dieldrin for *four* hours: eight dead after 24 hours.

(3) Six exposed to four per cent dieldrin for *thirteen* hours: all dead.

For *barbirostris:*

(1) Four exposed first to two per cent dieldrin for one hour, none dead after 24 hours; then again to four per cent dieldrin for one hour, also none dead after 24 hours; and again to 0·8 per cent dieldrin for *twentyfour* hours, one dead.

(2) Three exposed to four per cent dieldrin for *four* hours: none dead after 24 hours.

(3) Seven exposed to four per cent dieldrin for *thirteen* hours: all dead.

For *vagus:*

(1) Eight exposed first to 0·8 per cent dieldrin for one hour, none dead after 24 hours; then again to four per cent dieldrin for one hour, also none dead after 24 hours.

(2) Nineteen exposed to four per cent dieldrin for *four* hours, five dead after 24 hours.

(3) Eight exposed to four per cent dieldrin for *thirteen* hours: all dead.

To D.D.T., these mosquitoes did not show any marked difference in their susceptibility, except for *subpictus subpictus*, which increased its tolerance four times, as may be seen in Tables IV and XI. This will be discussed later in more detail.

TABLE IV.

Susceptibility tests at various concentrations of D.D.T. in risella oil with anophelines *caught from Surabaja City in March-April* 1958.

D.D.T. per cent	*barbirostris*	*vagus*	*subpictus subpictus*	*subpictus malayensis*
0·5:—				
Number tested ..	4	43	40	1
Number dead ..	0	14	11	1
Percentage mortality ..	..	35	28	..
1·0:—				
Number tested ..	21	83	61	23
Number dead ..	4	56	27	12
Percentage mortality ..	20	68	44	52
2·0:—				
Number tested ..	9	84	62	12
Number dead ..	3	72	30	12
Percentage mortality ..	3 out of 9	86	49	12 out of 12
4·0:—				
Number tested ..	61	25	29	1
Number dead ..	38	25	28	1
Percentage of mortality	95	100	97	1 out of 1

TABLE V.

Susceptibility tests at various concentrations of D.D.T. and dieldrin in risella oil with anophelines *caught from Sugihwaras and its vicinity.*

	annularis	*vagus*	*subpictus subpictus*	*subpictus malayensis*
Dieldrin per cent.				
0·0 (check):—				
Number tested ..	30	95	20	9
Number dead ..	2	1	0	0
Percentage mortality ..	6·7	1	0	0
0·8:—				
Number tested ..	20	122	18	8
Number dead ..	1	24	7	3
Percentage mortality .	0*	20	40	3 out of 8
2·0:—				
Number tested ..	43	107	32	7
Number dead ..	11	54	15	5
Percentage mortality ..	20*	50	47	5 out of 7
4·0:—				
Number tested .	32	114	36	11
Number dead ..	20	63	25	8
Percentage mortality ..	60*	55	70	8 out of 11
D.D.T. per cent.				
0·0 (check):—				
Number tested ..	..	76	20	9
Number dead ..	..	0	0	0
0·5:—				
Number tested ..	..	82	23	9
Number dead ..	..	40	3	5
Percentage mortality .	..	49	35	5 out of 9
1·0:—				
Number tested .	..	80	23	16
Number dead ..	..	64	11	13
Percentage mortality .	..	80	48	13 out of 16
2·0:—				
Number tested ..	..	60	37	13
Number dead ..	..	55	33	13
Percentage mortality ..	..	92	89	13 out of 13
4·0:—				
Number tested ..	..	60	15	3
Number dead ..	..	60	15	3
Percentage mortality ..	..	100	15 out of 15	3 out of 3

*Corrected mortality

TABLE VI.

Susceptibility tests at various concentrations of dieldrin in risella oil with Anophelines *caught from Sumberedjo and its vicinity in two periods: I—Middle of February, and II—End of March 1958.*

Dieldrin per cent	*vagus*		*subpictus malayensis*		*annularis*		*barbirostris*	
	I	II	I	II	I	II	I	II
0·0 (check):—								
Number tested	73	14	..	3	20	1	..	1
Number dead	0	0	..	0	0	0	..	0
0·025:—								
Number tested	115	..	4	..	30	..	11	..
Number dead	46	..	0	..	6	..	0	..
Percentage mortality	40	..	0	..	20	..	0	..
0·05:—								
Number tested	86	27	7	2	35	14	12	1
Number dead	42	2	5	0	15	0	4	0
Percentage mortality	48	7	5 out of 7	0	46	0	4 out of 12	0
0·1:—								
Number tested	60	30	5	6	26	10	4	5
Number dead	28	3	4	0	16	2	0	0
Percentage mortality	47	10	4 out of 5	0	61	2 out of 10	0	0
0·2:—								
Number tested		11		3	..	9	..	4
Number dead		3	..	3	..	0	..	0
Percentage mortality		3 out of 11	..	0	..	0	..	0

(2) IN AREAS SPRAYED THREE TIMES WITH D.D.T. AND ONCE WITH DIELDRIN (SUGIHWARAS, TUBAN).

Village Sugihwaras and its surrounding villages (along the coastal area, belonging to Sub-district Djenu, Regency Tuban, 110 km. from Surabaja City) were sprayed with D.D.T. at 2 gm./sq. m. for three rounds in January 1953, April 1954 and April 1955, but this was changed to dieldrin at 0·5 gm./sq. m. in July 1957, as the vector of malaria in that area is *A. sundaicus*. It was reported that the density of *A. subpictus subpictus* and a few other species increased to a considerable number about six months after dieldrin spraying, although no *sundaicus* was

found. Tests were therefore made on these species in the middle of February and at the end of March 1958, and all of them showed evidence of resistance to dieldrin but not to D.D.T. Table V gives the results of tests with dieldrin and D.D.T. Table V shows that *annularis*, *vagus*, *subpictus subpictus* and *subpictus malayensis* all have developed resistance to dieldrin, but the degree of resistance is not so high as in Surabaja City, which has been sprayed more often. However, they seem still to be susceptible to D.D.T., although *subpictus subpictus* has about a two-fold increase in tolerance.

TABLE VII.

Susceptibility tests at various concentrations of D.D.T. in risella oil with anophelines *caught from Sumberedjo and its vicinity.*

D.D.T. per cent	*vagus*	*annularis*	*barbirostris*
0·0 (check):—			
Number tested	77	21	1
Number dead	0	0	0
0·25:—			
Number tested .	70	27	8
Number dead . ..	19	2	0
Percentage mortality ..	27	7	0
0·5:—			
Number tested ..	89	17	4
Number dead . ..	37	3	2
Percentage mortality ..	41	3 out of 17	2 out of 4
1·0:—			
Number tested . .	64	22	22
Number dead	42	14	9
Percentage mortality ..	66	63	40
2·0:—			
Number tested .	6	.	..
Number dead	4	..	..
Percentage mortality ..	4 out of 6	.	..
4·0:—			
Number tested .. .	15	..	..
Number dead	15	..	..
Percentage mortality ..	100	..	..

TABLE VIII.

Susceptibility tests at various concentrations of dieldrin in risella oil with anophelines *caught from Blawi in three periods: I—End of January, II—End of February, III—End of March* 1958.

Dieldrin per cent	*barbirostris*	*vagus*			*subpictus malayensis*		
	III	I	II	III	I	II	III
0·0 (check):—							
Number tested	13	38	112	30	1	23	21
Number dead	0	2	1	3	0	0	15
Percentage mortality	0	5	1	10	0	0	
0·05:—							
Number tested	2	24	81	..	.	21	..
Number dead	0	5	11	..	..	10	..
Percentage mortality	0	16*	13	..	..	50	..
0·1:—							
Number tested	7	42	58	31	4	20	24
Number dead	0	20	12	3	2	5	15
Percentage mortality	0	47*	20	0*	2 out of 4	25	
0·2:—							
Number tested	7	7	71	36	15	16	28
Number dead	0	5	21	2	14	8	12
Percentage mortality	0	5 out of 7	30	0*	14 out of 15	8 out of 16	
0·4:—							
Number tested	8	14	49	34	8	7	29
Number dead	0	14	20	0	8	5	12
Percentage mortality	0	14 out of 14	40	0	8 out of 8	5 out of 7	
0·8:—							
Number tested	42	..	50	74	..	14	33
Number dead	4	..	26	13	..	11	17
Percentage mortality	10	..	52	9*	..	11 out of 14	*
2·0:—							
Number tested	..	..	31	..	..	9	.
Number dead	.	..	27	..	..	9	..
Percentage mortality	.	..	87	..	..	9 out of 9	..
4·0:—							
Number tested	32	..	36	40	..	11	4
Number dead	13	..	36	14	..	11	3
Percentage mortality	40	.	100	28*	..	11 out of 11	3 out of 4

* Corrected mortality

TABLE IX.

Susceptibility tests at various concentrations of D.D.T. in ricella oil with anophelines *caught from Blawi in January-March* 1958.

D.D.T. per cent	*barbirostris*	*vagus*	*subpictus malayensis*
0·0 (check):—			
Number tested	10	110	16
Number dead	0	2	0
Percentage mortality	0	2	0
0·5:—			
Number tested	25	86	11
Number dead	0	37	5
Percentage mortality	0	43	5 out of 11
1·0:—			
Number tested	44	77	22
Number dead	19	55	14
Percentage mortality	43	71	64
2·0:—			
Number tested	32	71	33
Number dead	26	68	33
Percentage mortality	80	96	100

(3) IN AREAS SPRAYED ONLY ONCE WITH DIELDRIN (SUMBEREDJO, TUBAN).

Village Sumberedjo (Sub-district Merak-urak, Regency Tuban) is situated inland, 3-4 km. away from village Sugihwaras. The malaria vector is *A. aconitus*, but dieldrin at 0·5 gm./sq. m. was sprayed in May 1957. No *aconitus* has so far been found since the spraying, and the other anopheline density was still comparatively low. Except for *vagus*, the number of mosquitoes tested was too small to enable definite conclusions to be drawn. *A. vagus* showed certain degree of resistance to dieldrin but was still susceptible to D.D.T. (Tables VI, VII, XI).

(4) IN AREAS SPRAYED ONLY THREE TIMES WITH D.D.T. (BLAWI AND ITS SURROUNDING SUB-VILLAGES).

Sub-village Blawi and its surrounding sub-villages (Sub-district Karangbinangun, Regency Lamongan) are an inland area, 45 km. north-west of Surabaja City and 65 km. south-east of Sugihwaras. As several sub-villages are situated near each other, their distance apart being less than ½ - 1 km., the whole area can be regarded as having at least three, if not six, rounds of spraying with D.D.T. at a dosage of 2 gm./sq. m. The date of spraying in different sub-villages is: for

TABLE X.

Susceptibility tests at various concentrations of dieldrin and D.D.T. in risella oil with anophelines *caught from Plaosan at end of January, early March and end of March* 1958.

	barbirostris	*vagus*	*subpictus malayensis*
Dieldrin per cent.			
0·0 (check):—			
Number tested	10	31	64
Number dead	2	2	9
Percentage mortality ..	2 out of 10	7	14
0·025:—			
Number tested	8	50	78
Number dead	8	37	61
Percentage mortality ..	8 out of 8	72*	74*
0·05:—			
Number tested	15	61	91
Number dead	13	49	76
Percentage mortality ..	13 out of 15	79*	80*
0·1:—			
Number tested	12	38	67
Number dead	11	32	57
Percentage mortality ..	11 out of 12	83*	83*
0·2:—			
Number tested	..	9	23
Number dead	..	9	22
Percentage mortality ..	..	9 out of 9	95*
0·4:—			
Number tested	..	8	17
Number dead	..	8	17
Percentage mortality ..	..	8 out of 8	100
D.D.T. per cent.			
0·0 (check):—			
Number tested	..	46	63
Number dead	..	2	4
Percentage mortality ..	..	4	6

(*Contd.*)

TABLE X.—(*Concld.*)

	barbirostris	*vagus*	*subpictus malayensis*
0·25:—			
Number tested ..	..	44	56
Number dead ..	..	20	29
Percentage mortality ..	.	45	47*
0·5:—			
Number tested	..	71	66
Number dead	..	49	47
Percentage mortality ..	..	70	68*
1·0:—			
Number tested	..	64	61
Number dead	..	59	52
Percentage mortality ..	..	92	84*
2·0:—			
Number tested	..	..	12
Number dead	..	..	12
Percentage mortality ..	..	..	100

* Corrected mortality

Blawi, December, 1956; for Ketapang-telu (less than ½ km. away from Blawi), December 1954 and June 1956; Moratjalan (less than ½ km. from Blawi), January 1957 and November 1957; and for Siko (1 km. from Blawi) September 1955. *A. aconitus*, the malaria vector, has so far not been found since the spraying, but other anophelines (first *subpictus malayensis*, later on *vagus*, and recently *barbirostris*) appeared since July 1957. Since the results of tests with dieldrin show some difference in different periods, Table VIII gives the details separately. *A. barbirostris* was tested mainly in Period III, when more specimens were available. As shown in Table VIII, development of dieldrin resistance in *vagus* was in progress as time went on, and it reached a very high level in Period III, i.e., end of March 1958, when *barbirostris* also showed high resistance to dieldrin. With the latter species an additional test was made: ten exposed first to four per cent dieldrin for one hour, no dead after 24 hours; then again to 0·8 per cent dieldrin continuously for *one hundred* hours, seven dead out of the ten mosquitoes tested. The number of *subpictus malayensis* tested was too small, and, unfortunately, the check mortality in Period III was so high that no attempt was made on its corrected mortality. The reason for including here the data of *subpictus malayensis* tests is just to give certain preliminary information showing that small degree of dieldrin resistance has also been developed in this species. However, all three species mentioned above still remained at a normal level of susceptibility to D.D.T., as shown in Table IX.

TABLE XI.

Dieldrin and D.D.T. LC_{50} of different species tested in different areas and at different lengths of time after the last spraying during the period of November 1957 to early April 1958 (the figures in brackets show the degree of increase in the LC_{50} as compared to those of normal strains).

AREA	*subpictus subpictus*	*subpictus malayensis*	*vagus*	*barbirostris*	*annularis*
	LC_{50} Dieldrin per cent in risella oil				
Unsprayed:	0·6	0·024	0·035	0·07	0·11
Sprayed					
1) 5×D.D.T. and 2×DLN:—					
4 months	4·0 (66x)	4·0 (160x)	3·2 (100x)	.	.
9 months	18·0 (300x)	6·6 (275x)	20·0 (600)	23·0 (330x)	..
Average	11·0 (200x)	5·0 (200x)	20·0 (600)	..	
2) 3×D.D.T. & 1×DLN:—	2·2 (35x)	1·1 (50x)	2·0 (60x)	.	3·4 (30x)
3) 1×DLN:—					
9 months	..	Normal(?)	0·11 (3x)	.	Normal (?)
10 months	.	Higher (?)	0·45 (13x)		Higher (?)
4) 3×D.D.T.:—					
13 months	..	0·1 (4x)	0·12 (4x)	.	..
14 months	..	0·2 (8x)	0·7 (20x)	.	..
15 months		..	13·0 (400x)	6·0 (85x)	..
5) 1×D.D.T.:—					
6 months	.	Normal	Normal	Normal	..
	LC_{50} D.D.T. per cent in risella oil				
Unsprayed	0·5	0·47	0·75	2·0	1·4
Sprayed:—					
1) 5×D.D.T. & 2× DLN	2·0 (4x)	1·0 (2x)	0·7	1·9	..
2) 3×D.D.T. & 1×DLN	1·0 (2x)	0·5	0·5	..	..
3) 1×DLN	..	..	0·63	1·0	0·8
4) 3×D.D.T.	..	0·6	0·6	1·4	..
5) 1×D.D.T.	..	0·3	0·3	..	.

Remark: DLN=dieldrin

(5) IN AREAS SPRAYED ONLY ONCE WITH D.D.T. (PLAOSAN, LAMONGAN).

Village Plaosan (Sub-district Babat, Regency Lamongan) is also an *aconitus* area and situated inland, about 30 km. west of Blawi and 35 km. south-east of Sugihwaras. The area was sprayed for the first time with D.D.T. at 2 gm./sq. m. in September 1957. Two months after the spraying, a few *subpictus malayensis* began appearing, and three months after spraying many *malayensis* and a considerable number of *vagus* were found; but those caught from window-traps fitted to the existing sprayed houses and stables were all dead within 24 hours. All three species, *barbirostris*, *vagus* and *subpictus malayensis*, tested at the end of January, in early March and at the end of March 1958 showed themselves to be very susceptible to both dieldrin and D.D.T., even more susceptible than the normal strains, as compared with those from unsprayed areas (Malang city, about 150 km. away, and Menongo, about 22 km. away). This may partly be for the reason that, as shown by the high mortality of the check, some of the mosquitoes caught at night in stables probably had contacted D.D.T., which was sprayed only three to six months before, and was still effective. Table X gives the results of the tests with dieldrin and D.D.T.

(6) COMPARISON OF THE MEDIAN LETHAL CONCENTRATION (LC_{50}) OF DIELDRIN AND D.D.T. BETWEEN DIFFERENT ANOPHELINE SPECIES TESTED IN DIFFERENT AREAS.

From the above results, the LC_{50} were obtained by reading from the regression line of logarithmic-probability graph. Some were interpolated from only two mortalities. Table XI gives the summary, from which may be seen that *subpictus subpictus*, *barbirostris*, *vagus* and *subpictus malayensis* in Surabaja City have developed resistance to dieldrin 200-600 times. In other sprayed areas, with the exception of Plaosan, these species (except for *vagus*) and also *annularis* have increased to a degree not yet so high.

DISCUSSION AND CONCLUSION.

Five more species are now being added to the world list of anophelines resistant to insecticides, *i.e. A. subpictus subpictus*, *A. subpictus malayensis*, *A. vagus*, *A. barbirostris* and *A. annularis* which have become highly resistant to dieldrin. It is somewhat unusual that so many anopheline species in an area showed resistance to the same insecticide (dieldrin) during more or less the same period and in the same locality. The relationships between the development of resistance and the insecticide spraying, the number of spraying cycles, etc. are discussed below.

(1) DEVELOPMENT OF RESISTANCE IN RELATION TO THE SPRAYING OF INSECTICIDES.

The dieldrin-resistant species reported above are all found in the sprayed areas whether sprayed with D.D.T., with dieldrin or with both. The limited examinations made in unsprayed areas did not reveal any resistant species even in a village, *e.g.* Menongo (Sub-district Surodadi, Regency Lamongan), which is only about 15 km. away from Blawi where the resistant species have been found.

The nearest surrounding area sprayed with D.D.T. is only about 5 km. away from village Menongo. In Menongo, *A. vagus* and *A. subpictus malayensis* were tested in March 1958, and the results show that the LC_{50} for the former species is 0·035 per cent dieldrin and 0·5 per cent D.D.T., and for the latter species about 0·029 per cent dieldrin and 0·25 per cent D.D.T. These figures are similar to those obtained in Malang city, another unsprayed area. On the one hand there is no positive evidence of natural dieldrin-resistant strains among the common anopheline species occurring in Java; on the other hand, as soon as a selection pressure by insecticide spraying is built up, a sufficient number of resistant individuals are detected by the susceptibility test. Another fact for consideration is the development of dieldrin-resistant individuals even after spraying with D.D.T. only, without any proportionate or even significant development of resistance to D.D.T.

It was informed by Zulueta (1958) that in Bombay State, as mentioned by T.R. Rao to the Expert Committee on Insect Resistance and Vector Control, which met in Geneva in November 1957, *A. subpictus* was found to be highly resistant to dieldrin also in areas treated with D.D.T. The explanation might be that dieldrin resistance was inherent in an appreciable proportion of the original natural population, and when it is subject to D.D.T. spraying, those susceptible to dieldrin which are also susceptible to D.D.T. were completely killed. Those which were resistant to dieldrin but susceptible to D.D.T., were mostly killed. On account of the selection pressure all the survivors represented dieldrin resistant individuals, but still remained susceptible to D.D.T.

As regards the order of emergence of the dieldrin-resistant strains of different species in a same locality, it shows that in the coastal areas *subpictus subpictus* appeared first, followed by *vagus*, *subpictus malayensis*, *barbirostris* and *annularis*; whereas in the inland areas, *subpictus malayensis* came out first, then followed by *vagus* and *barbirostris*. This difference in the order of emergence may be due to seasonal prevalence of the mosquitoes rather than to the degree of development of resistance.

(2) DEGREE AND SPEED OF DEVELOPMENT OF RESISTANCE IN RELATION TO THE CYCLE OF SPRAYING AND THE LENGTH OF TIME AFTER SPRAYING.

In general, the greater the number of spraying cycles undertaken, the higher the degree of resistance developed in the mosquitoes, as shown in Table XI. After more selection pressure, the survivors are of a purer strain of resistance. As to the speed of developing resistance to dieldrin, it seems that, as shown in village Sugihwaras a minimum period of ten months, after the first round of dieldrin spraying, would be required for the detection of the resistant strain.

In Surabaja City, where more sprayings were undertaken, the degree of resistance is much higher, up to 600 times, whereas in village Sugihwaras, which was sprayed also with both D.D.T. and dieldrin but in fewer spraying cycles, the degree of resistance is much lower, having an increase of only 30-60 times. However, during a short trip made in January 1958 to Demak, Middle Java, which had only one round each of D.D.T. and dieldrin spraying, it was possible to test a small number of *subpictus subpictus* as follows, the results giving a dieldrin LC_{50} of 24

per cent, or an increase of 400 times above the normal level:

Dieldrin per cent	Number of mosquitoes tested	Number dead
0·0 (check)	9	0
0·8	26	1
4·0	23	4

In Blawi and its surrounding villages, where only D.D.T. was sprayed in an average of three rounds, *A. vagus* increased four times above its normal level of dieldrin susceptibility thirteen months after the last spraying; 20 times one month later, and 400 times another month later. This big difference cannot be explained as being due to seasonal variations influencing the tests, because the temperature prevailing in that locality varies very slightly throughout the whole year. Moreover, the tests were made by the same workers, using the same materials and equipment, in the same field laboratory, and during a period of only one season (January to March, 1958). The experience gained from Malang City (unsprayed) was that the LC_{50} of dieldrin and D.D.T. for the mosquitoes tested during a period of half a year (September 1957 to March 1958) did not show too big difference from month to month.

(3) DIELDRIN-RESISTANT ANOPHELINES IN COMPARISON WITH D.D.T.-RESISTANT *A. SUNDAICUS*.

The development of D.D.T resistance in *A. sundaicus* in Semarang City was first noticed after a period of two years with three rounds of D.D.T. spraying (Chow and Soeparmo, 1956), and in Surabaja City after a period of about four years with four to five rounds of D.D.T. spraying (unpublished data). However, the dieldrin resistant anopheline species in East Java were first noticed after a period not counting D.D.T. spraying, of only a little longer than a year with two rounds of dieldrin spraying, as in Surabaja City, and after a period of less than half a year with one round of dieldrin, as in village Sugihwaras. In village Suberedjo, where only dieldrin was sprayed once in May 1957, a low degree of resistance to dieldrin was noticed ten months after the spraying. It shows that dieldrin-resistant anophelines are much quicker to appear in the field than are the D.D.T.-resistant species. Furthermore, the degree of resistance in dieldrin-resistant species is also much higher (certain species up to 600 times above the normal level) than D.D.T.-resistant *sundaicus* (only about 20 times) (Davidson, 1957*c*).

As reported by Davidson (1957*b*), the resistance to D.D.T. in *sundaicus* is virtually recessive, and the heterozygote resembles the susceptible homozygote, while the hybrids of dieldrin-resistant *A. gambiae* in Nigeria are significantly resistant. In the hope of knowing something about the resistance mechanism of the dieldrin-resistant strains in East Java, efforts are being made to isolate the pure dieldrin-resistant individuals and to cross-mate them with the susceptible strains. However, based on the very high degree of resistance and quick emergence of the resistant strains, one is inclined to presume at present that the dieldrin-resistant species in Java might also possess the same genetical characters of resistance to dieldrin as possessed by *A. gambiae*.

(4) DIELDRIN-RESISTANT STRAINS IN COMPARISON WITH SUSCEPTIBLE STRAINS.

No detailed study has yet been made of a comparison between the dieldrin-resistant and the susceptible strains of the mosquitoes reported in East Java. However, it was found that the resistant strains were much easier to breed in the laboratory than the susceptible ones.

Adults hatched out from eggs laid by dieldrin-resistant *vagus* caught from village Sugihwaras lived nicely in the cage (about 1 cu. m.) and fed on man. No eggs were laid, however, up to the ninth day after emergence, when the colony was, unfortunately, destroyed by ants.

(5) INCREASE OF D.D.T. TOLERANCE IN CONNECTION WITH RESISTANCE TO DIELDRIN.

The dieldrin-resistant species, as reported from East Java, seem still to be susceptible to D.D.T., except for *subpictus subpictus* which has increased four times above its normal level of susceptibility to D.D.T., as shown in Surabaja City.

It was learnt that in Semarang City, Mid-Java, this species had developed resistance not only to dieldrin but probably also to D.D.T. (Ronnefeldt, 1957). In one part of India this species was reported to be highly resistant to D.D.T. (Sharma and Krishnamurthy, 1957).

During a brief visit to Semarang in January 1958, tests were made with a small number of unfed and day-old mosquitoes (*A. subpictus subpictus*) hatched out from the larvæ collected from Semarang City, where three rounds of D.D.T. and two rounds of dieldrin had been sprayed. The following were the results, which show a D.D.T. LC_{50} of 2·2 per cent, having an increase also of about four times above the normal level:

D.D.T. per cent	Number of mosquitoes tested	Per cent mortality
0·0 (check)	12	0
1·0	23	4·3
2·0	19	47·4
4·0	17	82·3

The above results show that, in agreement with the view of Viswanathan (1958) and Zulueta (1958), there is no indication of the same true resistance to D.D.T. in *subpictus subpictus* in Semarang as in Surabaja, and this is probably only a case of tolerance (" vigour tolerance "?) either due to the previous use of D.D.T. or as a collateral effect of dieldrin resistance. Evidence of increased tolerance to D.D.T. in dieldrin-resistant *gambiæ* in Nigeria was mentioned by Davidson (1957).

(6) DEVELOPMENT OF DIELDRIN RESISTANCE IN ANOPHELINES IN RELATION TO THE MALARIA ERADICATION PROGRAMME.

It is well known that the main vector of malaria along the coastal areas of Java is *A. sundaicus*, which developed resistance to D.D.T. but has been, fortunately, well under control after a change of insecticide was made from D.D.T. to dieldrin.

The extent to which malaria transmitted by *A. aconitus*, the important vector in inland areas of Java, has been effectively controlled with the use of D.D.T. or dieldrin, is still under study. One redeeming feature is that so far we do not know if it has any increased tolerance to D.D.T. or dieldrin. If the dieldrin-resistant species as reported in East Java would assume any role as vector of malaria—at present there is no indication of their exercising any active vectorial role—it will certainly interfere with the malaria eradication programme. As far as we can know from the literature and from our own experience, *A. subpictus malayensis*, *A. vagus* and *A. annularis* are fortunately not vectors of malaria in Indonesia. *A. barbirostris* has been found naturally infected with malaria parasites in Malaya; in Indonesia, an infection rate of 0·5 per cent was reported (Bonne-Wepster, 1953), but this mosquito has been considered not important in malaria transmission, although it is much concerned in the transmission of *Wuchereria malayi*. *A. subpictus subpictus*, however, has long been regarded as a vector of secondary importance in malaria transmission along coastal areas of Java, where its associated species, *A. sundaicus*, is the main vector. Sundararaman dissected some 10,000 *subpictus subpictus* in the south coastal area of Mid-Java between 1951 and 1954, and found a 0·1 per cent sporozoite rate (as quoted in the report of Davidson, 1957*a*). But one has to consider if this mosquito may play more important role in the transmission of malaria in the absence of the main vector, *A. sundaicus*, which has now been controlled.

SUMMARY.

On susceptibility tests to D.D.T., carried out with *Anopheles aconitus* and *A. annularis*, a comparison was made between the Busvine and Nash method and the new World Health Organization test kit. It seems that the latter method gives a higher kill of mosquitoes than the former.

Normal levels of D.D.T. and dieldrin-susceptibility in common anophelines in Java were worked out.

A. subpictus subpictus, *A. subpictus malayensis*, *A. vagus*, *A. barbirostris* and *A. annularis* were found highly resistant to dieldrin in certain localities of Java. It is reported for the first time in this paper that many local anopheline species developed resistance to dieldrin in the same locality and at more or less the same period after dieldrin spraying. Dieldrin resistance was noted even in an area treated only with D.D.T., but such resistance has not so far been demonstrated in unsprayed areas. The rapidity of development of resistance, the degree of resistance developed and the development of dieldrin resistance after only D.D.T. has been sprayed, point to an inherent presence of natural resistance to dieldrin in the original population, which becomes accentuated by insecticidal spray. The greater the number of cycles of spray, the higher the development of resistance. It seems that a minimum period of ten months after the first round of dieldrin spraying would be required for the detection of resistant strains. All the dieldrin-resistant species were still susceptible to D.D.T., although *A. subpictus subpictus* showed an increase of four times its normal level of susceptibility to D.D.T. This is probably only a case of tolerance, not of true physiological resistance. The development of dieldrin resistance in a good number of anophelines in relation to malaria eradication programme in Java is discussed. They are all, as far as known at present,

not important vectors of malaria in Java. Of the two important vectors, *A. sundaicus* though resistant to D.D.T. in many places is still susceptible to dieldrin. The status of *A. aconitus* still requires precise determination both in its possibility of developing resistance, if any, to D.D.T. and dieldrin, and in its consequent vulnerability to insecticide application in a malaria eradication programme.

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A PRELIMINARY NOTE ON A PROBABLE CASE OF DIELDRIN RESISTANCE IN *ANOPHELES CULICIFACIES* IN THANA DISTRICT, BOMBAY STATE.

BY

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[November 8, 1958.]

PENDING further detailed investigations, over a much wider area and in a larger number of villages, we wish to record a case of what appears to be resistance to dieldrin in *Anopheles culicifacies* Giles, in five villages in Murbad Taluka, Thana District, Bombay State, India.

Using the World Health Organization test kit for adult mosquitoes, tests were carried out in these villages from October 13 to October 30, 1958. Adult females, which had taken full blood meals during the previous night were collected from the mixed dwellings early in the mornings and used for the tests before 12 noon. Dieldrin and D.D.T. impregnated papers supplied by World Health Organization (impregnated in January-February 1958) were used. The exposure given was one hour and the period of holding 24 hours. The tests were carried out at first with three replications for each dose in Potgaon village and at one replication in four other villages for confirmation. D.D.T. was tested in Potgaon village only. The data are summarised in Table I.

Probit regression lines were fitted to the data, and goodness of fit tested by the methods of Litchfield and Wilcoxon (1949) by using the nomograms provided by them. The LC_{50} and confidence limits were also estimated by their methods. The best of five lines was selected.

The results are—

	Dieldrin: All five villages	Dieldrin: Potgaon village only	D.D.T.: Potgaon village only
LC_{50}	3·1 per cent	3·0 per cent	0·84 per cent.
Confidence limits: Upper .	1·393 per cent.	5·358 per cent.	0·855 per cent.
" " Lower .	2·193 per cent.	1·700 per cent.	0·825 per cent.
chi^2 for the line	5·58	4·89	5·01
chi^2 for degrees of freedom (= 4) ..	9·49	9·49	9·49

The LC_{50} of 3·1 per cent for dieldrin has been estimated by extrapolation. This may not be satisfactory as the highest mortality recorded is only 39·7 per cent. Therefore, the figure should be used with caution. However, the fact that resistance or lower susceptibility is there, is proved by the fact that even the 1·6 per cent paper yielded only 39·7 per cent mortality.

The need for these tests arose when reports of an 'epidemic' of malaria were received this year from this area in which malaria control by indoor residual spraying was continuously in operation from 1948 and where very good control had been obtained. From 1948 to 1952, D.D.T. in the form of an aqueous emulsion was employed in three rounds each year at a dosage of 56 mg. per sq. ft. From 1953 to 1956, D.D.T. in the form of a 75 per cent water wettable powder was used in two rounds each year at a dosage of 100-112 mg. per sq. ft. In 1957, dieldrin 50 per cent water wettable powder was used in two rounds at 28 mg. dieldrin per sq. ft. In 1958 also, one round of dieldrin 50 per cent water wettable powder has been given at the same dosage in the latter part of May.

The LC_{50}'s for dieldrin so far reported from different parts of India for *A. culicifacies* have been around 0·05 per cent. The LC_{50}'s for dieldrin for most malaria vectors in the world which are susceptible have been less than 0·05 per cent as seen from the second global review made by the World Health Organization (1958). The LC_{50} of 3·1 per cent therefore represents approximately a 60-fold increase. The LC_{50} for D.D.T., viz., 0·84 is slightly higher than the usual ones recorded so far in India, but is well within the probable natural variations and may be regarded as merely a slight increased tolerance.

Until the investigations are extended to many more places, we do not wish to categorically state that the high LC_{50} for dieldrin now noticed is actually a case of *development* of resistance, because no LC_{50} determination for dieldrin for this species had been made in this particular area before. But the figure is much higher than that recorded for this species anywhere else and has been confirmed by observations in five different villages. The nearest place where the LC_{50} for D.D.T. was recorded earlier for this species was in 1956 in Revti village about six miles from Potgaon (Ramachandra Rao and Bhatia, 1957). The LC_{50} then was 0·412 per cent as determined by the Busvine/Nash test.

We do not also wish to enter at present into a detailed description of this epidemic' of malaria nor on the other entomological aspects, as a comprehensive

TABLE I

Twenty-four hour mortalities in A. culicifacies females, using World Health Organization test kits, with exposures of one hour.

Place: Thana District, Bombay State — Dates: October 15 to 30, 1958.

	Dieldrin														D.D.T.			
	Potgaon		Naudani		Mohop		Avola		Waghivli		Total				Potgaon only			
Dose per cent.	R	N	R	N	R	N	R	N	R	N	R	N	Adjusted mortality per cent.	Dose per cent.	R	N	Adjusted mortality per cent
Control ..	3	1/41	1	0/12	1	0/14	1	0/10	1	0/11		1/88	..	Control	3	0/49	
0·05 ..	3	1/35	1	0/15	1	2/15					5	3/65	3·5	0·25	3	3/45	11·1
0·1 ..	3	3/37	1	1/14	1	3/15					5	9/66	12·6	0·5	3	9/46	19·6
0·2 ..	3	10/41	1	4/15	1	3/16			1	2/11	6	19/82	22·3	1·0	3	23/45	51·1
0·4 ..	3	13/42	1	6/15	1	3/13			1	4/11	6	26/82	30·9	2·0	3	35/45	84·4
0·8 ..	3	13/31	1	5/15	1	3/16	1	4/10	1	3/11	7	28/103	26·4	4·0	3	44/44	100·0
1·6 ..	3	17/40	1	7/17	1	7/16	1	3/10	1	4/11	7	38/94	39·7	8·0	3	47/47	100·0

R = Replications
N = Number

NOTE.—
The number dead and number exposed are shown as 2/15, 19/82 and so on.

investigation on all aspects of the epidemiology of this incident is in progress and will be fully described in due course. We have, however, hastened to record these preliminary results only in order to alert the other workers in the country to the need for vigilance wherever dieldrin is being used. This perhaps is the first instance of *A. culicifacies*, the major vector of India, showing resistance to an insecticide.

SUMMARY.

The LC_{50}'s of 3·1 per cent for dieldrin and 0·84 per cent for D.D.T. are recorded from five villages in Thana District, Bombay State, India. The LC_{50} for dieldrin is very high being 60 times higher than any recorded for the species so far in India and was noticed after an outbreak of malaria had been reported in an area in which the disease had been well under control for over eight years.

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SUSCEPTIBILITIES OF MOSQUITOES TO D.D.T. AND DIELDRIN IN SPRAYED AND UNSPRAYED AREAS OF BOMBAY STATE, INDIA*.

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[November 10, 1958.]

An appraisal of susceptibilities of *Culex fatigans* (an important vector of filariasis in Bombay State and a nuisance mosquito), three important malaria vectors, viz. *Anopheles culicifacies*, *A. fluviatilis* and *A. stephensi* and other anophelines, was made during 1957 from various regions of the Bombay State, both from sprayed and unsprayed areas. The results of earlier findings had been reported by Ramachandra Rao and Bhatia (1957).

The technique adopted was essentially that of Busvine and Nash as recommended by World Health Organization (Tech. Report Series 80, 1954) except that the recovery cages used in the experiments were specially designed by the senior author, which consisted of wire gauze cylinders with a hole at the top for transfer of mosquitoes, lined with clean paper and provided with moist lint discs at the bottom and top of the cages. These cages maintain favourable conditions of temperature and humidity inside the cages as judged by the negligible mortalities in the controls. Mosquitoes used in the experiments were wild-caught females which were fully engorged with blood on the night previous to exposure. An exposure of one hour was given in all the experiments except in some *C. fatigans*, where an exposure of 24 hours was also tried. The solvent used was white oil supplied by Messrs. Burmah Shell Oil Distributing and Storage Company, Ltd., Bombay. The D.D.T. used was of technical grade supplied by the Malaria Institute of India, Delhi. Dieldrin used in the experiments was of technical grade and was supplied by the above mentioned company.

Fifty-two dosage-mortality curves were obtained on various species of mosquitoes both from unsprayed areas as well as areas where residual domiciliary

* These studies were carried out by the Malaria Organisation, Bombay State, as a part of the country-wide studies on the susceptibility of mosquitoes to insecticides recommended by the Indian Council of Medical Research.

adulticidal spraying with D.D.T. has been done for the past five to eight years. The D.D.T. spraying in these areas is done at 112 mg. per sq. ft. (from 1953 onwards) and 56 mg. per sq. ft. before 1953. Usually two rounds of D.D.T. spraying were done with the higher dosage and three rounds of spraying with the lower dosage in the monsoon period, June to November, each year. No anti-larval method of controlling mosquitoes is done in any of these areas except the urban areas of Poona, Nagpur and Surat, where weekly spraying of all surfaces of water was done with one per cent D.D.T. in malariol before 1953 and with malariol only after 1953. In Nagpur, B.H.C. water wettable powder has also been used as larvicide for some time. The areas of Katechiwadi, Manjri and Kolwadi of Poona District, were sprayed with one round of dieldrin at 28 mg. per sq. ft. during the years 1955 and 1956 instead of usual two rounds of D.D.T. D.D.T. used for the residual domiciliary spraying, was in the form of 75 per cent water wettable powder from 1953 onwards while it was used as five per cent Aromax-soap-emulsion before 1953. Dieldrin used for the spraying was in the form of 50 per cent water wettable powder.

At least four dosages of D.D.T. or dieldrin were tried for each of the dosage-mortality curves and about 36 mosquitoes exposed in six batches at each of the dosages. The mortality counts were taken 24 hours after the end of the exposure period. D.D.T. or dieldrin could be dissolved in the white oil up to four per cent only. For the higher concentrations the requisite quantity of the insecticide was first dissolved in the minimum quantity of benzene and then the requisite volume made up with the white oil. These higher concentrations of the insecticides in the mixture of benzene and white oil could not be strictly compared with those in the white oil alone. But, however, the increase in concentrations above four per cent did give higher mortalities of approximately the same order as those obtained with comparable concentrations of D.D.T. (tried up to ten per cent) in some of the vegetable oils like cotton oil.

Much care was taken in handling the mosquitoes in the experiments. The mosquitoes were picked up one by one in both end open tubes having a plug of cotton at one end. The female mosquitoes were immediately identified and selected for the proper stage of gonotrophic cycle. Only females which had fully fed on the previous night were selected. Partially-fed and gravid and semi-gravid females and those with amputated legs or damaged wings were discarded. The mosquitoes were immediately transferred from the tube to the recovery cages mentioned above after identification and selection. The recovery cages with the mosquitoes were carefully kept in wooden barrauds boxes and protected from heat by moist lint provided at bottom and top. The mosquitoes thus packed were carefully brought in a jeep or other vehicle to the field laboratory which was usually within ten miles from the places of collection. This proper handling helped in bringing down the control mortalities to about zero even with a most delicate mosquito like *A. culicifacies*. The slightest contamination of the lining paper or the lint discs of the recovery cages will vitiate the results to very great extent and therefore scrupulous care was taken to transfer exposed mosquitoes to perfectly clean and uncontaminated recovery cage.

The LC_{50}'s obtained are given in the accompanying Table I and Chart I. The LCs were determined visually from log dosage probit mortality curves. A detailed discussion of the findings will be made later on but the data are now presented, as they are, so that they may be available to other workers for comparison.

TABLE I

LC_{50} figures of D.D.T. and Dieldrin for different mosquitoes of Bombay State for 1957. Exposure period—One Hour (unless otherwise stated)

Serial Number	Species of mosquitoes	Place of test	District	Sprayed since with D.D.T.	Sprayed since with Dieldrin	LC_{50} D.D.T. per cent.	LC_{50} Dieldrin per cent.	Remarks
1.	*A. culicifacies*	Chorbavli	Nagpur	Unsprayed		0·2	0·02	
		Kada	Bhir	Unsprayed		0·3	0·05	
		Manjari	Poona	1953	1955	0·2	..	
		Kolwadi	Poona	1953	1955	0·6	..	
		Popatpura	Panchmahals	1950	..	0·5	0·08	
2.	*A. stephensi*	Lab. colony*	Poona	Unsprayed		2·1	0·10	* Origin in Poona District
		Kada	Bhir	Unsprayed		0·9	0·35	
3.	*A. fluviatilis*	Kallapur	Dharwar	Unsprayed		0·5 (i)	..	
		Katechiwadi	Poona	1953		0·6 (ii)	..	
		-do-	-do-	1953		0·8 (iii)	.	
		-do-	-do-	1953		0·6 (iv)	..	
		-do-	-do-	1953		0·4	0·7	
4.	*A. subpictus*	Ramtek	Nagpur	Unsprayed		0·5	5·0	
		Kada	Bhir	Unsprayed		2·8	6·0	
		Manjari	Poona	1953	1955	0·6	7·0	
		Katechiwadi	Poona	1953	1955	2·0	9·0	
		Nagpur City	Nagpur	1953	*	3·0	>20·0	* Part of the city was sprayed with dieldrin in 1956 and 1957.

(*Contd.*)

TABLE I.—(*Concld.*).

Serial Number	Species of mosquitoes	Place of test	District	Sprayed since		LC_{50}		Remarks
				with D.D.T.	with Dieldrin	D.D.T. per cent	Dieldrin per cent.	
5.	*A. annularis*	Popatpura	Panchmahals	1950	..	2·7	..	
		Chitrodia	Panchmahals	1950	..	..	0·07	
6.	*A. turkhudi*	Kada	Bhir	Unsprayed		1·4	..	
7.	*C. fatigans*	Lab. colony*	Poona	Unsprayed		>20·0 (1)	0·6 (1)	Exposure period in hours is shown in brackets
						1·2 (24)	..	* Origin Poona District
		Kada	Bhir	Unsprayed		>20·0 (1)	0·7 (1)	
						1·4 (24)	..	
		Ramtek	Nagpur	Unsprayed		>20·0 (1)	0·3 (1)	
						0·4 (24)	0·01 (24)	
		Surat City	Surat	1950	1956	>20·0 (1)	..	
						3·2 (24)	..	
		Kirkee	Poona	1950	..	>20·0 (1)	..	
						1·3 (24)	..	
		Poona City	Poona	1950	*	>20·0 (1)	6·0 (1)	* Part of the city was sprayed with dieldrin in 1956.
						1·7 (24)	..	
		Nagpur City	Nagpur	1953	*	>20·0 (1)	>20·0 (1)	* -do-
						1·4 (24)	1·5 (24)	

NOTE.—

(i) Experiments done in 1955 with p,p'-D.D.T.
(ii) Experiments done in 1955 with p,p'-D.D.T.
(iii) Experiments done in 1956 with p,p'-D.D.T.
(iv) Experiments done in 1956 with Tech. D.D.T.

CHART 1.

BOMBAY STATE
LC50 FOR DIFFERENT MOSQUITOES
(1957)
VILLAGE
DISTRICT
SPRAYED WITH DDT SINCE
1 SURAT SURAT 1950
2 CHORBAVDI NAGPUR UNSPRAYED
3 RAMTEK " "
4 NAGPUR " 1953
5 KOLWADI POONA
6 MANJRI "
7 KATECHWADI "
8 KIRKEE "
9 LAB. COLONY " 1950
10 POONA " UNSPRAYED
11 KADA BHIR 1950
12 POPUTPARA P. MAHALS UNSPRAYED
13 CHITRODIA " 1950
14 KALAPUR DHARWAR UNSPRAYED
SPRAYED AREA DIELDRIN
DDT
UNSPRAYED " DIELDRIN
DDT
LC50
% INSECTICIDE
A. CULICIFACIES
A. FLUVIATILE
A. STEPHENSI
A. ANNULARIS
A. TURKHUDI
A. SUBPICTUS
C. FATIGANS (1 HOUR)
C. FATIGANS (24 HOURS)

The chief interesting points, however, are:—

(1) Low LC_{50} for *A. culicifacies* both for D.D.T. and dieldrin, both in sprayed and unsprayed areas,

(2) A comparatively high LC_{50} for D.D.T. for *A. stephensi* maintained in a laboratory colony,

(3) A high LC_{50} for *A. annularis* for D.D.T. in a sprayed area in association with a low one for dieldrin,

(4) Very high LC_{50} of dieldrin for *A. subpictus*, whether the area was previously sprayed or not,

(5) Very high LC_{50} of D.D.T. to *Culex fatigans* in almost all places and of dieldrin in a few places.

The significance of these findings will be discussed elsewhere.

SUMMARY.

LC_{50}'s of D.D.T. and dieldrin as determined in 1957 for *Culex fatigans*, *Anopheles culicifacies*, *A. fluviatilis*, *A. stephensi*, *A. subpictus*, *A. annularis* and *A. turkhudi* from several places in Bombay State are recorded.

ACKNOWLEDGEMENT.

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SUSCEPTIBILITY TO D.D.T. OF PRINCIPAL MALARIA VECTORS OF WEST BENGAL.

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[September 8, 1958.]

INTRODUCTION.

THE development of resistance to insecticides has been reported in a number of anopheline vectors from different parts of the world. Thus, Brown (1957) quoting Quarterman and Schoof mentions about the development of D.D.T. resistance in *A. philippinensis* but wanted further confirmation. Similarly resistance in *A. sundaicus* has been reported from Indonesia — its homeland — and coastal Burma by Crandell (1954), Chow and Soeparmo (1956) and Brown (1958). Bertram (1950) recorded no true resistance but reduced mortality after D.D.T. spraying in *A. minimus* area of Assam in India.

All the three above mentioned species are well-known malaria vectors of this part of India. It was thought desirable to find out whether similar change in susceptibility to D.D.T. has taken place in any of these. Vector adult catches, collected from different areas, and larval densities in certain selected localities of the State (West Bengal) where large scale indoor residual spraying operations were carried out for a number of years, have been studied. The findings are presented in this paper.

METHODS.

During the period 1950-1952, two rounds of D.D.T. in the dosage of 50 mg. per sq. foot were applied in portions of *A. philippinensis* areas. But since 1953 this was modified so that the *philippinensis* areas of the State received a dose of 100 mg./sq. foot during the first round and 50 mg./sq. foot D.D.T. during the second

round. In the *sundaicus* and *minimus* areas systematic spraying operations have been carried out only since 1953. Dosage employed against the former species was the same as in the case of *A. philippinensis* but against *A. minimus* on the other hand this was 100 mg. D.D.T./sq. foot for both the rounds.

Adult anopheline catches were regularly made from selected catching stations in sprayed areas. The results of these catches in respect of the three vectors have been shown in Tables I, II and III. Studies were carried out in a few selected localities under spraying on the larval density as well. The *sundaicus* areas have been under observation from prior to control operations whereas *A. philippinensis* areas for the last two years only. The results are shown in Tables IV and V. Studies on larval densities in *minimus* areas are in progress.

TABLE I.

Monthly density of A. philippinensis *adults per ten man-hour catch in the sprayed areas according to years of spraying.*

Period of observation: 1950-1957.

Month	Year of spraying							
	1st year	2nd year	3rd year	4th year	5th year	6th year	7th year	8th year
January ..	—	0·0	0·0	0·0	0·0	0·0	0·0	0·0
February ..	—	6·6	0·0	0·0	0·0	0·0	0·0	0·0
March ..	—	1·9	0·9	0·0	0·0	0·0	0·0	0·0
April ..	—	1·5	0·0	0·0	0·0	0·0	0·0	0·0
May ..	—	0·0	0·0	0·0	0·0	0·0	0·0	0·0
June ..	—	0·0	0·0	0·0	0·0	0·0	0·0	0·0
July ..	— *	2·4	0·0	0·0	0·0	0·0	0·0	0·0
August ..	—	1·2	0·0	0·0	0·0	0·0	0·0	0·0
September	2·3	1·2	0·0	0·0	0·0	0·0	0·0	0·0
October ..	15·2	0·0	0·0	0·0	0·0	0·0	0·0	0·0
November	1·5	0·0	2·5	0·0	0·0	0·0	0·0	0·0
December	4·3	0·0	1·1	0·0	0·0	0·0	0·0	0·0

— Indicates no observation made. * First starting of anti-adult spraying with D.D.T.

RESULTS.

The results obtained showed that during the first three years of operation, only a few *A. philippinensis* adults could still be found in the sprayed dwellings. After three years of continuous spraying, however, no adult of this species could

TABLE II.

Monthly density of A. sundaicus *adults per ten man-hour catch in the sprayed areas according to year of spraying.*

Period of observation: 1953-1957.

Month	Year of spraying				
	1st year	2nd year	3rd year	4th year	5th year
January ..	35·4	32·2	0·0	1·0	1·1
February ..	60·5	0·9	0·0	0·0	0·0
March ..	78·0	6·9	0·0	0·0	0·0
April ..	0·0	1·0	0·0	0·0	0·0
May	37·8	0·0	0·0	0·0	0·0
June	5·2	1·2	0·0	0·0	0·0
July	21·2	0·0	0·0	0·0	0·0
August ..	1·0	0·0	0·0	0·0	0·0
September ..	6·6*	0·0	0·0	0·0	0·0
October .	0·0	0·0	0·0	1·3	0·0
November .	5·0	1·2	1·1	0·0	0·0
December ..	26·7	0·6	0·0	1·2	1·0

* First starting of anti-adult spraying with D.D.T.

be recovered from the houses. Similar reduction in density of the other two vectors was also noticed but there were occasions when few adults of these species could be caught. As in the case of adults, there was a corresponding decline in the larval catches of *A. philippinensis* and *A. sundaicus* as well.

DISCUSSION.

Preliminary investigations, carried out in collaboration with the Malaria Institute of India in an unsprayed area in Burdwan District in this State, indicated that the original level of susceptibility of *A. philippinensis* adults to D.D.T. was similar to that for other anopheline species of India. The entire *A. philippinensis* area of West Bengal has been under residual spraying for a number of years — in certain areas for more than five seasons. The reduction of adult vector population to zero level has been maintained in the sprayed areas, indicating thereby that there has been no change so far in its susceptibility to D.D.T.

TABLE III.

Monthly density of A. minimus *adults per ten man-hour catch in the sprayed areas according to years of spraying.*

Period of observation: 1953-1957.

Month	Year of spraying				
	1st year	2nd year	3rd year	4th year	5th year
January ..	—	0·0	0·0	0·0	0·0
February	—	0·0	0·0	0·0	0·0
March ..	—	2·4	1·9	1·8	0·0
April	—	1·8	1·2	0·0	0·0
May	—	0·0	0·0	0·0	0·0
June	—	0·0	0·0	0·0	0·0
July	—	0·0	0·0	0·0	0·0
August ..	—	0·0	0·0	0·0	0·0
September ..	—*	0·0	0·0	0·0	0·0
October ..	3·8	2·6	0·0	1·7	0·0
November ..	1·8	0·0	2·5	0·0	0.0
December ..	1·4	0·0	0·0	0·0	0·0

— Indicates no observation made. * First starting of anti-adult spraying with D.D.T.

D.D.T. susceptibility level of *A. sundaicus* adults of West Bengal, prior to residual spraying operation, has been previously determined by Ganguli, Sinha and Duttaray (1958). An LC50 of 0·55 per cent was obtained in an area subject to six years continuous larvicidal treatment from the ground, compared to this the LC50 of the same species in a neighbouring untreated area was more or less the same being 0·50 per cent. The initial susceptibility to D.D.T. of *A. sundaicus* in Indonesia and Burma, where the species is reported to have developed resistance, was also similarly low—being of the order of LC50 of 0·50 per cent and 0·31 per cent respectively according to the data collected by the Malaria Section, World Health Organization (1957). Indoor spraying for five continuous years does not seem to have altered the susceptibility of the species in West Bengal. In this connection, it may be mentioned that West Bengal is at the periphery of the distribution range of *A. sundaicus* where it has perhaps not been able to adapt itself properly and is thus particularly susceptible to the methods of control employed.

Bertram (1950 *loc. cit.*) no doubt recorded reduced mortality of *A. minimus* in D.D.T. sprayed dwellings of Assam [dosage 45 mg. D.D.T. per sq. foot] and

TABLE IV.

Monthly density of A. philippinensis *larvæ per* 100 *dips in sprayed areas according to year of spraying.*

Period of observation: 1956 and 1957.

Month	Year of spraying	
	7th year	8th year
January ..	0·0	0·0
February ..	0·0	0·0
March ..	0·0	0·0
April	0·0	0·0
May	0·0	0·0
June	0·0	0·0
July	0·0	0·0
August	0·0	0·0
September	0·0	0·0
October ..	0·0	0·0
November	0·0	0·0
December ..	0·0	0·0

TABLE V.

Monthly density of A. sundaicus *larvæ per* 100 *dips in sprayed areas according to year of spraying.*

Period of observation: 1953 - 1957.

Month	Year of spraying				
	1st year	2nd year	3rd year	4th year	5th year
January	4·0	3·3	0·8	0·5	0·0
February ..	0·9	0·2	0·0	0·0	0·0
March ..	0·3	3·0	0·0	0·0	0·0
April ..	0·1	1·5	0·1	0·0	0·0
May ..	1·3	0·3	0·2	0·0	0·0
June ..	0·1	0·2	0·0	0·3	0·0
July ..	0·0	0·1	0·9	0·1	0·0
August ..	0·1	0·4	0·8	1·3	0·0
September ..	1·7*	0·1	0·0	0·0	0·0
October ..	0·0	0·0	0·2	0·0	0·0
November ..	8·1	0·9	1·3	0·0	0·0
December ..	4·7	1·0	0·5	0·0	1·1

* First starting of anti-adult spraying with D.D.T.

suggested excito-repellant action of D.D.T. to be the cause. But Macdonald (1950), recording observations of Gilroy in Assam, noted 100 per cent mortality in *A. minimus* trapped from houses treated with D.D.T. [45 mg. and 90 mg. per sq. foot]. Observations carried out in the foot-hill regions of West Bengal indicate that the insecticide continues to be highly lethal to *A. minimus*.

CONCLUSION.

The initial susceptibility levels of *A. philippinensis* and *A. sundaicus* — two out of three vectors of West Bengal — were low.

Field observations indicate that the three species *A. philippinensis*, *A. sundaicus* and *A. minimus* continue to be susceptible even after continuous insecticidal treatment for five years or more.

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PRESENT STATUS OF SUSCEPTIBILITY OF SOME SPECIES OF MOSQUITOES TO D.D.T., B.H.C. AND DIELDRIN.

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[October 30, 1958.]

INTRODUCTION.

LARGE-SCALE use of chlorinated hydrocarbon insecticides has resulted in the decline of some of the insect-borne diseases throughout the world, but at the same time the number of insects showing resistance to insecticides has increased. Appearance of resistant strain of vector species has resulted in the failure of control measures by chemicals (Brown, 1958). Serious attention has, therefore, been paid to the study of this problem. The results of field observations and laboratory investigations on resistance phenomenon suggest that resistance of insects to insecticides is probably more a rule than an exception. As such it has been emphasized that whenever feasible control measures should be intensified to achieve an eradication of the diseases before the appearance of resistance strain of vector species. Side by side with the eradication programme, continuous vigilance for early detection of cases of resistance should be maintained. With the later object in view specific tests were carried out with some species of the anopheline mosquitoes in India to collect the base line data about their susceptibility to D.D.T., B.H.C. and dieldrin by various workers, Krishnan (1956), Shamasashtry *et al.* (1956), Ramachandra Rao and Bhatia (1957), Sharma and Krishnamurthy (1957*a*:1957*b*), Sharma *et al.* (1957*a* and 1957*b*), Sharma and Kalra (1958), Ganguli *et al.* (1958), Sunder Rao *et al.* (1958). Subsequent to these publications some more information about the susceptibility of anopheline, culicine and *Aedes* mosquitoes has been collected. Both published and unpublished data have been analysed, the results of which are presented in this paper.

MATERIAL AND METHODS.

Susceptibility test with twelve different species of anopheline, two species each of culicine and *Mansonioides* and one species of *Aedes* mosquitoes, were carried out in different States of the Indian Union (Map 1) against D.D.T., B.H.C. and dieldrin. Tests with all the species except *A. stephensi* and *Aedes aegypti* were

MAP I.

carried out in the field with wild-caught female gravid mosquitoes. *A. stephensi* adults were reared in the field laboratory from wild-caught larvæ. Two to four days old blood-fed mosquitoes were tested. Similarly two to four days old colony strain of *Aedes ægypti* fed on fowl blood were used for the tests. In addition to adult tests, larval tests were also carried out with *A. stephensi* larvæ in Erode and *A. subpictus* larvæ in Delhi.

Busvine-Nash method (Busvine and Nash, 1953:1954) was followed for testing the susceptibility of adult mosquitoes. In some cases the test method was slightly modified by replacing the cork with mosquito netting. In such cases the filter papers were cut to the size of the specimen tubes. In the tests with culicine mosquitoes, higher concentration of D.D.T. was prepared either in benzine-B.O.C. mixture or in aromax*. For larval tests 1000 cc of water in enamel bowls were treated with 1 cc of varying concentration of D.D.T. solutions in alcohol. 30 larvæ were exposed in each bowl. Test insects found dead beyond doubt at the end of observation period were recorded dead. Mortality data thus obtained for each experiment were analysed by probit analysis method and MLCs were generally estimated by graphic method. In some cases arithmatic method (Finney, 1952) was also followed to estimate the MLC values. MLC values of different insecticides for anopheline, culicine, *Aedes* and *Mansonioides* mosquitoes so far observed in India are consolidated for ready reference in Table I.

SUSCEPTIBILITY TO D.D.T.

It may be seen from Table I that the MLC of D.D.T. for anopheline mosquitoes from unsprayed areas varied between 0·125 per cent and 2·6 per cent. World Health Organization has recently observed that the MLCs of D.D.T. for majority of vector species varied between 0·5 and 1·5 per cent (World Health Organization, 1957:1958). Higher MLC value than 1·5 per cent was observed only with *A. stephensi* (colony strain) at Poona, Bombay and with *A. subpictus* in the unsprayed villages of Udaipur, Meerut and Bhir. The high MLC value, 2·1 × 1, for *A. stephensi* (colony strain) is 2·3 times more than that observed in the unsprayed area of Bhir District of Bombay (Table I). Higher MLC in the colony strain was also reported by Davidson (as quoted by Brown, 1958). Probably the higher MLC values of the colony strain were due to the effect of colonization. In case of *A. subpictus* the higher MLC values of D.D.T. in the unsprayed areas of Meerut District were due to infiltration of D.D.T.-resistant mosquitoes from the nearby sprayed areas of Delhi State (Sharma and Kalra, 1958). In Udaipur, the susceptibility tests with *A. subpictus* were carried out in different seasons of the year. The MLCs of D.D.T. were found to be 2·57 × 1, 1·62 × 1 and 0·44 × 1 in August, October and November-December respectively. However no significant difference in the slope of the ld-pr lines could be observed. It, therefore, appears that the higher MLC, 2·57 × 1, was probably due to effect of temperature and humidity. The species most susceptible to D.D.T. were *A. culicifacies* and *A. minimus*. The MLC values of these species were found to be less than that of the lower limit of 0·5 per cent D.D.T.

Increase in the MLC values in the D.D.T. sprayed villages was observed for *A. annularis* (in Udaipur and Ranchi), *A. culicifacies* (Udaipur and Poona), *A. fluviatilis* (Poona, Bombay) and *A. minimus* (Assam). However, the higher tolerance was never more than five times of the susceptible strains from nearby areas. It may be considered, therefore, that the higher tolerance observed in these species may be due to vigour tolerance or other experimental conditions such as temperature, nutritional status, age etc.

* These solvents were not found ideal as insecticide crystallises on the impregnated filter papers when higher concentrations are used.

TABLE I.

Susceptibility of Indian Mosquitoes to D.D.T., B.H.C. and Dieldrin.

Species of mosquitoes	Locality	Sprayed/Unsprayed	Median Lethal Concentration × hrs. of exposure			Reported/Tests carried out by
			D.D.T. (Tech.)	B.H.C. (gamma)	Dieldrin (Tech.)	
Unpublished data						
A. culicifacies. (adults)	Uchana, Karnal, Punjab.	Unsprayed	0·31 × 1	..	..	Malaria Institute of India team. M. I. D. Sharma.
,, ,,	Palad Pur, Morena Distt., Madhya Pradesh.	,,	0·35 × 1 (0·34 × 1)	0·0035 × 1 (0·0032 × 1)	0·076 × 1 (0·077 × 1)	Malaria Institute of India team. Babu Ram.
,, ,,	Nelligeri, Mandya Distt., Mysore.	,,	0·158 × 1	0·0026 × 1	0·03125 × 1	Malaria Institute of India team. M. L. Mamman.
,, ,,	Kailashpuri, Udaipur Distt., Rajasthan.	Sprayed	0·78 × 1	..	..	Malaria Institute of India team. J. S. Khamre.
,, ,,	,, ,,	Unsprayed	..	0·00667 × 1	..	
Published data						
A. culicifacies (adults)	Kailashpuri, Udaipur Distt., Rajasthan.	,,	0·19 × 1 (0·222 × 1)	0·0078 × 1 (0·0071 × 1)	0·037 × 1 (0·04365 × 1)	Malaria Institute of India team. Sharma *et al.* (1957a, 1957b)
,, ,,	Arthala, Meerut Distt., Uttar Pradesh.	,,	0·43 × 1 (0·4285 × 1)	0·0045 × 1 (0·0039 × 1)	0·035 × 1 (0·03214 × 1)	
,, ,,	Mandya Distt., Mysore.	,,	0·25 × 1	..	..	Sunder Rao *et al.* (1958)
,, ,,	Baramati, Poona Distt., Bombay.	Sprayed with D.D.T. since June, 1953	0·535 × 1*	..	..	T. Ramachandra Rao and S. C. Bhatia (1957)
,, ,,	Dhond, Poona Distt., Bombay.	Unsprayed	0·24 × 1*	..	..	
,, ,,	Beliary, Dharwar Distt., Bombay.	,,	0·379 × 1*	..	..	
,, ,,	Haldur, Bijapur Distt., Bombay.	Sprayed with D.D.T. since July, 1949	0·472 × 1*	..	..	

* p'p' D.D.T. was used.

(*Contd.*)

TABLE 1 (*Contd.*)

Species of mosquitoes	Locality	Sprayed/Unsprayed	Median Lethal Concentration × hrs. of exposure			Reported/ Tests carried out by
			D.D.T. (Tech.)	B.H.C. (gamma)	Dieldrin (Tech.)	
A. culicifacies (adults)	Dasagaon, Kolaba Distt., Bombay.	Unsprayed	0·365 × 1*	..	..	
,, ,,	Ladapur, Kolaba Distt., Bombay.	Sprayed with D.D.T. since June, 1953	0·41 × 1*	..	..	
,, ,,	Revti, Thana Distt., Bombay.	Sprayed with D.D.T. since July, 1949	0·412 × 1* 0·571 × 1*	..	..	T. Ramachandra Rao and S.C. Bhatia (1957)
,, ,,	Khojwa, Panchmahal, Bombay.	Sprayed with D.D.T. since 1950	0·501 × 1			
,, ,,	Manjari, Poona Distt., Bombay.	Sprayed with D.D.T. since June, 1953	0·453 × 1* 0·536 × 1			
,, ,,	Dhond, Poona Distt., Bombay.	Sprayed with D.D.T. since July, 1956	0·598 × 1			
,, ,,	Chorbarti, Nagpur, Bombay.	Unsprayed	0·2 × 1	..	0·02 × 1	
,,	Kada, Bhir Distt., Bombay.	,,	0·3 × 1	..	0·05 × 1	Bhatia *et al.* (1958)
,,	Manjari, Poona Distt., Bombay.	Sprayed with D.D.T. since 1953, also sprayed with dieldrin in 1955	0·2 × 1	..	..	
,, ,,	Kolwadi, Poona Distt., Bombay.	,,	0·6 × 1	..	..	
,, ,,	Popatpura, Panchmahal Distt., Bombay.	Sprayed with D.D.T. since 1950	0·5 × 1	..	0·03 × 1	
,, ,,	Pargaon, Thana Distt., Bombay.	Sprayed with D.D.T. during 1948-56 and with dieldrin in 1953 and 1957			> 1·6 × 1 3·0 × 1 (by extrapolation)	Patel *et al.* (1958)

*p'p' D.D.T. was used.

(*Contd*)

TABLE I (*Contd.*)

Species of mosquitoes	Locality	Sprayed/Unsprayed	Median Lethal Concentration × Hrs. of Exposure			Reported Tests carried out by
			D.D.T. (Tech.)	B.H.C. (gamma)	Dieldrin (Tech.)	
Unpublished data						
A. subpictus (adults)	Kailaspuri, Udaipur Distt., Rajasthan.	Unsprayed	0·44 × 1 (Nov. Dec., 57) 0·066 × 24	0·016 × 1	0·0195 × 1	Malaria Institute of India team. J. S. Khanre.
			2·57 × 1 (Aug., 1958)	0·01123 × 1	..	
			1·620 × 1 (Oct. 1958)			
A. subpictus (Larvae)	Kailaspuri, Udaipur Distt., Rajasthan.	Unsprayed	0·0331 p.p.m.	0·005 p.p.m.	..	
„ (Adults)	Bansdroni, 24 Paraganas, West Bengal.		3·16 × 1	0·15 × 1	2·8 × 4	Malaria Institute of India team. D. M. Venkoba Rao and S.C. Bhalla.
„ (Larvae)	„		0·0794 p.p.m.	0·0708 p.p.m.	0·0891 p.p.m.	
Published data						
A. subpictus (adults)	Arthala, Meerut Distt., Uttar Pradesh.	Unsprayed	0·28 × 1 (0·264 × 1)	0·023 × 1 (0·0216 × 1)	0·25 × 1 (0·256 × 1)	Malaria Institute of India team. Sharma *et al.* (1957a, 1957b)
„ „	Jwalaheri, Delhi.	Sprayed	3·1 × 24 (3·02 × 24)	0·014 × 1 (0·0158 × 1)	0·16 × 1 (0·161 × 1)	
„ „	Nangli Sahidan, Delhi.	„	2·2 × 24 (2·296 × 24)	..	..	Malaria Institute of India team. Sharma and Kalra (1958)
„ „	Arthala, Meerut Distt., Uttar Pradesh.	Unsprayed	2·6 × 1 (0·12 × 24)	..	..	
„ „	Nelothi and Sultanpur, Delhi.	Sprayed with D.D.T.	(6·00 × 24)	..	..	
„ (Larvae)	„	„	(1·2 p.p.m. × 24) tested in field. (0·63 p.p.m. × 24) tested in Lab.	(0·26 p.p.m. × 24) tested in Lab.	.	
„ „	Arthala, Meerut Distt., Uttar Pradesh.	Unsprayed	(0·28 p.p.m. × 24) tested in field. (0·34 p.p.m. × 24) tested in Lab.	(0·21 p.p.m. × 24) tested in Lab.		

(*Contd.*)

TABLE I (*Contd.*)

Species of mosquitoes	Locality	Sprayed/Unsprayed	Median Lethal Concentration × hrs. of exposure			Reported/ Tests carried out by
			D.D.T. (Tech.)	B.H.C. (gamma)	Dieldrin (Tech.)	
A. subpictus (Adults)	Ramtek, Nagpur, Bombay.	Unsprayed	0·5 × 1	..	5·0 × 1	Bhatia *et al* (1958)
,, ,,	Kada, Bhir Distt., Bombay.	,,	2·8 × 1	..	6·0 × 1	
,, ,,	Manjari, Poona Distt., Bombay.	Sprayed with D.D.T. since 1953 and with Dieldrin since 1955	0·6 × 1	..	7·0 × 1	
,, ,,	Katachiwadi, Poona Distt., Bombay.	,,	2·0 × 1	..	9·0 × 1	
,, ,,	Nagpur City, Nagpur, Bombay.	Sprayed with D.D.T. since 1953 and also part of the city sprayed with dieldrin in 1957	3·0 × 1	..	> 20·0 × 1	
,, ,,	Delhi.	Unsprayed	0·3 × 1 (1955)	..	..	Indian Council of Medical Research Report (1955)
Unpublished data						
A. fluviatilis (Adults)	Hindipuri, Ranchi Distt., Bihar.	Unsprayed	0·56 × 1 (Sept., 1957) 0·44 × 1 (Nov., 1957)	0·007 × 1	0·19 × 1 (Oct., 1957) 0·18 × 1 (Mar., 1957)	Malaria Institute of India team. P. V. Patil and S. Ramalingam
,, ,,	Jalmari, Singhbhum Distt., Bihar.	Sprayed with D.D.T. since 1945	0·52 × 1 (Sep., 1957) 0·35 × 1 (Nov., 1957)	0·01 × 1	0·18 × 1	
,, ,,	Mandya Distt., Mysore.	Unsprayed	0·125 × 1	0·003 × 1	0·0398 × 1	Malaria Institute of India team. M. L. Mamman
Published data						
A. fluviatilis (Adults)	Kallapur, Dharwar Distt., Bombay.	,,	0·6 × 1* (1955)	..	..	Bhatia *et al.* (1958)
,, ,,	Katechiwadi, Poona Distt., Bombay.	Sprayed with D.D.T. since 1953	0·6 × 1* (1955)	..	..	

*p′p′ D.D.T. was used.

(*Contd.*)

TABLE 1 (*Contd.*)

Species of mosquitoes	Locality	Sprayed/Unsprayed	Median Lethal Concentration × Hours of Exposure			Reported Tests carried out by
			D.D.T. (Tech.)	B.H.C. (gamma)	Dieldrin (Tech.)	
A. fluviatilis (adults)	Katechiwadi, Poona Distt., Bombay.	Sprayed with D.D.T. since 1953	0·8×1* (1956)	..	..	Bhatia *et al.* (1958)
,,	,,	,,	0·6×1* (1956)	..	..	
,, ,,	,,	,,	0·4×1	..	0·7	
,, ,,	Kailashpuri, Udaipur Distt., Rajasthan.	Unsprayed	0·30×1 (0·35×1)	0·021×1 (0·0224×1)	0·22×1 (0·238×1)	Malaria Institute of India team. Sharma *et al.* (1957a, 1957b). Sunder Rao *et al.* (1958).
,, ,,	Mandya, Mysore.	,,	0·35×1	..	..	
A. fluviatilis (adults)	Baramati, Poona Distt., Bombay.	Sprayed with D.D.T. since 1953	0·5×1* (1955) 0·65×1* (1956) 0·9×1* (1956)	..	..	Ramachandra Rao and Bhatia (1957)
Unpublished data						
A. splendidus (adults)	Jate, Ranchi Distt., Bihar.	Unsprayed	0·631×1	0·018×1	1·0×1 (1·005×1)	Malaria Institute of India team. P. V. Patel and S. Ramalingam
,, ,,	Jalmai, Singhbhum Distt., Bihar.	Sprayed with D.D.T.	0·794×1	0·0251×1	3·55×1 (3·73×1) 0·631×1	
,, ,,	Jate, Ranchi Distt., Bihar.	Unsprayed	0·631×1	0·018×1	0·269×1 (Feb., 1958)	Malaria Institute of India team. B.S. Krishnamurthy
,, ,,	Jalmai, Singhbhum Distt., Bihar.	Sprayed	0·79×1	0·025×1	0·71×1 (Feb., 1958)	
A. stephensi (adults)	Erode, Madras.	Sprayed	3·16×1	..	..	Malaria Institute of India team. M.L.Mammen
,, (larvae)	,, ,,	,,	5·6 p.p.m.×24+24 8·5 p.p.m.×24	0·01585 p.p.m.×24+24 0·302 p.p.m.×24	0·00398 p.p.m.×24+24 0·05 p.p.m.×24	
,, ,,	Coimbatore, Madras.	Unsprayed	0·0085×24+24 0·0085 p.p.m.×24	0·0112 p.p.m.×24+24 0·0112 p.p.m.×24	0·00063 p.p.m.×24+24 0·00126 p.p.m.×24	

*p'p' D.D.T. was used.

(*Contd.*)

TABLE 1 (*Contd.*).

Species of mosquitoes	Locality	Sprayed/Unsprayed	Median Lethal Concentration × Hours of Exposure			Reported/ Tests carried out by
			D.D.T. (Tech.)	B.H.C. (gamma)	Dieldrin (Tech.)	
Published data						
A. stephensi (larvae)	Colony strain, Poona, Bombay.	..	2·1 × 1	..	0·1 × 1	Bhatia *et al.* (1958)
„ „	Kada, Bhir Distt., Bombay.	Unsprayed	0·9 × 1	..	0·35 × 1	
Unpublished data						
A. minimus (adults)	Tangla, Assam.	Unsprayed	0·282 × 1	0·0066 × ½	0·0079 × ½	Malaria Institute of India team. Venkoba Rao
„ „	Khoirabari, Tangla, Assam.	Sprayed only once with D.D.T. in 1955	0·75 × 1	0·0072 × ½	0·079 × ½	
A. annularis „	Hindipuri, Ranchi Distt., Bihar.	Unsprayed	0·76 × 1	0·0166 × 1	0·083 × 1	Malaria Institute of India team. P. V. Patel and S. Ramalingam
„ „	Jalmari, Singhbhum Distt., Bihar.	Sprayed	1·2 × 1	0·0178 × 1	0·427 × 1	
„ „	Kailashpuri, Udaipur, Rajasthan.	Unsprayed	0·71 × 1	..	..	Malaria Institute of India team. J. S. Khamre
„ „	„ „	Sprayed	1·26 × 1	..	..	
Published data						
A. annularis (adults)	„ „	Unsprayed	0·56 × 1 (0·6295 × 1)	0·039 × 1 (0·033 × 1)	0·42 × 1 (0·414 × 1)	Malaria Institute of India team. Sharma *et al.* (1957*a* and 1957*b*); Bhatia *et al.* (1958).
„ „	Poparpura, Panchmahals Distt., Bombay.	Sprayed with D.D.T. since 1950	2·7 × 1	..	..	
„ „	Chirodia, „ „	„	..	..	0·07	
A. turkhudi (adults)	Kada, Bhir Distt., Bombay.	Unsprayed	1·4 × 1	..	..	
Unpublished data						
A. hyrcanus (adult)	Hindiperi, Ranchi Distt., Bihar.	„	0·794 × 1	..	..	Malaria Institute of India team. P. V. Patel and S. Ramalingam

(*Contd.*)

TABLE I (*Contd.*)

Species of mosquitoes	Locality	Sprayed/Unsprayed	Median Lethal Concentration × hrs. of exposure			Reported/ Tests carried out by
			D.D.T. (Tech.)	B.H.C. (gamma)	Dieldrin (Tech.)	
A. hyrcanus (adults)	Sonerpur, 24 Paraganas, West Bengal.		0·89 × 1	..	..	Malaria Institute of India team.
A. barbirostris (adults)	,, ,,		0·98 × 1	..	..	Venkoba Rao and S.C.Bhalla.
A. sundiacus (adults)	Salt water lakes, Kalikapur, Calcutta.		0·39 × 1	..	..	Malaria Institute of India team. M. L. Roy.
Published data						
A. sundaicus (adults)	Natunabad, North Salt Lake area, Calcutta.	Unsprayed	0·55 × 1	..	..	Ganguli *et al.* (1958)
,, ,,	Kalikapur, South Salt Lake, Calcutta.	,,	0·50 × 1	..	..	Malaria Institute of India team.
Unpublished data						
A. pallidus (adults)	Hindiperi, Ranchi Distt., Bihar.	,,	0·955 × 1	..	0.21135 × 1	P. V. Patel and S.Ramalingam.
C. fatigans	Rudrapur, Nainital Distt., Uttar Pradesh.	Sprayed with D.D.T. since 1947	4·0 × 24	0·083 × 1	0·398 × 1	Malaria Institute of India team. B.S. Krishnamurthy and J. S. Khamre.
,,	,, ,,	Sprayed with D.D.T. since 1947 and also with dieldrin in 1956-57	..	..	0·479 × 4	Malaria Institute of India taem.
,,	Kichha, ,,	Sprayed with D.D.T. since 1947	..	0·05 × 4	0·195 × 4	V. N. Bhatnagar.
,,	Shakarpur, Delhi State.	,,	7·07 × 24	..	..	Malaria Institute of India team.
,,	Babarpur, Delhi State.	,,	19·95 × 24	..	..	M. I. D. Sharma and
,,	Taharpur, Delhi State.	,,	> 4·0 × 24	..	..	B. S. Krishnamurthy

(*Contd.*)

TABLE I (*Contd.*)

Species of mosquitoes	Locality	Sprayed/Unsprayed	Median Lethal Concentration × hrs. of exposure			Reported/ Tests carried out by
			D.D.T. (Tech.)	B.H.C. (gamma)	Dieldrin (Tech.)	
C. fatigans (larvae)	Mangalore.		..	0·5248 p.p.m. × 24	..	
" "	Ernakulam, Kerala (eggs collected from wild caught female, larvae reared and tested in Lab.)		0·174 p.p.m. × 24 (July, 1958) 0·224 p.p.m. × 24 (Aug. 1958) 0·188 p.p.m. × 24 (Sept., 1958)	0·1819 p.p.m. × 24 (July, 1958) 0·1006 p.p.m. × 24 (Aug., 1958) 0·2114 p.p.m. × 24 (Sep. 1958)	0·1995 p.p.m. × 24 (July, 1958) 0·1883 p.p.m. × 24 (Aug., 1958) 0·1811 p.p.m. × 24 (Sept., 1958)	Malaria Institute of India team. S. Ramalingam and P.V. Patel.
Published data	Lab., Colony, Poona Distt., Bombay.	..	>20·0×1 1·4×24	..	0·6×1	
	Kada, Bhir Distt., Bombay.	Unsprayed	>20·0×1 1·4×24	..	0·7×1	
	Ramtek, Nagpur Distt., Bombay.	"	>20·0×1 0·4×24	..	0·3×1 0·01×24	
	Surat City, Surat, Bombay.	Sprayed with D.D.T. since 1950 and with dieldrin in 1956	>20·0×1 3·2×24	..	..	Bhatia *et al.* (1958)
	Kirkee, Poona, Bombay.	Sprayed with D.D.T. since 1950	>20·0×1 1·3×24	..	.	
	Poona City, Bombay.	Sprayed with D.D.T. since 1950. Part of the city sprayed with dieldrin in 1956	>20·0×1 1·7×24	..	6·0×1	
	Nagpur City, Bombay.	"	>20·0×1 1·4×24	.	20·0×1 1·5×24	
	Khurda Town, Orissa.	Sprayed with D.D.T., B.H.C. and dieldrin since 1953.	>20×1	..	.	Malaria Institute of India team. Krishnan (1956).
	Ganjam Distt. Orissa.	Unsprayed	>5×1	..	.	

(*Contd.*)

TABLE 1 (*Concld.*)

Species of mosquitoes	Locality	Sprayed/Unsprayed	MEDIAN LETHAL CONCENTRATION × HRS. OF EXPOSURE			Reported/ Tests carried out by
			D.D.T. (Tech.)	B.H.C. (gamma)	Dieldrin (Tech.)	
Unpublished data						
C. gelidus	Near Calcutta.		1·3×1	..		Malaria Institute of India team. Venkoba Rao and S.C.Bhalla.
Aedes aegypti	Colony Strain, Delhi.	..	0·426×1	0·0141×1	0·35×1	Malaria Institute of India team. Rajendra Pal and N. N. Singh.
Aedes aegypti	Colony Strain, Bombay	..	1·6×1	..	..	Personal communication from T. Ramachandra Rao.
M. uniformis	Thuravoor, Ernakulam, Kerala	Unsprayed	0·174×1 (Aug., 1958) 0·190×1 (Oct., 1958)	0·0133×1 (Aug. 1958) 0·0158 (Oct., 1958)	0·2239×1 (Aug. 1958) ..	Malaria Institute of India team. P. V. Patel and S.Ramalingam.
M. annulifera	,, ,,	,,	0·468×1	0·0071×1	0·126×1	

NOTE.—

Figures in parenthesis were estimated by arithmatical technique.

In the larval tests mortalities were recorded at the end of 24 hours of exposure and at the end of further 24 hours of observation period. MLC estimated from these data were shown as.....p.p.m ×24 and.....p.p.m. ×24+24 respectively.

Specific D.D.T. resistance was observed in *A. stephensi* of Erode (Madras), *A. subpictus* of Delhi and of 24 Parganas (West Bengal) and in *A. annularis* of Papatpura (Bombay).

The susceptibility test results with female adults *A. stephensi* against D.D.T. in Erode (Madras) showed MLC of 3·16 per cent. Similar data for this species could not be obtained from the unsprayed area due to paucity of test insects. The higher MLC, 3·16 per cent, observed in Erode indicates 1·5 times and about 3·4 times higher tolerance when compared with MLC values observed with colony strain in Bombay and field strain of Bhir District respectively (Table I).

In the larval susceptibility test, MLCs of D.D.T. in uncontrolled area was found to be 0·0085 p.p.m. The corresponding MLC values of the controlled areas were 5.6 p.p.m. From these MLC values it appears that *A. stephensi* larvæ from the controlled area were roughly 657 times* resistant to D.D.T. The most interesting feature of these observations is that the degree of resistance in the adult stage was much less than that observed in the larval stage.

A. subpictus was reported to be resistant to D.D.T. in Delhi in 1956 (Sharma and Krishnamurthy, 1957*a*:1957*b*) and the degree of resistance was stated to be more than ten times when compared with the MLC value of the same species from a nearby unsprayed village of Uttar Pradesh (Sharma *et al.*, 1957). Subsequent investigation showed that the degree of resistance of *A. subpictus* was more than 50 times than the susceptible strain (Sharma and Kalra, 1958). The larvæ of *A. subpictus* of sprayed area were also found to be two to four times more tolerant to D.D.T. than those from the unsprayed village (Sharma and Kalra *loc. cit.*). In the case of *A. subpictus* the degree of resistance is of lower order in the larval stage than the adult stage. This finding is just opposite to what has already been mentioned for *A. stephensi*. However, D.D.T.-resistant *A. subpictus* did not show any decrease in its susceptibility to B.H.C. and dieldrin. In 24 Paraganas (West Bengal) the MLC of D.D.T. for *A. subpictus* was found to be 3·16 per cent. When compared with the MLC, 0·3 per cent, reported for this species in the unsprayed area of Delhi, the degree of resistance was found to be ten times.

C. fatigans was found to be more tolerant to D.D.T. than the anopheline mosquitoes. The MLC values of D.D.T. for this species tested in unsprayed villages were found to vary between 0·4 and 1·4 per cent with 24 hours exposure. *C. fatigans* was the first species of mosquitoes reported to be resistant to D.D.T. in India (Pal *et al.*, 1952). The MLC fort his species of D.D.T.-sprayed villages were found to vary between 1·3 and 19·9 per cent when exposed to D.D.T. for 24 hours. This species has now been observed to be resistant to D.D.T. in Uttar Pradesh, Delhi, Orissa and Bombay (Table I).

Aedes ægypti, *C. gelidus*, *M. uniformis* and *M. annulifera* were found to be as susceptible to D.D.T. as the anopheline mosquitoes. However, the MLCs of D.D.T. for the two colony strains *Aedes ægypti* were found to be 0·43 and 1.6 per cent. This difference in the M.L.C values is probably due to the variations in the rearing and testing conditions in the two laboratories.

*The degree of resistance was found to be 1,000 times when the mortalities, soon after the exposure, were considered.

SUSCEPTIBILITY TO B.H.C.

A. minimus was found to be the most susceptible species to B.H.C. (MLC 0·007 × ½). MLCs of B.H.C. for other anopheline mosquitoes from unsprayed villages were found to vary between 0·003 and 0·04 per cent. The limits of normal variations reported by World Health Organization were 0·005 to 0·015 per cent (World Health Organization, 1958). Apparently higher MLCs than 0·015 per cent were observed with *A. subpictus*, *A. fluviatilis*, *A. splendidus* and *A. annularis*. There were no significant differences between the MLCs for these species tested in sprayed and unsprayed areas; as such these species may be considered to possess somewhat natural tolerance to B.H.C. as compared to the vector species referred to by the World Health Organization (World Health Organization, 1958, *loc. cit.*). However, D.D.T.-resistant larvæ of *A. stephensi* (Erode, Madras) were observed to be crossresistant to B.H.C. The degree of resistance was found to be about 27 times more than the larvæ of susceptible strain (Table I).

The MLC value of B.H.C. for D.D.T.-resistant *C. fatigans* was found to be 0·083 per cent. In the absence of base line data for the susceptible strain it is difficult to say whether the higher tolerance is a natural one or it is due to vigour selection. *Aedes ægypti* (colony strain), *M. uniformis* and *M. annulifera* were found to be as susceptible to B.H.C. as the anopheline mosquitoes from unsprayed villages. The M.L.C. of B.H.C. for these mosquitoes were found to vary between 0·014 and 0·0071 per cent.

SUSCEPTIBILITY TO DIELDRIN.

The MLC of dieldrin for the anopheline mosquitoes tested in the unsprayed areas were found to vary between 0·02 to 6·0 per cent. The MLCs ranged from 0·05 to 0·15 per cent of dieldrin for the vector species in the unsprayed areas of different parts of world (World Health Organization, 1957:1958). MLCs above 0·15 were found for *A. fluviatilis* (Bihar and Rajasthan), *A. pallidus* (Ranchi, Bihar), *A. splendidus* (Ranchi), *A. annularis* (Rajasthan), *A. stephensi* (Bombay) and *A. subpictus* (Bombay and Delhi) from unsprayed areas. The degree of higher tolerance in these species suggested that they are somewhat naturally tolerant to dieldrin.

Increase in tolerance to dieldrin was also observed in *A. annularis*, *A. fluviatilis* from D.D.T. sprayed villages. The increase in tolerance was found to be of low order when compared with the MLC values for these species tested in nearby unsprayed areas; as such they may be attributed to vigour selection.

Resistance to dieldrin was observed in *A. splendidus* (D.D.T. sprayed areas in Ranchi, Bihar), *A. culicifacies* (D.D.T. and dieldrin sprayed areas of Bombay) and *A. subpictus* in 24 Parganas (West Bengal). The MLC of dieldrin for *A. splendidus* of D.D.T.-sprayed area (Ranchi, Bihar) was observed to be 3·5 per cent against 1·0 per cent in the unsprayed area. This indicated 3·5 times higher tolerance. The susceptibility data was further analysed by probit analysis method following the arithmatic technique. The MLCs of dieldrin thus estimated for this species from sprayed and unsprayed areas were found to be 4·4 - 3·1 per cent and 1·1 - 0·8 per cent respectively. The slope of the ld-pr line was found significantly less for the

sprayed area than the unsprayed area (Fig. 1). The significantly higher MLC with less slope of ld-pr line indicated the presence of resistant mosquitoes in fairly higher proportion in that population. The higher tolerance to dieldrin in *A. splendidus* was not found to be a stable one. The MLC values of dieldrin for this species from D.D.T. sprayed and unsprayed areas were found to be $0{\cdot}71 \times 1$ and $0{\cdot}27 \times 1$ respectively when tests were carried out after two months. Recently Patel *et al.* (1958) have reported higher MLC value, 3·0 per cent, in *A. culicifacies* of Patgaon village (Thana District, Bombay) sprayed with D.D.T. (during 1948 to 1956) and dieldrin (during 1953 and 1957). The resistance to dieldrin was found to be associated with an epidemic of malaria in that village. Fairly high tolerance to dieldrin (6·6 times) was also observed in *A. stephensi* larvæ of Erode.

Fig. 1. Fitted ld-pr lines for *A. splendidus* ♀ of D.D.T. sprayed (S) and unsprayed (U/S) areas.

C. fatigans was found to be naturally tolerant to dieldrin. The MLCs of dieldrin for this species from unsprayed and D.D.T.-sprayed villages were found to vary between 0·3 to 0·7 and 0·4 per cent respectively when exposed for one hour.

This species was found to be resistant to dieldrin in dieldrin sprayed areas of Nainital (Uttar Pradesh), Poona and Nagpur (Bombay). However the MLCs for *Aedes ægypti*, *M. uniformis* and *M. annulifera* were found to vary between 0·13 and 0·35 per cent. This suggests that *Aedes ægypti*, *M. uniformis* and *M. annulifera* were quite susceptible to dieldrin.

CONCLUSION.

From the above account it may be concluded that the susceptibility of the vector species (except *A. stephensi*) to D.D.T. and B.H.C. remained practically unchanged. *A. culicifacies* was found to be highly susceptible to D.D.T. and B.H.C. and *A. minimus* was found to be susceptible to all the three insecticides tested. In addition to the already reported cases of D.D.T.-resistance in *A. subpictus*, *A. stephensi* and *C. fatigans*, sporadic cases of high dieldrin tolerance have been observed in *A. splendidus* (Bihar), *A. subpictus* (West Bengal and Bombay). Specific cases of dieldrin-resistance were also observed in *A. culicifacies* (Bombay) and *C. fatigans* (Uttar Pradesh and Bombay) as a result of dieldrin spraying in those areas.

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LABORATORY AND FIELD DEVELOPMENT OF RESISTANCE IN MOSQUITOES*.

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INTRODUCTION.

AMONGST insects of public health importance, more attention has been paid to investigations on different aspects of insecticide resistance in houseflies than in mosquitoes. In mosquitoes most of the work has been confined to the determination of their insecticide tolerance in the field. These investigations provided valuable information on the selection of resistant strains of mosquitoes in sprayed areas or on their continued susceptibility to the insecticide in use. Laboratory studies, however, have been inadequate on the whole and confined to a few aspects of the phenomenon only.

The interest taken by the World Health Organization in the study of resistance phenomenon in insects has given a new impetus to the laboratory and field investigations in all countries covered by this agency.

It would be the intention here to bring to the notice of the workers salient points regarding what has been achieved in respect of insecticide resistance in mosquitoes, to point out the various lacunae which still exist in our knowledge and to emphasise the urgency of undertaking studies on particular aspects of the problem which may yield fruitful results.

LABORATORY INVESTIGATIONS ON DIFFERENT ASPECTS OF INSECTICIDE RESISTANCE IN MOSQUITOES.

Attempts in the laboratory, to induce resistance in *A. quadrimaculatus* by rearing under D.D.T. pressure, produced only a slight change in the tolerance of the species (Fay *et al.*,1949). Recently studies carried out with *A. gambiæ* and *A. stephensi*(Davidson, 1957*a*; Weyer, 1957) in the Ross Institute of Tropical Hygiene,

* Paper read in the Seminar on *Resistance of Insects to Insecticides* sponsored by the World Health Organization in collaboration with the Government of India, New Delhi, February 27 to March 7, 1958. Additional information has been incorporated to make the publication up-to-date till August, 1958.

London, and the Tropen Institute, Hamburg, showed that the insects became only slightly more tolerant to D.D.T. on selection and this increased tolerance disappeared immediately on withdrawal of the D.D.T. selection pressure. Similar results were obtained in India with *A. stephensi* and *A. fluviatilis*. Selection with D.D.T. did not change the insecticide susceptibility of the species significantly (Mohan, 1958). These studies showed that if insecticide selection pressure was exerted on a limited population of insects, in the absence of resistant individuals in the original population, any increased insecticide tolerance was not due to specific insecticide resistance but due to what has been termed as ' vigour tolerance ' (Hoskins and Gordon, 1956). These studies thus indicated that the appearance of resistant strains would depend not only on the intensity of selection but also on the size of the original insect population and absence or presence of individuals with resistant genes in it, and in the latter case their frequency of occurrence and effectiveness.

It has been the general contention that insecticide resistance in mosquitoes would appear quicker if the same chemical is used against both the larval and adult stages. From laboratory investigations on *A. atroparvus* where D.D.T. selection pressure was applied on larvæ only, on adults only and on both adults (females) and larvæ, it was observed that resistance appeared quicker when the insecticide was applied against both larvæ and adults than in the case of other two categories (Mosna, 1957). However, on critical perusal of the data the three strains did not appear to differ very much from each other in their susceptibility to D.D.T. It would, therefore, seem that besides other factors the level at which insecticide selection pressure is applied, is also important. In view of this it is desirable that studies are conducted with as many species of mosquitoes as possible to determine the speed of development of resistant strains, with varying degrees of insecticides selection pressure and elucidate correlation, if any, of resistance and selection pressure applied on larval and/or adult stages.

Observations have shown that resistant strains of *A. gambiæ* and *A. sundaicus* were more hardy than the susceptible strains and were easier to colonize and rear in the laboratory. Longevity of resistant *A. sundaicus* was also found to be more than that of the susceptible population (Davidson, 1957*a*). It is desirable, therefore, that thorough investigations are undertaken on the susceptible and resistant strains of different species with regard to their reproductive capacity, length of different stages and longevity etc.

Genetical experiments on field strains of dieldrin resistant *A. gambiæ* (Davidson, 1956), D.D.T. resistant *A. sundaicus* (Davidson, 1957*a*:1957*b*) and D.D.T. resistant *Aedes aegypti* (Coker, 1957), have shown that insecticide resistance is a monofactorial phenomenon. Extension of such studies to other species of *Anopheles*, *Aedes* and *Culex* would be of great value.

Studies on *A. gambiæ* and *A. sundaicus* have shown that by making use of discriminating dosages of the insecticide, it is possible to estimate the proportion of resistant and susceptible individuals in the original population of the species (Davidson, 1957*a*). Similar studies on other species will provide very valuable data. The information so obtained may help in the initial choice of an insecticide for the control of a particular species. An insecticide to which the maximum

proportion of individuals of the species are susceptible, may be used against it effectively.

Biochemical studies on D.D.T.-resistant strain of *C. fatigans* have shown that its larvæ, pupæ and adults contained 50 per cent more citric acid than the non-resistant strain. The phenomenon of resistance could be closely associated with the acceleration of certain metabolic reactions resulting in the accumulation of certain metabolites, citric acid being one. The question whether increase in citric acid *per se* has anything to do with resistance, is worth exploring (Dikshit *et al.*, 1956).

Studies on the dehydrochlorination of D.D.T. in *C. fatigans* (Bami *et al.*, 1957) and *A. sundaicus* (Kearns, 1957) adults and *Aedes aegypti*, *Aedes taeniorhynchus* (Brown and Perry, 1956) and *C fatigans* (Hoskins, 1958) larvæ have shown that metabolism of D.D.T. into D.D.E. was more in the case of D.D.T.-resistant strains as compared to susceptible strains. However, no such difference in the metabolism of B.H.C. could be observed in the B.H.C. and dieldrin resistant and susceptible strains of *A. gambiæ* (Bradbury and Standen, 1956). Metabolism of insecticide alone, therefore, does not seem to explain the resistant phenomenon in different strains of insects. Extremely low susceptibility to D.D.T. was observed in *A. maculipennis* (Iran) prior to hibernation when the fat body was developing (Zulueta *et al.*, 1957*a*). Therefore, factors like decreased insecticide absorption and its storage in the insect fat, may also be important and require detailed studies.

STUDIES ON INSECTICIDE RESISTANCE IN MOSQUITOES IN THE FIELD.

In the field, due to the extensive use of insecticides, number of resistant species of mosquitoes has increased considerably during the past few years. At present there are about eight species of *Anopheles*, three of *Culex*, five of *Aedes* and two of *Psorophora* which have been established to be resistant (Tables I to IV). In almost all instances of resistance, the particular insecticide has been used as a larvicide and/or as an adulticide. There are instances where selection of resistant mosquitoes has been attributed to the use of insecticides in the agricultural field. *A. quadrimaculatus* and very likely *Psorophora confinis* and *Psorophora discolor* in Mississippi have been reported to have become dieldrin-resistant mainly due to the use of this insecticide against agricultural pests (Mathis *et al.*, 1955: 1956). Insecticide resistant strains of mosquitoes have been recorded from almost all parts of the world where these chemicals have been used on a large scale. In all, over 20 countries have so far reported resistant strains of mosquitoes.

Field observations seem to indicate that selection of resistant strains by exposure of adults or larvæ alone did not confer the same degree of insecticide tolerance on one stage as it did on the other. For example, the degree of resistance was found to be not of the same magnitude in adults and larvæ of *A. subpictus* (Delhi, India) and *A. stephensi* (Madras, India) where insecticide selection was exerted on adults in the former and larvæ in the latter. D.D.T.-resistant *A. subpictus* adults showed a greater degree of tolerance to D.D.T. as compared to larvæ, whereas *A. stephensi* larvæ were more tolerant to the insecticide than adults (Sharma and Krishnamurthy, 1957*a*:1957*b* and Mammen, 1958). Detailed investigations are needed to understand this aspect of the problem.

TABLE I.

Insecticide resistance in different species of Anopheles.

Species	Adult or larvæ	Insecticide	Country	Year reported	Authority	Remarks
A. albimanus	Adults	D.D.T.	Panama	1954	Trapido (1954)	Resistance to control was due to acquisition of hyper-irritability to D.D.T. (Behaviouristic resistance).
A. darlingi	"	"	Southern Brazil	1953	Pinotti (1953)	Behaviouristic resistance suspected.
	"	"	North Argentina	1953	Hess (1953)	
A. gambiæ	"	Dieldrin	Nigeria	1956	Elliot and Ramakrishna (1956); Busvine (1956); Davidson (1956); Elliot and Armstrong (1957).	Not resistant to D.D.T.
	Adults and larvæ	"	Haute Volta	1957-58	Hamon (1957); Adam *et al.*, (1958).	
	Adults	"	Liberia	1957	Ramsdale (1957)	Only slightly more tolerant to D.D.T.
A. maculipennis	"	D.D.T.	Greece	1954	Belios (1954)	Resistance suspected.
	"	"	Iran	1957	Zulueta *et al.* (1957)	
A. quadrimaculatus	"	Dieldrin	Mississippi (U.S.A.)	1956	Mathis *et al.* (1956)	Not resistant to D.D.T. Dieldrin resistance was considered to be due to the contamination of breeding places with cotton dusting insecticides.
	"	"	"	1957	Quarterman (1957)	
A. sacharovi	"	D.D.T.	Lebanon	1954	Garrett-Jones and Gramiccia (1954)	
	"	"	Greece	1953-55	Livadas and Georgopoulos (1953); Georgopoulos (1954); Livadas (1955)	Also showed tolerance to B.H.C., dieldrin and chlordane when these insecticides replaced D.D.T for its control.
A. sacharovi	"	D.D.T. and Dieldrin	Greece	1956–57	Hadjinicolaou (1956); Belios as quoted by Brown (1958).	
	Larvæ	Chlordane and Dieldrin	Greece	1956	Belios and Fameliaris (1956).	
	Adults	D.D.T.	Iran	1957	de Zulueta *et al.* (1957)	
	"	"	Turkey	1958	de Zulueta (1958)	Normal susceptibility to dieldrin.

(*Contd.*)

TABLE I.—(*Concld.*).

Species	Adult or larva	Insecticide	Country	Year reported	Authority	Remarks
A. stephensi	Adults	D.D.T.	Saudi Arabia	1955	Davidson (1955*a*)	Normal susceptibility to dieldrin and gamma B.H.C.
	Larvæ	,,	South India	1956	Rajagopalan *et al.* (1956)	
	Adults	,,	Iran	1957	Mofidi *et al.* (1957)	
	Adults and larvæ	,,	Iraq	1957	Gramiccia *et al.* (1957)	
	Adults	,,	Persian Gulf (Dhahran)	1957	Daggy (1957)	
A. sundaicus	Adults	,,	Indonesia	1954	Grandel (1954)	Normal susceptibility to dieldrin and gamma B.H.C.
				1955	Davidson (1955*b*)	
	,,	,,	Java (Indonesia)	1956	Chow and Soeparno (1956)	
	,,	,,	Akyab (Burma)	1957	Delphin (1957)	
	,,	D.D.T. and Dieldrin	Java (Indonesia)	1957	Sundararaman (1957)	Behaviouristic resistance.
A. subpictus	,,	D.D.T.	Delhi (India)	1957	Sharma and Krishnamurty (1957*a*:1957*b*)	Not resistant to dieldrin and gamma B.H.C.
	,,	D.D.T. and Dieldrin	Bombay (India)	1957	Ramachandra Rao and Bhatia (1957)	In unsprayed areas and areas sprayed with D.D.T.
	,,	Dieldrin	Indonesia	1957	Davidson (1957*b*) and Ronnefeldt (1957).	Also showed tolerance to D.D.T.
A. superpictus	,,	,,	Greece	1954	Belios (1954)	Resistance suspected.

TABLE II.

Insecticide resistance in different species of Culex.

Species	Adults or larvæ	Insecticide	Country	Year reported	Authority	Remarks
C. fatigans (*quinquefasciatus*)	Adults	D.D.T.	India	1952	Pal *et al.* (1952)	
	Adults and larvæ.	,,	Reunion	1953-54	Hamon (1953) Hamon and Dufour (1954)	
	Larvæ	B.H.C.	South India	1954	Rajagopalan *et al.* (1953)	
	,,	,,	Malaya	1955	Reid (1955)	Also resistant to dieldrin to which never exposed.
	,,	D.D.T. and Dieldrin	Singapore	1956	Collins (1956)	
	,,	D.D.T.	Taiwan	1957	Liu (1957)	
	,,	D.D.T., B.H.C Chlordane and Dieldrin	Okinawa	1957	Gentry and Hubert (1957)	Susceptible to Malathion.
	,,	D.D.T.	Venezuela	1957	Blazques (1957).	
	Adults and larvæ.	Dieldrin and B.H.C.	Malaya	1957–58	Wharton (1957 and 1958)	Susceptible to D.D.T.
	,,	,,	Haute Volta	1958	Hamon *et al.* (1958)	
C. molestus (*pipiens*)	Adults	D.D.T.	Italy	1948	Mosna (1948)	Could be controlled by chlordane and B.H.C.
	,,	,,	Greece	1953	Livadas and Georgopoulos (1953)	
	Larvæ	,,	Ohio and Massachusetts (U.S.A.)	1953	Hess (1953)	
	Adults	Chlordane	Italy	1953	Simmons (1953)	
	,,	D.D.T.	Greece	1954	Hadjinicolaou (1954)	
	Larvæ	,,	Massachusetts (U.S.A.)	1955	Armstrong (1955)	
	,,	D.D.T., dieldrin and B.H.C.	Israel	1958	Gratz (1958)	
C. tarsalis	,,	D.D.T.	California (U.S.A.)	1950	Bohart and Murray (1950)	
	,,	B.H.C.	,,	1952	Gjullin and Peters (1952)	
	Larvæ and adults.	Malathion	,,	1957	Gjullin and Isaak (1957)	Susceptible to Parathion.
	Larvæ	D.D.T.	Oregon (U.S.A.)	1957	Eddy *et al.* (1957)	

TABLE III.

Insecticide resistance in different species of Aedes.

Species	Adults or larvæ	Insecticide	Country	Year reported	Authority	Remarks
A. nigromaculis *A. dorsalis*	Larvæ	D.D.T.	California (U.S.A.)	1950–52	Bohart and Murray (1950) Gjullin and Peters (1952)	
A. sollicitans	,,	,,	Florida (U.S.A.)	1950	Deonier *et al.* (1950)	
A. taeniorhynchus	,,	,,	,,	1950	King (1950)	
A. aegypti	,,	,,	Dutch Guiana	1953	Pinotti (1953)	
	,,	,,	Trinidad	1956	Brown and Perry (1956); Gillete (1956); Fay (1956)	No cross-tolerance to B.H.C. and Dieldrin
			Venezuela	1956	Gabaldon (1956)	
	,,	,,	Haiti	1956	Sautet and Vuillet (1956)	
	,,	,,	Colombia	1957	Pan-American Sanitary Bureau (1957) PASB Quarterly, 1 (7).	Susceptible to B.H.C.

TABLE IV.

Insecticide resistance in different species of Psorophora.

Species	Adults or larvæ	Insecticide	Country	Year reported	Authority	Remarks
P. confinis and *P. discolor*	Adults and larvæ	Dieldrin	Mississippi (U.S.A.)	1955	Mathis *et al.* (1955)	Appearance of the resistance was due to use of dieldrin as a larvicide in the ricefields as well as its widespread use on cotton crops in the region. No cross-resistance to D.D.T.

Field observations also indicate that like houseflies, as far as the chlorinated hydrocarbon insecticides are concerned, resistant mosquitoes can be grouped under two categories:

(*i*) Resistant to D.D.T. and its analogues but susceptible to gamma B.H.C. and dieldrin. Examples under this heading may be cited as those of D.D.T.-resistant *A. sundaicus* in Indonesia, *A. stephensi* in Saudi Arabia, Iran and Iraq, *A subpictus* in India which showed normal susceptibility to dieldrin and gamma B.H.C. (Crandel, 1954; Davidson, 1955*a*, Mofidi *et al.*, 1957; Gramiccia *et al.*, 1957 and Sharma *et al.*, 1957). Similarly D.D.T.-resistant *Aedes ægypti* larvæ in Trinidad showed no cross tolerance to dieldrin or gamma B.H.C. (Brown and Perry, 1956 and Busvine, 1957).

(*ii*) Resistant to gamma B.H C. and dieldrin etc., but susceptible to D.D.T. and its analogues as has been observed in the case of dieldrin and B.H.C. resistant *A. gambiæ* (Davidson, 1956) in Nigeria, *A. quadrimaculatus* in Mississippi and *C. fatigans* in Malaya which were susceptible to D.D.T. Similarly *A. quadrimaculatus* larvæ in Mississippi and *C. fatigans* larvæ in Malaya resistant to dieldrin and gamma B.H.C., showed normal susceptibility to D.D.T. (Mathis *et al.*, 1956; Reid, 1955; Wharton, 1957:1958).

But unlike the houseflies, instances of multi-resistant strains of mosquitoes are few. D.D.T.-resistant *A. sacharovi* in Greece also showed tolerance to B.H.C., dieldrin and chlordane when these insecticides replaced D.D.T. for its control (Georgopoulous, 1954). Similarly D.D.T.-resistant larvæ of *C. fatigans* in Okinawa and Singapore, *C. tarsalis* in California and *C. molestus* in Israel became resistant to B.H.C. and dieldrin etc., when these compounds were used on a large scale for their control (Gentry and Hubert, 1957; Collins, 1956; Gjullin and Peters, 1952; Gratz, 1958).

The only instance of resistance with organophosphorus insecticides is the malathion-resistant strain of *C. tarsalis* (larvæ and adults) in California which appeared after the use of malathion as a larvicide for two-and-a-half years (Gjullin and Isaak, 1957).

Above are the instances of physiological resistance in mosquitoes. The only confirmed example of behaviouristic resistance is that of *A. albimanus* in Panama against D.D.T. (Trapido, 1954). Recently similar observations have been reported about *A. sundaicus* in Indonesia (Sundararaman, 1957).

Besides resistant mosquitoes, there is every possibility in the field of selection of strains showing 'vigour tolerance' to insecticides. The repercussion, if any, of such selection on disease control operations requires detailed investigations.

As against this somewhat gloomy picture, observations in a number of countries failed to show any change in insecticide susceptibility in mosquitoes after D.D.T. had been in use for several years. Years of house spraying have evidently not changed the susceptibility of *A. atroparvus* in Spain, Sardinia and Italy and of *A. culicifacies* in Ceylon and India, of *A. pseudopunctipennis* in Mexico or of *A. darlingi* in British Guiana (Busvine, 1957). The recent investigations in Bombay State, India, on *A. culicifacies* showed that there was no correlation between the level of LC50 of D.D.T. and the number of years of insecticidal application (Ramachandra Rao and Bhatia, 1957). Complete disappearance of

A. minimus after D.D.T. spraying has been reported from Thailand (Jaswant Singh *et al.*, 1954 and Griffith *et al.*, 1957). Similarly observations carried out in a number of sprayed areas of India have shown almost complete absence of vector species like *A. minimus*, *A. philippinensis* and *A. fluviatilis* along with efficient malaria control, thereby indirectly indicating their continued susceptibility to the insecticides in use. It seems that it would be possible to eradicate at least the most important mosquito-borne disease, malaria, before the insecticides have a chance to become ineffective. The strategy of malaria control has, therefore, shifted to that of eradication within a few years, so that the disease is eliminated prior to the appearance of resistance.

It is, however, prudent to emphasize that it is necessary to keep a continuous vigilance for immediate detection of variation in the susceptibility to insecticides in mosquitoes or any change in their behaviour pattern in areas where insecticides are employed. This will ensure implementation of remedial measures for the control of resistant insects at the proper time.

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THE PRACTICAL IMPLICATIONS OF STUDIES ON INSECTICIDE RESISTANCE IN ANOPHELINE MOSQUITOES.

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CASES of resistance among anopheline mosquitoes to the chlorinated hydrocarbon insecticides are becoming more and more numerous. At the present time the World Health Organization in its weekly epidemiological records recognises six species as resistant in eleven different countries (Table I)*. There is one encouraging feature of this list and that is that five of the species show either resistance to the D.D.T. group of insecticides or to the dieldrin-B.H.C. group, but not to both groups simultaneously. *Anopheles subpictus* is resistant to D.D.T. in India but susceptible to dieldrin (Sharma and Krishnamurthy, 1957). In Java the same species is resistant to dieldrin and B.H.C., but not to D.D.T. (Davidson, 1957, unpublished report to World Health Organization). *A. sacharovi* is the only species in which resistance to both groups is indicated in one and the same population of mosquitoes. However, some doubt remains as to whether resistance to D.D.T. in this species is true specific resistance or natural or induced "vigour tolerance". The *A. maculipennis* group of anophelines have been shown elsewhere (Raffaele and Coluzzi, 1956: 1957) to have a high natural tolerance to D.D.T. in areas where insecticides have never been used and very high tolerances indeed have been recorded in members of this group when in the hibernating state (de Zulueta *et al.*, 1957).

Resistant *A. gambiæ* have been shown to be resistant to chlordane, dieldrin, aldrin, isodrin, endrin and gamma-B.H.C. in that order of degree of resistance (Davidson, 1956). To dieldrin they are about 800 times as resistant as the susceptible strain while to gamma-B.H.C. they are only some 30 times as resistant. Both *A. sundaicus* and *A. stephensi* have been shown to be resistant to D.D.T., D.D.D.

* Since this article was written numerous other cases of anopheline resistance to insecticides have come to light. Among the malaria vectors, *A. albimanus*, *A. aquasalis* and *A. pseudopunctipennis* in the New World have shown resistance to dieldrin while in the Old World *A. sergenti*, *A. culicifacies* and *A. fluviatilis* have shown resistance to the same insecticide. From reports so far given, it seems possible that *A. albimanus* is resistant to both D.D.T. and dieldrin in El Salvador. *A. sacharovi* in Turkey, while remaining susceptible to dieldrin, has become highly resistant to D.D.T. Among non-vectors, records of dieldrin-resistance have been made in *A. barbirostris*, *A. subpictus malayensis*, *A. vagus* and *A. annularis* from Java and in *A. pulcherrimus* and *A. constani* from Saudi Arabia.—*Author.*

TABLE I.

Proven cases of resistance in anopheline mosquitoes

Species	Countries	Resistant to	Susceptible to
A. gambiae	French West Africa Liberia Nigeria	Dieldrin and B.H.C.	D.D.T.
A. quadrimaculatus	U.S.A.	Dieldrin and B.H.C.	D.D.T.
A. sacharoni	Greece	D.D.T., Dieldrin and B.H.C.	..
A. stephensi	Iraq Iran Saudi Arabia	D.D.T.	Dieldrin and B.H.C.
A. subpictus subpictus	India	D.D.T.	Dieldrin
A. subpictus subpictus	Java	Dieldrin and B.H.C.	D.D.T.
A. sundaicus	Java Burma	D.D.T.	Dieldrin and B.H.C.

(dichloro-diphenyl-dichloroethane), methoxychlor and "Perthane" (diethyl-diphenyl-dichloroethane). The degree of resistance to D.D.T. is some 40 times in *A. sundaicus*, but somewhat lower in *A. stephensi*.

The first practical implication of these findings is that a change of insecticide from the D.D.T. group to the dieldrin-B.H.C. group or vice versa should result in effective control of resistant species. This has, in fact, already been shown in parts of the Middle East, in Java and in Northern Nigeria. In the first two areas where D.D.T. resistance in *A. stephensi* and *A. sundaicus* respectively occurs, a change of insecticide has been made with dramatic reductions in mosquitoes and malaria. In Northern Nigeria, D.D.T. has replaced dieldrin and B.H.C. in the areas of resistance with satisfactory results.

The manner in which dieldrin-B.H.C. resistance in *A. gambiæ* and D.D.T. resistance in *A. sundaicus* is inherited is now precisely known (Davidson, 1958). In both cases a single gene is involved but whereas the hybrid *A. gambiæ* shows an intermediate degree of resistance (partial dominance), the hybrid *A. sundaicus* is virtually as susceptible as the susceptible strain (resistance is a recessive character).

In *A. gambiæ* three phenotypes can be recognized and two discriminating dosages of insecticide can be used to separate them. In terms of the adult testing technique of Busvine and Nash (1953), 0·4 per cent dieldrin in Risella oil will kill all susceptible *A. gambiæ* after one hour's exposure. This has been established for five susceptible strains of this species from Nigeria and the Belgian Congo, maintained as colonies in the Ross Institute in London. Four per cent dieldrin will kill

almost all hybrid *A. gambiæ* after one hour's exposure and certainly all after two hours' exposure. At this latter dosage and exposure time none of three resistant strains of *A. gambiæ* from Western Sokoto and Kano in Northern Nigeria and from Bobo Dioulasso in French West Africa, again maintained as colonies in London, show any mortality at all.

With *A. sundaicus*, only two phenotypes are recognisable and only a single, slightly imperfect, discriminating dosage is possible. In terms of the Busvine test, four per cent D.D.T. in Risella oil wil kill nearly all susceptibles and hybrids and only a very few resistants after one hour's exposure.

D.D.T. resistance in *A. stephensi* has yet to be fully worked out. Certainly the degree of resistance is even lower than that in *A. sundaicus* and even a single discriminating dosage is impossible to find. A susceptible strain of this species from India and maintained as a laboratory colony for some ten years is almost completely killed by four per cent D.D.T. for one hour, which dosage kills about 50 per cent hybrids and some ten per cent of a resistant strain from Iraq.

A knowledge of these discriminating dosages and of the mode of inheritance of resistance immediately suggests a practical means of detecting resistance in the field, if not before insecticides are used then certainly before resistance is so well established that renewed transmission of malaria is occurring. The means involves, first of all, establishing the minimum insecticide dosage (for the Busvine technique) which will consistently kill 100 per cent of a susceptible strain of the species concerned. The susceptibility of most of the anopheline vectors of the world has already been established and shows remarkable consistency and so we are almost in a position to predict the LD100 of dieldrin and D.D.T. for susceptible strains. It is thought highly probable that 0·4 per cent dieldrin and 4·0 per cent D.D.T. in Risella oil and one hour's exposure time will cover most species though the larger mosquitoes of the *maculipennis-quadrimaculatus-hyrcanus* group may require higher dosages or longer exposure time.

Whether or not resistant mosquitoes are common enough in unselected populations to be detected remains to be seen. In a survey in the Western Sokoto area of Northern Nigeria one unsprayed area showed as many as six per cent of *A. gambiæ* surviving 0·4 per cent dieldrin for one hour (Armstrong *et al.*, 1956). Obviously if it were possible to detect resistance before spraying campaigns are launched the choice of insecticide could be determined at the outset.

Be that as it may, it is suggested that when spraying campaigns are started, mosquitoes reappearing after the use of a particular insecticide should be exposed to the LD100 of this insecticide. The normal methods of capturing adult mosquitoes in the field yield already fed and fertilized females. If these are exposed and any survive (even a single one) it is a simple thing to rear from them. Most gravid females, when confined in a small tube over water, will lay their eggs. The rearing of larvæ is a comparatively easy thing to do with local breeding water and fine food. If the offspring of these survivors are exposed to the same dosages as their parents then a very different mortality should be shown if resistance is present and relatively rare. For example, supposing the population showed one per cent survivors to 0·4 per cent dieldrin and the type of resistance was

the same as in *A. gambiæ*, the offspring of the survivors should show at least a 50 per cent survival after exposure to the same dosage. A 50 per cent survival would occur if the surviving female was a hybrid and had previously mated with a susceptible male. A 25 per cent mortality would result where the surviving female was a hybrid and had previously mated with a hybrid male. Any other mating than these two would result in no mortality in the offspring when they are exposed to the dosages of insecticide which kills only susceptibles. Table II summarises possible matings and expected mortalities in the offspring of the survivors showing resistance of the *A. gambiæ* type to dieldrin and related compounds.

TABLE II.

Semi-dominant resistance as in Anopheles gambiæ.

Expected mortalities at the lower discriminating dosage in the offspring of female mosquitoes surviving that dosage.

Possible crosses	RS♀×SS♂	RS♀×RS♂	RS♀×RR♂
Expected mortality in F_1	50	25	0

Possible crosses	RR♀×SS♂	RR♀×RS♂	RR♀×RR♂
Expected mortality in F_1	0	0	0

If resistance is already present and of the type already determined in *A. sundaicus* to D.D.T., the offspring may show 0, 50 or 100 per cent mortalities depending on whether the surviving female (which, if resistant must be a homozygote) has mated previously with a resistant, a hybrid or a susceptible male (Table III). If the latter mating had taken place the offspring would be all heterozygotes and therefore appear apparently susceptible when exposed to the discriminating dosage. It might therefore be presumed that resistance was not present and that the surviving females were actually susceptible at the extreme range of susceptibility. To eliminate this possibility it would then be necessary to interbreed some of these offspring to obtain a second generation. If the parents of this second generation were indeed hybrids, then the mortality in the offspring, when exposed to the discriminating dosage, would be 75 per cent. It is realised that this is a difficult proposition involving as it does the colonisation of the mosquito species but with base laboratory facilities it should not prove impossible.

TABLE III.

Recessive resistance as in Anopheles sundaicus.

Expected mortalities at the discriminating dosage in the offspring of female mosquitoes surviving that dosage.

Possible crosses	RR♀×RR♂	RR♀×RS♂	RR♀×SS♂
Expected mortality in F_1	0	50	100
Expected mortality in F_2	0	44	75

Once specific resistance is confirmed by this discriminating dosage method, it should be possible with adequate laboratory facilities to actually isolate the homozygous resistant strain.

Apart from the detection of resistance, the discriminating dosages can be used to determine the proportions of phenotypes in a population and to record the extent and spread of resistance and fluctuations in its prevalence (after cessation of insecticide pressure or after the change of insecticide, for example). Where three phenotypes can be distinguished using two such dosages as in *A. gambiæ*, it was previously thought permissible to expose a sample of a mixed population to the lower discriminating dosage, to determine the proportion of susceptibles in the sample and then to expose the survivors to a higher dosage to ascertain the proportions of hybrids and resistants. Recent work has shown, however, that when hybrid *A. gambiæ* are exposed first to 0·4 per cent dieldrin for one hour on one day and then to 4·0 per cent for one hour on the next day, instead of the near 100 per cent mortality obtained when they had not previously been exposed to dieldrin, only a 30 per cent mortality results.* It is as though exposure to the dosage, insufficient to kill the hybrid, stimulates the production of a defence mechanism in sufficient quantity or strength to enable many of them to survive a later higher dosage which, without previous stimulation by a lower dosage, would kill the mosquitoes before they could produce sufficient defence mechanisms to deal with the higher dosage all at once. The problem remains to be studied in more detail and the physiological implications considered. Thus it is necessary with this type of resistance to expose one sample to the lower dosage and separate sample to the higher one to accurately assess the phenotypic constitution of the population.

By this discriminating dosage technique it should be possible to distinguish between the non-specific increased tolerance to insecticides (sometimes referred to as "vigour tolerance") and true specific resistance. If the survivors from the discriminating dosages are "vigour tolerant" individuals, then their offspring should show mortalities similar to those recorded among the parents.

Contrast this direct method of detecting resistance with the present method of exposing mosquitoes from the sprayed and unsprayed areas to serial dosages of insecticide and comparing the dosages producing a 50 per cent kill. The difference between median lethal concentrations (M.L.C.) will depend on the proportion of susceptible, hybrid and resistant individuals in the population and where resistant individuals are few in number, only a slight, probably statistically-insignificant, difference will be shown. Furthermore, it would be difficult to distinguish between true specific resistance and "vigour tolerance" by this method.

The serial dosage technique, if carried out with the utmost care using very large numbers of mosquitoes and several closely spaced dosages, could in itself be used to determine discriminating dosages. Graphs 1 and 2 show log-probit regression lines drawn for mixed populations of known composition of *A. gambiæ* exposed to gamma-B.H.C. and dieldrin, based on actual mortalities recorded among the individual genotypes. A tripartite segregation of the graph is indicated in

*It has now been shown that the lower kill of hybrid *A. gambiæ*, exposed to 4 per cent. dieldrin for one hour after previous exposure to 0·4 per cent dieldrin for one hour, can also be produced by previous exposure to Risella oil alone. Thus the proposed explanation given above should be ignored.—*Author.*

Graph 1 and the discriminating dosages separating susceptibles, hybrids and resistants can be extrapolated. This is not too obvious and the points could actually be taken as lying on a single straight line. This is because the degree of resistance to gamma-B.H.C. is only some 30 times and the gaps between the LD_{100} of the susceptibles and the LD_0 of the hybrids and the LD_{100} of the hybrids and the LD_0 of the resistants are very small. If a straight line is drawn nearest to the points recorded for the F_2 generation, the M.L.C. would be read off as 0·044 per cent

GRAPH 1.

Log-probit regression lines for susceptible (SS), hybrid (RS) and resistant (RR) strains of A. gambiae *exposed for 1 hr. in varying concentrations of gamma-BHC in risella oil and calculated percentage mortalities in a theoretical F_2 generation composed of 25 per cent susceptibles, 50 per cent hybrids and 25 per cent resistants (C).*

gamma-B.H.C. as compared with 0·007 per cent for the susceptible strain and the degree of resistance of the population would be recorded as some six times. In actual fact, the pure resistant strain is some 30 times resistant.

GRAPH 2.

Log-probit regression lines for susceptible (SS) and hybrid (RS) strains of A. gambiae exposed for 1 hr. to varying concentrations of dieldrin in risella oil and calculated percentage mortalities in the theoretical backcross offspring (50 per cent susceptibles and 50 per cent hybrids) of a hybrid to the susceptible parent (C).

Resistance to dieldrin is much higher (some 800 times) and Graph 2 shows the regression line for a population composed of 50 per cent susceptibles and 50 per cent hybrids. A definite step in the line separating the susceptible part of the population and the hybrid part is apparent where an increase in dosage produces no increase in kill (0·1 to 0·4 per cent dieldrin). As the degree of resistance to dieldrin is so high it is not possible to show the regression line of the resistant strain on this graph, where mortalities have been recorded after one hour's exposure to various concentrations of the insecticide. At this exposure time, no kill at all is recorded with the resistant strain even on a saturated solution of dieldrin in Risella oil.

Failure to recognize such steps in the regression line recorded in Nigeria in 1955 by Elliott and Ramakrishna (1956) as the result of the use of only small numbers of mosquitoes on only a few serial dosages, resulted in the recognition of a degree of resistance to dieldrin in *A. gambiæ* of only eight times in a mixed population containing some 15 per cent resistants, actually 800 times resistant to this insecticide. The recognition and use of discriminating dosages would have enabled the selection of the pure resistant strain to be made.

The present World Health Organization test kit for estimating the susceptibility of adult mosquitoes is based on the serial dosage technique. The number of dosages are few and widely spaced and would be scarcely adequate for the determination of discriminating dosages in mixed populations. Test kits, containing only the two discriminating dosages of dieldrin and the one of D.D.T., would be simpler to produce and in the light of present knowledge should suffice for the detection of resistance. One such kit, designed for the detection of dieldrin resistance in *A. gambiæ* and containing the lower discriminating dosage of dieldrin only, was produced temporarily but now seems to have been abandoned.

These same arguments concerning discriminating and serial dosages would apply equally to larval resistance. Discriminating dosages for the prevalent 24-hour larval test remain to be worked out but for the one hour larval test of Elliott (1958) they have been ascertained for dieldrin-resistant *A. gambiæ* and D.D.T.-resistant *A. sundaicus* and *A. stephensi*. The monofactorial mechanisms controlling the inheritance of resistance in adult *A. gambiæ* and *A. sundaicus* have been found to operate in the larvæ also. Details of this work are to be published shortly.

The finding of two types of specific resistance in anopheline mosquitoes and the solution of their modes of inheritance have other more general implications. Where the hybrid is partially resitant as in *A. gambiæ* and can be presumed to survive field dosages of dieldrin, selection of resistance by this insecticide will be very rapid. Such has apparently been the case in all areas of Africa where resistance has appeared. The same may be true to some extent of *A. stephensi* where, in the laboratory, the dosage killing susceptibles kills only some 50 per cent of hybrids. It is now known (Gramiccia 1957, unpublished report to World Health Organization) that in some areas in Iraq D.D.T.-resistant *A. stephensi* appeared after only one spraying with this insecticide. In Saudi Arabia, on the other hand (Daggy, 1957), it was some five years before the accumulation of D.D.T.-resistant *A. stephensi* began to cause an increase in malaria transmission. These differences, however, may be due to differences in selection pressure in the two areas (see below).

In the case of *A. sundaicus* where the hybrid is virtually as susceptible as the susceptible strain, selection would be expected to be very much slower. This seems to fit the facts as recorded in Java where resistance was not apparent for some three years after the first house spraying with D.D.T.

One thing is clear now that the inherent nature of specific resistance is established and that is that the higher the selection pressure the quicker any resistance present in the population will appear. Thus a more efficient insecticide or a combined attack on both larvæ and adults could all increase the speed of selection. Conversely, a less efficient insecticide, or a lower dosage, or a partial attack only on the insect population would delay the appearance of resistance, though, of course, such incomplete measures may not achieve the purpose of disease control. The habits of the mosquitoes themselves may also delay the appearance of resistance. If, for instance, the species is one that bites man relatively infrequently and rests only occasionally and for short periods in houses, selection will be limited. This may account for the absence so far of records of resistance in such species as *A. culicifacies* in India, *A. maculatus* in Malaya and even *A. gambiæ* in some parts of Africa. Alternatively of course the explanation may simply be that the gene for resistance is absent in these particular populations.

The use of mixtures of the two groups of chlorinated hydrocarbon insecticides to prevent the appearance of resistance to either one of them seems a logical indication. The choice at present would of necessity lie between D.D.T. and dieldrin or D.D.T. and gamma-B.H.C. The irritant properties of D.D.T. are well known and the indications are that these properties may in some cases result in mortalities too low to intercept malaria transmission. In a mixture, D.D.T. still continues to irritate and it seems highly probable that where the second insecticide is dieldrin, departure may be caused by D.D.T. before the mosquito acquires a lethal dose of dieldrin. Gamma-B.H.C. on the other hand, is much quicker acting and very short contact indeed on this insecticide is sufficient for the acquisition of a toxic dose. Mixtures of D.D.T. and B.H.C. have, in fact, shown the high initial kill of B.H.C. corresponding to the short persistence of this insecticide followed by the more moderate but more persistent kill of D.D.T. (Davidson, 1953; Burnett, 1957). Evidence of escape from dieldrin unharmed where this insecticide has been sprayed on top of D.D.T. has recently been given in Java and was actually interpreted as evidence for selection of behaviouristic resistance in *A. sundaicus* by the use of D.D.T.

The results of recent laboratory work (Davidson and Pollard, 1958) further favour the choice of B.H.C. as the second insecticide for use in mixtures with D.D.T. Here it was found that B.H.C., applied as in the field, was capable of killing *A. gambiæ* hybrids for some weeks after application and even homozygous resistants when the deposit is fresh. Dieldrin similarly applied had little effect on hybrids and none on pure resistant individuals. It will be recalled that the degree of resistance in *A. gambiæ* to gamma-B.H.C. is very much lower than that to dieldrin. This laboratory work has further implications which have recently been borne out in the field, namely that selection of resistance in *A. gambiæ* by B.H.C. will be somewhat slower than by dieldrin.

The very recent case of dieldrin resistance in *culicifacies* in Bombay State belies the statement made on page 421 above.—*Author.*

Latest reports from the Western Sokoto Pilot Project show that malaria transmission in areas sprayed with B.H.C. was less than in those sprayed with dieldrin.

In conclusion, as well as these practical implications, there are those of more academic interest arising from these studies of anopheline resistance and especially relevant to this year, 1958, when the centenary of the Darwin-Wallace conception of evolution has been celebrated. Here we have prime examples of evolution in actual progress in the form of pre-adapted mutations only brought to light by the agency of man and equally comparable with the classical example of melanism in the British peppered moth (Kettlewell, 1958).

SUMMARY.

1. Five of the six anopheline species recognized as resistant to insecticides at the present time show either resistance to the D.D.T. group or to the dieldrin-B.H.C. group of chlorinated hydrocarbons but not to both. The position of the sixth species, *A. sacharovi*, remains in doubt. A change of insecticide within the chlorinated hydrocarbons will thus suffice in most cases to ensure malaria control.

2. A knowledge of the modes of inheritance of resistance enables a recognition of the phenotypes to be made by the use of discriminating dosages instead of the current method of comparing median lethal concentrations in unsprayed and sprayed areas. The use of such discriminating dosages as have been found applicable to dieldrin-resistant *A. gambiæ* and D.D.T. resistant *A. sundaicus*, is discussed in detail.

3. The influence of dominance of the resistance factor on the speed of selection is mentioned as well as other general implications of the inherent nature of resistance.

4. The use of mixtures of D.D.T. and dieldrin and D.D.T. and gamma-B.H.C. for the possible prevention of the appearance of resistance, is considered.

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RESISTANCE OF HOUSEFLIES (*MUSCA DOMESTICA* L.) IN THE UNITED STATES TO ORGANOPHOSPHORUS INSECTICIDES.

BY

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[October 1, 1958.]

After several years of intensive use of D.D.T., houseflies began to develop resistance to this insecticide and subsequently to lindane, chlordane, and other chlorinated hydrocarbons. Fortunately, by the time resistance became so acute that these insecticides would no longer control houseflies satisfactorily, effective new insecticides, the organophosphorus compounds, had been found. These materials were first employed in baits, then as residuals and space sprays, and finally as larvicides. Each method of use proved so effective that the principles of sanitation were neglected, as in the early days of the use of D.D.T. However, from the outset research workers recognized the possibility of resistance to the organophosphorus insecticides and began laboratory experiments to see if it could be developed through selection and watching for signs of increased tolerance in the field.

Early laboratory studies showed that flies developed 7- to 10-fold resistance to parathion in 10 to 55 generations of selection (March, 1952; Pimentel *et al.*, 1953). By the fall of 1954, flies in several dairies in the vicinity of Orlando, Florida, exhibited 5- to 7-fold resistance to malathion (LaBrecque *et al.*, 1958*a*). By the fall of 1955 and early 1956, flies in these locations showed 5- to 20-fold resistance to malathion and also to dipterex (Lindquist, 1957). Thereafter resistance swiftly increased to the point that malathion would not provide effective fly control (LaBrecque and Wilson, 1957). LaBrecque *et al.* (1958*a*) found high resistance to dipterex, diazinon, and parathion, as well as malathion, in Florida houseflies. Reports from other areas indicate that resistance to the organophosphorus compounds has now become widespread throughout the United States. Malathion resistance

has been reported from California (Bohart *et al.*, 1958) and Georgia and Arizona (Fay *et al.*, 1958).

Hansens (1958) found diazinon resistance in several strains of houseflies in New Jersey, with cross-resistance extending to malathion, parathion, dipterex and other organophosphorus insecticides. J. G. Matthysse (personal communication) has reported the failure of diazinon to control houseflies in several barns in New York, with indications that the failures were following the classical picture of resistance development.

At the United States Department of Agriculture Laboratory at Orlando, Fla., tests to determine the degree of resistance of houseflies to organophosphorus compounds are run with contact sprays, baits, and residues. In the tests with contact sprays, female flies four to five days old are exposed to various concentrations of the insecticides in a wind tunnel. This apparatus consists essentially of a cylindrical tube 4 inch in diameter through which a column of air is moved at 4 m.p.h. by a suction fan. The flies in a cylindrical screen cage are placed in the centre of the tube. One-fourth milliliter of insecticide solution is atomized at a pressure of 1 p.s.i. into the mouth of the tube, and the flies are exposed momentarily as it is drawn through the cage. They are then transferred to untreated (screen) holding cages and furnished honey solution. The mortality is recorded after 24 hours. All tests are run in duplicate.

In the bait tests, the desired concentrations of the toxicants in acetone are added to dry sugar and the mixtures allowed to dry. Ten-gram samples are then spread over the bottoms of Petri dishes, and cylindrical screen cages, each containing 20 female flies, are placed on top of them so that the insects can feed *ad libitum*. The mortality is determined after 24 hours.

In the residue tests, the insecticides in acetone solutions are sprayed on plywood panels at the rate of 100 mg. per square foot. Twenty female flies, under half-sections of petri dishes, are exposed to the treated panels for intervals ranging from 15 minutes to 24 hours. At the completion of the exposure period, if less than 24 hours, the flies are transferred to untreated holding cages and given food and water. Mortality counts are made 24 hours after the beginning of the exposure. When a 24-hour exposure fails to cause complete mortality, the exposure is continued for 48 or 72 hours. All tests are replicated at least twice. Whenever possible, comparisons are made at an LC or LT 50 and 90. Occasionally there is less than 50 per cent mortality at the highest concentration tested, or more than 90 per cent at the lowest.

In a survey of the resistance of Florida houseflies in 1957, the authors (LaBrecque *et al.*, 1958*a*) found that, based on the LC-50's in tests with contact sprays, houseflies collected in eight dairies and six poultry houses in central and southern Florida were 3 to 133 times as resistant as normal flies to malathion, 11 of the strains were 1·3 to 72 times as resistant to dipterex, 5 strains were 5 to 38 times as resistant to diazinon, and 7 were 3 to > 18 time as resistant to parathion. In tests with poisoned baits 10 strains were 13 to > 105 times as resistant as normal flies to malathion, and four of these strains were 3 to 19 times as resistant to dipterex. In 1954 and 1956 the resistance in these four strains to dipterex or malathion baits did not exceed eight times normal.

Resistance was believed to be mainly physiological in nature. It was observed, however, that most strains showed a higher resistance to poisoned baits than to contact sprays. Kilpatrick and Schoof (1958) reported a strain of flies in which resistance to malathion baits was behavioral as well as physiological. Schmidt and LaBrecque (1959) conducted laboratory studies to determine the degree of behavioral resistance in the malathion-resistant Grothe colony. Radioactive baits were prepared by mixing P^{32}-phosphoric acid with sugar containing 1 per cent of malathion and were tested by the bait-test technique. Unpoisoned sugar was labelled with P^{32} and offered to flies in the same manner. After 24-hour exposures to the bait, all susceptible flies, 40 per cent of the resistant female flies, and 85 per cent of the resistant male flies were dead. The radioactivity in each fly was determined and converted to milligrams of bait consumed. The surviving males and some of the surviving females showed moderate behavioral resistance, as they consumed much less bait than unpoisoned sugar. However, they consumed more bait than the normal flies that died. Half the surviving females showed an extreme avoidance by feeding but slightly on the poisoned bait. No avoidance was evident in tests with dipterex baits.

Studies were conducted to determine the effect of selection with an organophosphorus and a chlorinated hydrocarbon insecticide when used individually, alternately and in combination, on the rate of development of resistance (LaBrecque *et al.*, 1958*b*). Colonies of houseflies from a susceptible strain were selected with contact sprays of D.D.T. and/or malathion for 32 generations. The resistance developed to D.D.T. was 40- to 100-fold when D.D.T. was used alone or in combination with malathion, 20- to 40-fold when the two were used in alternate generations, and 10- to 30-fold when malathion was used alone. The resistance developed to malathion was more than 2-fold when this insecticide was used alone or in combination with D.D.T. Flies that developed D.D.T.-resistance by selection with malathion only, showed a high rate of conversion of D.D.T. to D.D.E.

Wilson *et al.* (1959) described the establishment and maintenance of five colonies of houseflies resistant to various organophosphorus compounds in connection with studies on resistance in field strains of houseflies—the *C* colony, resistant to dipterex; a colony resistant to Bayer 21/199; the Grothe colony, resistant to malathion; and the Tropical *D* and *P* colonies, resistant to four organophosphorus compounds.

The *C* colony was established in December 1954 from flies collected at four barns that had been intensively treated with various organophosphorus baits. Each generation was fed the usual diet to which 0·02 per cent of dipterex was added. When the colony was established, the flies were about ten times as resistant as the regular-colony flies to dipterex in bait tests but showed no resistance in residue tests. No additional resistance was developed through 98 generations of selection at a high level, after which the colony was discontinued.

The Bayer 21/199 colony was established in February 1956 from the 101st generation of the L.D.D. colony (moderately resistant to lindane, D.D.T., and dieldrin). Selection was accomplished by exposing flies to contact sprays of the insecticide at a concentration that killed approximately 90 per cent of them. At the 55th generation, this colony was more than 70 times as resistant to Bayer

21/199 as the regular colony at the LC-50 level. The highest concentration of Bayer 21/199 that went into solution (8 per cent) killed less than 50 per cent of these flies.

The Grothe colony was established in February 1957 from a strain of houseflies that had developed a high resistance to malathion in the field. Selection by malathion contact sprays was maintained in each generation. By the 20th generation, the Grothe flies were about 100 times as resistant to malathion as regular flies at the LC-50 level.

The Tropical *D* and *P* colonies were established from a strain of flies that had developed a high resistance to malathion, diazinon, parathion, and dipterex in the field. The *D* colony was selected by contact sprays of diazinon, and at the 20th generation showed resistance of 60-fold at the LC-50 level. The *P* colony was selected with parathion, and at the 20th generation showed resistance of about 20-fold at the LC-50 level.

New reports of failure to control houseflies with the organophosphorus insecticides are constantly being received, and cause deep concern regarding the prospects of effective fly control in the next few years. The situation appears especially critical in Florida, particularly in poultry houses, where prompt disposal of manure is a formidable economic problem. Elsewhere additional emphasis on sanitation may help alleviate the problem, although some chemical control is always required.

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A REVIEW OF INSECTICIDE RESISTANCE IN THE BLUE TICK, *BOOPHILUS DECOLORATUS* KOCH IN SOUTH AFRICA.

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[September 15, 1958.]

INTRODUCTION.

In Africa the *Acarina* are represented by a large variety of tick species, many of which are parasitic on man and his domestic animals. As is common with many parasites, ticks are responsible for the transmission of pathogenic organisms capable of causing considerable economic loss and for this reason more satisfactory methods for the control of ticks are continually sought after.

In South Africa, tick control measures take the form of insecticidal treatment of cattle, sheep, goats and horses either by total immersion in large open dipping tanks or by spraying with specially designed mechanical sprayers. The method and frequency of treatment, and the nature of the insecticide used, is dependent on the tick species existing in the particular locality.

For most species of tick, adequate control measures are now available although the problem of tick control has been complicated by the development of insecticide resistance in one species, namely the blue tick, *Boophilus decoloratus* Koch.

THE BLUE TICK.

B. decoloratus, which is also parasitic on a variety of indigenous fauna, is a serious ectoparasite of domesticated animals. Large infestations of the blue tick can cause a general debility of stock but it is economically more important as a vector of several disease-causing organisms. The diseases known to be transmitted by the blue tick are red water fever (*Piroplasma bigeminum*) and gallsickness (*Anaplasma marginale*) in cattle, spirochætosis (*T. theileri*) in cattle, horses and other animals, and tick bite fever (*Dermacentroxenus rickettsi* var. *pijperi*) in man and various animals.

A map of the distribution of *B. decoloratus* shows that the species is most abundant in the eastern areas of South Africa. The distribution can be related

directly to the annual rainfall. Blue tick populations are greatest in areas receiving an annual average rainfall of more than 25 inches and does not exist in areas receiving an average annual rainfall of less than 10 to 15 inches (Theiler, 1949).

Of all species of ticks of economic significance in South Africa, the blue tick is apparently the most easily controlled. It is commonly termed a "one host" tick because of the characteristic of attaching to a host as a larva and remaining on the same host until mature. In the female, the period of time taken from the attachment of larva to full engorgement of the adult is an average of twenty-one days. The general practice of insecticidal dipping or spraying of cattle at seven-day intervals ensures that the blue tick will come into contact with the insecticide three times during its parasitic life. In consequence, the blue tick is more easily controlled than a variety of other cattle ticks, many of which possess "two or three host" characteristics and can remain on a single host for as short a period as six days. However, the control of the blue tick has become involved as a result of the facility to develop resistance to a variety of insecticides.

INSECTICIDE RESISTANCE DISPLAYED BY THE BLUE TICK.

For many years, blue tick infestations on cattle were effectively controlled throughout the country by weekly treatment with sodium arsenite at a concentration of 0·16 per cent As_2O_3. It was demonstrated on many farms that such a practice could reduce the blue tick population to negligible proportions and that failure to maintain arsenic treatment would soon result in a re-infestation, presumably from the indigenous fauna.

Resistance to sodium arsenite was shown to have developed in the blue tick population in the East London area in 1939, thirty to forty years after the introduction of sodium arsenite as a control measure (Du Toit, Graf and Bekker, 1941). In a short period of time, the arsenic-resistant blue tick covered a narrow coastal area stretching from Port Elizabeth in the south through Natal in the north. Gamma-B.H.C., which was exceedingly effective initially, was introduced for the control of the arsenic-resistant strain but after eighteen months of general use, this treatment became ineffective. This was shown to have resulted from the development of a high degree of resistance to gamma-B.H.C. Ticks, resistant to gamma-B.H.C., covered the same geographical area originally covered by the arsenic-resistant strain and from this area no examples were found of ticks resistant to gamma-B.H.C. which were not also resistant to sodium arsenite (Whitnall, Thorburn, McHardy, Whitehead and Meerholz, 1952).

In the face of the development of resistance to arsenic and B.H.C. in the blue tick from the eastern coastal areas, D.D.T. was introduced as a control measure. The first indications of resistance to D.D.T. were recorded during 1954, five to six years after the introduction of D.D.T. in the field (Whitehead, 1956). D.D.T. resistance occurred initially in isolated districts of the East London area but more recently reports of the failure of D.D.T. have been received from adjacent areas. Ticks showing resistance to D.D.T., remained resistant to both arsenic and gamma-B.H.C. Although resistance to sodium arsenite, gamma-B.H.C. and D.D.T. can be found in a single population, it is clear that the development of resistance in each case arose independently and it may be tentatively concluded that the mechanism of resistance to these compounds is not related.

An examination of the response to a number of insecticides, by several strains of the blue tick in laboratory tests, using both fully engorged adult female and unengorged larval ticks (Whitehead, 1959), revealed a pattern of cross-tolerance to insecticides similar in many respects to that found in houseflies and mosquitoes (Metcalf, 1955).

It was shown (Whitehead, 1959) that resistance to sodium arsenite was not related to resistance to any other insecticide. Resistance to B.H.C. resulted in an automatic cross-tolerance to toxaphene, chlordane, dieldrin and aldrin, all of which are chlorinated cyclic hydrocarbons. D.D.T.-resistance was not related to resistance to either sodium arsenite or the chlorinated cyclic hydrocarbons but conferred a substantial cross-tolerance to dilan, a mixture of nitro-paraffin analogues of D.D.T. In houseflies, resistance to D.D.T. does not confer a cross-tolerance to dilan (Metcalf, 1955), and this fact has been accepted as support for the dehydrochlorination theory of D.D.T. resistance. The relationship between D.D.T. resistance and resistance to dilan suggests that the mechanism of D.D.T. resistance in blue ticks is different from that in houseflies.

We have detected no resistance to the organo-phosphorus insecticides, malathion and diazinon in blue ticks which are resistant to sodium arsenite, B.H.C. or D.D.T., although Fiedler (1952) reported a cross-tolerance to parathion conferred by a combined resistance to arsenic and B.H.C. Our attempts to confirm Fiedler's (1952) results have been unsuccessful.

The degree of resistance that has developed to a variety of insecticides in some strains of the blue tick, can be observed in Table I. The L.C. 50 values were obtained from log concentration-probit mortality regression lines obtained with unengorged blue tick larvæ in a test described by Whitehead (1959).

TABLE I.

The LC_{50} values and factors of increased tolerance obtained with an immersion test procedure using two strains of unengorged blue tick larvæ.

Insecticide	LC_{50} Per cent Insecticide Concentration		Factor of increased tolerance
	Sensitive larvæ	As-gamma-B.H.C. resistant larvæ	
pp'D.D.T.	0·0049	0·0034	—
gamma B.H.C.	0·0002	0·2395	8×10^3
Chlordane	0·0042	30156·0*	7×10^6
Toxaphene	0·0008	0·8292	9×10^4
Dieldrin	0·0012	26971·0*	2×10^7
Aldrin	0·0017	71013·0*	3×10^7
Malathion	0·1034	0·0819	—
Diazinon	0·00074	0·00075	—

*Computed from results obtained with the highest concentrations used.

The results, from a study of cross-tolerance to insecticides in blue ticks, suggest a grouping of insecticides as follows:

1. Sodium arsenite.
2. D.D.T. and dilan.
3. Chlorinated cyclic hydrocarbons: gamma-B.H.C., toxaphene chlordane, dieldrin and aldrin.
4. Organo-phosphorus insecticides: diazinon and malathion.

Resistance to one insecticide in a group resulted in automatic resistance to all insecticides in the same group. Although resistance to several of the above groups of insecticides exists in ticks from some localities, the development of resistance to each group arose independently.

THE DEVELOPMENT AND DISTRIBUTION OF THE DIFFERENT STRAINS OF RESISTANT BLUE TICKS.

Apart from a few isolated occurrences of gamma-B.H.C. resistance in ticks from inland areas of South Africa, insecticide resistance appears to be confined to a narrow area along the eastern coast. The development of strains resistant to sodium arsenite, gamma-B.H.C. and D.D.T. has always been recorded first from the East London area and later north and south along the coast. The high humidities of the coastal belt favour the development of the blue tick which, in consequence, necessitates the frequent application of control measures. These two factors, *viz.*, a high reproductive potential and frequent insecticidal treatment, appear to have favoured the development of resistant strains. If it is assumed that the factor for resistance to any particular insecticide exists in all populations of *B. decoloratus* then the rapidity of the development of a resistant strain will depend on the number of generations per year and the frequency of insecticidal treatment. There is no reason to assume that there is any difference between the blue tick from the east coast and the same species occurring inland. In consequence, resistance to insecticides might be expected to develop in blue ticks from inland areas in much the same way as it has done along the coast. However, the time taken for the establishment of resistant populations in blue ticks from inland areas, would be extended because of reduced reproductive potential resulting from lower atmospheric humidity and consequent reduced frequency of treatment. This is substantiated by the fact that gamma-B.H.C. resistance has developed inland in the Transvaal four to five years after the first use of B.H.C., whereas resistance developed in blue ticks from the coast after eighteen months of the field use of gamma-B.H.C. preparations.

THE MECHANISMS OF INSECTICIDE RESISTANCE IN THE BLUE TICK.

The toxic action of arsenicals has been associated with thiol-containing enzymes in insects and a variety of other organisms (Voegtlin, 1923; Fink, 1927; Forgash, 1957). With the advantage presented by the availability of both arsenic-resistant and arsenic-sensitive strains of the blue tick, we have investigated the levels of some sulphydryl containing compounds in the two strains. An

examination of tick eggs from an arsenic-resistant strain showed a sulphydryl level twice that found in eggs from arsenic susceptible ticks (Thompson and Johnston, 1958).

Our determination of cystine, cysteine, glutathione and free sulphydryl in blue tick larvæ showed an increased level of these compounds in arsenic-resistant larvæ as compared with arsenic-sensitive larvæ. These results suggest that the mechanism of resistance to sodium arsenite in ticks may be a result of an increased level of several –SH containing compounds.

In view of the differences between the cross-tolerance conferred by D.D.T. resistance in blue ticks and that in houseflies, we have attempted to extract D.D.T.-dehydrochlorinase from D.D.T.-resistant ticks. Using the method described by Sternburg (1954), no "in vitro" breakdown of D.D.T. could be detected, although the results could not be checked with extracts from D.D.T.-resistant houseflies as such a strain was not available to us. It is significant, however, that Roulston (1957) working with a D.D.T.-resistant strain of the Australian cattle tick, *Boophilus microplus* (Canestrini) could not detect the D.D.T.-dehydrochlorinase enzyme whereas he was able to obtain it from a strain of D.D.T.-resistant houseflies.

Repeated attempts to detect the mono-dechlorination product of B.H.C., hexachlorocyclohexene detected by Sternburg (1956) and later by Bradbury and Standen (1958) could not be detected by us in gamma-B.H.C. treated B.H.C.-resistant adult female blue ticks. However, these results should not be considered conclusive as no B.H.C.-resistant houseflies were available for comparative purposes.

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FIELD EFFECTIVENESS OF MALATHION DEPOSITS AGAINST DIELDRIN-RESISTANT *ANOPHELES QUADRIMACULATUS*.

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In tests conducted under simulated field conditions in 1957 against dieldrin-resistant *Anopheles quadrimaculatus*, malathion suspensions applied at rates of 100 or 200 mg. per square foot were found to give essentially 100 per cent kill of adult females for four to five months (Mathis and Schoof, 1959). In 1958, the studies were continued and expanded to include treatment of both vacant and occupied houses in the State of Mississippi, U.S.A.

METHODS.

The treated removable plywood panels used in the 1957 experiment at Savannah, Georgia, U.S.A., were subjected to further evaluation in the same shed-type huts (6′×6′ with a 7′ front) (Mathis and Schoof, 1959). The huts were equipped with window traps on two sides and were fitted with an entrance cage (2′×1′×1′) at the rear wall of the hut two feet above the floor level. An opening (1″×20″) permitted the mosquitoes to enter the hut, which contained a domestic rabbit as an attractant and a source of food. The panels had been sprayed in 1957 with the following treatments: (*a*) D.D.T. (200 mg./sq. ft.); (*b*) dieldrin (25 or 50 mg./sq. ft.); (*c*) malathion (100 or 200 mg./sq. ft.); (*d*) malathion: D.D.T. (200:100 or 100:200 mg./sq. ft.); (*e*) malathion:dieldrin (100:25 or 50 mg./sq. ft.); (f) parathion (12·5 mg./sq.ft.); (g) parathion: D.D.T. (12·5:200 mg./sq. ft.); and (*h*) EPN:DDT (25:200 mg./sq. ft.).

In the vacant house treatments in Mississippi, the rooms were lined with cardboard prior to the experiment to insure an insecticide-free pre-treatment surface

and to mosquito-proof the rooms. Treatments were applied to the cardboard-lined walls and ceiling, using a hand sprayer equipped with a nozzle calibrated to deliver 0·4 gal./min. (type 8004). After treatment, the floors were covered with wrapping paper to aid in the recovery of dead mosquitoes. In each room, two windows were screened and allowed to remain open constantly for ventilation. Each formulation was tested in two rooms, with a third untreated room serving as a check. Treatments were applied on March 26 and 27, 1958, and included toxicants and dosages as follows: (*a*) malathion (50, 100, and 200 mg./sq. ft.), (*b*) D.D.T. (200 mg./sq. ft.) and (*c*) malathion:D.D.T. (50:100 and 100:100 mg./sq. ft.). Two rooms were treated with each formulation. The suspensions used were prepared from 25 per cent malathion and 75 per cent D.D.T. wettable powders.

The occupied homes were the residences of rural families, each dwelling being treated by an operational spray crew* which used a power sprayer equipped with an 8004 nozzle. With the exception of the dosage of 200 mg. of malathion/sq. ft., each of the same dosages applied to unoccupied houses also was applied to five occupied homes (total of 25 houses treated).

The appraisal of the effectiveness of the treatments in the huts at Savannah, Georgia, was conducted at monthly intervals by introducing 100 specimens of dieldrin-resistant or susceptible *A. quadrimaculatus* females into the entrance cage at 4·00 p.m. and removing the dead and living specimens from the house and window traps from 8 to 9 a.m. the following morning. The live adults were collected and held for 24 hours for mortality determinations.

The vacant room treatments were evaluated by the release of 100 field-collected female *A. quadrimaculatus* in each room at four-week intervals beginning June 1 and continuing until October. The specimens were liberated in the rooms at 7.00 p.m. and recovered at 8.00 a.m. the following day. Specimens recovered alive were held 24 hours for mortality determinations.

For additional evaluation in the unoccupied houses, wall cage tests were made using polished transparent plastic funnel-shaped cones† (8·5 cm. in diameter at the base and 5·5 cm. high) in which the stem of the funnel had an aperture 1·0 cm. in diameter. The base of the funnel had a circular flange 1·5 cm. in width to which an adhesive type tape could be fitted which would permit the cone to be attached to uneven surfaces. An aspirator with an angular tip (135°) was used to introduce and remove the specimens from the cones‡. In each room, five cones (each with ten females) were attached to the walls at five different locations for a 30-minute exposure period. After exposure, the specimens were removed and held for a 24-hour mortality count. Both release and wall cage tests were made during the same week.

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* The Bolivar County Health Department, Cleveland, Mississippi, U.S.A., kindly provided the services of one of their regular spray crews which was treating approximately 6,000 homes on country-wide basis using a malathion : DDT spray.

† These cones were furnished through the courtesy of Mr. James W. Wright, Division of Environmental Sanitation, World Health Organization, Geneva, Switzerland.

‡ To prevent contamination of the aspirator by the malathion deposits, a separate aspirator was used for exposing adults in the untreated rooms. Both the cones and the aspirator, used for the treated surfaces tests, were decontaminated between successive evaluations by washing them in soapy water.

In the occupied houses, evaluation of the treatments was limited to use of the plastic cones. Five sites were tested in each of three houses treated with a given formulation, beginning one week after treatment and continuing at four- to five-week intervals until October.

RESULTS.

Since insufficient numbers of dieldrin-resistant *A. quadrimaculatus* were available, the tests at Savannah, Georgia, were evaluated chiefly with a susceptible laboratory strain. The results (Table I) show that parathion alone at 12·5 mg./sq. ft. was ineffective at the first test in 1958. In combination with D.D.T., the same dosage of parathion gave essentially equivalent results to D.D.T. alone at 200 mg./sq. ft. at 14 months. Therefore, both parathion formulations were discarded. Malathion at 100 or 200 mg./sq. ft. gave excellent results for 15 months but at 16 months the lower dosage showed signs of failure. Combinations of malathion with either D.D.T. or dieldrin produced complete mortalities at the last tests (15 months). Since the D.D.T. applications gave a much lower kill at this same

TABLE I.

Per cent mortality of susceptible Anopheles quadrimaculatus *females exposed to residual treatments in animal baited huts.*

Material, water wettable	Dosage (mg./sq.ft.)	Per cent mortality at months after treatment					
		3–5*	12	13	14	15	16
D.D.T.	200‡	68	..	..	74†	57	71§
Dieldrin	50	99	..	95†	85	..	63
Dieldrin	25	100	79†	51	62	12	
Malathion	200	.	..	..	100†	100	100
Malathion	100	..	100†	100	100	91	57
Malathion: D.D.T.	100:200	.	..	100†	89	100	
Malathion: D.D.T.	200:100	..		96†	100	100	
Malathion: dieldrin	100:50	100	.	99†	100	100	
Malathion: dieldrin	100:25	..	94†	100	91	100	
Parathion	12·5	100	41†	.	.	..	..
Parathion: D.D.T.	12·5:200	100	..	82†	..	.	
EPN : D.D.T.	25:200	98		100†	78	99	

* September 1957
† June 1958
‡ Actual dosage estimated as 175 mg./sq. ft.
§ At 17 and 18 months, mortality was 57 and 11 per cent, respectively

time interval, the effectiveness of the combination treatments presumably was due to the malathion component. Dieldrin at 25 mg./sq. ft. showed a definite break in effectiveness after 12 months, and the 50 mg./sq. ft. dosage was ineffective at the last test.

When dieldrin-resistant mosquitoes were available, tests were run against dieldrin (25 and 50 mg./sq. ft.), D.D.T. (200 mg./sq. ft.) and malathion (100 mg./sq. ft.). At four and twelve months, the dieldrin dosages gave kills of 40 and 19 per cent (25 mg./sq. ft.), and 46 and 7 per cent (50 mg./sq. ft.); the D.D.T. gave kills of 54 and 32 per cent; and the malathion 100 and 96 per cent, respectively.

In the unoccupied houses in Mississippi, tests through 25 weeks after treatment resulted in complete mortalities of either released specimens or those exposed in the plastic cone, with the exception of one room which had been treated with malathion at the rate of 50 mg./sq. ft. With released mosquitoes, the mortalities in this room were 80, 87, 93, 92, and 86 per cent at 10, 14, 18, 22, and 25 weeks, respectively, after treatment. With specimens in the cone, the mortalities were 75, 85, 76, 76 and 50 per cent at the same intervals after treatment. As the other room treated with this dosage gave 100 per cent mortality for the duration of the experiment, the reduced kills in the one room were considered a reflection of inadequate treatment. Since the release test at 25 weeks gave essentially the same mortality as in previous tests, the data indicate that an insufficient amount of toxicant was present rather than its deterioration.

In the occupied houses, the cone tests, started in the second week after treatment in June 1958, showed that all formulations gave complete mortalities of dieldrin-resistant *A. quadrimaculatus* for the duration of the experiment (12-16 weeks). In twenty-five houses treated as part of the routine spraying of rural dwellings by the Bolivar County Health Department*, similar tests also gave complete mortalities of female mosquitoes for the entire evaluation period (June-September).

DISCUSSION.

The results of treatments in either occupied or unoccupied houses in Mississippi verify the data from the 1957 Savannah tests in the animal baited huts, which showed that malathion at 100 mg./sq. ft. and malathion: D.D.T. at 100:200 or 200:100 mg./sq. ft. gave 100 per cent kill of dieldrin-resistant *A. quadrimaculatus* for four to five months. The continued evaluation of the plywood panels treated in 1957 revealed that the residues of malathion at 100 mg./sq. ft. gave essentially 100 per cent kill of dieldrin-resistant *A. quadrimaculatus* for 12 months (maximum period tested) and the same kill of a susceptible strain through 15 months with evidence of failure at 16 months.

The 1958 data also indicated that malathion:D.D.T. (50:100 mg./sq. ft.) was equivalent in effectiveness to the malathion treatment of 100 mg./sq. ft. for 25 weeks in the unoccupied houses. Malathion residues from the 50 mg./sq. ft. treatment likewise showed promising results which contrast sharply with earlier laboratory tests in which this dosage was ineffective.

*Treatment at a rate of 50:100 mg. of malathion:DDT per square foot.

In the two years of study, malathion has been shown to be an extremely effective residual insecticide against *A. quadrimaculatus*. The surfaces involved in these tests have included wood (painted and unpainted), cardboard, and paper. How durable the deposits would be on mud surfaces in the field is a question which can be resolved only through field tests inside such structures. The only previous data on such field treatments are from tests against culicine and anopheline adults in India by Krishnan *et al.*, who reported that diazinon (25 mg./sq. ft.) and malathion (25 mg./sq. ft.) gave control for only three weeks in houses with walls of bamboo matting or coconut leaves. Studies involving mud, leaf, and rice-straw surfaces are now being planned in Central America against *Anopheles albimanus*.

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COMPARATIVE SUSCEPTIBILITY OF D.D.T.-RESISTANT AND NORMAL *CULEX FATIGANS* LARVÆ TO DIPHENYL METHANE DERIVATIVES, CHLORINATED HYDROCARBONS AND ORGANOPHOSPHORUS INSECTICIDES.

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INTRODUCTION.

THE number of insects resistant to insecticides of different chemical groups have been steadily increasing. Attention has, therefore, been paid to the synthesis of newer insecticides; the outstanding example of which is the discovery of negatively correlated insecticides (Ascher and Kocher, 1954; Mitlin *et al.*, 1956). With this object in view, some newer compounds, *i.e.*, derivatives of diphenyl methane were evaluated for their toxicity against normal and resistant *C. fatigans* larvæ. Moreover, the comparative susceptibility of D.D.T.-resistant and normal *C. fatigans* larvæ to the commonly used insecticides such as D.D.T., D.D.D., dieldrin, B.H.C., malathion and phosdrin was also critically examined.

MATERIALS AND METHODS.

1. INSECTICIDES.

The following derivatives of diphenyl methane were evaluated.

1. 2-hydroxy-5-methyl-2′-chlorodiphenyl methane.
2. 4-methyl-4-hydroxy-2′-chlorodiphenyl methane.
3. 4-hydroxy-2′-chlorodiphenyl methane.
4. 2-methyl-4-hydroxy-3′-chlorodiphenyl methane.
5. 2-hydroxy-4-methyl-2′-chlorodiphenyl methane.
6. 2-hydroxy-5-methyl-3′-chlorodiphenyl methane.
7. 4-hydroxy-3′-chlorodiphenyl methane.
8. 2-hydroxy-3′-chlorodiphenyl methane.
9. 3-methyl-4-hydroxy-2′-chlorodiphenyl methane.

Besides these, D.D.T. (p'p'), B.H.C. (gamma), dieldrin (HEOD), D.D.D. (Technical), malathion (Technical 99 per cent purity) and phosdrin (63 per cent purity) were also tested.

2. TEST LARVÆ.

Late third- or early fourth-stage *C. fatigans* larvæ were obtained from the village Khuraji of Delhi State which is being sprayed with D.D.T. since 1947. *C. fatigans* adults from this village were reported to be resistant to D.D.T. (Pal *et al.*, 1952) and were 20 times as resistant as the normal species (Bani *et al.*, 1957). Susceptible *C. fatigans* larvæ were obtained from the colony maintained, for many years, at the Malaria Institute of India.

3. TEST PROCEDURE.

The World Health Organization method for assessing the susceptiblity of larvæ was followed (Brown, 1957). Various concentrations of alcoholic solution of the insecticide were pipetted at the rate of 1 ml./bowl into enamel bowls each containing 1 litre of water to obtain the desired concentration of the insecticide in parts per million. Thirty late third-stage larvæ were introduced into each bowl and exposed to the insecticide for 24 hours after which the mortality was recorded. The water temperature varied between 20-24° C. A minimum of six replicate tests were carried out with each compound.

4. BIOMETRIC TECHNIQUE.

The results of the experiments were analysed by the probit analysis method (Finney, 1952). The median lethal concentration with its fiducial limits, and regression coefficient values with standard error, were calculated.

RESULTS AND CONCLUSIONS.

(a) *Toxicity of diphenyl methane derivatives to normal and D.D.T.-resistant* C. fatigans *larvæ.*

The MLCs with fiducial limits and regression coefficients of the derivatives of diphenyl methane for normal and D.D.T.-resistant *C. fatigans* larvæ are given in Table I. The results indicated that these compounds were toxic to both susceptible and D.D.T.-resistant larvæ. The most active of these compounds was 4-hydroxy-3'-chlorodiphenyl methane, the M.L.C. of which was 1·7 and 3·4 p.p.m. for normal and D.D.T.-resistant larvæ respectively. This compound was found to be about four times more toxic to D.D.T.-resistant *C. fatigans* larvæ than D.D.T. The range of dosages required to obtain from very low to high mortalities with some of these compounds was found to be significantly lower than the range of the dosages of commonly used insecticides (regression coefficient values of these compounds varied fom 2·6 to 8·5, whereas in the case of commonly used insecticides these varied from 1·6 to 4·9).

From Table I it may be seen that the MLC (1·7 p.p.m. with fiducial limits 1·6-1·9) of 4-hydroxy-3'-chlorodiphenyl methane, [Fig. I(I)] was significantly less

TABLE I.

LC_{50} with fiducial limits and Regression coefficients of diphenyl methane derivatives for normal and D.D.T. resistant C. fatigans *larvæ.*

Compound	Normal larvæ			D.D.T. resistant larvæ		
	LC_{50} (in parts per million)	Fiducial limits	Regression coefficient	LC_{50} (in parts per million)	Fiducial limits	Regression coefficient
2-hydroxy-4-methyl-2′-chlorodiphenyl-methane	8·3	7·9—8·7	5·9±0·4	9·7	9·6—9·8	6·6±0·4
2-hydroxy-5-methyl-2′-chlorodiphenyl methane	9·8	9·6—10·0	4·1±0·3	10·3	10·2—10·4	6·0±0·3
4-hydroxy-2′-chlorodiphenyl methane ..	9·0	9·0—9·9	5·9±0·5	15·6	14·9—16·3	7·1±0·6
3-methyl-4-hydroxy-2′-chlorodiphenyl methane	100 per cent mortality at 20·0 p.p.m.	..	..	100 per cent mortality at 20 p.p.m.	..	..
2-methyl 4-hydroxy-3-chlorodiphenyl methane	12·6	11·7—13·6	6·7±0·6	14·8	14·3—15·3	8·5±0·6
2-hydroxy-5-methyl-3′-chlorodiphenyl methane	4·3	4·0—4·7	4·6±0·4	4·5	4·0—4·5	4·7±0·4
4-hydroxy-3′-chlorodiphenyl methane ..	1·7	1·58—1·87	4·0±0·3	3·4	2·5—4·1	3·0±0·3
2-hydroxy-3′-chlorodiphenyl methane ..	3·9	3·4—4·4	2·6±0·2	10·7	10·3—11·2	6·6±0·5
3-methyl-4-hydroxy-2′-chlorodiphenyl methane	100 per cent kill at 10 parts per million.	..	..	100 per cent kill at 10 parts per million.		..

than the MLC value (9·4 p.p.m. with fiducial limits 9·0-9·9) of its isomer 4-hydroxy-2′-chlorodiphenyl methane [Fig. I(II)]. It is apparent that with the change in position of chlorine from 2′ to 3′ the toxicity of the compounds increased by about six times. From this it is evident that structural configuration seems to have a bearing on the toxicity of the compound. Similar difference in toxicity between p-p′-D.D.T., [Fig. I(III)] and its isomers o-p′-D.D.T. [Fig. I(IV)] and o-o′-D.D.T. [Fig. I(V)] has been observed by Cristol *et al.* (1946).

(*b*) *Toxicity of some chlorinated and organophosphorus insecticides to D.D.T. resistant* C. fatigans *larvæ.*

The detailed results of the probit analysis of the susceptibility of D.D.T.-resistant *C. fatigans* larvæ to various chlorinated and organophosphorus insecticides are given in Table II. From Table II it is seen that malathion was the most

FIG. I.

Structural formulae of diphenyl methane derivatives (I and II), D.D.T. and its isomers (III, IV and V).

toxic of all the insecticides tested. The MLC of malathion was 0·044 p.p.m. The order of toxicity of various insecticides was malathion> phosdrin> D.D.D.> dieldrin> B.H.C.> D.D.T. From Table II, it may also be seen that malathion had the highest regression coefficient value (4·86±0·3). The regression coefficient value of dieldrin, B.H.C. and phosdrin varied from 3·24 to 3·74 whereas D.D.T. had the least value i.e. 1·6±0·1. The results indicated that malathion and phosdrin, the two organophosphorus compounds tested, were more effective than the chlorinated hydrocarbon insecticides.

TABLE II.

Probit analysis results of susceptibility tests of D.D.T.-resistant C. fatigans *larvæ to various insecticides.*

Insecticide	Equation of the fitted lc-pr line Y=	MLC (in parts per million)	Fiducial limits	Regression coefficient	χ^2 (Degree of freedom)*	X=log† (z)
D.D.T. (p-p')	—0·0970+1·61X	14·97	12·50—17·94	1·61±0·15	4·84 (23)	X100
D.D.T. (Technical)	1·6254+2·57X	0·20	0·19— 0·22	2·57±0·14	3·77 (38)	X100
Dieldrin (HEOD)	0·3580+3·24X	0·27	0·26— 0·31	3·24±0·14	7·03 (49)	X100
B.H.C. (gamma)	0·5103+2·88X	0·36	0·33— 0·40	2·88±0·19	2·66 (28)	X100
Malathion	1·8797+4·86X	0·044	0·041—0·047	4·86±0·3	6·79 (22)	X100
Phosdrin (Shell)	1·6353+3·74X	0·079	0·076—0·083	3·74±0·11	9·55 (55)	X100

* Degree of freedom is number of batches in all concentrations minus two.
† Dosage is multiplied by the factor to give a positive value of X.

(c) *Toxicity of some chlorinated and organophosphorus insecticides to normal* C. fatigans *larvæ.*

The results of the biometric analysis of the susceptibility of normal *C. fatigans* larvæ to D.D.T., dieldrin, B.H.C., D.D.D., malathion and phosdrin are given in Table III. From Table III, it may be seen that the MLC of malathion and D.D.D. were 0·028 and 0·032 p.p.m. which were not significantly different from one another. Both of these insecticides were found to be more toxic than the other insecticides tested. The MLC of pp'-D.D.T., which was the least effective of the insecticides tested, was 0·23 p.p.m. D.D.D., malathion and phosdrin had very high regression coefficient values ranging from 3·1 to 3·5 whereas pp'-D.D.T., which was least toxic of all the insecticides tested, had the lowest regression coefficient value i.e. 1·6 ± 0·1. It may, therefore, be concluded from Table III that the order of effectiveness of various insecticides was malathion = D.D.D. > phosdrin > B.H.C. = dieldrin > D.D.T.

TABLE III.

Biometric analysis results of the susceptibility tests of normal C. fatigans *larvæ to various insecticides.*

Insecticide	Equation of the fitted log-pr line Y =	MLC (in parts per million)	Fiducial limits	Regression coefficient	χ^2 (degree of freedom)*	X = log† (z)
D.D.T. (p-p')	3·0878 + 1·4 X	0·23	0·19—0·28	1·4 ± 0·11	0·92 (20)	X100
D.D.D. (Technical)	0·3781 + 3·06 X	0·032	0·030—0·035	3·06 ± 0·14	9·22 (42)	X1000
Dieldrin (HEOD)	2·9080 + 1·87 X	0·13	0·12—0·14	1·87 ± 0·12	2·32 (54)	X100
B.H.C. (gamma)	2·4487 + 2·48 X	0·11	0·09—0·12	2·48 ± 0·18	1·87 (21)	X100
Malathion (Technical)	3·5772 + 3·16 X	0·028	0·026—0·030	3·16 ± 0·27	6·99 (16)	X100
Phosdrin (Shell)	2·3858 + 3·47 X	0·057	0·053—0·061	3·47 ± 0·14	19·28 (65)	X100

* Degree of freedom is number of batches in all concentrations minus two.
† Dosage is multiplied by the factor to give a positive value of X.

(d) *Comparative susceptibility of D.D.T.-resistant and normal* C. fatigans *larvæ to various insecticides.*

The MLC value of D.D.T. for normal *C. fatigans* larvæ and resistant larvæ was found to be 0·23 and 14·97 p.p.m. respectively indicating that the resistant larvæ were about 65 times as resistant as the normal larvæ. It has been reported that the degree of resistance exhibited by the adults and larvæ was not of the same magnitude in the case of *A. subpictus* (Sharma and Kalra, 1958); *A. stephensi* (Mammen, 1957—personal communication) and *A. aegypti* (Busvine and Coker, 1958). But, in the case of *C. fatigans* it would appear that the degree of resistance exhibited by the larvæ was also of very high order as is the case with the adult

mosquitoes. The MLCs of D.D.D. for susceptible and resistant *C. fatigans* larvæ were 0·032 p.p.m. with fiducial limits 0·030-0·035 and 0·20 with fiducial limits 0·19-0·22 respectively. The D.D.T.-resistant *C. fatigans* larvæ were about six times more tolerant to D.D.D. than normal *C. fatigans* larvæ. The regression coefficient value for D.D.D. for resistant larvæ is significantly less than that of the normal larvæ. About six times higher tolerance with flatter lc-pr line in D.D.T.-resistant larvæ (Chart 1) clearly showed that D.D.T.-resistant strain was also cross-tolerant to D.D.D. The MLCs of dieldrin, B.H.C., phosdrin and malathion for susceptible and D.D.T.-resistant *C. fatigans* larvæ were 0·13, 0·11, 0·057 and 0·028 and 0·27, 0·36, 0·079 and 0·044 parts per million respectively. D.D.T.-resistant larvæ were about 1·5 to 3·0 times more tolerant to these insecticides and regression coefficient values were significantly higher than those of susceptible larvæ (Chart 1). A higher MLC with a steeper slope, as compared to the MLC and slope of homogeneous susceptible population indicates vigour tolerance (Hoskin and Gordon, 1956).

CHART 1.

Slopes of lc-pr lines of dieldrin (HEOD) and D.D.D. (technical) for susceptible and resistant *C. fatigans* larvæ.

From the results discussed above, it would appear that D.D.T. residual spraying for about ten years has resulted in the selection of a strain which exhibited

resistance to D.D.T. and its analogue D.D.D., but only vigour tolerance to organophosphorus insecticides, B.H.C. and cyclodiene insecticides.

SUMMARY.

Various derivatives of diphenyl methane were evaluated as insecticides. These were found to be toxic to both susceptible and D.D.T.-resistant *C. fatigans* larvæ. The most toxic of these compounds was 4-hydroxy-3′chloro-diphenyl methane. Evidence is presented to show that a slight change in the structural configuration results in alteration of their toxicity.

The susceptibility of D.D.T.-resistant and susceptible *C. fatigans* larvæ to D.D.T., D.D.D., dieldrin, B.H.C., malathion and phosdrin was also determined. Malathion was found to be the most toxic of all the insecticides tested, and was 300 and 10 times more toxic than D.D.T. to resistant and susceptible *C. fatigans* larvæ respectively.

It has been observed that D.D.T.-residual spraying for about ten years has resulted in the selection of *C. fatigans* strain which showed resistance to D.D.T. and its analogue D.D.D. but only vigour tolerance to B.H.C., organophosphorus and cyclodiene insecticides.

The degree of resistance exhibited by the larvæ to D.D.T. was also of very high order.

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BEHAVIOUR OF VARIOUS SUSCEPTIBLE AND RESISTANT STRAINS OF *MUSCA DOMESTICA* L. TOWARDS CETYL AND MYRISTYL BROMO- AND CHLOROACETATES.

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[November 3, 1958.]

In a preceding work (Bettini, Boccacci and Natalizi, 1958) it has been demonstrated that in order to evaluate toxicity differences, even between substances having a similar chemical nature, and therefore the same mode of action [iodo-, bromo- and chloroacetic acid and some of their esters (Boccacci and Bettini, 1956; Bettini and Boccacci, 1958)] it is important to depend on data which give a clear picture of knock-down against time, since between the substances under test there might be very marked differences in their speed of action. For instance, the ratio between the LD_{50} values of cetyl bromoacetate and cetyl chloroacetate is 1 : 2 at 24 hours while it is 1 : 9 at two hours.

In the present work, we have tested the toxicity of cetyl and myristyl bromo- and chloroacetates on various susceptible and resistant strains of *Musca domestica*, determining the LD_{50} values at different times from exposure, for a period of 24 hours.

Ascher (1958*a*:1958*b*) in a preceding research, had found that cetyl bromoacetate showed an enhanced knock-down in various resistant strains, compared with susceptible strains, employing, however, a method [continuous tarsal contact (Kocher, Roth and Treboux, 1953)] which considers knock-down within two hours only.

Only limited data, representative of the numerous tests carried out in this research, are reported in the present note. The statistical analysis of all data derived from these experiments which are still under way, will be reported elsewhere and will include the results of other tests carried out on other products.

MATERIAL AND METHOD.

The following strains of *M. domestica* raised in our laboratory, according to the usual technique, have been employed.

S-Roma	.. Susceptible.
Svizzera	.. Susceptible.
K_1	.. Highly resistant to D. D. T. and resistant to other chlorinated hydrocarbons (Geigy).
R-Sardinia	.. Highly resistant to chlordane.
P_2	.. Resistant to diazinone (Geigy).
Ac—R	.. R-Sardinia selected with chloroacetic acid for 80 generations.

Three-day old females, average weight mg. 20·0 ± 0·8, have been used for toxicity tests. Experiments have been carried out at room temperature (24° -28° C.). The contact technique has been described in a previous paper (Bettini, Boccacci and Natalizi, 1958) and it consists in exposing flies for a period of 30 minutes to different concentrations of the substance in petri dishes.

RESULTS AND DISCUSSION.

As can be observed from Tables I (A, B) and II, the LD_{50} values at 24 hours for each substance are substantially similar for the various strains tested; the only exceptions are strains R-Sardinia which results more susceptible to cetyl bromoacetate, and K_1 which results more susceptible to myristyl bromoacetate.

TABLE I.

LD_{50} values g/m² at different times after exposure for various strains of Musca domestica.

(A) *Cetyl bromoacetate.*

Strain	Hours									
	1	2	3	4	5	6	7	8	9	24
R-Sardinia	0·40	0·28	0·26	0·24		0·24	..	..	..	0·22
Svizzera ..	..	0·56	0·52	0·45	0·39	0·37	0·38	..	0·31	0·28
S-Roma ..	0·65	0·46	0·42	0·40	0·36	0·34	..	..	..	0·30
AcR ..	..	0·38	..	0·35	0·34	0·34	0·33	..	..	0·31
K_1 ..	0·73	0·45	0·40	0·37	0·36	..	..	0·34	..	0·33
P_2 ..	0·46	0·44	0·42	0·40	0·38	0·36	..	..	..	0·34

(B) Cetyl chloroacetate.

Strain	Hours									
	1	2	3	4	5	6	7	8	9	24
AcR ..	..	..	0·83	0·70	0·58	0·55	0·48	..	..	0·38
R-Sardinia	..	4·60	1·70	1·00	0·73	0·66	..	..	..	0·52
Svizzera ..	..	2·30	1·00	0·83	0·73	0·62	0·62	..	0·58	0·53
K_1 ..	..	3·20	1·27	1·05	0·84	..	..	0·68	..	0·62
P_2 ..	..	..	1·25	0·90	0·71	..	..	..	..	0·64
S-Roma ..	..	..	1·75	1·30	1·00	0·87	..	..	..	0·73

TABLE II.

LD_{50} values (g/m²) at 24 hours for various strains of Musca domestica *exposed to myristyl bromo- and chloroacetate.*

Strain	LD_{50} at 24 hours	
	Myristyl bromoacetate	Myristyl chloroacetate
S-Roma (susceptible)	0·26	0·42
Svizzera (susceptible)	0·25	0·47
R-Sardinia (resistant)	0·27	0·42
K_1 (resistant)	0·20	0·42
AcR (resistant)	0·30	0·49
P_2 (resistant)	0·24	0·44

Our results seem to show that there is no correlation between resistance, *sensu lato*, to insecticides* and susceptibility to the substances tested. Considering cetyl bromoacetate in particular, it can be observed that only the strain R-Sardinia, out of the four resistant strains, has shown an LD_{50} lower than that of both the susceptible strains; while strains P_2, AcR and K_1 have shown LD_{50} values equal or higher than those of susceptible strains.

These results are not wholly in agreement with those reported by Ascher (1958*a*:1958*b*), who observed higher knock-down action of cetyl bromoacetate for the four resistant strains P_2, K_1, R-Sardinia and AcR, compared with the two susceptible strains S-Rome and London. (The strains used by the above mentioned

*In the present paper, the term "insecticides" refers only to the products commonly employed and to which field strains have shown resistance.

author are those employed in the present work, with the exception of the susceptible strain London in place of which the susceptible strain Svizzera has been used).

As can be observed from Chart 1, where the reciprocals of LD_{50} values against time have been reported for cetyl bromoacetate, the speed of action may differ from strain to strain, inasmuch that, while at two hours strain AcR, for instance, shows an LD_{50} lower than that of the two susceptible strains, at 24 hours, however, it shows an LD_{50} equal or even higher.

CHART 1.

Reciprocals of LD_{50} values against time for female house-flies from different strains in contact with cetyl bromoacetate.

These differences in speed of action could explain the differences between our results and those obtained by Ascher (1958*a*:1958*b*) since this author has employed a method by which knock-down values within two hours only of continuous contact were considered.

It is highly probable that the nature of the differences, in speed of action and in susceptibility of some strains to some of the susbtances tested, is related to peculiar characteristics of that particular strain rather than to a general phenomenon, connected with resistance, induced by any type of insecticide. In view of this, it is

interesting to note that strain R-Sardinia, in the case of cetyl bromoacetate, shows to be the most susceptible one; strain K_1, in the case of myristyl bromoacetate; while in all cases the other resistant strains show an LD_{50} for the substances tested almost equal to that of susceptible strains.

In any case, for the resistant strains R-Sardinia and K_1, which result more susceptible to the substances tested, it is clear that one cannot speak of " negative correlation " between these substances and, respectively, chlordane and D.D.T., for, according to the definition accepted by the World Health Organization's experts " a negatively-correlated pair of insecticides *A* and *B* is such that as resistance to *A* develops, generation by generation, so does susceptibility to *B* increase " (World Health Organization, 1958).

Therefore, in order to obtain data on the eventual " negative correlation ", the strains from which the resistant ones have originated should be available.

In this regard, it is useful to recall the work by Mitlin *et al.* (1956) who observed that the diisopropyl ester of 1, 2, 2, 2-tetrachloroethyl phosphoric acid was 2·6 times more toxic (LD_{50} at 24 hours) to a D. D. T.-resistant strain than to a susceptible strain, and that the substance was the only one, out of a series of esters of the corresponding acid, to show a " negative correlation ". In this work, however, the data are referred only to two strains, a resistant one, Orlando Beltville, and a susceptible one, 1948 NAIDM, of different origin. According to the above mentioned definition, therefore, in this case also one cannot speak of " negative correlation ". The same can be said for the data, reported by Ascher and Kocher (1954), in connection with their experiments with KBr.

On the other hand, clear examples of " negative correlation " can be ascribed to the work by Ogita, as reported by Tsukamoto (1958), who found that a D.D.T.-resistant strain of *Drosophila melanogaster* was always susceptible to phenyl thiocarbamide and that a single dominant gene, located in the region of the D.D.T.-resistant gene on the 2nd chromosome, was responsible for the susceptibility to phenyl thiocarbamide; and to the work by Kearns (1957) who reported that a D.D.T.-resistant strain of *M. domestica*, following exposure to unpurified isopropyl-tetrachloroethyl phosphate for three generations, was transformed into a susceptible strain.

SUMMARY AND CONCLUSIONS.

The speed of action and the LD_{50} values at 24 hours for cetyl and myristyl bromo- and chloroacetates, have been determined on six strains (two susceptible ones, S-Roma and Svizzera and four resistant ones R-Sardinia, Ac-R, P_2 and K_1) of *Musca domestica*.

According to the present results it can be excluded that, in general, a higher susceptibility exists for insecticide-resistant strains toward the substances tested. It is believed that for the two strains R-Sardinia and K_1, which are exceptions to the said rule, the difference in susceptibility, respectively toward cetyl and myristyl bromoacetates, are due to the characteristics of the strains.

In any case, for these two strains, there are no elements to state that a " negative correlation " (according to the above mentioned definition) exists, for,

in order to demonstrate such a "negative correlation" one should necessarily have available the strains from which the resistant ones have originated, or to have available genetically controlled strains.

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THE RELATION BETWEEN RESISTANCE TO INSECTICIDES AND THE EFFECT OF CETYL BROMOACETATE IN DANISH STRAINS OF *MUSCA DOMESTICA* L.

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[December 9, 1958.]

INTRODUCTION.

In recent discussions of countermeasures against insecticide resistance, much attention has been given to compounds showing a higher toxicity to strains resistant to D.D.T. and other insecticides than to susceptible strains.

Thus, at the Seminar on the Resistance of Insects to Insecticides in New Delhi, 1958, Brown considered the discovery of such compounds to be " the most promising recent development in research that might give us an answer to the resistance problem ".

One of the compounds Brown referred to, and perhaps the most promising one, was cetyl bromoacetate, which Ascher (1957: 1958) had tested on house-flies by a tarsal contact method and found that the rate of knock-down was greater in strains selected for resistance to D.D.T. and other insecticides than in susceptible strains.

At the request of the Division of Environmental Sanitation, World Health Organization, our laboratory undertook to test the effect of cetyl bromoacetate (which in the following will be designated as *C.B.A.*), on the D.D.T.-resistance of wild fly populations on farms.

Preliminary to the field tests, it was planned to investigate the correlation between susceptibility to C.B.A. and resistance to D.D.T. etc. in various Danish strains kept in our laboratory. Some results of these laboratory experiments are reported in the present paper.

They comprised (*a*) tarsal contact tests with C.B.A. comparing the speed of knock-down in different strains, and (*b*) selection experiments with two heterogeneous D.D.T.-resistant strains submitted to C.B.A.-pressure for several generations.

MATERIAL AND METHODS.

FLY STRAINS.

As the purpose was to investigate the correlation between resistance to insecticides and other physiological characters, we considered it important to compare strains of the same origin (field sample), but with different history of insecticidal pressure in the laboratory. Furthermore we preferred as usual to use strains which had acquired their insecticide resistance in the field.

For the experiments reported in this paper, three groups of strains were used namely No. 17 (*ba*, *bb*, *bc*, *f* and *e*), No. 150 (*a* and *b*), and No. 203 (*a*, *ac*, and *b*).

The history of these strains and their present pattern of resistance is given below:

Strains 17 ba, 17 bb, 17 f, and 17 e.

Origin: About 50 flies collected on a farm (Tillitze, Lolland) in July 1950 (Keiding, 1953).

Following the use of D.D.T., B.H.C., chlordane and toxaphene during the years 1945-50, Strain 17 developed resistance to these and other chlorinated hydrocarbons. At the time of collection, however, it was heterogeneous as to D.D.T.-resistance segregating in about 30 per cent knock-down susceptible, and 70 per cent resistant. After ten generations in the laboratory without insecticidal pressure, several substrains were submitted to selective pressure with various insecticides (Keiding 1957).

17 *ba* (previously called 17*b*).—D.D.T.-pressure from January 1951 to March 1954. Newly hatched flies (less than one day old) were exposed to D.D.T. deposits for three hours in 31 out of 64 generations. The D.D.T.-dosage was kept constant from the 4th generation, the aim being to keep the D.D.T.-resistance at a constant high level. As a result, there was very little or no mortality after the first generations. All the flies were knockdown-resistant (assumed to be homozygous for the gene *kdr.* (Keiding, 1957)). In the same period, resistance to B.H.C. and the cyclodien-group decreased considerably.

Since March 1954 the strain has not been exposed to insecticidal pressure with the result that some reversion (by segregation) to susceptibility took place. Thus at the beginning of the present investigations only about 30 per cent were knock-down-resistant (*kdr.*).

17 *bb*.—Strain 17 *ba* exposed to selective D.D.T.-pressure during February-April 1958 for four generations.

Males and females were separated before mating and exposed to D.D.T.-deposits. Already in F_1 (date of test March 5, 1958) the strain was almost homozygous for the *kdr*-gene. D.D.T.-selection was repeated in the three next generations and then discontinued when the strain was considered purely D.D.T.-resistant.

17 *bc*.—Strain 17 *ba* exposed to selective pressure with C.B.A. from April, 1958. The details of the selections are described below.

17 *e*.—The history of selective pressure with insecticides is similar to 17 *ba*, 17 *bb*, except that in Strain 17 *e* the flies have been exposed to a mixture of D.D.T., lindane, and chlordane from January 1951 to March 1954, and, after a decline

in resistance, to a mixture of lindane and chlordane only from February 1958 to June 1958.

At the time when it was used for test with C.B.A., it was highly resistant to B.H.C. and the cyclodien-group and partly resistant to D.D.T.

17*f* (Keiding, 1957; Bøggild and Keiding, 1958) was developed from Strain 17 by selecting for knock-down susceptibility to D.D.T. from December 1951 to March 1955. Newly hatched flies were exposed to D.D.T. at low temperature and the first flies affected removed for recovery in the breeding cage at higher temperature. It is now susceptible to D.D.T. and other insecticides tested.

Strains 150 (a and b)

Origin.—About 1,000 flies collected in October, 1955 on a farm (Vejløgaard) in South Zealand where the flies had developed some resistance to diazinon (about 15 times normal) and other organo-phosphorus insecticides (Keiding, 1956). Moreover it was partly resistant to D.D.T., lindane, and chlordane.

150 *a* has been kept without exposure to insecticides. The diazinon-resistance has decreased and is now only about five times normal (topical application).

150 *b.*—Diazinon-pressure from March, 1956 by exposure of flies to treated aluminium-foil usually in every other generation.

Strains 203 (a, ac and b)

203 *a.*—

Origin: About 1,200 flies collected in July, 1957 in a calf barn near Hillerød, North Zealand. The barn had been treated frequently with diazinon and in July, 1957 the flies showed a considerable resistance to diazinon (about 60 x) and parathion (about 15 x), while the strain was heterogeneous as to resistance to D.D.T., B.H.C., and chlordane.

The strain has been kept in the laboratory without selective pressure with the result that the resistance to diazinon has decreased and is now only about six times normal.

203 *ac.*—

Derived from 203 *a* in April 1958, when put on selective pressure with C.B.A. (see below).

203 *b.*—

Origin: 450 flies collected in October, 1958 in the same barn as strain 203 *a*. During the summer, the barn had been treated with both diazinon sprays and dipterex-baits, and the flies showed a high resistance to diazinon (about 80 x normal) and a considerable resistance to parathion, dipterex and malathion (about 12-15 x).

Strain En Ø.

Origin: About 50 flies collected on a farm in South Zealand in June 1955.

It was the most susceptible strain found among more than 100 strains tested from collections in 1955 on Danish farms. No selection in the laboratory.

Strain B.P.M.

A susceptible strain obtained as pupæ from University of Leiden, Holland, in August, 1955 and since kept without selection.

The pattern of resistance of the strains in the period when tests with C.B.A. took place, is summarized in Table I.

TABLE I.

Resistance to D.D.T., lindane and diazinon in the fly strains used for C.B.A.-investigations. Explanation of symbols: s = susceptible, (r) = slightly resistant, r = moderately resistant, rr = resistance intermediate, rrr = high resistance, s→rrr = the strain contains flies ranging from susceptible to highly resistant, kdr = knock-down-resistant. 5 × = LD_{50} 5 times normal.

Strain	Tarsal contact		Topical application
	D.D.T.	Lindane	Diazinon
	kdr		
Fn Ø	s	s	s
BPM	s	s	s
17 f	s	s	s
17 ba	s→rrr 30-40 per cent	s—r	s
17 bb	rrr 100 per cent	rr—rrr	s
17 c	s→rrr about 5 per cent	rrr	(r) 2x
150 a	s→r 0 per cent	s—r	r 5x
150 b	s→rr 0 per cent	s—r	rr 30x
203 a	s→rrr 40-50 per cent	s→rrr	r 6x
203 b	rr—rrr about 35 per cent	r→rrr	rrr 80x

BREEDING METHOD.

Breeding of flies took place in a room kept at 27-28° C. and 70-75 per cent RH. In order to avoid selection for short pre-imaginal development, eggs for the next generation were not taken until all flies in the breeding stock had passed their pre-oviposition period. The larvæ were reared in earthen-ware jars containing a mixture of wheat bran (400 g.), alfalfa meal (200 g.), malt (15 g.), live baker's yeast (10 g.) and 1 litre of water. As far as possible, each jar was seeded with about 3,000 eggs. After 9-10 days, the pupæ were separated from the medium by floating in water, and distributed in cages for hatching.

The peak-emergence was normally 12 days after egg-laying, and the "average age" of flies was calculated from that day.

When the hatching of adults was completed, the flies to be used for testing were kept at room temperature (about 22°C.).

Flies to be used for tarsal contact tests were given water and sugar only, while flies for insecticide-selection and topical application tests also received milk (whole milk powder or fresh milk).

TESTING METHODS.

TARSAL CONTACT ON SURFACES TREATED WITH C.B.A.

A method similar to Ascher's "tarsal contact of long duration" was used for *C.B.A.* in the experiments reported in the present paper. However, instead of using treated petri-dishes we used pieces of aluminium-foil (28·5 cm. × 8·5 cm. = 240 cm²) sprayed as cylinders on a turn-table with 25 ml. one per cent C.B.A.* in acetone. When the acetone had evaporated, the foil, evenly covered by tiny drops of C.B.A., was used to line the inner side and bottom of metal cylinders 8·5 cm. high and 8·5 cm. in diameter.

The cylinders were used for test one day after treatment.

The day before test the flies were sexed and counted under light ether anaesthesia into small cages each containing 50 ♀♀ or 50 ♂♂. By the counting, care was taken to make each group a balanced sample of all the flies from the particular culture. Next morning, the flies were knocked-down with CO_2 and transferred to the treated cylinders, which were covered by a loose lid of cellophane distended on a ring. After the transfer the flies quickly recovered from the CO_2 anaesthesia, normally in less than a minute. Usually 100 females and 100 males, distributed in eight treated cylinders, were used for each strain, with 50 ♀♀ + 50 ♂♂ as controls in untreated cylinders.

The number of flies knocked-down (KD, unable to walk) was noted every 15 minutes for the first two hours and then every 30 minutes until all were knocked-down.

During the test the flies were practically in continuous contact with the treated surface. The tests were carried out at a temperature of about 22° C. Flies not paralysed after six hours contact were fed water and sugar.

Age of the flies.—In some tests young flies (two to three days old) were used, but in most tests we used flies at a higher average age (Tables II and III) because the variation in susceptibility due to differences in rate of pre-imaginal development is less than in the young flies.

Mortality or knock-down in the controls was negligible.

D.D.T.

As in previous investigations of the pattern of D.D.T.-resistance in fly populations (Barbesgaard and Keiding, 1955; Keiding, 1957) flies were exposed continuously for 24 hours to blotting paper sprayed with a five per cent acetone solution of D.D.T. (dosage 5 g. per m²). The tests were carried out in the same way as with C.B.A.

*The cetyl bromoacetate used was prepared by Prof. E. D. Bergmann, Israel, and obtained through World Health Organization., Geneva.

The preparation contained 95·5 per cent cetyl bromoacetate as determined both by saponification and analysis of the bromine content.

RESULTS.

RATE OF KNOCK-DOWN IN TARSAL CONTACT TESTS WITH CBA.

When the percentage of flies knocked down was plotted on probability paper with time (not log time) as abscissa, the points usually fitted a straight line well (Graph 1). From regression lines drawn by hand the time (in minutes) for 50 per cent and 90 per cent knock-down, KDT_{50} and KDT_{90} respectively, was read.

TABLES II AND III.

Knock-down effect of cetyl bromoacetate (CBA) in 9 fly strains (Table 1). Method: Continuous tarsal contact with CBA on aluminium foil. KDT_{50-90} = time in minutes for 50 per cent and 90 per cent knock-down estimated graphically from time-probit response lines based on about 100 flies (Graph 1). 1. Curve bending downward. 2. Curve bending upward.

(*i*) TABLE II.

Date (Year 1958)		Females								
		EN Ø	BPM	17 f	17bb	17 e	150a	150b	203 a	203 b
Mar. 5	Age (days) ..	..	..	6	8	..	..	..	8	..
	Weight (mg) ..	..	..	22·5	15·0	..	..	..	16·5	..
	KDT 50–90 ..	..	..	*64–81*	*83–105*	..	..	..	*94–119*	..
Mar. 25	Age	8	..	9	8	..	..	..	..	..
	Weight ..	20·1	..	19·6	19·4	..	..	..	..	..
	KDT 50–90 ..	*60–93*	..	*57–81*	*75–97*	..	..	..	..	..
Apr. 9	Age	..	10	..	..	..	..	9	..	..
	Weight ..	..	16·5	..	..	..	..	24·6	..	..
	KDT 50–90 ..	..	*54[1]–95*	..	..	..	..	*125–175*	..	..
Apr. 24	Age	12	..	..	11	11	..	..	12	..
	Weight ..	20·2	..	..	20·3	20·6	..	..	18·8	..
	KDT 50–90 ..	*72–101*	..	..	*94–128*	*113–142*	..	..	*88–126*	..
May 1	Age	..	..	..	..	..	10	10	..	..
	Weight ..	..	..	..	..	..	18·7	17·6	..	..
	KDT 50–90 ..	..	..	..	..	..	*90–122*	*96–131*	..	..
June 10	Age	..	..	..	13	12	..	..	..	..
	Weight ..	..	..	..	14·0	15·9	..	..	..	..
	KDT 50–90 ..	..	..	..	*92[1]–135*	*130[2]–159*	..	..	..	..
July 11	Age	..	..	3	3	3	..	..	3	..
	Weight ..	..	..	18·9	17·8	16·9	..	..	19·4	..
	KDT 50–90 ..	..	..	*94–125*	*97–126*	*159–218*	..	..	*103[1]–139*	..
July 12	Age	..	2	..	2	..	..	..	..	..
	Weight ..	..	19·5	..	16·4	..	..	..	..	..
	KDT 50–90 ..	..	*91[1]–155*	..	*92[1]–140*	..	..	..	..	..
July 16	Age	..	..	..	8	8	..	..	..	..
	Weight ..	..	..	..	17·8	16·9	..	..	..	..
	KDT 50–90 ..	..	..	..	*95–119*	*150[2]–167*	..	..	..	..
Nov. 27	Age	..	..	9	..	..	..	..	..	9
	Weight ..	..	..	20·6	..	..	..	..	..	21·3
	KDT 50–90 ..	..	..	*73–101*	..	..	..	..	..	*100–123*

(*ii*) TABLE III.

Date (Year 1958)		Males								
		EN Ø	BPM	17 f	17 bb	17 e	150	150 b	203 a	203 b
Mar. 5	Age (days) ..	..	..	6	8	..	..	..	8	..
	Weight (mg) ..	..	..	15·4	11·5	..	..	..	12·6	..
	KDT 50-90 ..	..	..	*40-57*	*47-59*	..	..	..	*62-72*	..
Mar. 25	Age	8	..	9	8	..	..	..	..	..
	Weight ..	13·4	..	13·6	13·3	..	..	..	..	..
	KDT 50-90 ..	*39-63*	..	*34[1]-59*	*47-80*	..	..	..	..	..
May 1	Age	..	..	..	..	..	10	10	..	..
	Weight ..	..	..	..	..	..	13·8	12·7	..	..
	KDT 50-90 ..	..	..	..	..	..	*52[2]-92*	*75-119*	..	..
June 10	Age	..	..	..	13	12	..	..	..	..
	Weight ..	..	..	..	11·8	13·3	..	..	..	..
	KDT 50-90 ..	..	..	..	*70[1]-140*	*78[1]-113*	..	..	..	..
July 11	Age	..	..	3	3	3	..	..	3	..
	Weight ..	..	..	14·3	13·4	12·6	..	..	14·8	..
	KDT 50-90 ..	..	..	*54-74*	*61-81*	*103-141*	..	..	*59-75*	..
July 12	Age	..	2	..	2	..	..	..	..	..
	Weight ..	..	14·4	..	11·8	..	..	..	..	..
	KDT 50-90 ..	..	*57[1]-90*	..	*65[1]-100*	..	..	..	..	..
July 16	Age	..	..	..	8	8	..	..	..	..
	Weight ..	..	..	..	13·4	12·6	..	..	..	..
	KDT 50-90 ..	..	..	..	*56-72*	*84-120*	..	..	..	..
Nov. 27	Age	..	..	9	..	..	..	..	..	9
	Weight ..	..	..	13·8	..	..	..	..	..	15·7
	KDT 50-90 ..	..	..	*60-90*	..	..	..	..	..	*69-91*

The results are given in Tables II and III. In some cases indicated in the Tables, a single straight line did not fit the data when plotted on the time-probit diagrams. KDT_{50} and KDT_{90} were then obtained from log-time-probit diagrams, or by drawing the broken curve (often with a break about 50 per cent knock-down) which was indicated by the data.

It appears from the Tables that the males were always more susceptible to C.B.A. than the females, but there was no indication of a greater effect of C.B.A., measured as rate of knock-down, on the susceptible strains than on the resistant ones.

On the contrary the data suggest a positive correlation of resistance to insecticides and tolerance to the paralyzing effect of C.B.A. in our strains. Thus, in tests where the susceptible strain 17 *f* was directly compared with the D.D.T.-resistant 17 *bb*, the resistant strain was always affected later by C.B.A. than the susceptible one even in tests where the 17 *f*-flies were bigger than the 17 *b*-flies (e.g. March 5). The difference in C.B.A.-tolerance was most distinct when the flies were old, and the results indicate that the tolerance to C.B.A. in D.D.T.-susceptible flies decreases with age, while it seems less dependent of age (and size) in the D.D.T.-resistant strain.

GRAPH 1.

Examples of time-probit-regression lines from a tarsal contact test with cetyl bromoacetate (CBA), July 11, 1958. 3 days old females of strains 17 f (susceptible) and 17 e (lindane-chlordane-resistant).

For comparison, data from petridish tests of Ascher (1958) has been plotted. 2-3 days old females of strains S-Rome (susceptible) and R-Sardin (chlordane-resistant).

The effect of age on C.B.A.-tolerance will be further investigated.

When Strain 17 *bb* was compared with the highly lindane-chlordane-resistant Strain 17 *e*, the latter showed a significantly higher tolerance to C.B.A. irrespective of age, and Strain 17 *e* was the most C.B.A.-tolerant strain of all tested.

In the test May 1, where strains differing in resistance to organic phosphates (diazinon) were compared, the most diazinon-tolerant strain, 150*b*, was also somewhat more tolerant to C.B.A. However, the comparison needs to be repeated before we can say whether Strain 150*a* that has not been under insecticidal pressure in the laboratory, is really less C.B.A.-tolerant than Strain 150*b* whose diazinon-resistance has been maintained.

In order to have a strain represented which showed high resistance to diazinon and other organic phosphates, a test with the second laboratory generation of Strain 203 *b* has been included. It is of interest to note that this strain does not show a particularly high C.B.A.-tolerance compared with strain 203*a* or the strains resistant to chlorinated hydrocarbons, but susceptible to the organic phosphates (*e.g.* 17 *bb* and 17 *e*).

SELECTION IN STRAINS 17 *BC* AND 203 *AC* WITH C.B.A.

As mentioned above two strains, 17 *ba* and 203 *a*, were submitted to selective C.B.A.-pressure to study the effect on their resistance to D.D.T. and other insecticides.

These strains were chosen because they were both heterogeneous in regard to D.D.T.-resistance, as is generally the case with the Danish field populations of house-flies which have not been exposed to D.D.T. for some time (Keiding, 1958).

Thus the effect of C.B.A.-selection on the proportion of highly D.D.T.-resistant flies (genotype *kdr/kdr*) could be followed within these two strains.

As appears from Table I, Strain 203 *a* was more tolerant to lindane (and chlordane) and to organic phosphates insecticides than 17 *ba*.

The procedure of C.B.A.-selection was as follows:

Usually 800 pupæ were used for the selection. Females and males were separated every day during the hatching period to avoid mating before exposure to C.B.A.

When all flies had emerged, the flies were given milk-powder for some days and then exposed to aluminium-foil sprayed with 0·2 per cent or 0·4 per cent C.B.A. in acetone.

The exposure period varied from a quarter of an hour to seven hours for the females and from 10 to 90 minutes for the males. After exposure the flies were kept in blotting paper cages for 24 hours, and then the flies that were not paralysed or badly affected, were transferred to the breeding room for mating and egg-laying.

The percentages of flies selected for breeding in each generation appear in Table IV.

In Table V the results of some C.B.A.-tests are given. They show that the C.B.A.-pressure did increase the tolerance appreciably in Strain 203 *ac* and in the females of Strain 17 *bc*, whereas it is more doubtful in the males of that strain.

TABLE IV.

Selection with C.B.A. in strains 17 bc and 203 ac through ten generations.

Per cent flies selected for breeding.

Generation	Strain 17 bc				Strain 203 ac			
	Date (Year 1958)	♀♀	♂♂	√♀♀×♂♂	Date (Year 1958)	♀♀	♂♂	√♀♀×♂♂
1	March 26	49%	22%	33%	March 27	60%	70%	65%
2	April 16	68—	51—	59—	April 18	23—	20—	21—
3	May 13	39—	53—	45—	No selection	..	..	100—
4	June 4	16—	8—	11—	June 4	28—	26—	27—
5	No selection	..	..	100—	No selection	..	..	..
6	July 16*	96—	81—	88—	July 16*	85—	81—	83—
7	August 13	42—	31—	32—	August 8	57—	46—	51—
8	No selection	..	..	100—	September 4	29—	16—	23—
9	October 1	21—	31—	25—	No selection	..	..	100—
10	No selection	..	..	100—	November 5	30—	30—	30—

*Dosage, by mistake, only one-fourth of that intended.

TABLE V.

Knock-down effect of C.B.A. in strains 17 bc and 203 ac, selected for C.B.A.-resistance, and the corresponding unselected strains, 17 ba and 203 a.

(For further explanation see Tables II and III.)

Date	Generation		Females				Males			
			17 ba	17 bc	203 a	203 ac	17 ba	17 bc	203 a	203 ac
May 13, 1958	3	Age (days) ..	10	10	..	..	10	10	..	..
		Weight (mg) ..	16·1	14·2	..	..	11·6	11·5	..	..
		KDT 50–90 ..	*83–120*	*105[2]–124*	..	..	*57–85*	*51–75*	..	..
June 6-10, 1958	4	Age ..	12	12	11	11	12	12	11	11
		Weight ..	17·4	16.5	19·5	18·5	12·3	11·8	13·9	14·3
		KDT 50–90 ..	*91–123*	*112[2]–160*	*82–115*	*122–156*	*78–121*	*64[1]–93*	*60–100*	*102[2]–140*
July 11, 1958	6	Age ..	3	3	3	3	3	3	3	3
		Weight ..	19·6	17·7	19·4	20·5	14·9	13·5	14·8	15·9
		KDT 50–90 ..	*97–120*	*128–165*	*103[1]–139*	*141–178*	*64–84*	*75–96*	*59–75*	*93–122*

The effect of C.B.A.-pressure on the D.D.T.-resistance of the two strains is shown in Table VI.

TABLE VI.

D.D.T.-resistance in two heterogeneous D.D.T.-resistant strains, 17 ba and 203 a, kept without insecticidal pressure, compared with the D.D.T.-resistance in two strains, 17 bc and 203 ac, under selective pressure with C.B.A.

Tarsal contact tests with continuous exposure to D.D.T.-treated surfaces.

Generation	Sex	Per cent knock-down-resistant (KDR)				Per cent survival after 24 hours			
		17 ba	*17 bc*	*203 a*	*203 ac*	*17 ba*	*17 bc*	*203 a*	*203 ac*
2	♀♀	25	31	39	90	8	24	22	70
	♂♂	31	43	44	88	7	18	17	52
	♀♀+♂♂	28	37	41·5	89	..	..	..	..
3	♀♀	29	48	..	..	20	22	..	..
	♂♂	25	39	..	..	9	19	..	..
	♀♀+♂♂	27	43·5	..	..	..	..	..	..
4	♀♀	22	39	53	61	14	34	35	49
	♂♂	28	37	45	50	6	20	16	24
	♀♀+♂♂	25	38	49	55·5	..	..	..	..
6	♀♀	35	57	53	90	29	49	36	75
	♂♂	35	55	44	88	12	35	18	59
	♀♀+♂♂	35	56	48	89	..	..	..	..
8	♀♀	..	..	39	90	..	..	23	59
	♂♂	..	..	42	90	..	..	5	53
	♀♀+♂♂	..	..	40·5	90	..	..	..	..
11	♀♀	37	27	..	..	35	24	..	..
	♂♂	26	38	..	..	22	24	..	..
	♀♀+♂♂	31·5	32·5	..	..	..	..	..	..

As mentioned above both strains were heterogeneous D.D.T.-resistant, and the percentage of flies that were knock-down-resistant (*kdr*) and therefore also resistant to the lethal action of D.D.T., could be estimated from time-knockdown diagrams of which some examples are shown in Graph 2.

It appears from Table VI that the proportion of resistant flies (both *kdr* and survivors after 24 hours) increased in the first generations under C.B.A.-selection, more in Strain 203 *ac* than in Strain 17 *bc*, and then did not increase further in later generations. In the last test shown (after 11 generations) the proportion of D.D.T.-resistant flies was practically the same in Strain 17 *bc* and 17 *ba*.

GRAPH 2.

Examples of knock-down tests with D.D.T., July 15, 1950. 6th generation of strains 17bc and 203ac selected by CBA in comparison with the unselected strains 17ba and 203ac.

If Table V and VI are compared, not a negative, but rather some positive correlation between C.B.A.-tolerance and D.D.T.-resistance is again indicated. In Strain 203 *ac* the flies clearly became more resistant to both compounds, in Strain 17 *bc* the effect was less pronounced.

The effect of the C.B.A.-selection on the resistance to other insecticides is being investigated.

DISCUSSION.

As mentioned above the reason for starting this investigation was the experiments by Ascher (1957; 1958) showing that flies of four resistant strains were more quickly knocked down by tarsal contact with cetyl bromoacetate than flies of two susceptible strains used for comparison.

Our results show rather the opposite as appears from the preceding section.

This *might* be due to differences in the method of testing the toxicity of C.B.A. or to differences in the breeding and selection procedures of the fly strains.

Some points concerning the *testing methods* have been discussed in correspondence with Mr. Ascher whom I thank for information and suggestions.

In both laboratories the type of test has been essentially the same: Continuous tarsal contact to C.B.A. applied to smooth non-absorbent surfaces, petri-dishes (Ascher), and aluminium foil cylinders (Keiding). In a preliminary test, we compared the two types of test containers and obtained parallel results.

A further indication of a similar reaction of the flies in both types of tests is obtained when the knock-down percentages are plotted on probability graph paper with time as abscissa, as the data from both laboratories give very similar straight lines (Graph 1).

The food of the flies has also been the same (sugar and water). No doubt there are certain differences in *handling the flies* before test; an obvious one is that we use ether *anæsthetization* when sexing and counting flies the day before test and CO_2 when transferring the flies to test surfaces, while Ascher only uses CO_2 or cooling for immobilizing the flies. If the rate of knock-down has some connection with the lipoid content in the tarsi, the use of ether *may* influence the reaction differently in susceptible and resistant strains. This point is under investigation.

In most of our tests we used flies of a higher *age* (8-13 days) than Ascher's (which were only two to three days old).

The influence of this has already been discussed in the preceding section.

In our tests with two to three days old flies the difference in rate of knock-down to C.B.A. between D.D.T.-resistant and susceptible flies was less than when older flies were compared, but still there was no case of the D.D.T.-resistant strain being affected earlier than the susceptible one. Even if the differences in testing methods have played some role, we do not think that they can explain why the results in the two laboratories show different cross-resistance patterns between C.B.A. and various insecticides.

This leads to the conclusion that tolerance to the knock-down action of C.B.A. is a character which varies from strain to strain, without being consistently

correlated, negatively or positively, to the investigated types of insecticide resistance.

This behaviour of C.B.A. shares with other physiological and biological characters such as cytochrome oxidase activity (Brown, 1958) and length of larval period (Bøggild and Keiding, 1958).

The fact that Ascher finds an enhanced susceptibility to C.B.A. in four strains that have been selected in the laboratory for resistance to insecticides (D.D.T., chlordane, diazinon, and chloroacetic acid) even when the selection has not increased the tolerance to the selective agent, while we generally find the opposite in our strains, indicates that it is the selection and breeding procedure as such that is influencing the susceptibility to C.B.A.

How selection for D.D.T.-resistance may influence the rate of larval development has been discussed by Bøggild and Keiding (1958). This will play a particular role when the strains have obtained homozygosity for the specific resistance genes, as further selection will act on more unspecific vigour genes.

Thus, for example, tolerance to C.B.A. *might* increase if the procedure of insecticide pressure favours short larval period, just as selection for long larval period often increases the D.D.T.-resistance (Kerr *et al.*, 1957).

As mentioned in the introduction, Ascher's results with cetyl bromoacetate nourished the hope that a compound "negatively correlated with D.D.T.-resistance" had been found which might be used in the control of resistant insect populations by reversing the resistance.

The results presented in this paper show that Ascher's results cannot be generalized, and in a recent paper Bettini *et al.* (1958) have also shown that when the *killing action after short exposures* is considered "it can be excluded that in general a higher susceptibility exists for insecticide-resistant strains to – (cetyl bromoacetate)".

For discussion of the concept of "negative correlation" between insecticidal compounds, see the paper of Bettini *et al.* (1958 *loc. cit.*).

SUMMARY.

The rate of knock-down to cetyl bromoacetate (C.B.A.) was determined in ten strains of house-flies by continuous tarsal contact with aluminium foil sprayed with acetone solutions of the compound.

The *strains* comprised two susceptible strains B.P.M. and Enø and eight strains which were derived from collections in three farms, where they had developed resistance to chlorinated hydrocarbons (Strain 17) or chlorinated hydrocarbons + organophosphorus compounds (strains 150 and 203).

By breeding with or without insecticidal selection, sub-strains with different combinations of insecticide-resistance had been obtained (Table I).

The results of tests with C.B.A., comparing such sub-strains of common origin, showed that the insecticide-resistant strain was also more resistant to the knock-down effect of C.B.A. both in females and males (Tables II and III). In some cases, especially when young flies were used, this difference in C.B.A.-tolerance was insignificant.

Selection with C.B.A. was carried out through ten generations in two strains, 17 *bc* and 203 *ac*, which were heterogeneous D.D.T.-resistant.

The effect of the C.B.A.-pressure on the D.D.T. resistance was determined by tarsal contact tests from which the percentage of knock-down resistant flies (*kdr*) was estimated.

The results (Table VI) showed that the proportion of D.D.T.-resistant flies (both *kdr* and survivors after 24 hours) increased in the first generations, but not further in later generations. At the same time, a certain increase of the rate of knock-down to C.B.A. was found.

When all the results are considered, they indicate a *positive* correlation of resistance to insecticides (D.D.T., lindane, etc.) and tolerance to the knock-down action of C.B.A., while Ascher's results (1958) indicated a *negative* correlation.

The possible reasons for these opposite results are discussed. Although differences in testing methods and age of the flies may have played some rôle, it is concluded that C.B.A.-tolerance (knock-down) and resistance to insecticides vary independently. When an apparent "correlation" is found for several strains, it may be a result of the selection and breeding procedure which selects characters that have no close connection with certain types of insecticide resistance.

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HALOGENIC INTOXICATION OF HEXAPODES. SOME EXPERIENCES ON THE VARIATIONS OF SUSCEPTIBILITY TO DDT: THE HIERARCHY OF INTEGUMENTS.*

BY

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[September 10, 1958.]

It is well-known, now a days, after years of researches that all the insects do not behave equally in the presence of a halogenic insecticide. This fact is universally recognized, but unfortunately we are almost ignorant from the point of view of mechanism and significance. Moreover, there is a genuine hierarchy in the resistance of hexapodes to the halogenic insecticides; certain species being resistant with all their individuals (the Hemipteres for example), in others there are some resistant strains (races according to some) dispersed in the midst of susceptible ones. It is as if the susceptible insect loses its susceptibility progressively. The Darwinian Laws of Selection have been invoked to explain this phenomenon.

There is more: in a determined insect, whose "global" susceptibility we know, it is possible to note further variations, which are of diverse origin, either extrinsic or intrinsic. We shall not go into details on the extrinsic factors of variations: these factors bring in modifications in the motor activity of the Hexapode. It is what we called formerly "Pseudo-resistances". There seems to be a close correlation between the motor activity of the insect and its susceptibility to the halogenic insecticide. The intrinsic factors are of different nature. We have published formerly, somewhere else, the observations and experiences as to the variations of susceptibility according to sex or the stage within the same species. These facts are very well recognized.

Today we would like to insist on the hierarchy of integumentary diverse parts of the insect. It is generally an unrecognized notion: certainly we know since long, this "tongue to paws" of flies, but, perhaps, on account of difficulties in making experiments, this process has not been explored completely. Besides, it is probable that here also specific variations exist.

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CHOICE OF MATERIAL AND EXPERIMENTAL TECHNIQUE.

The study of cutaneous susceptibility of hexapodes requires the utilisation of a species presenting the following special characteristics:

1. "Global" susceptibility almost constant, neither too strong, nor too feeble. It has appeared to us that an insect showing signs of intoxication, ranging between 10 and 20 hours, would constitute a material of choice.

2. The captured insect should be of good size so as to permit easy manipulations.

3. For the same reason, it is necessary to utilize an insect which is relatively slow in its movements or easily immobile.

Our choice fell on the Acridiens and for practical reasons on *Oedipoda coerulescens coerulescens* (K. Linnaeus, 1758). This locust is found in abundance in the paleoarctic region and shows a relatively constant "global" susceptibility which we can estimate briefly as under (at 20° C.):

Males.....pp' D.D.T. pure.....tremor 2 - 5 hours, death 16 - 18 hours.
D.D.T. 0·5 per cent.....tremor 7 hours, death 20 hours.

Females....pp' D.D.T. pure.....tremor 3 - 5 hours, death 17 - 24 hours.
D.D.T. 0·5 per cent.....tremor 7 hours, death 20 hours.

The males therefore appear to be more susceptible than the females: the point in question is, perhaps, a phenomena directly correlated with the mass of the insects.

The D.D.T. (D.D.T. chemically pure) is deposited at an exact point of the integument of the insect and is maintained in contact with the latter using a solution diluted with arabic gum: the D.D.T. is therefore in suspension in the arabic gum. In fact, such a procedure brings in some causes of errors: the insect finds its movements limited by the arabic gum and it is not possible to determine the exact quantity of D.D.T. which is in contact with its integuments. It must be made clear that in order to avoid any error due to the normal movements of the insect, the susceptible organs to come into contact accidentally with the D.D.T., were amputed or paralysed by paraffin. Thus the D.D.T. was deposited on the femur after amputation of the tibia, on the tibia after amputation of the tarsi, on Tibia III after immobilisation of the knee with paraffin, on the lateral side of the elytron after amputation of the third leg.

RESULTS.

Results are contained in Table I. Dichlorodiphenyl-trichloromethylmethane (D.D.T.) penetrates by transparietal route at the points where the integument is not thick, at the level of articulations in particular (Fig. 1). It is certain that the movements of the insect play a most important role : an immobile articulation like the Sutura pronotomesonotalis is not suitable for the penetration of the insecticide. In the same way, the proximity of nervous centres is of great importance: the most sensible zones are unnerved by the cerebral complex pro-, meso- and metathoracic ganglions, and abdominal ganglions.

FIG. 1.

Intoxication to D.D.T. of integuments of Oedipoda coerulescens coerulescens.

TABLE I.

Deposit of chemically pure D.D.T. on Oedipoda coerulescens coerulescens.

	Males		Females	
	Tremor (Hours)	Death (Hours)	Tremor (Hours)	Death (Hours)
MASSIVE INTOXICATION	3	16	5	17
CAPUT				
Proboscis	10	25	20	48
Vertex	20	28	30	48
Ocellus	20	28	30	48
Antenna I, II, III	10	20	22	45
Antenna, Flagellum	12	22	30	48
CERVIX	12	20	16	48
THORAX				
Pronotum	24	48	36	48
Propleura	20	36	24	48
Prosternum	12	36	15	48
Coxa I	6	36	6	36
Femur I	6	20	18	30
Tibia I	24	48	36	72
Tarsus I	18	26	24	48
Sutura pronotomesonotalis	24	56	30	96
ARTICULUS ALO I MESONOTALIS	20	36	24	50
ARTICULUS ALO II METANOTALIS	18	48	18	80
Ala I, Ala II	19	48	36	48
Ala I, external face	10	24	15	48
Mesonotum	30	50	12	24
Mesosternum	15	30	20	80
Metanotum	18	30	20	50
Metasternum	12	30	12	72
Coxa II	10	24	12	72
Femur II	8	15	12	24

TABLE I—(*Contd.*)

	Males		Females	
	Tremor (Hours)	Death (Hours)	Tremor (Hours)	Death (Hours)
Tibia II	18	25	12	48
Tarsus II	8	15	12	24
Coxa III	10	48	10	50
Femur III, external face	18	72	20	50
Femur III, internal face	8	15	10	24
Tibia III	24	50	24	96
Tarsus III	12	30	12	18
ARTICULUS COXOFEMORALIS I	8	30	10	60
" " II	8	15	10	24
" " III	6	14	10	30
ARTICULUS FEMOROTIBIALIS I	10	24	10	30
" " II	8	15	10	24
" " III	10	24	10	48
ARTICULUS TIBIOTARSALIS I	10	24	10	48
" " II	8	15	10	48
" " III	8	20	10	30
ABDOMEN				
Uronotum	15	30	12	24
Urosternum	12	25	10	15
ARTICULUS URONOTO UROSTERNALIS I-II . .	8	15	10	24
" " " V-VI .. .	8	15	10	24
" .. " VII-VIII ..	8	15	10	24
ARTICULUS UROSTERNO UROSTERNALIS I-II ..	8	15	10	24
" " " V-VI . .	8	15	8	15
Genitalia	24	48	40	50

NOTE.—

This Table indicates the average figures observed in the author's experiences. As far as possible, insects of comparable size were utilised. For the anatomic Latin nomenclature, refer to thesis by R.M. NICOLI for D.Sc. of Marseilles University (1955).

For this purpose, it is possible to distinguish three types of cutaneous zones, as illustrated in Fig. 1:—

(1) Regions where the D.D.T. deposited brings in a rapid intoxication: Proboscis, antennae, Coxae I, II, III, Femur I, II and III on the internal face; Tarsus II, articulations of legs, abdominal articulations, Alae I on the external face.

(2) Regions where the intoxication is slow: Cephalic capsule, pro-, mesa- and metasternum, urosternum, Tarsi I and III, Femur III on the external face, Alae I and II, Sutura pronotomesonotalis.

(3) Regions where the intoxication is tardy: Tibia outside the articulations, pro-, meso- and metapleura, pro-, meso and metanotum.

There exists, therefore, a genuine hierarchy in the susceptibility of different cutaneous parts. Subsequent studies should enable to establish a true picture of cutaneous susceptibility.

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PHYSIOGENETIC RESEARCHES ON RESISTANCE OF INSECTS (MOSQUITOES, FLIES, TERMITES AND SILKWORMS) TO INSECTICIDES. THE EFFECTS OF PRETREATMENT WITH NEUROTROPIC DRUGS IN *BOMBYX MORI*.

BY

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[November 24, 1958.]

In our Institute, one laboratory is chiefly specialized for the study of resistance to D.D.T. and other insecticides: the laboratory of domestic fly genetics, research field of Dr. Riccardo Milani.

Other laboratories, however, are giving increasing attention to such a problem: fascinating both for general biological interest (evolutionary genetics and comparative physiology) and for practical application in the fields of medical and agricultural entomology.

The laboratory of genetics of malaria mosquitoes, *Anopheles* — research field of Dr. Guido Frizzi — is fully specialized, since many years — in the salivary cytogenetics: with a thorough investigation of the *maculipennis* group — in his paleoarctic and neoarctic species — and with a comparative study of other complexes such as *aquasalis*, *albimanus*, *stephensi*, *gambiæ*.

In Brazil by Ricciardi and Schreiber and in India by Rishikesh, the method of salivary cytogenetics is being applied to the research on some of most dangerous malaria vectors.

In the most recent investigations of Dr. Frizzi — in our Institute in Pavia and also in cooperation with the Sicilian Centre of Malaria Control — attention has been focussed on possible relationship between chromosomic arrangement and insecticide resistance, extending in this direction the researches previously undertaken in order to define the biological value of chromosomic mutations and the meaning of the polymorphism in relation to the plasticity of adaptation to environment.

Everybody knows what a formidable danger for the success of malaria control — as for the control of many other tropical diseases transmitted by insects and ticks —

is given by the formation of strains resistant to D.D.T. and other synthetic insecticides. It is quite possible that in a very near future similar methods of cytological investigation — based on the examination of the chromosomic set in other somatic tissues having polytenic nuclei — could give similar advantages for studying other *Diptera* like flies and phlebotomi and also insects of quite different kind, like *Triatomidæ*.

Also other laboratories of the Institute "L. Spallanzani" are undertaking researches on insecticide resistance in insects that constitute the specific material of their experimental activity.

The laboratory for the study of termites and cockroaches (*Blattoidea* and *Isoptera*), research field of Dr. Antonio Springhetti and Dr. Paola Bernardini-Mosconi, is beginning to investigate the effect of several insecticides on the two indigenous species, *Calotermes flavicollis* and *Reticulitermes lucifugus:* as a means of physiological testing and in connection with the practical aims of termite control.

The method of application of insecticides to the control of species like Sotterranean termites is much more apt to hasten than to avoid the outcoming of physiological resistance in these social insects: in them, however, because of caste differentiation, is rather difficult to explore — and even to foresay — the mechanisms of formation of genetically resistant strains.

We are engaged in investigating the comparative endocrinology in termites, trying to follow — through the families of the ordo *Isoptera* — the evolution of the endocrine system, more or less parallel to the evolution of the social system, starting from the level of *Blattoidea*, till to higher *Termitidæ*.

We hope that the close association of endocrinological and toxicological investigation shall open, in a near future, a new path of research: in order to clarify the experimental analysis of extrinsic and intrinsic factors modifying the insect resistance to the insecticide.

Another material for experimental investigation in our "Spallanzani" Institute is given by silkworms: *Bombyx mori* chiefly, but also other silk producing *Lepidoptera*, like "eriworm" of India, *Phylosamia ricini*.

On these silkworms we are investigating chiefly physiogenetics: biochemical mechanisms through which mendelian factors realize, during ontogenesis, their potentialities, giving origin to morphological and physiological peculiar features.

After long and thorough investigations on specific differential permeabilities — to substances deriving from food — and on nitrogen metabolism, associated with researches on resistance to parasites like the fungus of "calcino" and the virus of the poliedric disease; we have recently undertaken investigations on D.D.T. and other insecticides resistance: being confident that silkworms are a very suitable material for the study of the biochemical and physiological basis of the resistance.

In this paper, a preliminary and short information on the kind of researches undertaken is given.

The genetical point of view has not been emphasised, notwithstanding researches were planned chiefly in view of their genetical interest.

My programme was as follows: to investigate if hybrids of first generation, between two strains of silkworms, show or do not show, in comparison with the parent breeds, an higher degree of resistance to the toxic effect of D.D.T. and other insecticides.

Such investigation was embodied on a long series of researches performed in silkworms in order to explore every possible manifestation of heterosis or hybrid vigour: in the degree of fertility, developmental capacities of virgin and fertilized egg, capacity and rate of growth of the larvae and of their serigenous glands.

Since half a century, in Italy heterosis of silkworms is utilized in industrial production of hybrid seed of silkworms, crossing indigenous and oriental breeds.

In these last years, however, in practical sericulture, double crosses — between four strains — have been synthetized and with them Japanese have conquered, for silkworms, a performance comparable to what Americans have done *in mais*.

Those crosses are made between breeds highly selected for their silk-production; so the advantages of crossing is not so much in raising to higher levels such productivity as in giving back sufficient vigour and vitality to the organism — weakened by such an artificial selection that has been pushed too far till physiological unbalance.

In 1957, at the International Symposium of Genetics in Tokyo, I considered the problems of relationship between heterosis and resistance : resistance to unfavourable environmental factors and to pathogenic agents or parasites.

I considered the problem in general terms and chiefly in reference to the utilization, in plant breeding, of the aspecific resistance giving to hybrids some superiority over their parent breeds.

Since I had occasion to promote researches on D.D.T.-resistance in silkworms, I began to pay attention to the opportunity of assuming D.D.T.-resistance as index for evaluating hybrid vigour.

Data collected for three couples of reciprocal hybrids, in comparison with their parent breeds, do not seem to show a clear cut superiority of hybrids in resistance.

In the present paper, however, I shall leave aside these aspects of the problem in order to consider in what direction our line of research could give a contribution to the investigation of a central problem " the mode of action of D.D.T.". Such a problem is unavoidably implied in the problem of D.D.T.-resistance if the latter is attached — besides as formal genetic analysis of the behaviour in inheritance of resistance genes — also on the basis of physiogenetic investigations searching to discover the physiological and biochemical mechanisms of their operation.

Without going in any detail of experimental technique, I will give only a brief and schematic account of my line of work.

I thought interesting to look if some previous treatment acting on the nervous system — in the direction of exciting or depressing it — could have a significant influence on the degree of D.D.T.-resistance.

Going a step further, I wanted to investigate the effects of drugs acting on different level and in a different manner on the nervous system: on brain, on other parts of the central system, on ganglia of the so called autonomic system, on motor or sensory nerves, on neuromuscular junction or the so called muscular plates.

So I tried a lot of substances of the type employed in human therapy and in physiological investigation of laboratory mammals: as tranquillizers, ganglioplegics and curarics.

I cannot dwell upon the details of our experiments. I will only quote some general results.

Pretreatment with curaro — like synthetic drugs — appears to make worser the intoxication by D.D.T.

We did assay, in a quite preliminary way, " true curarics " like 'laudolissin' that must act by defect of acetilcoline (in the sense that acetilcoline is prevented to reach the neuromuscular plates: what is known or presumed in the case of vertebrates or human organism).

We did assay, however, also "pseudocurarics" that are presumed to act by excess of acetilcoline: in the sense that their structures induce a lasting depolarization of the neuromuscular plate.

With drugs of different type, however, a significant or even a quite sharp and strong antagonistic effect resulted, making lighter the consequences of D.D.T. intoxication.

So happened with strychnine, and atropine: alkaloids very well known in neurophysiology and assayed in silkworms by me and Fois many years ago (1937) in researches that were perhaps the very first of the kind.

The graphs relative to the experiments of pretreatment with strychnine or atropine show quite sharply a lowering of the mortality curves, comparing with the controls treated only with D.D.T.

The effect it is clear for every one of the strains: AP (golden Chinese), R_2 (Italian yellow) ♀AP♂R_2,♀R_2♂AP. It is quite possible that closer analysis could reveal distinctive features — like a smaller or greater advantage gained by hybrids in confront with pure bred ones — from the pretreatment.

Pretreating with chlorpromazine (known as Largactil in Italian pharmaceutical trade) the effect antagonistic to D.D.T. intoxication is rather questionable: with lower doses such an effect appears; with higher ones, however, the intoxication is so strong that no amelioration is obvious except, perhaps, some delay of the lethal effect.

I cannot discuss here the complex pharmacological effect of chlorpromazine. I can say only that probably enlightening results should be reached comparing with promazine and other derivates of the phenotiazine group.

My plan is to research, both widely and deeply, investigating the effects of modification of molecular structure both in the insecticide and in the neurotropic

drug used in pretreatment. I include in my programme histological control of nervous tissue and of endocrine organs; and biochemical researches on metabolic aspects of the toxication. For instance my co-worker and colleague Prof. Manunta has tried to inject sulphurated aminoacids — like methyonine, cistine, and cysteine — and aromatic aminoacid, like istidine, in silkworms before D.D.T. treatment: in order to see if any detoxicating conjugation happens; apparently, however, this is not the case.

As concluding remark, I wish to point out that this line of research could prove interesting in these three directions.

(1) to deepen the analysis of some physiological aspect of heterosis, chiefly of relationship between hybrid vigour and resistance.

(2) to deepen the analysis of the physiological and biochemical mechanisms of intoxication by synthetic insecticides and of detoxication and resistance.

(3) to open the way to the analysis of genetic differences in the functionalities of nervous system in insects.

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OUTLINE FOR THE STUDY OF POPULATION GENETICS OF RESISTANCE.

BY

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[November 29, 1957.]

INTRODUCTION.

RESIDUAL insecticides have made it possible to control insect disease vectors and during the past ten years spectacular success has been achieved in the control of malaria and other insect-borne diseases in many parts of the world. In the wake of this success, the problem of insect resistance to insecticides has arisen to such an extent that in some parts of the world, for example, in Greece, it has become difficult to control malaria as the insecticides in use are no longer effective. The alarming growth of the resistance problem can be judged by the steady increase annually in the number of resistant species of public health importance from 2 in 1946 to 38 in 1956.

Considerable work has been done on the resistance problem as is evident from reviews recently published (Hoskins and Gordon, 1956; Kearns, 1956; Crow, 1957; Milani, 1957). The picture, however, still remains confusing.

The appearance of resistant strains of insects can be explained only under one condition that resistant individuals already existed in the population and by application of insecticides gradual selection has been made in favour of such individuals. The possibility of an induced mutation, arising as a result of insecticidal application, is ruled out.

This being the basis of our discussion it may be pointed out that studies on the resistance problem can yield successful results when the genetic background is known. In the literature all possible kinds of inheritance of resistance are cited; mono-factorial, poly-factorial, autosomal, sex-linked, maternal and cytoplasmic

inheritance. Even on cursory examination, it is certain that there is more than one genetic mechanism responsible for the transmission of resistance.

For detailed investigation of the resistance problem firstly, one would have to determine whether the resistance is due to mono- or poly-factors or any other genetic mechanism. Secondly, the frequency of resistance genes in a natural population must be determined as this information would indicate the probable time required for the development of resistance. Thirdly the provision of a homozygous resistant strain is a primary pre-requisite for any physiological study on resistance. In fact it seems that the results achieved so far, both on the inheritance and on the physiological basis of resistance, are diverse and inconclusive because of the lack of this insight.

It seems to us that resistant strains of insects have emerged because the dosages of insecticides used were only on the consideration of achieving successful disease control without reference to the nature of the genetic processes involved in the selection of resistant strains. In almost all cases the insecticidal dosages used have been massive with the result that the homozygous susceptibles and a large proportion of heterozygous insects are killed and further crossing took place only between the remaining heterozygous and homozygous resistant individuals. With each generation the resistant individuals appeared in a larger proportion so that in very short time a totally resistant strain was evolved.

By making judicious use of insecticides in such a manner that only a part of the homozygous susceptible insects are killed, the appearance of a totally resistant strain may be postponed for a longer period of time. It may, however, be pointed out that the mortalities amongst the insect population, resulting with low dosage of insecticides, should be consistent with a certain degree of disease control. Under such circumstances, disease control may be achieved before the resistant strains of vectors appear.

It seems to be the objective of practical application of genetics to postpone appearance of resistant strains as long as possible. Resistance in mosquitoes has been cited as an example for purposes of this paper.

The paper is intended to place at the disposal of entomologists and malriologists a brief account of the scheme for carrying out such genetical investigations and to determine the mortality levels amongst a population so that a compromise can be made in the choice of the insecticidal dosage which is consistent at the one side with the disease control and at the other side with the postponement of selection of resistant individuals.

MEDIAN LETHAL DOSE AND THE LEVEL OF RESISTANCE.

The first thing to do would be to determine for a particular species the median lethal dose for the insecticides in as many parts of the area as possible. In every case care should be taken to have as representative a part of the natural population as possible. The strain with the lowest susceptibility should be indicated as the *reference strain.*

However, a strain from an area which has not been sprayed with insecticides, if such an area is available, would give an ideal reference strain. This strain should be bred in the laboratory taking care that no undue mortalities occur while inbreeding and that inadvertently selection is not made for any particular trait.

If evidence is available that in a certain area, where insecticides have been used for some time, a resistant strain of that particular species has already appeared, the susceptibility level of this resistant strain and the reference strain should be compared. In situations where a resistant strain has not appeared in the field, selection of such a strain should be attempted in the laboratory. For selection of a resistant strain, mortality of 90 per cent* or over should be obtained in the first generation and kept to that level in subsequent generations until a resistant strain is selected. If attempt is not met with success, it may not be interpreted that a resistant strain would not emerge in the field. The sample under selection may not have had individuals with resistant genes. Further samples should then be obtained from natural populations and tried in the same manner.

CROSSING OF SUSCEPTIBLE AND RESISTANT STRAINS.

Reciprocal crossing (♀ strain A × ♂ strain B; ♀ strain B × ♂ strain A) of the two strains should be carried out and the susceptibility level of the F_1 generation determined. On theoretical considerations, it is expected that the F_1 generation would be intermediate between the susceptibility levels of the reference and the resistant strains or like the one or the other parent strain, depending on the dominance relationship of the involved genes. If there is no dominance at all between the susceptibility gene (s) and the resistance gene (r), the F_1 generation will be intermediate as regards insecticide susceptibility. If s is dominant over r then F_1 is as susceptible as the reference strain. If *r* is dominant over s, then F_1 is fully resistant like the resistant parent strain. The F_1 generation should be inbred and if the resistance is mono-factorial the F_2 generation should follow Mendelian segregation : 25 per cent susceptible, 50 per cent intermediate and 25 per cent resistant in susceptibility level, *or* 75 per cent susceptible and 25 per cent resistant *or* 25 per cent susceptible and 75 per cent resistant. Any significant deviation from these ratios should rule out mono-factorial inheritance. In the case of poly-factorial inheritance for a single trait such as resistance or susceptibility two or more genes may be involved. If the genes are located in all three chromosomes, eighty-one different types of combinations would be expected in the F_2 generation after inbreeding.

The reference and resistant strains should then be carefully studied for any morphological differences. Very likely no such differences may exist, but there is a remote possibility that some morphological character may be linked with resistance gene. Any departure from the normal crossability, for instance, fertility in one direction only, disturbance in the normal sex ratio or infertility of one of the sexes, points to the fact that these strains are different systematic units and perhaps different species. Although no morphological differences may be detected yet it

*This would hold true only in the case of mono-factorial resistance.

is probable that differences may exist in the bionomics and habits of these two strains, such as the period of maturation of ovaries, number of ova and other changes in the gonotrophic cycle or feeding, resting and biting habits etc.

It is evident from the above that individuals with varying susceptibilities (most susceptible, intermediate susceptibility and resistant) can be differentiated by carrying out susceptibility tests but unfortunately there is no means morphologically to sort out individuals which belong to these three groups. Hence it is desirable that some morphological characters which are inherited should somehow be linked with the resistant gene. In order to be able to do so the species will have to be exposed to X-Ray radiations or any other mutagenic agent to obtain some gross mutations. Their inheritance should then be carefully studied. Preferably more than one mutation lying in the same chromosome should be investigated. The experience with culicine mosquitoes has shown that exposure to X-Rays of 4-6000 r should give sufficient numbers of mutations. Such mutations are used as markers to differentiate between different chromosomes (Laven, 1956: 1957).

LOCALIZATION OF THE RESISTANCE GENE.

By crossing experiments it is possible to investigate the inheritance of the mutations and to find out whether they are sex-linked or autosomal. The sex-linked mutations should be left alone and investigations concentrated on autosomal mutations, because from available data it may be concluded that at least in mosquitoes the resistance gene is not sex-linked. Whether the resistance gene is located in one of the autosomal chromosomes, and in which of them, should be investigated by crossing experiments.

The resistant strain should be crossed with a susceptible strain, which has one autosomal mutation in homozygous condition, preferably a dominant mutation. The F_1 generation would be heterozygous in respect to the mutation as well as to the susceptibility to insecticides. If the F_2 generation after inbreeding of the F_1* generation results in segregation of 25 per cent susceptibility (at the same time homozygous for the mutation), 50 per cent of intermediate susceptibility (heterozygous for the mutation) and 25 per cent resistant (without the mutation), it would indicate that the resistance gene is located in the same chromosomes as the mutation (Fig. 1*a*). If the resistance gene is dominant or recessive this proportion would be different as has been discussed earlier . In the event of results being different in F_2 (i.e. independent segregation of the mutation and resistance gene) it can be concluded that the resistance gene is located in a different chromosome than the mutation (Fig. 1*b*).

Unfortunately in such an event one will have to start over again with another mutation which is known not to be located in the same chromosome as the first mutation used.

LINKAGE OF RESISTANCE GENE WITH ONE OR SEVERAL MARKS.

(*a*) *One mark*:

Experiments on localization of the resistance gene, will give heterozygous insects both in respect of the mark (mutation) and the resistance gene.

* Assuming that no crossing-over has taken place.

The aim of subsequent experiments is to bring together the mark and the gene for the resistance in the same chromosome. So long they are located only in homologous chromosomes. In order to be able to make such an experiment, the heterozygous strain, as referred to above, should be crossed with the reference strain. The probability of crossing-over so that the marker gene and the resistance gene are transferred to the same chromosome, has to be explored. As far as is known in Culex crossing over takes place probably only in the males. The schematic figures (2*a*, *b*), therefore, would refer only to the culicine mosquitoes and would have no bearing on anopheline mosquitoes or flies. The following discussion applies to culicine mosquitoes only.

FIG. 1*a*.

Localisation of resistance-gene with one marker-gene: a, resistance-gene and mark-gene in the same chromosome.

The frequency of crossing-over will depend upon the distance between the mark and the resistance gene in homologous chromosomes and as a result of this, 'cross-over insects', with mark and resistance gene located in the same chromosome, may be expected. When crossing-over takes place besides the two types of 'non-cross-over insects', two types of cross-over insects will be observed in the following generation. The 'non-cross-over types of insects', would be heterozygous for

FIG. 1*b*.

Localisation of resistance-gene with one marker-gene; b) resistance-gene and mark-gene in different chromosomes.

resistance or heterozygous for the mark. The 'cross-over types' would be homozygous normal or heterozygous in respect of both the mark and the resistance gene (Fig. 2*a*).

FIG. 2*a*.

Linkage of resistance-gene with one mark-gene, test strain I (continuation from 1a).

The last type of 'cross-over insects' i.e. heterozygous both for the marker and the resistance gene should then be in-bred. In F_2, segregation would result in Mendelian ratios so that 25 per cent of the insects would be of the normal reference type, 50 per cent heterozygous both in the marker and the resistance gene and 25 per cent homozygous both in respect of the marker and the resistance gene provided there is no crossing-over which will upset this ratio. The latter would be referred to as the *test strain* for further discussion.

b) More than one mark:

In order to be able to distinguish morphologically homozygous susceptibles, heterozygous and homozygous resistant insects, it is preferable that more than one marker gene should be linked with the resistance gene. Reference may be made to Fig. 2*b* in which the linkage of the resistance gene with several marker genes has been worked out. Unlike the test strain referred to above which has only

one marker gene linked with resistance gene, the test strain No. 2, as derived in this experiment, would have multiple marker genes linked with the resistance gene.

Fig. 2*b*.

Linkage of resistance-gene with several mark genes, test strain II [partly continuation from (1a).]

It may be pointed out that to obtain a multiple marked test strain would not be a simple process but would involve a number of experiments extending over many generations before crossing-over would result in evolving such a strain.

TESTING NATURAL POPULATIONS FOR PRESENCE OF RESISTANCE GENE.

The ultimate objective of this study is to be able to determine the frequency of the resistance gene in a natural population. Crossing experiments between the test Strain I and insects from natural population, would indicate in the F_1 generation whether the natural population contains individuals which are completely susceptible or of intermediate susceptibility or completely resistant. For the sake of simplicity we refer to test Strain I which has only one marker gene linked with the resistance gene (Fig. 3).

Reciprocal crossing of test Strain I with insects from natural population, if completely susceptible, would in F_1 generation give only individuals of intermediate susceptibility. On the other hand if individuals from natural population are intermediate in resistance in F_1 generation they will give rise to individuals 50 per cent of which are of intermediate susceptibility and 50 per cent completely resistant. The third possibility is that the natural population is completely resistant. Under such circumstances the F_1 generation of the individuals would be completely resistant. This experiment would indicate the *frequency of the resistance gene in the natural population.* These data are essential for setting up an artificial population which simulates natural conditions.

STUDIES ON POPULATION GENETICS OF RESISTANCE.

With reference to Fig. 2(*a*), it can be seen that individuals homozygous for the susceptibility gene with no mark, heterozygous both for the mark and the resistance and homozygous both for the mark and the resistance, can be produced and differentiated by morphological characters. These three types of individuals thus obtained should then be mixed in a proportion which has been previously worked out on the frequency of the resistance gene in the natural population. This artificial population should now be exposed to a varying degree of selection pressure with insecticides by setting up a number of replicates.

For the sake of discussion, in Fig. 4, three types of populations are visualized (1) with 20 per cent heterozygous individuals, (2) 10 per cent heterozygous individuals and (3) 1·98 per cent hetrozygous individuals and two types of selection pressures (1) 25 per cent mortality and (2) 50 per cent mortality amongst the population. When the selection pressure is high (50 per cent mortality) the percentage of homozygous resistant individuals in the composition of the population would rapidly increase with each generation. If the original composition of the population is 20 per cent, 10 per cent, 1·98 per cent heterozygous individuals, completely resistant strain is expected to evolve in about 3, 5 and 9 generations respectively.

Fig. 3.

Testing natural populations for presence of resistance-gene.

On the other hand if the selection pressure is low (25 per cent mortality) with the 10 and 1·98 per cent composition of heterozygous individuals, the appea-

rance of a resistant strain would be deferred for a number of generations as is evident from the Graph 1.

GRAPH 1.

Studies on population genetics of resistance.

The data thus obtained would indicate the *level of selection pressure which should be applied under natural conditions* so that evolution of a resistant strain is postponed as far as possible, provided the selection pressure is sufficient to give a certain degree of disease control.

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CROSSABILITY AND INTER-SPECIFIC FERTILITY OF MOSQUITOES *CULEX P. MOLESTUS* AND *CULEX FATIGANS*.

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[October 24, 1958.]

INTRODUCTION.

INDIVIDUALS, with varying susceptibility to insecticides, can be differentiated in a population by carrying out susceptibility tests but unfortunately there is no means morphologically to sort out individuals which are (*a*) most susceptible, (*b*) of intermediate susceptibility and (*c*) resistant. Hence it is desirable that some morphological characters, which are inherited, should somehow be linked with the resistant gene or genes. In order to be able to do so, the species has to be exposed to X-ray radiations or any other mutagenic agent to obtain some gross mutations. Their inheritance can be studied and such mutations can be used as 'markers' to locate the resistance gene in the chromosomes (Laven, 1956:1957). Crossing experiments were, therefore, undertaken between *C. fatigans* resistant strain obtained from Malaya and *C. p. molestus* strain obtained from Germany which had 'wing length' mutation in order to determine whether cross between *C. fatigans* and *C. p. molestus* was possible, and if so, whether the resistant gene or genes could be linked with the wing length mutation.

MATERIALS AND METHOD.

The materials used for the studies consisted of the following three strains of *C. p. molestus* of European origin and a 'marker strain' with wing length mutation. The strains were : (*1*) Oggelshausen (Og), (*2*) Immenrode (Im), (*3*) Latina (La) and the 'marker strain' Hamburg (Ha). These were obtained from West Germany through the courtesy of Dr. H. Laven of the Max-Planck-Institute, Tübingen, who initiated these studies when he was at the Malaria Institute of India as World Health Organization Consultant. A dieldrin-resistant strain of

C. fatigans was obtained from Kuala Lumpur (Malaya) through the courtesy of Dr. J. A. Reid. These strains were crossed with susceptible strain of *C. fatigans*, being maintained in the Laboratory of the Malaria Institute of India, Delhi.

The technique adopted for the crossing experiments was that followed by Laven (1956:1957) which consisted of isolating the pupæ singly and using the freshly-hatched males and females for mating. The mosquitoes were mated in cylindrical glass jars (6 inches × 3 inches); later the females were individually fed either on man or on bird and then isolated in specimen tubes (3 inches × 1 inch) singly for egg laying. Egg rafts, when layed, were transferred into small glass tubes (1·5 inches × 0·25 inch) and were allowed to hatch in the tubes. The eggs and larvæ were counted and recorded. The results of the crossing and back crossing experiments are summarised in Chart 1 by denoting the percentage of viable eggs obtained in each cross.

RESULTS AND CONCLUSIONS.

In all the crossing experiments, the following data was recorded:—

(1) The total number of eggs in each egg raft (obtained by isolating the female singly in specimen tube);

(2) Number of larvæ hatched;

(3) Number of eggs embryonated but not hatched; and

(4) Number of unfertilized eggs.

Details of the type of crossing and other data obtained are summarized in Table I.

From the data it is evident that the cross, between *C. p. molestus* (of European origin) and *C. fatigans* susceptible Delhi strain, did not materialize. Few eggs were obtained in three instances but were found to be unfertilized. The female mosquitoes, when dissected, also showed no sperms in the spermathecae. The inter-specific cross between *C.p. molestus* and *C. fatigans* does not seem to be possible. Ghelelowitch (1952) and Laven (1956:1957 *loc. cit.*) have demonstrated the specificity of certain strains of *C.p. molestus* in respect of their crossability between the different geographical strains of *C.p. molestus* and with *C. fatigans*. Laven (1956:1957 *loc. cit.*) has convincingly proved that the crossability type of a particular strain does not change, even if the chromosomal constitution was completely altered by repeated back crosses with another strain.

The crossing experiments with susceptible and resistant strains of *C. fatigans* from Delhi and Kaula Lumpur respectively have given the following results:

(*a*) The reciprocal cross of ♀ Ku × ♂ De was half as successful (46·07 per cent viable eggs) as that of ♀ De × ♂ Ku (99·02 per cent viable eggs).

(*b*) The maximum number of 98 and 99 per cent viable eggs were obtained in the crosses ♀ De × ♂ Ku and F_1 hybrid of this when back crossed with ♂♂ of De (♀ De/Ku × ♂ De) respectively.

(*c*) The back crosses of the hybrids ♀ De/Ku and ♀ Ku/De with ♂♂ Ku were not successful as in the former case none of the eggs hatched and in the latter only 17 per cent hatched out.

CHART I.

Details regarding the crossability of various strains as indicated by the percentage of viable eggs.

		C. p. molestus				C. fatigans			
	♀ \ ♂	Ha	Og	Im	La	DE	KU	DE/KU	KU/DE
C. p. molestus	Ha					0·0			
	Og					0·0			
	Im					0·0			
	La					0·0			
C. fatigans	DE	0·0	0·0	0·0	0·0		99·2		
	KU					46·07			
	DE/KU					98·66	0 0	54·56	
	KU/DE					67·93	17·8		65·46

C.p.molestus

Laboratory culture
Ha - Hamburg Strain; Marker with Kuf/+ for wing length
Og - Oggelshausen strain
Im - Immenrode strain
La - Latina strain

C. fatigans

DE - Delhi susceptible strain
Ku - Kaulalcmpur (Malaya) Dieldrin resistant strain
DE/KU- hybrid of ♀ DE x ♂ KU
KU/DE- hybrids of ♀ KU x ♂ DE

TABLE I.

Details regarding the number of female mosquitoes used for each type of crossing, total number of rafts and eggs obtained, number of larvæ hatched and percentage of viable eggs, etc.

Serial number	Type of cross	Number of females used	Total number of egg rafts	Number of rafts hatched	Total number of eggs obtained	Number of larvæ hatched	Number of eggs embryonated but not hatched	Number of eggs unembryonated	Percentage of viable eggs
1.	♀Ha × ♂DE ..	4	..	..	..	..	..	..	..
2.	♀De × ♂Ha ..	7	1	0	26	0	0	26	0·0
3.	♀Ha × ♂Ha ..	12	..	..	..	..	..	..	..
4.	♀Og × ♂De ..	2	..	..	..	..	..	..	..
5.	♀Jm × ♂De ..	3	2	0	39	0	0	39	0·0
6.	♀La × ♂De ..	1	2	0	25	0	0	25	0·0
7.	♀De × ♂Ku ..	29	9	9	926	917	8	1	99·02
8.	♀Ku × ♂De ..	26	8	4	1276	557	12	707	46·07
9.	♀De/Ku × ♂De/Ku	17	8	4	1316	718	19	579	54·56
10.	♀De/Ku × ♂De ..	11	11	11	2481	2448	15	18	98·16
11.	♀De/Ku × ♂Ku ..	9	1	0	36	0	0	36	0·0
12.	♀Ku/De × ♂Ku/De	17	9	5	1376	901	2	473	65·46
13.	♀Ku/De × ♂De ..	21	4	3	524	356	5	163	67·93
14.	♀Ku/De × ♂Ku ..	33	14	3	3620	618	2	3002	17·08

The results indicate specific affinity in the crossability type of different strains chiefly associated with the female mosquito of the strain. It has also exhibited the specificity of a strain in respect of inter-fertility with another strain.

SUMMARY.

Efforts were made to explore the feasibility of crossing between *C. p. molestus* with wing length mutation and a resistant strain of *C. fatigans*, in order to link the wing length mutation with the resistant gene or genes. The results obtained indicated that inter-specific crosses between *C.p. molestus* (of European origin) and *C. fatigans* (Delhi and Malayan strains) did not materialise.

Crossability type differs with each strain and there are specific crossing affinities chiefly associated with the female mosquito.

The authors are grateful to Dr. H. Laven of the Max-Planck Institute, Tübingen, Germany, for supply of material and necessary guidance and to Dr. J. A. Reid for supplying a resistant strain of *C. fatigans* from Malaya.

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INHERITANCE OF D.D.T.-RESISTANCE IN *CULEX FATIGANS*

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INTRODUCTION.

WITH the intensive and extensive use of chemicals to control insect-pests, the number of insect species resistant to insecticides has also increased (Brown, 1958; Quarterman and Schoof, 1958). Of late, this phenomenon has attracted attention of a large number of research workers, and systematic studies on different aspects of the resistance problem have been undertaken. By and large, it has been accepted that increase in the frequency of resistant individuals in any insect population is neither due to mutation in insects caused by the insecticide, nor due to the acquired ability of the insects to adapt themselves to the changed environment. The development of resistance was due to selection, by insecticide, of a few resistant individuals already present in the population. The selected individuals possess an innate ability to withstand the hazard of toxicant. Studies on the inheritance of resistant factors have, therefore, been carried on on a number of species. The results of such studies on *Aonidiella aurantii*, *Blattella germanica*, *Musca domestica* and *Drosophila melanogaster* have been reviewed by Crow (1957), Milani (1957*a*, 1957*b*), and Busvine (1957). It would appear from these reviews that very little work has been undertaken on mosquitoes. Davidson (1956 and 1958) published work on the genetical aspects of dieldrin and B.H.C. resistance in *A. gambiæ* and D.D.T. resistance in *A. sundaicus*. In India, the first report of resistance in mosquitoes to D.D.T. was that of *C. fatigans* (Pal *et al.*, 1952). Since then, this species has been reported to be resistant to D.D.T. in a number of areas in this country. So far no work has appeared on the inheritance of resistance in *C. fatigans*. Genetical investigations were started during March, 1958 on this species, the results of which are presented in the present communication.

MATERIAL AND METHODS.

D.D.T. resistant *C. fatigans* (Strain R) was collected from the village Khuraji of Delhi State and colonised in the insectary of the Malaria Institute of India, Delhi, for one generation. The progeny of this strain and that of the *C. fatigans* susceptible strain (Strain S), maintained at the Institute, over a number of years, were used for the present study.

Adults of both the strains were obtained by hatching single pupa separately in specimen tubes (3" × 1"). This assured virginity of the females of the two strains. When sufficient number of adults were available reciprocal crossings, in mass, were carried out. The hybrids, RS* (F_1) and SR† (F_1) were again inbred to obtain RS (F_2) and SR (F_2) strains. One more strain RRS(F_1) was obtained by back crossing female R-strain with male RS(F_1) strain. The plan of crossing experiments is shown in Fig. 1.

FIG. 1.

Fig 1 : Showing the plan of the different crossing made with the Susceptible and Resistant Strains of c.fatigans.

R = Resistant Strain

S = Susceptible Strain

In addition to the above five strains, Strain X comprising 50 mosquitoes of Strain R, 100 of Strain SR (F_1) and 50 of Strain S was also tested for purposes of comparison. Female adults of equal ages, reared under identical conditions, of different strains were used.

* Progeny of resistant female × Susceptible male.
† Progeny of susceptible female × Resistant male.

TEST METHODS.

To determine the level of tolerance of these strains to D.D.T., susceptibility tests were carried out both on adults and larvae. In the adult-tests 4 - 8 days old, glucose-fed female mosquitoes were treated with D.D.T. by topical application method. For this purpose mosquitoes were anaesthetised with carbon dioxide, and D.D.T. (technical) alcoholic solution of varying concentrations was applied to the mosquitoes on dorsal surface of the thorax at the rate of 0·001 ml. of the solution per mosquito. Dosages of D.D.T. applied were expressed as μg./mosquito. 0·001 ml. alcohol was applied to the mosquitoes for purposes of comparison. The treated mosquitoes were kept under observation for 24 hours in glass chimneys and were provided with glucose solution. Mosquitoes found ' dead beyond doubt ' were recorded as dead after the holding period.

Larval susceptibility tests were carried out in enamel bowls (of 1850 ml. capacity and 26 cm. surface diameter) containing 1000 ml. tap water. The temperature of water was found to vary between 20° - 30° C. D.D.T. (technical) alcoholic solution of varying concentrations was applied to each bowl at the rate of 1 ml. of the solution per bowl. The dosages of D.D.T. were expressed as parts per million (p.p.m). 30 larvae of late 3rd or 4th instars were exposed to each concentration. Mortality counts were made after 24 hours of exposure. Larvae found ' dead beyond doubt ' were recorded as dead. Pupation rate was found to vary in different replicates, hence the number of pupae were excluded from the total number of larvae exposed while calculating the mortality rates. Controls, using 1 ml. absolute alcohol, were set up with each batch of experiment.

The mortalities in control, both in the adult and larvae, were used for correcting mortality rates observed at different concentrations.

DESIGN OF THE EXPERIMENTS.

To estimate mean response rates of different strains to D.D.T., both adults and larval tests were, as far as possible, designed and planned following the suggestion of Finney (1952*a*: 1952*b*). The susceptibility tests were carried out both by the Scalar dosage method and Discriminating dosage method wherever applicable. In the susceptibility tests with RS (F_2) and SR (F_2) strains and X strain, the concentrations of D.D.T. used included the D.D.T. concentration (median lethal) for R and S strains respectively.

BIOMETRIC TECHNIQUE.

The mortality data obtained by the ' Scalar dosage ' method were analysed by Probit analysis technique*. The mean response rates, MLDs, and the regression coefficients, b_s, of the different strains were estimated following the " Maximum likelihood" estimation method outlined by Finney (*loc. cit.*). The standard errors, SE_s, of MLDs and their fiducial limits with 95 per cent probabilities, were calculat-

*It was doubted whether 'probit analysis' method would be applicable in case of heterogeneous populations RS (F_2), SR (F_2) and X strains. The non-significant values of χ^2, however, showed that the probit analysis method was valid in the present study.

ted for each strain. Furthermore χ^2 tests were also done to see (*a*) the validity of the test methods, (*b*) the goodness of fit of the ld-pr line to the observed data and (*c*) homogenity of response in mosquitoes of different batches treated with the same dose.

In order to test the segregation ratio in F_2 generations and the offspring of hybrid F_1 parent backcross, the expected heterogeneous population was exposed to discriminating dosage to estimate the individuals of different groups, namely susceptible, hybrid and resistant.

It may be seen from Graph 1 that all the different populations were exposed to varying degrees of risk of dying at any of the discriminating dosages. This point was, therefore, given due consideration in calculating the expected mortalities in different strains based upon Mendelian segregation. In order to be able to calculate mortalities in different strains, some simple statistical formulae were applied (Appendix I). The calculated mortalities were then compared with the actual mortalities observed by toxicological tests.

GRAPH 1.

The fitted ld-pr lines for *C. fatigans* (adult-female) of different strains.

RESULTS.

The basic data on the susceptibility tests with adults and larvae of different strains are given in Tables I and II. The results of statistical analysis are given in Tables III, IV and V.

TABLE I.

Basic data of tests of susceptibility to D.D.T. with different strains of C. fatigans *(Adults).*
(Topical application method).

Strains of *C. fatigans*	Dosage of D.D.T. µg./mosquito																							
	10·0			5·0			2·5			1·25			0·625			0·3125			0·15625			0·078125		
	n	r	p	n	r	p	n	r	p	n	r	p	n	r	p	n	r	p	n	r	p	n	r	p
Strain R ..	166·6	114·6	68·8	199·0	101·0	50·7	193·1	55·1	28·5	125·4	21·4	17·1												
„ S ..	..	..	..	..	..	..	72·1	65·1	90·3	81·8	59·8	73·1	96·0	52·0	54·2	107·0	25·0	23·4	39·9	2·9	8·8	..	..	..
„ X ..	18	17	93·9	31	27	85·8	..	..	..	..	..	..	34	13	32·2	36	6	8·7	..	..	..	..	..	..
„ RSF_1	..	..	..	..	..	..	42·9	31·9	74·3	30·2	14·2	47·0	67·0	17·0	25·4	19·1	1·1	5·7	..	..	..	..	..	..
„ SRF_1	..	..	..	..	..	..	68·6	56·6	82·5	64·6	41·6	64·4	130·8	62·8	48·0	65·2	15·2	23·3	86·1	12·1	14·0	..	..	..
„ RSF_2	..	..	..	149·7	115·8	77·3	99·3	70·3	70·8	..	..	..	101·2	21·2	20·9	102·2	22·2	21·9	..	..	..	75·0	6·0	8·0
„ SRF_2	..	..	..	78·7	71·7	91·1	73·6	61·6	83·7	..	..	..	73·2	32·2	44·0	82·8	17·8	21·5	..	..	..	64·9	0·9	1·0
„ $R \times RSF_1$ (Back cross)	..	..	..	54·4	40·4	74·3	30·5	19·5	63·9	49·5	21·5	43·4	20·0	6·0	30·0	..	..	..	..	..	..	..	..	..
„ Z ..	77	58	75·3	78·6	49·2	62·6	75·0	26·0	34·7	73·6	18·5	25·1												

n = number of mosquitoes treated (after correction).
r = „ „ „ dead „ „
$p = \frac{100\,r}{n}$

NOTE.—
The control mortality rates in different replicate tests were found to vary between 0–26 per cent. Since in different replicates the same dosages were not always tried there was a danger of either over-estimating or under-estimating the true mortality rates in different dosages if Abbott's formula is applied to summed up results. Therefore, n and r were corrected for the control mortality rates of each test and summed up. These values were then used to estimate the mortality rates in different dosages. In such cases, weighting coefficient value for further calculations were read from Table II (Finney, 1952) for C = 0.

TABLE II.

Basic data of tests of susceptibility to D.D.T. with different strains of C. fatigans *larvæ.*

Dosages of D.D.T. in parts per million (p.p.m.).

Strains of *C. fatigans*	20·0			10·0			2·5			1·25			0·625			0·3125			0·078125			0·0390625			CONTROL			REMARKS
	n	r	p	n	r	p	n	r	p	n	r	p	n	r	p	n	r	p	n	r	p	n	r	p	n	r	p	
Strain R	641	579	89·5	1130	1001	85·9	1126	771	66·9	..	..	..	563	272	45·7	..	..	..	..	..	..	..	..	..	273	13	4·8	2 ml. of 1 per cent D.D.T. solution in alcohol was used for getting 20 p.p.m. dose.
„ S	..	..	..	..	..	..	..	..	..	1271	1196	93·9	..	..		1751	1396	79·1	1620	824	50·2	..	..	..	300	8	2·7	
„ SR(F_1)	..	..	..	..	..	..	..	..	..	148	146	98·6	..	..		144	120	88·3	141	78	55·3	139	10	7·2	26	..	0·0	
„ RS(F_1)	..	..	..	..	..	..	..	..	..	..	..	..	..	..		141	117	83·0	143	68	47·5	136	20	14·7	28	..	0·0	
„ SR(F_2)	..	..	..	..	..	..	..	..	..	517	499	96·1	542	444	79·9	..	..	..	365	189	46·4	309	112	29·1	80	8	10·0	
„ RS(F_2)	..	..	..	..	..	..	267	255	95·2	466	431	92·1	96	81	83·5	..	..	..	422	112	22·2	285	41	9·4	73	4	5·5	
„ Z	..	..	..	67	60	89·5	65	34	52·3	..	..	..	30	5	16·7	..	..	..	..	..	..	..	..	..	28	..	0·0	11·8 per cent mortality rate was also observed in 0·15625 p.p.m. Number of dead was 4 out of 37 exposed.

n = number of larvæ exposed.
r = „ „ „ dead.
p = corrected mortality rate.

TABLE III.

Equation of the fitted ld-pr line and the parameters with their standard errors estimated from the susceptibility test data of different strains of C. fatigans *adults to D.D.T.*

Strains of *C. fatigans*	Equation of the fitted ld-pr line. (X = log 100xZ)	χ^2 (d. f.)*	m (log. 100 μg/mosquito)	Variance of m	MLD μg/mosquito	Fiducial limits of MLD (μg./mosquito)	Regression Coefficient (b)
Strain R	Y = 1·5957X + 0·6746	3·57 (19)	2·711 ± 0·012	0·000148	5·14	5·42—4·87	1·6 ± 0·2
„ S	Y = 2·1734X + 1·0926	0·86 (13)	1·798 ± 0·032	0·001064	0·63	0·73—0·54	2·2 ± 0·2
„ X	Y = 1·8404X + 1·1007	0·43 (2)	2·119 ± 0·092	0·008426	1·31	1·99—0·87	1·8 ± 0·3
„ RS(F_1) ..	Y = 2·2495X + 0·2061	0·23 (8)	2·131 ± 0·05	0·002815	1·35	1·72—1·06	2·2 ± 0·4
„ SR(F_1) ..	Y = 1·7016X + 1·8212	0·27 (14)	1·8681 ± 0·039	0·001590	0·74	0·88—0·62	1·7 ± 0·2
„ RS(F_2) ..	Y = 1·4141X + 1·9798	8·11 (18)	2·14 ± 0·04	0·002080	1·38	1·66—1·15	1·4 ± 0·1
„ SR(F_2) ..	Y = 2·0727X + 0·9514	6·46 (12)	1·953 ± 0·039	0·001513	0·90	1·07—0·75	2·1 ± 0·1
„ RxRS(F_1)(back-cross strain) ..	Y = 1·2844X + 2·1468	0·18 (4)	2·221 ± 0·085	0·007281	1·66	2·44—1·13	1·28 ± 0·3
„ Z	Y = 1·6484X + 0·7699	1·24 (10)	2·566 ± 0·046	0·002129	3·68	4·5—3·0	1·6 ± 0·2

* Degree of freedom (d. f.) = *SB*—2
where *SB* = Total number of batches of mosquitoes for all dosages in the experiment.

TABLE IV.

Equation of the fitted ld-pr line and the constants with their standard errors estimated from the susceptibility test data of different strains of C. fatigans *larvæ to D.D.T.*

	Strains of *C. fatigans*	Equation of the fitted ld-pr line (X = log 100 Z)	χ^2 (d. f.)*	*m* in log 100 dose 1 (p.p.m.)	Variance of m	MLD (p.p.m.)	Fiducial limits of MLD (in p.p.m.)	Regression coefficient b
Strain	R	Y = 0·9536X + 3·1553	6·25 (122)	1·934 ± 0·042	0·001776	0·859	1·037—0·711	0·9 ± 0·05
,,	S	Y = 1·2689X + 3·8698	1·14 (160)	0·891 ± 0·024	0·000576	0·078	0·087—0·0698	1·3 ± 0·05
,,	SR(F_1) ..	Y = 2·3756X + 2·5845	8·36 (22)	1·016 ± 0·052	0·002703	0.104	0·131—0·082	2·4 ± 0·18
,,	RS(F_1) ..	Y = 1·9625X + 2·979	0·50 (13)	1·030 ± 0·045	0·001191	0·107	0·131—0·087	1·96 ± 0·20
,,	SR(F_2) ..	Y = 1·3768X + 3·5655	17·44 (62)	1·041 ± 0·035	0·001255	0·110	0·129—0·094	1·4 ± 0·09
,,	RS(F_2) ..	Y = 1·7100X + 2·6775	2·3(56)	1·358 ± 0·028	0·000800	0·228	0·259—0·201	1·7 ± 0·07
,,	Z	Y = 1·4428X + 1·6922	4·1 (6)	2·293 ± 0·070	0·004931	1·963	2·67 —1·43	1·4 ± 0·17

* Degree of freedom (d. f.) = *SB*—2
where *SB* = Total number of batches of larvae for all dosages in the experiment.

TABLE V.

Results of testing of hypothesis of segregation of characters in different strains.

Strains	Hypothesis of segregation (phenotypic ratio of characters in the population)	Discriminating dosages											
		MLD of S strain				MLD of RS (F_2) strain				MLD of R strain			
		d	n	s	c	d	n	s	c	d	n	s	c
RS (F_2) (Adult)	SS:RS:RR = 1:2:1	4·8	101·2	4·4	1·1	..	..	..	..	7·1	149·7	4·7	1·5
SR (F_2) (Adult)	(SS+SR) : RR = 3:1	3·4	73·2	4·2	0·81	..	..	..	..	4·0	78·7	3·1	1·3
X (Adult)	–do–	0·8	31	2·7	0·03	..	..	..	..	2·2	34·0	2·0	1·1
RRS (F_1) (Adult)	RS:RR = 1:1	..	..	..	..	4·7	49·5	3·3	1·4	2·2	54·4	3·4	0·65
RS (F_2) (Larvae)	(SS+SR):RR = 3:1	77	398·8	9·3	8·2*	..	..	..	..	..	..	..	..
SR (F_2) (Larvae)	–do–	16	328·5	8·9	1·81	..	..	..	..	..	..	..	..

* Significant at P.— 0·05

d = deviation of observed mortalities from the expected mortalities.

n = total number under observation.

$s = \sqrt{pqn}$, where p is probability of dying of an individual, q is 1-p.

$c = \frac{d}{s}$

Before discussing the results, two points need clarification (*a*) whether or not the strains used for these experiments were homogeneous in respect of susceptible or resistance character and (*b*) whether or not the toxicological techniques used were sensitive enough.

The MLD of D.D.T. for the resistant strain was found to be 5·0 μg./mosquito (Bami *et al.*, 1957) and the regression coefficient was found to be 1·7 (Kalra, 1958—personal communication) by the topical application method. After 9 months interval, the strain was tested again and the MLD and regression coefficient were found to be 5·14 μg. per mosquito and 1·6±0·2 respectively. There has been no change in the susceptibility and homogenity of this strain during all these months; hence it may be considered to be homogeneous.

The susceptible strain likewise may also be considered to be homogeneous, as this strain failed to develop resistance when subjected to D.D.T. selection pressure in the laboratory for a number of generations. The MLD and regression coefficient values for this strain were found to be 0·63 and 2·2±0·2 respectively (Table III). The population of both the strains appears to be almost of equal homogenity as there was no significant difference between the regression coefficient values for the two strains. In the adult test the resistant strain was found to be 8·2 times more resistant than the susceptible strain, and in the larval test 11 times as resistant (Tables III and IV).

The values of regression coefficient $2{\cdot}2 \pm 0{\cdot}2$ and $1{\cdot}27 \pm 0{\cdot}05$ obtained in the adult and larval tests respectively for the susceptible strain suggest that the larval test method was not as sensitive as the adult test method*. It was found, therefore, that the topical application method showed a significantly higher MLD for RS (F_1) strain than the susceptible strain tested under almost identical conditions, but in the larval test with this strain, such a difference was not brought out clearly (Tables III and IV). Crow (1954) and Busvine (1957) have also pointed out that different values of MLD and regression coefficient may be observed for the same biological material by using different toxicological techniques.

S, *R* AND RECIPROCAL CROSSES.

The MLD of D.D.T. for susceptible, resistant and their reciprocal crosses are given in Table III.

It would be evident that MLDs for the hybrid strains, differed significantly from that for the resistant parent strain. Resistant strain was about 3·8 and 7·0 times more tolerant than the RS (F_1) and SR (F_1) strains respectively. When the MLD value of susceptible parent strain was compared with the MLD value of RS (F_1) strain, it was found that the RS (F_1) strain is 2·1 times as tolerant as the susceptible strain. On the other hand, SR (F_1) strain showed equal susceptibility to D.D.T. In the larval test, both RS (F_1) and SR (F_1) strains were found to be equally susceptible to D.D.T. and their MLD values did not differ significantly from similar value obtained for the susceptible strain (Table IV). The differences in the results of adult and larvae tests may be considered, as has been discussed earlier†, due to the varying sensitivity of the two methods used.

The observation recorded above that the hybrid (F_1) strains were almost as susceptible to D.D.T. as the parent susceptible strain, would suggest that the resistance is almost or completely a recessive character. Davidson (1958) has also observed that resistance to D.D.T. in *A. sundaicus* is almost completely recessive.

SEGREGATION OF RESISTANT FACTORS.

The MLD and the regression coefficient values for RS (F_2) strain were 1·38 μg./mosquito and $1{\cdot}4 \pm 0{\cdot}1$ respectively. It may be seen from Table III that the regression coefficient value for RS (F_2) strain was significantly lower than the similar value of *S* strain and apparently lower than that of RS (F_1) strain, suggesting that the population was more heterogeneous (Graph 2).

In view of the heterogenity of the population, the apparent higher MLD of D.D.T. for RS (F_2) strain than RS (F_1) may be considered as an indication of the presence of highly tolerant individuals in this population.

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* Lack of proper standardization of the test larvae may also influence the value of regression coefficient and therefore the results of adult tests were given primary importance. Information obtained from larval tests was used as supporting evidence.

† The possibility that larvae may not be exhibiting the defence mechanism operating in adults, is less in this case, because significantly higher MLD was observed with RS (F_2) than SR (F_2) larvae.

GRAPH 2.
The fitted ld-pr line for *C. Fatigans*-RS (F_2) (Adult).
N. B:—The dotted curve suggests a mixed population.

Assuming that the segregation was in the ratio 1:2:1 the MLD for RS (F_2) strain was calculated as 1·56 μg./mosquito.*

This value was closely corresponding to the experimental value obtained for RS (F_2) strain by toxicological experiments.

Further confirmation of the segregation of resistance factor in the ratio of 1:2:1 was obtained by discriminating dosage method, the results of which are given in Table V. The mortalities observed in RS (F_2) strain at MLD of D.D.T. for S and R strains, were 21·2 and 115·8 respectively. These values are in consonance with the expected mortalities of 26·0 and 122·9 respectively based on 1:2:1 segregation ratio. (*vide* Table V).

The larval tests also gave identical results. The MLD of D.D.T., 0·23 ppm was significantly higher than the similar value for S, and RS (F_1) strains. Assuming that the RS (F_2) population was composed in the ratio of 1 (S); 2 (hybrids) and 1 (R), the MLD was calculated as 0·17 p.p.m., which is almost the same as has been observed in the actual larval tests on RS (F_2) strain.

* Log 100MLDs. of susceptible strains $\frac{1{\cdot}798 + \text{RS } (F_1) - 2{\cdot}131 \times 2 + \text{R} - 2{\cdot}711}{4} = 2{\cdot}193$. Antilog of $2{\cdot}193 = 155{\cdot}96$; therefore estimated MLD $= \frac{155{\cdot}96}{100} = 1{\cdot}5595$ μg./mosquito.

By the discriminating dose method, at D.D.T. concentration (MLD of S strain), however, the results of significance test did not show the close agreement between the observed mortality and the expected mortality based on the above segregation ratio. The observed mortality was lower [but such was not the case with the SR (F_2) strain] which may be due to the influence of cytoplasmic factor (*vide infra*).

The MLD of D.D.T. for SR (F_2) strain was found to be 0·9 μg./mosquito and this value was significantly higher than the MLD value for S strain which was found to be 0·63 μg./mosquito. The regression coefficient value for this strain was 2·1 $\pm$0·1 but it did not differ significantly from that of the S strain. The presence of resistant individuals in the F_2 generation, raised the value of MLD; but unexpectedly enough, the regression coefficient value was not effected.

These observations were further confirmed by the data collected on the X strain [simulated SR (F_2) population as mentioned earlier]. The MLD of the X strain was significantly higher than the MLD for the S strain; but the regression coefficient values for the X and S strains were not found to differ significantly (Table III).

GRAPH 3.

The fitted ld-pr lines for SR (F_2) and X strains of *C. fatigans*.

The MLD and regression coefficient values of X strain and the SR(F_2) strain did not differ significantly from each other (Table III and Graph 3.). This indicated that the population of the two strains were of identical

composition i.e. 1:2:1 ratio of S, hybrid SR(F_1) and resistant. Discriminating dosage method also confirmed the segregation in the above ratio (Table V).

The expected number of mosquitoes dead at the LD_{50} dosage for strains S and R were 28·8 and 67·7 against 32·2 and 71·7 actually observed in the tests. The differences between the calculated and observed mortality were not found to be significant. In the larval tests also, the MLD value 0·11 p.p.m. was significantly higher (1·4 times) than the similar value for the S strain. This may be considered due to the presence of resistant larvæ in the population of SR (F_2) strain. The results of the significance test on the data collected by discriminating dose method confirmed the presence of larvæ of the S, SR(F_1) and R strains in the ratio of 1:2:1 (Table V).

RRS (F_1) — BACKCROSS STRAIN.

The MLD and regression coefficient values for the RRS (F_1) were found to be 1·66 μg./mosquito and 1·28±0·3 respectively. The significantly lower value of *b* and higher MLD for this strain than the S strain indicated the heterogenity of the RRS (F_1) strain due to the presence of resistant mosquitoes in its population. The results of significance test with the data obtained by discriminating dose method confirmed 50:50 ratio of R and RS(F_1) in RRS (F_1) population.

CONFIRMATION BY BIOLOGICAL EXPERIMENTS.

The presence of resistant individuals in the SR (F_2) and RS (F_2) strains, as detected by both the toxicological and statistical methods, was further confirmed by biological experiments. For this purpose, fourth instar larvæ of SR (F_2) and RS (F_2) strains were subjected to D.D.T. selection pressure (at a concentration of 1·25 p.p.m. which is slightly higher than the LD_{50} for the R strain)* by employing larval susceptibility test technique. The surviving larvæ of both the strains were pooled and reared to adult mosquitoes. The adults were inbred for raising F_3 generation (Z strain). The adults and larvæ of the Z strain were tested for their susceptibility to D.D.T. The MLD of D.D.T. for the adults of this strain was found to be 3·7†/μg./mosquito. This value was significantly higher than the similar value observed for S strain. The results of larval test also showed that the larvæ of Z strain were 25·2 times as resistant as the S strain. These results confirmed that some proportion of individuals were resistant to D.D.T. in SR (F_2) and RS (F_2) strains.

The appearance of susceptible, hybrid and resistant genotype in the ratio 1:2:1 in F_2 generation of the reciprocal crosses of the R and S strains; and the 1:1 ratio of R and RS (F_1) phenotype in the progeny of the 'resistant hybrid' backcross, RRS (F_1) strain, is possible only when the gamets for a 'single factor' are pure and when they (gamets) combine in a random way to form zygote. This suggests that the D.D.T.-resistance is controlled by a single recessive gene or a 'radical'‡ and that this factor is inherited according to the Mendelian Laws.

* Mortality rates were 96 and 86 per cent when 1320 and 51 larvae of SR (F_2) and RS (F_2) strains respectively were exposed to DDT concentrations of 1·25 p.p.m.

† This lower MLD value than that of *R.* strain (5·1 μg./mosquito) may be due to survival of some susceptible and hybrid larvae when they were subjected to selection pressure in F_2 generations.

‡A number of genes completely clustered together as to be inheritable as a unit.

LOCATION OF RESISTANCE FACTOR.

In view of the fact that:

(*a*) the MLD level for female adults has remained static over a number of months,

(*b*) the segregation of susceptible and resistant individuals (phenotypes) is in the ratio of 3:1 in SR (F_2) strain and,

(*c*) the segregation of susceptible, hybrid and resistant individuals (phenotype) is in the ratio of 1:2:1 in RS (F_2) strain;

it may be concluded that the resistance factor was not linked with the sex chromosome. It would, therefore, appear that the R-factor is associated with one of the autosomes. It is, however, not possible to specify the autosomes from the data collected by us.

In *Drosophila* presence of the resistant factor (resistant to D.D.T., B.H.C., Diptrex, Parathion etc.) in the 2nd chromosome, has been reported by Tsukamoto (1958).

MATERNAL OR CYTOPLASMIC EFFECT.

The inheritance of D.D.T.-resistance in *C. fatigans*, however, cannot be explained completely by the presence of a single recessive gene or a 'radical' in one of the autosomes as the results of the reciprocal crosses are not identical (*vide* Table II). As stated earlier, the mean tolerance levels of the female mosquitoes of RS (F_1) and RS (F_2) were significantly higher than the corresponding values of SR (F_1) and SR (F_2) strains respectively. In the larval test also, higher MLD was observed in RS (F_2) strain than the SR (F_2) strain. The association of higher tolerance in F_1 generation and its persistency in the F_2 generation in the line originated from the crosses of female R and male S strains, also suggest the contribution of cytoplasmic or maternal factor in the inheritance of D.D.T.-resistance in *C. fatigans*. The contribution of cytoplasmic factor in the inheritance of resistance has also been reported in *Blattella germanicas* (Cochran *et al.*, 1952) in *Musca domestica* (Johnston *et al.*, 1954) and in *Drosophila* (Tattersfield and Kerridge, 1953). A critical examination of the susceptibility test results reported by Davidson (1958) also suggest the influence of cytoplasmic or maternal factor in the inheritance of D.D.T.-resistance in *A. sundaicus*.

It may be seen from Table III that the RS (F_1) and RS (F_2) strains were 1·8 and 2·0 times more resistant than the SR (F_1) and SR (F_2) strains respectively. Similarly lower degree of tolerance, contributed by cytoplasmic factor, was also found in *Blattella germanica* and *A. sundaicus* (in F_2 generation).

SUMMARY.

The inheritance of resistance to D.D.T. in *C. fatigans* was studied by crossing susceptible and resistant strains of this species. It has been observed that D.D.T. resistance in *C. fatigans* is due to a single recessive gene or a 'radical'. This gene does not appear to be associated with the sex chromosome but one of the autosomes. In addition to this factor, cytoplasmic or maternal influence has also been observed.

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APPENDIX I.

Evolution of simple equations for estimation of expected mortality rates at discriminating dosage based on the Mendelian Law of segregation of a character in F_2 generation.

When inheritance of one character determined by a single gene is under study, according to 'Mendelian Law', the expected genotype ratio in the population of F_2 generation is 1:2:1. If the character is completely recessive, the phenotype ratio of the individuals possessing the characters is 3 : 1, but when it is completely dominant, the ratio is 1 : 3. In case of intermediate dominance, the phenotype ratio is 1:2:1.

In the resistance study, characters are estimated by the mean response rates, of the populations. As such, the MLD values for the parents and hybrid populations are used as discriminating dosages to study the segregation of characters in F_2 generations.

If a random sample of size *n* is drawn from the population of F_2 generation, according to the Mendelian Law, the sample is likely to consist of $0{\cdot}25_n$ susceptible, $0{\cdot}5_n$ hybrids and $0{\cdot}25_n$ resistant individuals.

When *n* number of mosquitoes are exposed to MLD (s) (mean response rate of susceptible strain) the expected mortality in the susceptible class is

$$\frac{0{\cdot}25n}{2} = 0{\cdot}125n \ldots\ldots\ldots\ldots\ldots\ldots (a)$$

Since this dose, MLD(s), also causes some mortality to the mosquitoes of hybrid and R-classes (*vide* Graph 1), it is essential to estimate these mortalities also.

The expected mortality of hybrid class at MLD(s) is

$$0{\cdot}5np^{hs} \ldots\ldots\ldots\ldots\ldots\ldots (b)$$

where p^{hs}, is the probability of dying of the hybrid individuals at MLD(s).

Similarly, the expected mortality of resistant class at MLD(s) is

$$0{\cdot}25np^{rS} \ldots\ldots\ldots\ldots\ldots\ldots (c)$$

where p^{rS} is the probability of dying of the resistant individuals at MLD(*s*). Therefore, the sum of the mortalities of different strains, expected number dead, i.e., E.M.(s), at MLD(s) is

$$E.M.(s) = 0{\cdot}125n + 0{\cdot}5np^{hS} + 0{\cdot}25np^{rS} \ldots\ldots\ldots\ldots (\text{sum of a, b and c}).$$

$$\text{or } E.M.(s) = n(0{\cdot}125 + 0{\cdot}5p^{hS} + 0{\cdot}25p^{rS}) \ldots\ldots\ldots\ldots (1)$$

In order to estimate the values of p^{hS} and p^{rS}, first probit values are worked out solving the ld-pr line equations of the hybrid and resistant populations respectively by substituting the log MLD value of the susceptible population for x with equations of the ld-pr lines. Probit values thus worked out are referred to Table I (Finney, 1952) and the probabilities of dying of different populations are estimated from the percentage mortalities read in the Table.

Similarly the equations for the expected number dead at MLDs of hybrid and resistant populations, EM(H) and EM(R), are evolved and given below:

$$EM(H) = n(0{\cdot}25\ p^{sH} + 0{\cdot}25 + 0{\cdot}25\ p^{rH}) \ldots\ldots\ldots\ldots (2)$$

$$\text{and } EM(R) = n(0{\cdot}25p^{sR} + 0{\cdot}5p^{hR} + 0{\cdot}125) \ldots\ldots\ldots\ldots (3)$$

where p^{sH} and p^{rH} are probabilities of dying of susceptible and resistant individuals respectively at LD 50 of hybrid strain and p^{sR} and p^{hR} are probabilities of dying of susceptible and hybrid strains respectively at LD 50 of resistant strain.

On the above rationale the following equations for estimating the expected mortalities are evolved when the character is (*a*) recessive and (*b*) dominant.

(*a*) *When the character (resistant) is recessive:*

$$EM(S) = n(0{\cdot}375 + 0{\cdot}25p^{rS}) \ldots\ldots (4)$$
$$EM(R) = n(0{\cdot}75p^{sR} + 0{\cdot}125) \ldots\ldots (5)$$

(*b*) *When the character (resistant) is dominant:*

$$EM(S) = n(0{\cdot}125 + 0{\cdot}75p^{rS}) \ldots\ldots (6)$$
$$EM(R) = n(0{\cdot}25p^{sR} + 0{\cdot}375) \ldots\ldots (7)$$

On the similar line, the formulæ for estimation of expected numbers dead, for the population obtained by the back cross of F_1 hybrid and either of the parent stock, are worked out and given below. In these cases, the MLD for hybrid and the parent (used for crossing) are used as discriminating dosage. According to theory 50:50 ratio of hybrid and parent (used for crossing) are expected and they can only be discriminated if they are phenotypically found different.

$$EM(H) = n(0{\cdot}25 + 0{\cdot}5P^{pH}) \ldots\ldots (8)$$
$$EM(P) = n(0{\cdot}5P^{hP} + 0{\cdot}25) \ldots\ldots (9)$$

where P^{pH} and P^{hP} are the probabilities of dying of parent class used for crossing and hybrid (F_1) individuals at LD_{50} of hybrid F_1 and parent strains respectively.

EXAMPLE.

The $RS(F_2)$ strain showed 20·9 per cent and 77·3 per cent mortality rates at LD_{50} for S strain and R strain respectively (*vide* Table I). In this case resistance was observed to be slightly dominant. It is intended here to test the hypothesis that resistance is determined by a single gene.

If resistance is controlled by a single gene, according to Mendelian Law, the susceptible, hybrid and resistant genotypes are expected to be present in $Rs(F_2)$ in the ratio of 1:2:1.

Owing to overlapping phenotypes, the expected mortalities at MLD(s) and $MLD(_R)$, are estimated, on these ratios, and are given below:

$$EM(S) = n(0{\cdot}125 + 0{\cdot}5p^{hS} + 0{\cdot}25P^{rS}) \ldots\ldots \text{equation (1)}$$

To solve the equation (1) the values of p^{hS} and p^{rS} are required, estimation of which are as follows:

Estimation of the probability of dying of hybrid class at LD50 of S strain, (p^{hS})

$Y = 2{\cdot}2495x + 0{\cdot}2061 \ldots\ldots$ equation of the fitted ld-pr. line for $RS(F_1)$ strain (vide Table III).

$Y = 2{\cdot}2495 \times 1{\cdot}798 + 0{\cdot}2061$ [substituting the log $MLD(_S)$ value for x].

or $Y = 4{\cdot}2507$* (in probit).

From Table I (Finney, 1952), 22·7 per cent response rate is read for the probit value 4·2507.

* The probit values can also be read approximately from the ld-pr lines of RS (F_1) and R strains for LD_{50} of S strain (Graph I).

The probability of dying of the RS(F_1) population at MLD($_S$) is 0·227.

Estimation of the probability of dying of R strain at LD_{50} for S strain, (p)rS.

$Y = 1{\cdot}5957x + 0{\cdot}6746$ ..... (equation of the fitted ld-pr line for R strain).
$Y = 1{\cdot}5957 \times 1{\cdot}798 + 0{\cdot}6746$ .... [substituting the log MLD($_S$) for x]. or
$Y = 3{\cdot}5437$* (in probit).

From Table I (Finney, 1952), 7·3 per cent response rate is read for the probit value 3·5437

the probability of dying of R strain at MLD($_S$) is 0·073. Putting the estimated values of p^{hS} and p^{rS} and n in equation (1),

$EM(S) = 101{\cdot}2\ (0{\cdot}125 + 0{\cdot}5 \times 0{\cdot}227 + 0{\cdot}25 \times 0{\cdot}0730) = 26$

Likewise, the expected mortality at MLD of R strain EM(R), is estimated as 122·9.

The number of mosquitoes observed dead when exposed to MLD_s for S and R strains were 21·2 and 115·8 respectively. The standard error, s, of the mortalities at MLD(S) and MLD(R) were $4{\cdot}4(\sqrt{pqn} = \sqrt{0{\cdot}257 \times 0{\cdot}743 \times 101{\cdot}2})$ and $4{\cdot}7(\sqrt{0{\cdot}821 \times 0{\cdot}179 \times 149{\cdot}7})$ respectively.

The normal deviate, c, is the ratio of d (deviation of observation from expectation) to s (Mather, 1951), and so in the present case at MLD($_S$) is $\frac{4{\cdot}8}{4{\cdot}4} = 1{\cdot}1$. Similarly the value of c for the data obtained at MLD($_R$) was calculated to be 1·5.

Both the values of c, i.e., 1·1 and 1·5 are less than 1·96 and therefore the differences between the observed and expected values are not considered significant at conventional level of probability (P.-0·05). The hypothesis of 1:2:1 ratio of susceptible, hybrid, RS(F_1) and resistant mosquitoes in RS(F_2) strain was therefore considered tenable.

* The probit values can also be read approximately from the ld-pr lines of RS (F_1) and R strains for LD_{50} of S strain (Graph I).

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BIOCHEMISTRY AND PHYSIOLOGY OF RESISTANCE BY INSECTS TO INSECTICIDES.

BY

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PRIOR to the reports of the development of resistance by houseflies and mosquitoes to D.D.T. in 1947, resistance had generally been considered a minor entomological problem affecting only a few agricultural pests. Indeed, there were many entomologists who did not believe the early reports of resistance and attributed the insecticide failures to poor application, adulterated insecticide, the weather, or ignorance on the part of the operator. Today, resistance is almost universally considered the most serious problem confronting the entomologist interested in the control of agricultural pests and arthropod vectors of disease.

Dr. George Decker (1958) quotes S. A. Forbes as saying "The struggle between man and insects began long before the dawn of civilization,..... We commonly think of ourselves as the lords and conquerors of nature, but insects had thoroughly mastered the world and taken full possession of it long before man began the attempt. ..... we can scarcely flatter ourselves that we have gained any very important advantage over them..... and since the world began we have never yet exterminated — and we probably never shall exterminate — so much as a single insect species." Many entomologists will undoubtedly disagree with the attitude expressed but the present state of our knowledge of how to control insects and related species, certainly indicates the need for serious thinking on the problem. It seems obvious that relaxation of our efforts even for a short time may have far-reaching consequences.

At the present time, there are only three genera of insects of public health importance in which resistance has not been found, Simulium, Phlebotomus, and Glossina. Resistance has appeared and has been authenticated to all the synthetic and natural insecticides now in widespread use. To date, the only apparent sure way to prevent the development of resistance is to stop the use of insecticides. This obviously is not a satisfactory solution. The literature on resistance is now most voluminous. However, most of the publications pertain to reports of new species becoming resistant, determining the build-up of resistant species, and to

ascertaining the efficacy of substitute insecticides in controlling species resistant to those materials in previous use. Only a small fraction of the publications deal with the biochemistry, physiology, ecology or genetics of resistance. Much genetical data relating to resistance that was obtained with *Drosophila melanogaster Meig.* has been used as the basis for conclusions on the inheritance of resistance in general. Whether or not such conclusions are always valid remains to be determined. The resistance developed by the housefly, *Musca domestica L.* to D.D.T. has been spectacular and widespread and for these reasons the housefly has been the insect of choice by many investigators who are attempting to elucidate the fundamental aspects of the resistance problem. D.D.T. has been the insecticide most frequently used, not only because of its importance but because it can be readily obtained in pure form and microanalytical methods are available both for D.D.T. and some of its common metabolic products.

It is the purpose of this paper to briefly summarize the present state of knowledge of the physiological and biochemical aspects of arthropod resistance to insecticides. For more extensive coverage, the reader is referred to the reviews by Brown (1958), Metcalf (1955), Winteringham and Barnes (1955), Hoskins and Gordon (1956), Crow (1957), Busvine (1957) or Lhoste (1955).

Much of the published evidence continues to be somewhat contradictory in nature. However, many of the differences are probably more apparent than real and are attributable to results indicating normal strain variation rather than being directly related to resistance. To date, none of the differences reported between the susceptible and resistant strains have universal applicability to all strains tested nor do any of the reported differences seem to offer a solution to the serious problems of how to control insecticide resistant arthropods once resistance is acquired, or how to control insects with insecticides and insure that resistance will not develop.

The physiology of resistance to the insecticides in general use prior to World War II is not discussed at this time. For a summary of the situation up to 1951, the review by Babers and Pratt (1951) is suggested.

Drug tolerance in humans has been known for many years. It has one aspect in common with arthropod resistance to insecticides — neither process is at all understood — but there most of the similarity ends. In man, the individual to whom the drug is administered is the one that develops tolerance but in arthropods it is the progeny of those that are treated that exhibit increased resistance.

Lindquist and Wilson (1948) were apparently the first to develop, from a normal laboratory colony, insects resistant to the post World War II insecticides. Since their report, many colonies, resistant to many insecticides and mixtures of insecticides, have been established. The general procedure has been to treat a large susceptible population with such a dosage of insecticide that about 90 per cent mortality will result. Eggs are obtained from the survivors and the treatment is repeated for succeeding generations. For the chlorinated hydrocarbons such as D.D.T., appreciable resistance is usually evident in about ten generations. Increase is then rapid and after about 25 - 30 generations, the colony may be virtually immune. With the pyrethrins and organophosphorus insecticides, resistance develops more slowly and generally to a lesser extent.

When the normal colony is treated with lower doses, a somewhat different effect is obtained. Hoffman *et al.* (1951) treated resistant and susceptible flies with sub-lethal doses of D.D.T. six times in three days. These flies were then more susceptible than unexposed insects. Hadaway (1956) obtained similar results with gamma isomer hexachlorocyclohexane (gamma-B.H.C.) on houseflies as did Beard (1952) working with D.D.T., nicotine, or pyrethrum treated *Galleria mellonella.* Harrison (1952) failed to produce a resistant strain of houseflies by treating susceptible insects with sub-lethal doses of D.D.T. but Eddy *et al.* (1955) produced highly resistant body lice, *Pediculus humanus corporis*, by treatment of a susceptible colony with D.D.T. that at first produced only about 5 per cent mortality. However, Cole *et al.* (1957) obtained only two-fold resistance in a louse colony after 26 generations exposure to a D.D.T. dosage that produced no mortality.

According to Brown (1958) insecticides do not produce mutations. Luers (1953) found no increase in the mutation rate of D.D.T.-treated *Drosophila.* It is doubtful, however, whether valid conclusions on the genetics of the whole resistance problem can be drawn from work with *Drosophila.* If one accepts that mutations may be caused by such chemicals as the nitrogen mustards, then the possibility must certainly exist that certain insecticides under favourable conditions may induce mutations. Crow (1957) concludes that " all the evidence still supports the original view: Insecticide resistance is an example of evolutionary change, the insecticide acting as a powerful selective sieve for concentrating resistant mutants that were present in low frequencies in the original population ".

Reports have frequently been made of strains of insects being from a few to several thousand times as resistant as normal strains. However, extreme care must be used in attempting to correlate or evaluate such figures with results from other laboratories since the results vary greatly with the method used to determine the degree of resistance. Busvine (1951) for example, found that when D.D.T. in acetone was applied topically to adult houseflies, an LD50 of 36 gamma per fly was obtained but, when a non-volatile mineral oil was used as a solvent, the LD50 was only 7·2 gamma/fly. When exposures are made to increasingly heavy residual deposits of an insecticide, one must question the effect of the layers of insecticide below the exposed surface. Considerable variation also occurs when different generations are tested under identical situations. If comparisons then are to be made, rigid adherence to standardized conditions must be the rule and conclusions should be drawn only after careful consideration of all facets of the data.

Many papers have been published in which attempts were made to establish morphological or biological differences between susceptible and resistant strains. Darker pigmentation, stiffer tarsal bristles, wider tarsal segments, thicker cuticle, and intersegmental membranes of the pulvilli, smaller diameter pulvilli, and differences in the ratio of the width to the length of the second abdominal sternum have all been reported as relating to resistance in houseflies but none seem to have universal applicability.

It has been reported from various laboratories (Brown, 1958) that houseflies emerging later were more resistant to D.D.T. than those emerging early and a resistant strain was developed by such selection. However, other reports indicate the

opposite to be true and it must be considered as not proven that length of larval period is directly related to resistance.

Other ratios determined between susceptible and resistant strains have shown no difference in pupal weights, egg production, viability of eggs, pupal or adult weights, sex variation, or length of egg stage. Sokal and Hunter (1955) made a detailed morphometric analysis of 16 body measurements of five resistant and four susceptible strains of houseflies and found no relationship to resistance.

That reduced rate of insecticide penetration is not the answer to resistance is clear from the fact that similar resistance is evident when the insecticide is applied by injection.

As pointed out by Metcalf (1955) "modern toxicological theory almost exclusively relates the mode of action of poisons to specific interference with biochemical systems, largely enzyme in nature. It is most probable that the secrets of insecticide resistance are to be found within this realm of biochemistry and enzymology.....".

At the present time the mode of action of no insecticide has been completely elucidated. Hence, it is not surprising that the biochemical and physiological nature of resistance have not been determined. Much progress, however, has been made. Evidence has been presented that at least in some cases, detoxification systems have been developed by the insect at the same time that resistance builds up so that the insect can cope with doses of toxicants that otherwise would be fatal.

In humans, according to Williams (1947), besides oxidation and reduction reactions, there are nine synthesis reactions or conjugation processes by which detoxication is accomplished. In a review on detoxication processes in insects, Smith (1955) lists 13 known processes by which poisons are eliminated but not all have been related to resistance. Detoxication processes are considered to be "all chemical changes which foreign organic compounds undergo in the animal body". The definition, of course, includes the change of non-toxic materials to toxic compounds as is the case with some of the phosphorus-containing insecticides.

Enzyme systems in arthropods are undoubtedly as numerous and as complicated as those in other living things. Only a few of these systems have been studied and none are completely understood.

The role of acetylcholine and cholinesterase in insects and related species has been frequently studied but is still not clear. However, the large amount of cholinesterase present in some tissues undoubtedly is there for a purpose. The housefly brain is one of the richest known sources of cholinesterase type enzymes but the amount present and the specific nature of the enzyme varies greatly between arthropod species. Spider mites apparently have little or no cholinesterase. Pratt and Babers (unpublished results), using about two milliliters of parathion resistant or susceptible *Tetranychus bimaculatus Harvey* could not demonstrate the presence of a cholinesterase in either strain. The insecticidal organophosphates generally are very active cholinesterase inhibitors, or through transformations in the animal or plant become so. It is frequently stated in the literature that in insects, the toxicity of the organophosphates is due to their anti-cholinesterase effect. Kearns (1956) in his review brings out the pros and cons of this theory and presents

weighty evidence that other enzyme systems are also involved. One can only conclude that the mode of action of the organophosphates is presently not known.

Houseflies, honeybees and the American roach contain other esterases in addition to cholinesterase. One esterase, not inhibited by metal salts, hydrolyzed a number of organophosphate insecticides such as paraoxon and parathion (Metcalf *et al.*, 1956).

Both an organophosphorus-resistant strain of houseflies and a susceptible strain metabolized paraoxon at a similar rate according to Lord and Solly (1956). No difference, other than variation in susceptibility to paraoxon, was noted between the strains. However, the LD50 for the resistant strain was only about twice that of the susceptible and for this reason the similarity in metabolic rates may not be meaningful.

Sternburg *et al.* (1950) and Perry and Hoskins (1950) and later numerous others showed that D.D.T.-resistant houseflies could much more rapidly metabolize D.D.T. than could susceptible strains. While the main metabolic product has not yet been isolated and its identity confirmed, excellent presumptive evidence indicates that the principal degradation product in houseflies is the relatively non-toxic 2,2 bis (p-chlorophenyl)-1,1 dichloroethylene commonly called D.D.E. Later work by Kearns and his co-workers has shown that the degradation process is enzymatic in nature, and they have called the responsible enzyme D.D.T.-dehydrochlorinase. Small amounts of D.D.E. have also been reported as being found in the tissues of D.D.T. treated susceptible strains. Hoskins and Gordon (1956) hypothesize that the small amount of D.D.E. in susceptible flies is due to the slow metabolism of D.D.T. by enzymes specific for other reactions or possibly by non-enzymatic chemical reactions.

Sternburg and Kearns and co-workers have been unable to demonstrate D.D.T.-dehydrochlorinase in susceptible insects and have concluded that the ability of resistant flies to degrade D.D.T. to D.D.E. is a major factor in the survival of poisoned flies. Apparently, only D.D.E. appears in the tissues as a metabolic product soon after resistant flies are treated with D.D.T., but after several days other metabolites are found according to Terriere and Schonbrod (1955).

Kerr *et al.* (1957), starting with a colony that had been established from wild houseflies in 1939 and had never been exposed to insecticides, developed two strains with resistance specific to D.D.T. One strain, " D ", was selected by exposing the larvæ to D.D.T. and the other, " L ", by selecting the late emerging adults from D.D.T. free media. When compared to the parent unselected strain, " U ", both " D " and " L " showed resistance and when acetone-ether extracted tissues were tested for D.D.T.-dehydrochlorinase activity, the same order of enzyme activity was present. The males were consistently somewhat less resistant than the females but exhibited slightly more D.D.T.-dehydrochlorinase activity. Thus " production of D.D.T.-dehydrochlorinase is not necessarily dependent on a stimulus from D.D.T.". The function of the enzyme in flies that had never been exposed to an insecticide, is not known. According to the authors, the enzyme activity of the unselected strain was negligible. However, the females of this strain produced 11·8 per cent of the amount of D.D.E. produced by females of the " D " strain and 9·4 per cent of that produced by females of the " L " strain. The significance of these amounts has yet to be determined.

Perry and Sacktor (1955) compared the absorption and metabolism of D.D.T. by three susceptible strains of houseflies with seven resistant strains and attempted to relate cytochrome oxidase activity with degradation of D.D.T. They reported that the susceptible strains metabolized little D.D.T. whereas the resistant strains rapidly metabolized the absorbed D.D.T. to D.D.E. The relative significance of this factor varied with each strain. Cytochrome oxidase activity varied between strains but there was no distinction due to resistance and no direct relationship between cytochrome oxidase activity and degradation of D.D.T. The authors conclude that "D.D.T. resistance among all strains cannot be characterized by a single common factor" and suggest that "each strain possesses a combination of attributes for resistance which may be different from that found in other strains". Miyake *et al.* (1957) consider that the internal tissues in houseflies have sufficient dehydrochlorinating ability to protect the vital site from those doses of D.D.T. brought to the site by the transport system.

In numerous cases, the amount of D.D.T. and metabolites recovered did not account for the D.D.T. applied to houseflies and the existence of other metabolites than D.D.E. has been suggested. In certain other strains, D.D.E. appears to be the only product since D.D.E. plus D.D.T. accounts for the D.D.T. applied. It is possible that the inability to account for all of the applied D.D.T. is a question of poor analytical technique as indicated by the work of Perry *et al.* (1955) who conclude that D.D.E. is the principal degradation product of D.D.T. by houseflies. It seems more probable that some strains of flies or the same strains under different conditions metabolize D.D.T. by different processes and to different end products.

In resistant *Musca domestica*, Sternburg and Kearns (1950) found that important sites of D.D.T. metabolism appear to be the hypodermal cells of the integument following topical application and the wall of the gut following ingestion. Miyake *et al.* (1957) found D.D.T.-dehydrochlorinase listed in order of decreasing abundance in the fat body, brain, cuticle, hæmolymph muscle, ovary, and intestinal tract.

Although most of the research with houseflies has been done with the adult insect, sufficient work has been done to indicate that the overall picture of D.D.T. metabolism in the immature stages is similar to that in the adult (Metcalf, 1955).

The presence of D.D.T.-dehydrochlorinase has not been demonstrated in susceptible houseflies. When resistant strains are developed from susceptible strains in which no dehydrochlorinase is seemingly present, apparently no attempt has been made to determine when the enzyme first appears nor whether it first appears in small quantities and then increases regularly with increased resistance.

It seems probable that the enzyme is present in susceptible insects in quantities too minute to be demonstrable by available assay methods and increases as resistance increases.

The effect of suspensions of D.D.T., D.D.E. and related compounds on the *in vitro* activity of the succinoxidase system of resistant and susceptible flies, was determined by Anderson *et al.* (1954). No pronounced differences were noted in the sensitivity to D.D.T. of enzyme preparations from the two strains. The inhibition of succinoxidase and its components by D.D.T., is not considered a primary

factor in the mode of action of D.D.T. or in the mechanism of D.D.T. resistance in the housefly.

Triosephosphate dehydrogenase in D.D.T. susceptible and resistant strains of houseflies is inhibited by iodo- and chloro-acetic acid according to Bettini and Boccacci (1956). There was little difference between the strains in enzyme activity or inhibition by the chemicals.

Synergists to enhance the activity of D.D.T. against resistant flies have been suggested and in some cases have at first shown promise. However, resistance soon develops to the combination and this line of attack has largely been abandoned. The more active synergistic compounds found so far are close structural relatives of D.D.T. The most effective seemed to be D.M.C. [1, 1-bis(para chlorophenyl) methyl carbinol] but at no time was the lethal dose of D.D.T. reduced to that of the susceptible strain. D.M.C. is rapidly metabolized by living flies and is excreted principally as a compound tentatively identified as D.D.A. [bis-(para chlorophenyl) acetic acid]. D.M.C. irreversibly inhibits D.D.T.-dehydrochlorinase and Moorefield and Kearns (1955) attribute the failure of D.M.C. to show a synergistic effect on susceptible flies to the lack of the enzyme in these strains. The dehydrochlorinase is also inhibited by other D.D.T. synergists.

Ability to rapidly metabolize an insecticide apparently is not always related to increased resistance. The American cockroach *Periplaneta americana* is normally susceptible to D.D.T. and as yet, no confirmed report of the development of resistance by this species has been noted. However, Vinson and Kearns (1952) report that at 15°C., 12 hours after treatment with D.D.T., 47 per cent of the D.D.T. absorbed is metabolized and this increases to 78·7 per cent, 96 hours after treatment. When the roaches were held at 35°C., the percentages metabolized increased to 60·8 per cent after 12 hours and 83·6 per cent after 96 hours. Although the weight of evidence indicates otherwise, Weisman and Reiff (1956) concluded that D.D.T. metabolism by houseflies had no bearing on resistance. The tarsi of their susceptible houseflies secreted twice as much lipoid onto the walking surface as did resistant insects and consequently picked up more D.D.T. They concluded that tissue lipoids provide a protective factor in resistance to insecticides. Continuing this line of investigation, Weisman (1957) reports that the epicuticle of resistant flies has 30 - 40 per cent more lipoids than that of susceptible flies. Resistant flies have inclusions of small fatty drops in the epidermal cells at the base of the setæ on the abdominal wall. The inclusions act as a barrier against the penetration of the active ingredient of D.D.T. because the substance entering through the papillæ of the setæ is dissolved in the lipoids and thus blocked. Butts *et al.* (1953) treated *Periplaneta* with C^{14} labelled D.D.T. and found that the main metabolic product was a conjugate not extractable from water by organic solvents. The conjugate was thought to be carbohydrate in nature and this would, according to Smith (1955), suggest a preliminary hydroxylation of D.D.T. followed by conjugation or else conversion of D.D.T. to the acid D.D.A. and conjugation of the carboxyl group. Neither oxidation of D.D.T. nor conversion to D.D.A. has been shown to occur in insects. Robbins and Dahm (1955) also used radioactive D.D.T. and found that about 75 per cent of the applied D.D.T. was excreted in the feces within 24 days. Little of the excretory product (less than 10 per cent) was D.D.T., D.D.E. or D.D.A.

The blood of D.D.T.-poisoned *Periplaneta* contains an ether insoluble toxin that is equally toxic to resistant and susceptible flies on injection. (Sternburg and Kearns, 1952).

Pratt and Babers (1953) applied D.D.T. directly to the thoracic ganglion of houseflies and found that leg tremors in resistant insects were produced less frequently than in susceptible ones and in the resistant insects, the induced tremors were of shorter duration. These authors (Babers and Pratt, 1953) also found that when massive doses of D.D.T. were injected into resistant insects, little or no metabolism of the insecticide occurred and the insects seemed unaffected. The argument has been advanced that, when large doses of insecticides are injected, the insecticide does not reach a site of action. The fact remains that large doses injected into susceptible insects are invariably fatal so at least some transportation of injected heavy doses of D.D.T. to vital sites does occur.

Weiant (1955) found that the sensory nerves of resistant flies are less sensitive to the direct action of D.D.T. than the nerves of susceptible insects, and the D.D.T.-resistant flies were less sensitive than susceptible flies to toluol vapour when this chemical was used as the evocative agent in electro-physiological studies.

According to Bradbury and Standen (1955 : 1956*a*), a resistant strain of flies metabolized gamma B.H.C. more rapidly than a susceptible strain with the formation of water soluble metabolites. Later, using radioactive insecticide, Bradbury (1957) detected eleven different metabolites by paper chromatography but radioactive CO_2 was not produced.

Oppenoorth (1956) also reported that gamma B.H.C. is rapidly broken down to a non-toxic derivative by a lindane (pure gamma B.H.C.) resistant strain of houseflies. The susceptible strain metabolized the material more slowly, and eventually metabolism ceases completely. This might be caused by toxic action of B.H.C. on the flies. Oppenoorth concludes that resistance to B.H.C. is due to a decreased rate of absorption and an increased ability to detoxify the material.

Sternburg and Kearns (1956) did not detect D.D.T. dehydrochlorinase in a B.H.C.-resistant strain of houseflies. However, both B.H.C. resistant and susceptible insects metabolized lindane and pentachlorocyclohexene was one of the intermediates in the metabolism.

At the Pest Infestation Laboratory in England [Anonymous (1958)] dieldrin-resistant flies *Musca domestica Vicina* were shown to excrete the Sulphur-35 analogue of dieldrin faster than do susceptible flies, especially during the first few critical hours following topical application of the insecticide. The dieldrin analogue appeared to undergo no extensive breakdown within the tissues and resistance was not due to greater impermeability of the cuticle. Since the dieldrin-resistant flies were also resistant to gamma B.H.C., carbon 14 labelled alpha- and delta-isomers were used to study the metabolism of these materials. In agreement with the work in other laboratories, both resistant and susceptible flies metabolized the materials to pentachlorocyclohexene and similar quantities of metabolite were found in each strain three hours after application. Significant quantities of a water soluble metabolite were found in the excreta. To the observers it seemed unlikely that either metabolism or a faster rate of excretion by resistant flies can account for the resistance.

Topically applied heptachlor is readily absorbed by heptachlor-resistant houseflies and is metabolized to heptachlor epoxide (Perry *et al.*, 1958). The epoxide is also toxic to susceptible houseflies. However, the susceptible insects also rapidly metabolize heptachlor, also to the epoxide. After 24 hours, the entire topically applied dose had been absorbed by both strains. About 53 per cent was recovered from the resistant insects and of this, 93 per cent was the epoxide. With the susceptible strains, 62 per cent of the applied dose was recovered and of this, 78 per cent was the epoxide. The authors conclude that "housefly resistance to heptachlor does not specifically involve a detoxication mechanism".

Reiser *et al.* (1953) noted a direct correlation between fat content and survival of cotton boll weevils (*Anthonomus grandi* Boh.) exposed to toxaphene, dieldrin, E.P.N., methyl parathion, and calcium arsenate, but the resistance was of the seasonal variety rather than true insecticide resistance. Hoffman and Lindquist (1952), using a bioassay technique, showed that toxaphene and chlordane were absorbed and metabolized by resistant houseflies but did not attempt to identify the metabolic products.

Alexander *et al.* (1958) attempted, without success, to relate phosphatase activity to resistance in houseflies and German roaches. March and Lewallen (1956) examined the fresh tissue extracts of four susceptible and five insecticide resistant strains of houseflies by means of paper chromatography. A number of differences in chromatographic patterns between the strains were noted but none could be directly correlated to susceptibility or resistance. Rather, origin of the strain seemed to be the important factor since a resistant strain showed the same pattern as the susceptible strain from which it was developed.

Resistant mosquitoes also metabolize D.D.T. to D.D.E. as shown by Brown and Perry (1956). Resistant *Aedes taeniorhynchus*, admixed with *Aedes sollicitans*, and *Aedes ægyptii* were compared with susceptible insects to determine their relative ability to produce D.D.E. Larvæ from each strain were exposed to D.D.T. and their D.D.T. uptake and *in vivo* formation of D.D.E. determined. One hundred and forty (140) susceptible *Aedes taeniorhynchus* larvæ took up 5·6 micrograms and produced 2·4 micrograms D.D.E. The same number of resistant larvæ took up 73·3 micrograms D.D.T. and produced 15·9 micrograms of D.D.E. These correspond to about 21 and 24 per cent conversion respectively. For *Aedes ægyptii*, two micrograms and 1,553 micrograms were absorbed by susceptible and resistant mosquitoes and 72 per cent and 11 per cent of that absorbed was converted to D.D.E. The authors conclude "Both normal strains produced an insignificant amount of D.D.E., their uptake of D.D.T. being small" but to the reviewer both the uptake and amount metabolized seem significant. Tissue homogenates of neither strain converted D.D.T. to D.D.E. under conditions suitable for housefly material. Dieldrin and gamma B.H.C. resistant *Anopheles gambiæ* absorbed no less B.H.C. than did susceptible insects. Only 11 per cent of absorbed B.H.C. was metabolized according to Bradbury and Standen (1956*b*).

In the German roach *Blattella germanica*, resistance is a serious problem in the United States. While the importance of cockroaches, as vectors of disease, has generally received little attention, a recent publication by Roth and Willis (1957) indicates that the medical importance of cockroaches may have been greatly underestimated. Chlordane-resistant German roaches more rapidly converted

D.D.T. and D.D.E. than did a susceptible strain (Babers and Roan, 1953) but most of the applied D.D.T. was converted to metabolites not appearing in analyses by the Schechter Haller procedure.

Certain chemicals have proven to be somewhat more toxic to resistant flies than to susceptible insects (Ascher, 1957; Mitlin *et al.*, 1956). While this interesting line of approach has not been fully explored, present indications are that probability for successful application to actual control procedures is low.

When the biological habits of the insect make it feasible, the release of large numbers of sterilized male insects to compete with the wild males offers interesting possibilities. Knipling (1955) has shown that with the screwworm, *Callitroga americana* (C. & P.), this method is indeed practical. None of the resistant species so far studied have the requisite biological habits, particularly single matings by the female, to permit the use of this procedure.

Insecticide failures, generally with mosquitoes, because of "behavioristic resistance" have also been reported (Brown, 1958). Little or no evidence has been published that relates these failures to a physiological response but such relationship must exist. Whether or not the insects' avoidance habits are due to increased sensitivity, or whether the insecticide acts as a repellent, is not clear. It does seem established that certain resting habits of the mosquitoes have changed so that control is not now accomplished by methods that were formerly successful. Yet, when the insecticide is applied directly to the insect, no increased tolerance is evident.

As indicated earlier, resistance by most arthropod vectors of disease, and by many agricultural and household pests, has already developed. However, little or no physiological or biochemical work with post-war insecticides has been done on other species than those already mentioned in this brief summary.

Experts, and the word is used loosely, have offered much advice on how to prevent the development of resistance. They have advised that space spraying will prevent development of resistance while residual treatments cause its development: only sufficient insecticide should be applied to effect prevention of disease transmission (this level, of course, is unknown); thorough coverage should be attained; treatment should be aimed at eradication; treatment should be aimed at achieving complete kill of a population etc. etc. As a matter of fact, insufficient experimental work has been done to determine what treatment process will minimize development of resistance and none so far proposed seems to offer a permanent solution to the problem.

The most effective procedure for controlling resistant insects has been to change to more toxic insecticides. Generally, the control pattern has been to first use D.D.T. or chlordane and when resistance develops, change to dieldrin, aldrin or B.H.C. Within a few generations, a switch to the organophosphates is necessary. At the present time, no new class of insecticides is available for use when the organophosphates become ineffective. It is for that reason that large scale investigation of all possible control measures is urgently indicated.

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BIOCHEMICAL MECHANISMS OF INSECTICIDE RESISTANCE.

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INTRODUCTION.

It has been known for the last fifty years that insects and other arthropods develop resistance towards certain chemicals used for their control (Chadwick, 1955). The problem of resistance attracted serious attention when it was observed in Northern Sweden in 1946 that the houseflies, *Musca domestica* L., did not respond as expected to D.D.T. Since then it has been shown in many parts of the world that several species of insects including houseflies; mosquitoes, *Culex fatigans*, *Aedes* spp., *Anopheles* spp.; human louse, *Pediculus humanus* L.; and several agricultural pests have developed resistance to one or more insecticides (Busvine, 1956). Within a short span of seven to eight years the literature on this subject has grown enormously. Besides hundreds of papers published in almost all the leading entomological and biochemical journals, several excellent reviews have appeared (Chadwick, 1955; Metcalf, 1955*a*: 1955*b*; Hoskins and Gordon, 1956; Crow, 1957; Brown, 1958).

ORIGIN AND FACTORS OF RESISTANCE.

The majority of workers in the field of genetics of insecticide-resistance believe that resistance is a pre-adaptive phenomenon (Crow, 1957), and resistance has been defined as the ability to withstand an insecticide, acquired by breeding from those individuals which survive exposures to that particular insecticide (Hoskins and Gordon, 1956). Thus insecticides act as "selective agents" (Metcalf, 1955*a*: 1955*b*; Crow, 1957), and not "mutagenic agents".

The earlier investigations with the phenomenon of resistance were primarily directed towards such factors as thickness of cuticle, length of life cycle, biotic potential, and preferential resting habits etc. None of these, however, gained much popularity because of inconclusive experimental evidences (Metcalf, 1955*a*: 1955*b*). Smyth and Roys (1955) reported that D.D.T.-sensitive flies, given a choice, spent a greater amount of time on D.D.T.-treated areas, whereas resistant flies did not. The failure of D.D.T. to affect reflex behaviour of this strain of

D.D.T.-resistant houseflies was not due to failure to penetrate the cuticle, but must be due to some mechanism intrinsic to the nervous system. Hardman and Craig (1941) stated that the resistant races of California red scale, *Aonidielia aurantii* Mask., kept their spiracles closed as long as HCN was in the environment and they attributed this as a factor of resistance. Yust (1952), however, conclusively demonstrated that tracheal closure was not responsible for resistance in the scale insects. These observations suggested that the basis for resistance must lie in factors other than simple ones mentioned above. The most attractive theories, now advanced to account for the resistance of insects to insecticides, have their bases in physiological and biochemical mechanisms.

BIOCHEMICAL MECHANISMS: METABOLISM OF INSECTICIDES.

Insecticides have been shown to undergo considerable metabolism in the body of the insects. Williams (1951) reviewed the work done in this area prior to 1951. Sternburg and Kearns (1950), Winteringham *et al.* (1951), Babers and Pratt (1953), Robbins and Dahm (1955) and Lindquist and Dahm (1956), using spectrophotometric and radiochromatographic techniques, studied the metabolism of D.D.T. in the insect body. These studies have shown that D.D.T. in the insect body is dehydrohalogenated to the non-toxic ethylene analogue D.D.E. Hoffman and Lindquist (1952) studied the absorption and metabolism of D.D.T., toxaphene and chlordane using a bioassay technique and showed that the resistant flies were capable of metabolizing D.D.T., toxaphene and chlordane to non-toxic metabolites. Winteringham *et al.* (1955) reported that significant and comparable fractions of all the applied Carbon^{14} labelled pyrethroids were metabolized to relatively non-insecticidal substances within 24 hours in the body of the houseflies. Schmidt and Dahm (1956) using Carbon^{14} labelled piperonyl butoxide, demonstrated that more than 50 per cent of this compound was metabolized and excreted by the Madeira roaches, *Leucophaea maderae* (F.), as water soluble metabolites. Oppenoorth (1954: 1955), Bradbury and Standen (1955), and Sternburg and Kearns (1956) studied the metabolism of γ B.H.C. in the houseflies. Pentachlorocyclohexene has been demonstrated as one of the metabolites of B.H.C. by the last two mentioned workers.

It became obvious that resistance must be related to the above phenomena. Sternburg *et al.* (1950), Perry and Hoskins (1950) and Babers and Pratt (1953) demonstrated that, whereas susceptible flies were either very slow or incapable of metabolizing D.D.T. to D.D.E., the resistant flies had a great capacity of converting D.D.T. to the non-toxic analogue. Sternburg and Kearns (1950) studied the site of D.D.T. degradation and found D.D.E. in almost all the areas of the body of the housefly after topical application. This suggested that D.D.T. might be metabolized during the time it is being absorbed by the resistant flies through the cuticle-hypoderm. They showed by careful experiments that detoxication apparently occurred in the cuticle hypoderm.

D.D.T. DEHYDROCHLORINASE.

Sternburg *et al.* (1954) announced the isolation and purification of an enzyme from various strains of D.D.T.-resistant houseflies, which was capable of catalyzing

the dehydrochlorination of D.D.T. to D.D.E. They found that this enzyme required activation by glutathione *in vitro*. Maximum activity was observed at pH 7·4 and 37°C. The enzyme had a high degree of specificity and appeared to attack only those analogues of D.D.T. which were sterically similar. It has been found in all the strains of resistant flies assayed and has not been found in the susceptible flies. The authors proposed the name 'D.D.T. Dehydrochlorinase' for this enzyme.

The fact that this enzyme was present in only the resistant varieties of houseflies lends strong support to the theory that dehydrochlorination is the chief mechanism in the resistance of houseflies to D.D.T.

Winteringham *et al.* (1951) pointed out that although an attractive possibility, the role of metabolism in the resistance of flies to D.D.T. is not clear. The quantity of unchanged D.D.T., recovered from surviving resistant houseflies, was in many cases more than enough to kill susceptible flies. They used Bromine* labelled analogue of D.D.T., assuming that it would follow essentially the same metabolic pattern as the normal D.D.T. They demonstrated that per cent absorption was the same in resistant and susceptible houseflies. When two μg. of the Bromine analogue of D.D.T. was injected into the resistant houseflies, up to 57 per cent was converted to the ethylene derivative in 24 hours, whereas no change was observed in the susceptible houseflies.

Anderson *et al.* (1954), considering that unchanged D.D.T. and lindane in the body of resistant houseflies were considerably more in quantity than the LD_{50} of these compounds, speculated that dehydrohalogenation was not sufficient explanation for the resistance phenomenon. They studied the effects of suspensions of D.D.T., D.D.E. and related compounds on the *in vitro* activity of the succinoxidase system and its components colorimetrically and manometrically. These compounds were found to inhibit cytochrome oxidase, succinoxidase and succinic dehydrogenase. No pronounced differences in the sensitivity to D.D.T. and related compounds were observed between enzyme preparations from the resistant and susceptible strains of flies. Hence they concluded that the inhibition of succinoxidase and its components by D.D.T. is not a primary factor in the mechanism of resistance.

Sacktor (1950) found that the cytochrome oxidase activity of the resistant strain of houseflies was considerably greater (64 per cent for the females and 36 per cent for the males) than that of the susceptible flies. He suggested that one of the explanations for the resistance of houseflies to D.D.T. may be the greater cytochrome oxidase activity.

Busvine (1951) found interesting differences in the type of tolerance of two strains of insecticide-resistant houseflies, Italian resistant strain and Sardinian resistant strain. Compounds which readily split HCl and leave ethylenic residues are tolerated by both strains, but the analogues which are more stable in this respect are relatively more toxic to the Sardinian strain. Relative resistance of the Sardinian strain to the normal strain is roughly proportional to the logarithm of the rate of dehydrohalogenation. The Italian strain is distinctly resistant to

* Radioactive bromine.

D.D.T. and pyrethrins but not to other insecticides, whereas the Sardinian strain is about as resistant to D.D.T. as the Italian, but in addition it is highly resistant to chlordane and several chlorinated hydrocarbons, even dieldrin, aldrin and toxaphene. He concluded that the dehydrohalogenation mechanism did not account for D.D.T.-resistance fully. The Sardinian strain relies mainly on dehydrohalogenation while the Italian strain had some unknown mechanism. Busvine (1953) showed by crossing the Sardinian strain with susceptible flies that there is a D.D.T.-resistance factor not concerned with dehydrohalogenation and it appeared that this factor is associated with resistance to D.D.T. paralysis. It has also been shown by Busvine (1954) that resistance to insecticides, B.H.C., chlordane, dieldrin, aldrin, and toxaphene is quite distinct from D.D.T.-tolerance. These compounds must have some factor in common, because selection by exposure to one of them raises the resistance of a fly strain to another.

SYNERGISTS FOR D.D.T.-RESISTANT FLIES.

'Synergism' and mode of action of synergists has thrown considerable light on the understanding of resistance mechanisms. When D.D.T.-resistance was reported in houseflies, a simultaneous search for D.D.T. synergists was made in many laboratories. Perry and Hoskins (1950:1951) showed that piperonyl cyclonene, a successful pyrethrin synergist markedly increased the toxicity of D.D.T. for the D.D.T.-resistant flies. They observed that the conversion of D.D.T. to D.D.E. was prevented in the presence of piperonyl cyclonene. Piperonyl cyclonene, however, exhibited no synergistic activity with the D.D.T.-susceptible houseflies. The optimum ratios of D.D.T. to synergist did not indicate any stoichiometric relation between them.

Winteringham *et al.* (1955) demonstrated that the metabolism of pyrethroids was considerably inhibited when piperonyl cyclonene was applied simultaneously. These observations suggest that synergism in these compounds involves an interference with the natural detoxication mechanisms.

The high ratio of piperonyl cyclonene to D.D.T., needed to initiate synergistic activity, limits its practical use. It was suggested that, since D.D.E. is the primary step in the degradation of D.D.T., addition of D.D.E. or compounds similar to that should decrease the rate of metabolism by the simple law of mass action.

Sumerford *et al.* (1951) studied compounds structurally similar to D.D.T. and found that D.M.C. [1,1-bis-(*p*-chlorophenyl) ethanol], was effective in increasing the toxicity of D.D.T. to the resistant houseflies. Perry *et al.* (1953) confirmed that D.M.C. acted as a synergist by competing with D.D.T. for the active site of enzyme by virtue of similar structure. Thus it is a case of competitive inhibition of D.D.T. dehydrochlorinase. Moorefield and Kearns (1955) showed that maximum inhibition of D.D.T. dehydrochlorinase occurred when the ratio of D.M.C. to D.D.T. was 1:1.

MODE OF ACTION OF D.D.T. AND RESISTANCE.

Winteringham (1956) studied the effect of D.D.T. on the levels of A.T.P., adenosine triphosphate, in the thoracic tissue of the houseflies. He showed that

D.D.T. did reduce the level of A.T.P. in a way which cannot be explained by glycolytic exhaustion of the insect. This observation has been corroborated by Pant (1958). This effect was not observed in a strain of D.D.T.-resistant houseflies. Therefore even though the rate of D.D.T. detoxication was very slow in resistant flies, it was quite adequate to protect them from this critical biochemical lesion. However, since very little information on this important aspect of mode of action of D.D.T. is known, it is not possible to make any definite speculations on this observation.

SUMMARY AND CONCLUSIONS.

In summary it can be stated that the most promising field for investigations on the phenomenon and problems of insecticide resistance is the biochemical one. Agreement has been reached by majority of the workers in this field that, at least in some of the chlorinated hydrocarbons, enzymatic dehydrochlorination is the basic mechanism of resistance. Genetics of insecticide resistance is still a controversial field.

One of the fundametal difficulties that is faced in attacking the problem of resistance, is the paucity of knowledge on the actual mode of action of some of the important and popular insecticides. It seems that until a little better understanding regarding the mode of action of insecticides is achieved, little progress will be made in facing this challenge. The use of synergists for D.D.T.-resistant flies has shown considerable promise in this field.

More and more studies on the basic biochemical aspects of insecticide toxicology and structure-enzyme relationships are needed. These studies have to be, of course, supplemented with the genetic findings in this field. Thus resistance offers an unlimited field for a coordinated research work by the geneticist and the toxicologist.

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BIOCHEMISTRY OF RESISTANCE TO ORGANOPHOSPHORUS COMPOUNDS*.

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1. INTRODUCTION.

COMPARATIVELY little is known about resistance to organophosphorus compounds because they have been less extensively used, especially for insects of public health importance. With the widespread appearance of resistant strains of flies and mosquitoes to chlorinated hydrocarbons, certain organophosphorus insecticides were substituted starting in 1953. As a result of limited laboratory experiments it was thought at that time that resistance was unlikely to develop (Brown, 1954) to this group of compounds and even if some selection took place, increase in tolerance level was likely to be of a much lower order (Brown, 1956) as compared to chlorinated hydrocarbons. Both of these assumptions have been modified recently as not only resistance has appeared in isolated areas, namely, Denmark, Italy, Switzerland, United States of America (where houseflies have shown resistance to a number of such compounds such as D.F.P., diazinon, resitox, malathion, parathion, etc., and *Culex tarsalis* to malathion), but it has also been pointed out by some workers that a ten-fold resistance to organophosphorus compounds may be sufficient to jeopardize the use of this group of insecticides, for instance for fly control in the field (Keiding, 1956). The question of resistance to organophosphorus compounds has thus assumed considerable importance during recent years.

2. PHOSPHATE-RESISTANT STRAINS.

The available information on the phosphate-resistant strains is briefly summarized in Table I.

*Reproduced from *World Health Organization/Insecticides/76*. Paper read to the seminar on "Resistance of Insects to Insecticides" sponsored by the World Health Organization in collaboration with the Government of India, New Delhi: February 27 - March 7, 1958.

TABLE I.

Resistance to organophosphorus compounds.

Insects	Compound	'Times' resistant	Generations	References
Houseflies (adults)	Paraoxon	11	40	Metcalf (1955). (United States of America).
	D.F.P.	10	70	
	Parathion	18	148	
	Diazinon	16	14	Meltzer (1956). (Holland).
	Malathion	12	16	March *et al.* (1956). (United States of America).
	Chlorthion	54	39	
	Diazinon	10	19	
	Parathion	3	3 years	Keiding (1956). (Denmark).
	Diazinon	5	3 years	
	Resitox	40	3 years	
	Malathion	5 - 20	4 years	Lindquist (1956). (United States of America).
	Dipterex	5 - 20	4 years	
	Diazinon	60	4 years	Sacca (1957). (Italy.)
	Malathion	23 - 37		Labrecque and Wilson (1957). (Florida, United States of America).
	Malathion	2 - 4 (behaviouristic)	2 years	Kilpatrick and Schoof (1957). (Georgia, United States of America).
Houseflies (larvæ)	Diazinon	30	..	Mer and Gwilich (1956). (Israel).
Chrysomyia putoria	Diazinon	300	2 years	Bervoets *et al.* (1957). (Belgian Congo).
Culex tarsalis (adults)	Malathion	93 - 100	2-1/2 years	Gjullin and Isaak (1957).
Culex tarsalis (larvæ)	Malathion	21 - 33	2-1/2 years	(California, United States of America).

It would be evident from Table I that the overall picture, as it emerges by and large, does not appear to be fundamentally different from what occurred with respect to the resistance to chlorinated hydrocarbons. Both physiological and behaviouristic resistance has also been observed. In certain instances, cross-resistance to other organophosphorus compounds and to the chlorinated hydrocarbons has also been detected.

The increase in tolerance of organophosphorus insecticides are not of a high order; for example, in the case of houseflies, the highest degree of resistance reported to organophosphorus compounds is about 54 times as compared to the thousand-fold resistance with chlorinated hydrocarbons. In the case of *Chrysomyia putoria* and *Culex tarsalis* the highest level of resistance reported is 300 and 100 times respectively. The number of generations required to select a resistant strain also seems to vary with different compounds, depending upon the intensity of the selection pressure applied.

The avoidance of malathion baits by houseflies is significant, since behaviouristic resistance to organophosphorus insecticides had not been reported previously (Kilpatrick and Schoof, 1957).

3. MODE OF ACTION OF ORGANOPHOSPHORUS COMPOUNDS.

Before discussing the mechanisms of organophosphorus resistance, it would be profitable to examine the physiological basis for the mode of action of organophosphorus compounds.

From a great deal of intensive study it may be concluded that the organophosphorus insecticides are toxic to animals because of their ability to interfere with the normal mechanism of nerve impulse transmission. This hypothesis supposes that a chemical mediator, i.e., acetylcholine (ACh) serves as the transmitter of nerve impulse at synaptic and neuromuscular junctions. Soon after the transmission of the nerve impulse it is hydrolysed with extreme rapidity by the enzyme cholinesterase (ChE)*. If then the ChE is inhibited, ACh will accumulate causing nerve block and finaly death through disfunction and failure of the neuromuscular or some other vital system. A more recent viewpoint is that ACh is associated with the protection of the nerve action potential by controlling the permeability of the nerve membrane to ions (Metcalf, 1956). As far as is known at present, all organophosphates that are directly toxic to insects, are active inhibitors of ChE†.

This hypothesis on the whole seems to have convinced mammalian physiologists of its validity. Among insect physiologists, however, doubts were first expressed about the ubiquity in insects of the elements for this hypothesis, i.e., the presence of ACh and enzymatic systems for both its synthesis and hydrolysis. The persence of ACh (Lewis, 1953; Augustinsson, 1954; Smallman and Chefurka, 1956) and enzymatic systems for its hydrolysis and synthesis [i.e. ChE (Richards and Cutkomp, 1945) and choline acetylase (Smallman, 1936; Smallman and Pal, 1957)] have, however, been recently demonstrated in the nervous tissue of insects in a convincing manner.

Even if ACh and ChE are associated with the nervous tissue of insects, there were difficulties in the way of assuming that they function in the same way as in the mammals. Hopf (1952:1954) and others have noted the striking insensitivity of insects to injected ACh and the ineffectiveness of atropine, adrenaline etc. However, O'Brien (1956) has recently obtained evidence which seems to reconcile this anomaly by demonstrating a permeability barrier in roaches which prevents entry of ionized substrates such as ACh and its analogues to the site of action. On the other hand, unionized substrates such as phenyl and ethyl acetate penetrate to the site of action without any difficulty and are hydrolysed readily. The barrier responsible may be identical with that shown for potassium ions by Hoyle (1952:1953).

*Several types of esterases which hydrolyse ACh are found in animals (Augustinsson, 1950; Nachmansohn and Wilson, 1951). Acetylcholinesterase has a greater affinity for ACh than for any other ester. It occurs in the nervous tissue of both vertebrates and invertebrates in large amounts and seems to be concentrated in the synaptic regions. Non-specific aliesterases which also hydrolyse choline esters, and an aromatic esterase which hydrolyses phenyl acetate and other aromatic esters, are also present (Aldridge, 1953).

†Although the action of organophosphorus insecticides on ChE has been studied most extensively, the other enzymes with esterase action, as mentioned above, are also inactivated. This hypothesis has been challenged on this ground. It has also been argued, that the toxic action of organophosphorus may be due to inhibition of other systems equally as vital as the ChE, such as chymotrypsin (Balls and Jansen, 1952). For detailed discussion regarding these points, reference may be made to earlier work reviewed by Kearns (1956).

Colhoun (1956) has been able to demonstrate an increase in the ACh content of thoracic cord of the roach following stimulation, but ACh which accumulated during stimulation did not appear in the perfusate under conditions where the ChE was inhibited. Smallman (1956) has summed up " that if O'Brien's barrier to exogenous ACh is identical with Colhoun's barrier against endogenous ACh, then the barrier would appear to be symmetrically impermeable to this compound. But we do not know the relation between them nor whether either is identical with the membrane described by Hoyle (1952-1953) ".

Twarog and Roeder (1956:1957) have recently demonstrated that synaptic transmission can be controlled by applying ACh under controlled conditions to the *desheathed* last abdominal ganglion of the roach. In these experiments, relatively high concentration of ACh (10^{-4} to 10^{-3}M) were required, which may indicate the presence of some additional unidentified barriers.

Still some doubt has been left in the minds of some workers who are of the opinion that the physiological mediator in insects is not ACh but some unidentified compound or compounds. Chadwick (1957) has recently pointed out that " no early solution of these problems is forecast unless (*a*) the blood toxins of Kearns* and co-workers turn out to be the natural mediator and (*b*) this material is successfully isolated and identified ". In this connection, it may also be mentioned that Pal and Smallman (1957) have demonstrated the presence of free ACh in the blood of T.E.P.P. treated roaches.

It may be concluded that the classical interpretation of nerve function based on the ACh system constitutes the best basis for interpreting the toxic action of organophosphorus compounds.

E. EFFECT OF ORGANOPHOSPHORUS INSECTICIDES ON ACh LEVEL AND ChE IN NORMAL AND RESISTANT INSECTS.

It is implicit in the hypothesis for the toxicity of these compounds that following the inhibition of ChE, ACh accumulates in the nervous tissue. Smallman and Fisher (1956) found that after treatment with several of these compounds, the ACh level in the heads of houseflies increased as much as 100 per cent above the normal level (*vide* their Fig. 1). After an initial increase, the ACh level fell again toward the normal level and it was possible to correlate these changes with changes in the ChE activity. The increase and duration of the supranormal levels of ACh were directly related to the dosage applied. Winteringham and Harrison (1956) have reported an initial increase and subsequent fall in ACh level in houseflies after a lethal dose of D.F.P. Pal and Smallman (1957) have adduced evidence to show that anti-ChE insecticides, besides inhibiting the enzyme ChE, affect the state of nerve tissue to bind ACh. As indicated earlier in support of this conclusion, free ACh has been demonstrated in the hæmolymph of T.E.P.P.-treated roaches by Pal and Smallman (1957).

*Sternburg and Kearns (*Science*, 1952, **116**, p. 144) found that the hæmolymph of *Periplaneta* in the prostrate stage of poisoning contains toxins other than insecticide (D.D.T. in this case). The nature of these toxins is not known.

Pal (1957) has further shown that unlike the normal strain, the malathion-resistant strain of houseflies collected at Savannah, Georgia, did not accumulate any ACh. These observations are corroborated by the Savannah laboratory, where it has been found that this strain contained 30 per cent more brain cholinesterase than the normal strain. Adults of the resistant strain suffered 30 per cent ChE inhibition in the first two hours, but later recovered to only 20 per cent inhibition, whereas the normal adults suffered 65 per cent inhibition increasing to 90 per cent from similar topical applications*. It has been further demonstrated that although both normal and resistant strains rapidly metabolize malathion, the resistant strain does so slightly more rapidly than the normal†.

5. RESISTANCE MECHANISMS.

From the foregoing account, it would appear that according to the preliminary studies carried on, the mechanism for resistance in the Savannah strain is due to its ability of malathion to rapidly metabolize this compound. However, no clear indications as yet are available of the resistance mechanism involved in other organophosphorus compounds which seem to vary with different compounds. From the available information regarding the relationship between the structure of these compounds to their toxicity, a number of possible resistance mechanisms may be considered:

(*i*) Decreased penetration through cuticle (D.F.P.);

(*ii*) Increased lipoid content (diazinon);

(*iii*) Specific detoxication systems (malathion and parathion);

(*iv*) Failure of the phosphorothioates to be oxidized to the toxic phosphates (parathion);

(*v*) Barrier preventing entry of the metabolite to the site (schradan);

(*vi*) Other biophysical or biochemical mechanisms.

(*i*) *Decreased penetration through the cuticle.*—Chadwick (1954) has reported that the D.F.P.-resistance in a laboratory strain of houseflies was due to the fact that the penetration of the insecticides was somewhat impeded. This hindrance may be due to an alteration of the properties of the integument, including presumably the tracheal lining or some more centrally located barrier such as the nerve sheath. Busvine (1957) also found that resistance was reduced if D.F.P. was injected instead of topically applied, which suggests that the cause is reduced penetration through the cuticle.

(*ii*) *Increased lipoid content.*—Reiff (1956) has found that a diazinon-resistant strain at Basle had twice the lipoid content of a normal strain.

(*iii*) *Specific detoxication systems.*—The toxicity of an organophosphorus compound to a species will naturally be affected by the presence of detoxication

*Cytochemical studies on the same strain also revealed that in the resistant strain only acetylcholinesterase were inhibited by malathion whereas aliesterases were not inhibited. Similar studies on susceptible flies indicated inhibition of all cholinesterases (Personal communication from Dr. L. Connell, Science Service Laboratory, London, Ontario, Canada). This may indicate the importance of other esterases than ChE in the normal mode of action [(Van Asperen, 1958) *Nature* (London, **181**, pp. 355-56)].

†Extracts from summary of investigations, Communicable Diseases Centre, Technology Branch, *Communicable Diseases Centre* No. 12: January-June, 1957.

systems such as enzymes capable of splitting the compound into inactive products. In mammalian tissues, the presence has been shown of enzymes capable of hydrolysing a number of these compounds (Augustinsson and Heimburger, 1954; Nazur, 1946; Mounter *et al.*, 1953; Mounter, 1954). In insects, Metcalf *et al.* (1958) have found an aromatic esterase capable of hydrolysing; for example parathion and paraoxon. There is evidence against the existence of insect enzymes hydrolysing schradan or its active metabolites (Metcalf and March, 1953; O'Brien and Spencer, 1956). It may, however, be pointed out that non-enzymatic hydrolyses cannot be ruled out.

Dipterex and malathion break down in mammals fairly rapidly, which accounts for their low mammalian toxicity. In insects, however, the metabolism is not extensive. As reported earlier in a malathion-resistant strain of houseflies, the breakdown was found to be slightly more than the normal.

(*iv*) *Failure of activation system.*—Certain phosphorothioates do not function directly as enzyme inhibitors, which is probably due to their greater stability to enzymatic hydrolysis. But these compounds were as toxic to animals as other compounds, which indicated that they underwent conversion to active anti-ChE in animals.

Compounds such as schradan, parathion and malathion, which as such are weak anti-ChE agents, are converted to powerful inhibitors by these enzyme activation systems. The conversions of several thiophosphates to the phosphates has been shown in roach gut by Metcalf and March (1953) and parathion activation in roach fat-body has been shown by Kok and Walop (1954). Similarly, O'Brien and Spencer (1953:1956) and Heath *et al.* (1955) have shown conversion of schradan in insects. Similar activation has been observed in the case of systox. Arthur and Casida (1955) have suggested that in dipterex, a non-enzymic dehydrochlorination produces the active D.D.V.P. which is ten times as toxic as dipterex itself.

Parathion is toxic to both the housefly and the honey bee, while the isopropyl analogue of parathion is toxic to the fly but non-toxic to the bee. Presumably one of the biochemical mechanism in bees is slightly different — either the esterase enzyme system for detoxication or the activating enzyme systems. The toxicity of some of the organophosphorus compounds, may, therefore, depend on the relative activity of the activating and detoxifying enzymes.

The rapidity of detoxication appears to be of great importance in poisoning with organophosphates. Unlike chlorinated hydrocarbons, these compounds exert an irreversible action on the ChE of the nervous system. D.D.T.-resistant flies may be knocked down by large doses of D.D.T. but will recover within a few hours because D.D.T. has not resulted in any permanent injury. High resistance to these compounds will be dependent upon a detoxication mechanism so efficient that it intercepts the entry of the insecticide even from reaching the nervous system.

Oppenoorth (1958) found that upon injection of parathion, much less paraoxon was found in the resistant strain as compared to susceptible strain. Injected paraoxon was also removed more rapidly in the resistant strain. They found no difference in ChE activity in the two strains. However, Lord and Solly (1956) found that a normal strain destroyed paraoxon as fast as a strain with twice the tolerance to organophosphorus compounds.

(*v*) *Decreased penetration through lipoid nerve sheath.*—In an attempt to account for the non-toxicity of schradan to the roach, Spencer and O'Brien (1957) observed that the roach and other naturally non-susceptible species rapidly converted schradan to an active anti-ChE metabolite as discussed above, but this metabolite could not penetrate to the active site. It is an attractive hypothesis that the resistant insects have a barrier preventing the entry of the compound (Hoyle's nerve lipoid sheath). Casida *et al.* (1953), however, believe that susceptibility in insects depends upon the sensitivity of the particular insect ChE to inhibition by the schradan metabolite. For example, ChE from *Trialeurodes* and *Blattella* required 6 - 24 times as much for inhibition as that for *Musca*.

(*vi*) *Other biophysical or biochemical mechanisms.*—The attractive idea (Babers and Mitlin, 1955) that D.D.T.-resistant flies, having a highly active enzyme capable of dehydrochlorinating D.D.T., might have a similar effect on dipterex, has not been confirmed.

It will, however, be clear that more than one mechanism may be responsible for the resistance phenomenon. Our knowledge of phosphate-resistance is meagre and inadequate, and there can be no question of the urgent need for definite information about the basic facts of the situation that now confronts us.

6. SUMMARY.

Comparatively little is known about resistance to organophosphorus insecticides because they have been less intensively used, especially for insects of public health importance. Instances of induced resistance to these compounds in the laboratory and emergence of phosphate-resistant strains of insects in the field, are cited. The increases in tolerance level of organophosphorus compounds are of a lower order than those in the case of chlorinated hydrocarbons.

The physiological basis for the hypothesis that organophosphorus compounds exert their insecticidal action by inhibition of cholinesterase, is examined. The lethal agent seems to be acetylcholine which accumulates in the tissues and causes nerve-block. The effect of organophosphorus compounds on cholinesterase activity and on acetylcholine levels, both in susceptible and resistant insects, is discussed.

The toxicity of an organophosphorus compound to a species is affected by the activity of (*a*) enzymes capable of converting the compound to a powerful inhibitor of cholinesterase and (*b*) enzymes capable of splitting the compound to inactive products. The rapid degradation of these compounds in resistant insects may account for the phenomenon of resistance. From the available information regarding the relationship between the structure of these compounds to their toxicity, a number of possible resistance mechanisms are discussed.

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THE WAXES AND LIPOIDS OF RESISTANT HOUSEFLIES AND MOSQUITOES.

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WHILST most biochemical studies of resistance of insects to insecticides are concerned with metabolism and detoxication of the poison by resistant strains, there is a school of thought which holds that distribution of the poison in the insect plays a dominant role in resistant mechanisms. The main exponent of this view is Wiesmann (1957) who, with his collaborators, has discussed at length the proposition that D.D.T.-resistance is achieved, in part, by changes in the quantity and nature of fatty tissues of the insect so as to direct the insecticide preferentially to fatty sites in the insect body where it cannot exert its toxic action. This theory receives support from the work of Langenbuch (1954) and Munson *et al.* (1954), and has been discussed in numerous review articles.

Work in Widnes on resistance of houseflies to γ-B.H.C. has been directed mainly to detoxication studies. It has been shown that conversion of γ-B.H.C. to water-soluble excretable products, proceeds more rapidly in γ-B.H.C. resistant strains than in susceptible ones. Although the magnitude of this difference, and hence its contribution to resistance to poisoning, is readily determined in the laboratory, its contribution to resistance in the field is difficult to assess on account of the necessarily artificial nature of laboratory experiments compared with field behaviour of insects. If the time required to remove 95 per cent of the dose of γ-B.H.C. absorbed in 15 minutes exposure to the vapour is taken as a measure, resistant flies may be said to have an eight-fold advantage over susceptible ones. If the dose were smaller, as it may well be in practice, the advantage might be greater (Bradbury and Standen, 1958).

It has seemed unlikely, however, that the difference in rate of detoxication, unsupported by other mechanisms, could be great enough to enable a resistant fly to survive a hundred-fold increase of the normal dose of insecticide. Miyake *et al.* (1957) have recently demonstrated that the enzyme concerned in D.D.T. detoxication, D.D.T. dehydrochlorinase, is widely distributed in the body of D.D.T.-resistant houseflies, especially in brain, fat body, and muscle, and they visualize detoxication as a dynamic localized process which protects by removing the poison as it arrives at the site of action. This is a development which could eliminate the apparent contradiction between the low overall rate of metabolism and the high degree of resistance to insecticide commonly encountered in resistant strains of houseflies. Nevertheless we thought it necessary to examine carefully the distribution theory to establish whether it contributed in any significant way to the γ-B.H.C. resistance of the strains of houseflies and mosquitoes with which we have been working. The point had been made elsewhere (Bradbury, 1957) that a distribution factor and a detoxication factor might together increase resistance by an effect which is the product and not merely the sum of the individual effects.

This paper records the results of these investigations. The results lend no support to the theory propounded by Wiesmann (1957). The findings are largely negative in character but they are published as a corrective to the tendency to generalize resistance theory on the lines proposed by Wiesmann (1957). This paper is written to demonstrate that insects can exhibit a high degree of resistance to γ-B.H.C. in the absence of any increase in their lipoid content or major change in the solvent properties of the lipoids towards the insecticide.

I. INSECTS.

The five strains of flies (*Musca domestica*) used in these experiments were: (S_1) the normal Jealott's Hill strain, (S_2) a susceptible strain from the Pest Infestation Laboratory, Slough, (R_1) a γ-B.H.C. resistant strain originating in Uruguay and subsequently bred at Jealott's Hill on a larval medium containing half per cent γ-B.H.C., (R_2) the original Uruguay strain maintained at Jealott's Hill without further selection, (R_3) the R_1 strain freed from selection pressure with γ-B.H.C. for twelve generations. The resistant fly strains were bred in Mr. J. F. Newman's laboratory at Jealott's Hill (Newman, 1957). A recent determination of resistance by Dr. H. H. S. Bovingdon, using a modification of the method of Busvine and Nash (1953), showed the R_1 strain to have a LC50 approximately 200 times greater than that of the S_2 strain. The resistance to γ-B.H.C. of R_2 was six-fold and R_3 22-fold when the LC50's were compared with that of S_1.

The mosquitoes were supplied by Mr. G. Davidson from Ross Institute cultures. Three susceptible strains were used, *i.e.*, Lagos (S_1), Maidihini (S_2) and Diggi (S_3) all of which originated in Nigeria. The resistant strains were Aunbursa (R_1) and Kano RR (R_2) both of which had originated in Northern Nigeria and which were resistant to dieldrin and to γ-B.H.C. R_1 showed a >800-fold resistance to dieldrin and a 25 to 32-fold resistance to γ-B.H.C. The R_2 strain was >800 times resistant to dieldrin and 38 to 49 times resistant to γ-B.H.C. compared with susceptible strains (Davidson, 1958).

2. ESTIMATION OF EPICUTICULAR WAX.

Wax was removed from flies by stirring 1,000 flies with 100 m.l. carbon tetrachloride, filtering, and washing the bodies with a further 50 m.l. of solvent. The carbon tetrachloride was removed by evaporation and the residue weighed.

The amount of epicuticular wax in mosquitoes was too small to be estimated by weighing; a surface pressure method was used for these insects. A number of mosquitoes (4 - 24) was placed in 30/40 petrol ether (1 m.l. for every four mosquitoes used) for 60 seconds. The petrol ether solution was fed on to the clean surface of 20 m.l. of water in a 2¼-inch diameter petri dish from a micrometer syringe. After each addition, the petrol was allowed to evaporate and the surface tension of the water measured using a Du Noüy tensiometer. Surface tension measurements and additions of petrol solution were continued until a distinct drop in surface tension was obtained, indicating the formation of a monomolecular film of wax on the water. For a fixed area of surface the weight of wax required to give a monomolecular layer was constant so that the concentration of the wax in the petrol solution was inversely proportional to the volume required to produce the observed drop in surface tension. By plotting the reduction in surface tension against the reciprocal of the volume of petrol solution added, a surface pressure/volume curve was obtained from which the volume of solution, containing an arbitrary fixed amount of wax, could be read. By reference to a similar curve, produced by addition of a petrol ether solution of cetyl alcohol to the same surface area, the concentration of wax in the unknown solution could be expressed as μg. of cetyl alcohol.

Whilst this method was very sensitive, it was liable to gross error if the exposure of mosquitoes to the solvent was continued long enough to permit extraction of fat from internal tissues. This was especially liable to occur with susceptible strains or with mosquitoes that had not been freshly killed. Such an occurrence was recognisable by a twenty to forty-fold jump in the surface activity of the extract and such solutions were discarded.

3. ESTIMATION OF FAT.

After rinsing with carbon tetrachloride, to remove epicuticular wax, the insects were ground in a mortar with an equal volume of sodium sulphate, then the mass was stirred for 15 minutes with chloroform/methanol (2:1) and filtered through a sintered glass filter. The residue was rinsed with a further 50 m.l. chloroform/methanol. The combined filtrates were evaporated on the water bath, under reduced pressure, until some solid was deposited, then the residual liquor was extracted six times with 15 m.l. 40/60 petrol ether. The residue, insoluble in petrol, was almost completely water soluble. The petrol ether was evaporated on the water bath and the remaining fat was weighed. The fat was a viscous brown oil which usually slowly crystallized on standing.

4. PHYSICO-CHEMICAL CONSTANTS OF FATS.

(*a*) REFRACTIVE INDEX.

The refractive index of extracted fats was measured at 20°C. in an Abbé refractometer.

(*b*) IODINE VALUES.

A solution of fat, in carbon tetrachloride, was re-acted with iodine monochloride/potassium iodide in glacial acetic acid (British Pharmacopoeia, 1958).

(*c*) SAPONIFICATION NUMBERS.

The saponification numbers were determined by hydrolysis of about 0·5 g. of fat with alcoholic potassium hydroxide and back titration of excess alkali (British Pharmacopoeia, 1958).

5. SOLUBILITY OF γ-B.H.C. IN FATS AND WAXES.

One per cent petrol ether solutions of susceptible and resistant fly fat and wax were prepared. The solutions were applied by an Agla micro-syringe to 1 c.m. diameter discs of Whatman No. 1 filter paper, mounted on the points of three pins, so that the contact of the filter paper with its supports was negligible. Petrol solution was placed uniformly on the paper at the same rate as it evaporated, until 1 m.g. of fat or wax had been applied. The disc of paper was allowed to stand in the air for five to ten minutes to remove all traces of petrol and put in a petri dish over a larger filter paper which had been impregnated with 100 μg. γ-B.H.C./cm^2. The two papers were separated by an interposed disc of filter paper so that wax or fat on the top paper was exposed only to the vapour of γ-B.H.C. Several treated discs of paper were put in the petri dish for periods of up to twenty-one days. The B.H.C., dissolved by individual wax or fat-treated papers, was estimated by putting the whole paper into a Schechter-Hornstein apparatus and performing the determination in the usual way (Schechter and Hornstein, 1952). When the amount of B.H.C. in the treated papers no longer increased, it was assumed that the wax or fat was saturated.

6. DISTRIBUTION OF FAT WITHIN THE FLY BODY.

The distribution of fat, within both susceptible and resistant flies, was studied in flies fed on a diet of sugar and water. The flies were killed and the abdomen was removed. The tip of the abdomen was cut off then, by pressing gently, the internal organs were made to protrude and were pulled out leaving the fat body attached to the abdomen cuticle. The organs of the abdomen were combined with the head and thorax and the fat was extracted from these, and from the cuticle plus fat body, as described above. Floating fat bodies in the fly-blood were not separated from the organs of the abdomen.

7. DISTRIBUTION OF γ-B.H.C. IN THE FLY BODY.

(*a*) The penetration of B.H.C. into whole flies was studied by confining 50 susceptible or resistant flies in a three-inch diameter petri dish over a filter paper treated with 1 mg. γ-B.H.C. applied as an acetone solution and freed from solvent by airing. The amount of B.H.C. in the flies was estimated by rinsing the flies with carbon tetrachloride to remove superficial contamination with B.H.C. and

determining the remaining B.H.C. in the bodies by the Schechter-Hornstein method (Schechter and Hornstein, 1952). The B.H.C., found inside the flies after 1, 4 and 24 hours, is recorded in Table IX.

A similar series of experiments was performed in which the fat body, plus the abdominal wall of the fly, was removed after the exposure to B.H.C. and the rinsing with carbon tetrachloride, and the B.H.C. in this part of the fly separately determined.

(*b*) The distribution of γ-B.H.C. between external wax ("outside") and "inside" the fly, and the amount metabolized, were determined using 14C-labelled γ-B.H.C. by methods described in detail by Bradbury and Standen (1955). S_1 and R_1 flies were exposed to saturated γ-B.H.C. vapour for fifteen minutes, then removed to clean flasks and analysed at different intervals of time of storage. The results, shown in Table X, have been adjusted for batch-to-batch variation.

RESULTS.

The results are set out in Tables I to X below.

TABLE I.

Comparison of wax obtained by petrol ether treatment of susceptible and resistant A. gambiæ.

	S_1 (Susceptible)		R_1 (Resistant)	
	♂	♀	♂	♀
Replicates ..	0·20	0·4, 0.4	0·19, 0·11	0·33, 0·45, 0·40, 0·30
Mean ..	0·20	0·40	0·15	0·37

Results expressed as μg of cetyl alcohol per mosquito.

TABLE II.

Comparison of fat contents of susceptible and resistant A. gambiæ.
(Number of mosquitoes shown in brackets).

Strain	♂			♀		
	μg. fat/ mosquito	Dry wt. μg. after fat extracted	Per cent fat	μg. fat/ mosquito	Dry wt. μg. after fat extracted	Per cent fat
S_1 ..	31 (55)	262	11	54 (31)	339	14
S_2 ..	33 (146)	208	14	37 (156)	250	13
S_3 ..	30 (50)	220	12	60 (121)	265	18
R_1 ..	18 (25)	260	6	66 (54)	200	25
R_2 ..	10 (254)	168	6	11 (284)	192	5
R_3 ..	30 (35)	260	10	—	—	—

TABLE III.

Comparison of wax contents of susceptible and resistant houseflies.

Strain	Number of flies	Wt. (g.)	Mean wt. of fly (mg.)	Wt. of wax (mg.)	Wt. of wax per fly (μg.)
S_1 (Susceptible)	1000	12·8	12·8	28	28
	1626	20·0	12·3	51	31
	2500	31·0	12·4	51	21
	2000	29·5	14·8	43	21
	4906	68·1	13·9	167	34
R_1 (Resistant)	2214	31·0	14·0	56	26
	2300	33·4	14·5	86	37
	1492	22·8	15·3	28	18
	2445	37·2	15·2	48	20

In these experiments, flies were not separated by sex or age.

TABLE IV.

Comparison of fat contents of susceptible and resistant houseflies.

Strain	Number of flies	Wt. of fat (mg.)	Wt. of fat per fly (mg.)	Refractive Index of fat
S_1 (Susceptible)	1000	320	0·32	1·4712
	1626	836	0·51	1·4720
	2500	1049	0·42	1·4706
	2000	719	0·36	1·4708
	3685	2065	0·56	1·4710
	4906	2513	0·51	1·4707
R_1 (Resistant)	2214	1085	0·50	1·4730
	2300	954	0·42	1·4776
	4300	1169	0·27	1·4773
	1492	921	0·38	1·4685
	2445	1084	0·44	1·4700

In these experiments, flies were not separated by sex or age.

TABLE V.

Distribution of fat in houseflies of different age-groups.

(Number of flies shown in brackets).

Strain	Approximate age	Weight of fat (mg. per fly)	
		Head and thorax	Fatbody and abdominal cuticle
S_1 (Susceptible)	2 days (204)	0·50	0·20
	2 „ (212)	0·49	0·16
	3 „ (150)	0·47	0·20
	4 „ (150)	0·47	0·19
	6–7 „ (150)	0·50	0·16
	8 „ (150)	0·34	0·11
R_1 (Resistant)	2–3 „ (245)	0·40	0·11
	3 „ (213)	0·34	0·10
	3 „ (218)	0·29	0·07
	4 „ (150)	0·43	0·12
	7 „ (198)	0·38	0·12
	8 „ (152)	0·29	0·07

All flies were de-waxed before extraction.

TABLE VI.

Distribution of fat in male and female houseflies of susceptible and resistant strains.

(Number of flies shown in brackets).

Strain	Weight of fat (mg. per fly)			
	♂		♀	
	Head and thorax	Fat body and abdominal cuticle	Head and thorax	Fat body and abdominal cuticle
S_1 (Susceptible)	0·36 (200)	0·10	0·37 (200)	0·14
S_2 do. ..	0·50 (114)	0·18	0·39 (200)	0·19
R_1 (Resistant)	0·33 (200)	0·09	0·39 (200)	0·10
R_2 do. ..	0·42 (200)	0·07	0·39 (139)	0·13
R_3 do. ..	0·35 (200)	0·17	0·42 (158)	0·20

All flies were de-waxed before extraction.
All flies were 2-3 days old.

TABLE VII.

Chemical properties of fats from susceptible and resistant houseflies.

Strain	Duplicate determinations on different batches of fat.									
	Iodine Value				Saponification Numbers					
S_1 (Susceptible)	68·2	75·4	73·0		93·9	86·2	86·3			
	66·1	75·5	73·4		93·8	86·6	85·0			
R_1 (Resistant)	67·1	73·9	85·1	76·3	105·6	93·8	93·4	96·3	104·5	101·7
	65·9	74·6	84·0	76·2	107·9	93·3	94·5	92·2	101·4	100·8

TABLE VIII.

Solubility of γ-B.H.C. (per cent w/w) in wax and fat of susceptible and resistant houseflies.

Strain	Wax.	Fat
S_1 (Susceptible) ..	6·7	4·1
R_1 (Resistant) ..	6·7	3·7

TABLE IX.

Distribution of γ-B.H.C. in susceptible and resistant flies.

Exposure to γ-B.H.C. vapour (Hours.)	μg. γ-B.H.C. inside 50 whole flies		μg. γ-B.H.C. inside fat body plus cuticle of 50 flies	
	S_1	R_1	S_1	R_1
1	15	8	0	0
4	17	26	0	0
24	21	24	4·5	0

DISCUSSION.

The results, tabulated in Tables I and III show that susceptible and resistant strains cannot be characterized by differences in the amounts of epicuticular wax recovered by solvent extraction. This applies equally to mosquitoes and houseflies.

The similar nature of the epicuticular waxes in S_1 and R_1 strains of flies is reflected in similar rates of absorption of γ-B.H.C. by the two strains. Table X shows the amounts of γ-B.H.C. per 20 flies found in the exterior wax layer (W), the amount found inside (F) (the bulk of which is assumed to be dissolved in interior lipoids) and the amount metabolized (R) at various intervals of time after exposure to the insecticide. Rates of absorption from the exterior wax layer into the interior of the fly for the S_1 and R_1 strains, were calculated from these figures as shown in Appendix I.

The two estimates of the limiting rate of absorption b were

for R_1 flies $b = 0{\cdot}050 \pm 0{\cdot}005$ μg./hr.
for S_1 flies $b = 0{\cdot}050 \pm 0{\cdot}025$ μg./hr.

TABLE X.

Amounts of exterior (W), interior unmetabolized (F) and metabolized (R) radioactive material expressed as μg. B.H.C./20 flies, adjusted for batch to batch variation.*

Storage time (Hours) ——→		3/8	3/4	1½	3	6	12	24
γ-B.H.C. resistant flies	(W) ..	0·56	0·26	0·12	0·06	0·02	0·02	0·06
	(F) ..	0·68	0·71	0·54	0·34	0·19	0·02	0·03
	(R) ..	0·30	0·57	0·88	1·14	1·33	1·50	1·45
γ-B.H.C. susceptible flies	(W) ..	0·72	0·56	0·29	0·16	0·10	0·05	0·05
	(F) ..	1·29	1·55	1·50	1·30	0·90	0·63	0·36
	(R) ..	0·35	0·25	0·57	0·90	1·36	1·68	1·95

*Adjustment was made by replacing the experimental results W′, F′, and R′, by W, F, and R given by $W = W'T/T'$, $F = F'T/T'$ and $R = R'T/T'$ where T is the mean value of T′, the total radioactivity recovered.

There is, therefore, no suggestion that penetration of the cuticle by γ-B.H.C. takes place less readily in resistant than in susceptible flies. The susceptible flies S_1 pick up approximately 1½ times as much γ-B.H.C. as the R_1 flies (Table X) because of the greater amount of internal fat in the S_1 strain (*see* below).

The lipoids extracted by solvents from three susceptible and two resistant strains of *A. gambiae* (Table II) show no consistent differences in weight between resistant strains on the one hand and susceptible strains on the other. Statistical analysis shows a difference between strains significant at the ten per cent level of probability (Appendix I, Table XI), and examination of the mean fat contents corrected to equal numbers in either sex (Appendix I, Table XII) shows that this effect is due to the very low fat content of the more highly resistant strain, R_2.

When S_1 and R_1 strains of housefly were compared in large numbers, ignoring age and sex, the batch variance was large and no significant difference

between the strains could be demonstrated. When, however, an experiment was designed which permitted the separation of age effect, there was a statistically significant difference between the strains (Table V), the R_1 strain having less lipoids than S_1. The difference averages about 0·1 mg./fly in the head and thorax and 0·07 mg./fly in the fat body and abdominal cuticle (Appendix I, Table XV) and is significant at the one per cent level in both cases (Appendix I, Table XIV).

In the analysis, the weights of lipoid isolated were used in preference to the alternative of considering the lipoids as percentages of body weight. This would not affect the argument for mosquitoes since no consistent difference between the body weights of the various strains was detected. The use of percentages would reinforce the conclusions drawn for houseflies since the *R* flies were slightly heavier than the *S* flies (Table III).

Table VI in which S_1 and S_2 are compared with the resistant strains R_1, R_2 and R_3, shows a difference in weight of fat in the fat body and abdominal cuticle between strains which is significant at the five per cent level (Appendix I, Table XVI). Examination of mean fat contents, corrected to equal numbers in either sex (Appendix I, Table XVII), shows that the difference in weights of fat extracted arises mainly from the low fat content of the fat body and abdominal cuticle of strains R_1 and R_2.

The so-called distribution theory of insect resistance to insecticides may be stated in this way. The potency of an absorbed dose of insecticide is reduced at the site of action by a changed distribution between phases in the resistant strain which results in a decreased concentration of the poison at the point of attack and an increased concentration in other phases where it exerts no toxic action. The fatty tissues are usually postulated as the reservoir for holding insecticide and this function is said to be enhanced in resistant insects both by an increase in the bulk of fat and chemical changes to increase the solubility of insecticide. Thus Wiesmann (1957) reports more fat in the D.D.T.-resistant strain of housefly (14·9 per cent of dry weight compared with 11·6 per cent in a susceptible strain) and increased cholesterol content which is said to increase the solubility of D.D.T. Munson *et al.* (1954) report a greater degree of unsaturation in the fatty acids to give rise to greater D.D.T. solubility.

Our experiments give no support to a distribution theory of resistance mechanism in the γ-B.H.C. resistant flies and mosquitoes examined. Our resistant strains of flies have the same or less fat than the susceptible strains and this is also true of comparisons of resistant and susceptible strains of *A. gambiæ*. Moreover, the R_1 resistant flies showed a marked deficiency of chloroform soluble matter when the abdominal cuticle and adhering fat bodies were separately compared with the same region of susceptible flies. The exposure of flies to γ-B.H.C. vapour for periods up to 24 hours, followed by analysis for γ-B.H.C. in the fat body tissue, showed no insecticide in this part of the resistant fly whereas on the distribution theory of resistance a build up of insecticide in this region might be expected.

Analysis of the extracted fats showed no major differences in chemical composition between resistant and susceptible strains. Iodine values showed a difference significant at the ten per cent level of probability, the resistant fly fats having slightly higher values indicating more unsaturation. Saponification

numbers for the lipoids of the two strains showed no statistically significant differences. Refractive indices varied from batch to batch but the range covered by values for resistant flies overlapped completely the range for susceptible fly fat values and analysis revealed no significant difference between the strains. This again points to no consistent differences in chemical composition.

The solubility of γ-B.H.C. in fats from the resistant and susceptible strains has been measured and found to be the same. This is consistent with the evidence of chemical similarity of fats discussed above.

It is tempting to advance an alternative theory based on the tendency towards a reduced fat in resistant strains of houseflies and mosquitoes compared with susceptible strains. For example, the reduced absorption of γ-B.H.C. by R_1 flies, when compared with S_1 (Bradbury and Standen 1956), might well be due to the lower lipoid content of the R_1 strain. Comparisons of this kind, however, between one laboratory strain and another of different origin may have no direct significance for resistant mechanisms. We prefer to regard the difference in fat content between strains as one aspect of difference in physiology which may or may not be linked with the ability to resist poisoning by insecticides. In the negative sense the results are, we believe, of value in showing that a strain of flies can resist large doses of insecticide whilst possessing the same kind of fat as strains which are susceptible to poisoning, and in equal or smaller amount.

A point of interest emerging from the work is the failure to find γ-B.H.C. in the fat body tissues of the fly. This could mean that the insecticide is not taken up by the fat body or, alternatively, that it is very rapidly metabolized in this region. Some support for the view that fat body and abdominal cuticle (which were not separated in our experiments) are regions of rapid metabolism, comes from the work of Tahori and Hoskins (1953) on the distribution of D.D.T. and its metabolite D.D.E. in resistant flies. Out of a total internal D.D.E. of 17·28 μg., 6·62 μg. was found in the abdominal cuticle and 2·81 μg. in the fat body, together making over half of the total metabolite. On the other hand, 0·19 μg., out of a total 0·53 μg. of unchanged D.D.T., were located in the fat body and abdominal cuticle in Tahori and Hoskins' (1953) experiments.

More recently Miyake *et al.* (1957) have shown that the fat body and cuticle of flies contain D.D.T. dehydrochlorinase which brings about the conversion of D.D.T. to D.D.E. It is possible that our failure to find γ-B.H.C. in fat body and cuticle of resistant houseflies is due to the presence in these organs of the enzyme responsible for B.H.C. metabolism.

It is not contended, of course, that an increase in the fat content of an insect would not increase resistance to poisoning by fat soluble insecticides. A recent review by Tahori (1958) cites many examples of reduction in susceptibility to D.D.T. poisoning accompanying increase in fat content of insects. It is to be expected from the theoretical thermodynamic treatment of biological action due to Ferguson (1939) that an insecticide will tend to be distributed in the insect tissues in such a way that its thermodynamic activity a is the same in all phases. Since $a = \gamma x$ where γ is the activity coefficient of the poison in the phase in question and x its mol fraction, it follows that the distribution of a fixed dose of an insecticide, at equilibrium, will be diminished in any biophase which may be the site of action

by the presence of another phase in which the insecticide has a low activity coefficient and that this effect will increase as the bulk of the second phase increases. It is to be expected, therefore, that susceptible flies fed on fat-rich diet will become more resistant to D.D.T. poisoning (Mer and Furmanska, 1953); likewise Wiesmann's finding that susceptibility was decreased by olive oil injection (Wiesmann and Reiff, 1956) is to be expected. What has emerged from the work described here is that neither in bulk nor in solubility properties do the fats of the resistant flies and mosquitoes examined show the kind of changes required to make this mechanism operative. Moreover the increase in lipoids reported by Wiesmann and the increase in D.D.T. solubility are not big enough to make an important contribution to resistance of the magnitude of 100-fold encountered in the field.

Another aspect of the thermodynamic treatment of toxicity arises if we consider the effect of changes in the nature of the biophase in which the poisoning process is initiated, say the nerve tissues. Chemical changes, which result in an increase in the activity coefficient of the insecticide in the nerve tissue, will have the effect of diminishing the concentration of poison in this phase when the system is in equilibrium. The protective role of the Hoyle nerve sheath, demonstrated by Twarog and Roeder (1957), may be due to a physical mechanism of this kind. The relative bulk of a phase, such as the nerve sheath, must be very small, with the result that changes in this part of the total lipoids between *S* and *R* strains would not be detected in analyses of the total extract.

The thermodynamic arguments advanced above presuppose a state of equilibrium in the distribution of insecticide between the phases of insect tissues. It is very likely that such a state is not reached in highly resistant strains of insects since destruction by metabolism must continually reduce the concentration of poison in those phases in which the metabolism takes place. Kearns and his colleagues (Miyake, Kearns and Lipke, 1957) have suggested that D.D.T. is metabolized by the nerve tissues as it arrives at the site of action. It is also possible that the insect fat bodies play a dynamic role in detoxication. Their relative bulk and solvent power for chlorinated hydrocarbons, ensure that a substantial proportion of the absorbed insecticide will reach them. They are centres of active metabolic processes in the insect and the failure to recover γ-B.H.C. from fat bodies, reported in this paper, suggests that they rapidly metabolise γ-B.H.C. It seems likely, therefore, that the floating bodies in the insect blood may function as mobile scouring agents for removal of insecticide from other insect tissues.

The mechanism of B.H.C. resistance in *A. gambiæ* strains remains obscure. The figures presented in this paper for wax and fat contents offer no support to a distribution mechanism, and previous work (Bradbury and Standen, 1956) has shown the resistant strain to have only slight capacity — no better than the susceptible — for metabolism of B.H.C. Another experiment, using this time the more resistant *A. gambiæ* strain, has confirmed the result reported earlier.

Fifty female *A. gambiæ* of the R_2 strain were exposed to 50 μg. of C^{14}-labelled γ-B.H.C. in a 100 m.l. conical flask for one hour. The mosquitoes were then transferred to a clean flask where they were kept for five hours, when they were ground with kaolin and extracted with 80 per cent alcohol which was also used to

extract the storage flask. The total radioactivity in this extract was determined and also its γ-B.H.C. content (by isotopic dilution).

(Total activity 7·4 μg. γ-B.H.C. 6·3 μg.).

If the difference is assumed to be metabolites, this gives an upper limit of 1·1 μg. or 15 per cent of the absorbed dose of B.H.C. metabolized in five hours. The figures found previously for susceptible *A. gambiæ* were 14·6 per cent and 14·8 per cent metabolized (Bradbury and Standen, 1956).

It seems, therefore, that detoxication by metabolism cannot account for γ-B.H.C. resistance in *A. gambiæ*. This is in contrast to the D.D.T.-resistance mechanism in *A. sundaicus* which has been reported (Brown, 1958) to be associated with the presence of D.D.T.-dehydrochlorinase. The present work does not support a resistance mechanism for *A. gambiæ* based on increased fat content. The possibility that the mechanism in this case is one of exclusion of the poison from the site of action by a physical process, is worthy of investigation.

SUMMARY.

The epicuticular waxes and the lipoids of five strains of *A. gambiæ* and of five strains of *M. domestica* have been examined. The weights of waxes were not found to differ between γ-B.H.C.-resistant and susceptible strains. Rates of penetration of γ-B.H.C. were calculated and found to be the same for a susceptible and a highly γ-B.H.C.-resistant strain of houseflies. The amounts of lipoids in resistant strains were found to be equal to or less than those of susceptible strains. The lipoids extracted from resistant and susceptible flies were very closely similar in chemical and physical properties including solubility of γ-B.H.C. The results lend no support to the theory that preferential distribution of insecticide to fatty tissues in the resistant strains contributes to the defence mechanism of γ-B.H.C.-resistant strains of mosquitoes and flies.

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APPENDIX I.

Statistical Analysis of Results.

Two special procedures were required to analyse these data, namely, non-orthogonal variance analysis and co-variance analysis.

The necessity for non-orthogonal variance analysis arises when there are two independent criteria of classification (in this case sex and strain) and the numbers of values in the sub-groups defined by these criteria vary in an irregular manner. The cases involved are Tables II and VI. The method of analysis used was that of Kendall (1951). In each case, seven constants were fitted to the data, one for each sex and one for each of the five strains. The sum of squares, associated with the seven constants, was evaluated by means of Kendall's formula and that associated with difference between sexes was obtained in the conventional manner, thus giving the "residual between strains" by substraction. The remainder of the total sum of squares between sub-groups then gave the "sex × strain" component. The results of this analysis are given in Tables XI and XVI.

When significant variation between strains is revealed, mean values for each strain corrected for any possible effect of sex difference, are needed to interpret this variation. These corrected means have been calculated from the fitted constants and the results are given in Tables XII and XVII.

Table XI.

Variance analysis of fat contents (μg./mosquito) of five strains of mosquito (Table II).

Source of variation	Sum of squares	Degrees of freedom	Mean square	"F" and Significance
Between sexes	41,733	1	41,733	2·66 Not significant.
Residual between strains ..	289,754	4	72,438	4·61 10 per cent.
Sex × strain	62,868	4	15,717	

Table XII.

Mean fat contents of five strains of mosquito. Corrected to equal numbers in either sex (Table II).

Strain	S_1	S_2	S_3	R_1	R_2
Fat content (μg./mosquito)	40·5	34·9	49·5	49·3	11·8

TABLE XIII.

Variance analysis of fat and wax contents of two strains of housefly (Tables III and IV).

Source of variation	Sum of squares	Degrees of freedom	Mean square	"F" and Significance
Wax content (μg./fly)				
Between strains	30,096	1	30,096	.. Not significant.
Within strains	901,906	7	128,844	
Fat content (mg./fly)				
Between strains	33·019	1	33·019	1·15 Not significant.
Within strains	257·564	9	28·618	

TABLE XIV.

Co-variance analysis of fat content and age for two strains of housefly (Table V).

(The dependent variable is fat content in mg./fly).

Source of variation	Sum of squares	Degrees of freedom	Mean square	"F" and Significance
Head and thorax				
Residual about one regression line (*A*) ..	10·9354	10	1·0935	
Residual about two parallel lines (*B*) ..	4·7779	9	0·5309	
Residual about two separate lines (*C*) ..	4·3935	8	0·5492	
A – *B*	6·1575	1	6·1575	11·60 1 per cent.
B – *C*	0·3844	1	0·3844	.. Not significant.
Fat body and abdominal cuticle				
Residual about one regression line (*A*) ..	3·8440	10	0·3844	
Residual about two parallel lines (*B*) ..	1·1501	9	0·1278	
Residual about two separate lines (*C*) ..	0·9165	8	0·1146	
A – *B*	2·6939	1	2·6939	21·08 1 per cent.
B – *C*	0·2336	1	0·2336	2·04 Not significant.

Co-variance analysis was needed in the analysis of Table V. Within each of the two sections ("Head and Thorax" and "Fat Body and Abdominal Cuticle") three regression analyses of fat content on fly age were made, denoted by *A*, *B* and *C*. In *A* one regression line was calculated, ignoring any difference between

TABLE XV.

Regression equations of fat content on age for two strains of housefly (Table V).

(The equation is $F = F_0 - b.A$, where F is the fat content in mg./fly and A the age in days).

Fat content of:	Strain.	F_0	b
Head and thorax	S_1	0·517	0·0109
	R_1	0·402	
Fat body and abdominal cuticle	S_1	0·193	0·0054
	R_1	0·122	

TABLE XVI.

Variance analysis of fat contents (mg./fly) of five strains of housefly (Table VI).

Source of variation	Sum of squares	Degrees of freedom	Mean square	"F" and Significance
Head and thorax				
Between sexes	0·0366	1	0·0366	Not significant.
Residual between strains ..	1·2020	4	0·3005	Not significant.
Sex × strain	1·7381	4	0·4345	—
Fat body and abdominal cuticle				
Between sexes	0·5453	1	0·5453	14·3 5 per cent
Residual between strains ..	2·7960	4	0·6990	18·3 5 „ „
Sex × strain	0·1527	4	0·0382	—

TABLE XVII.

Mean fat contents of five strains of housefly, corrected to equal numbers in either sex (Table VI).

Strain	S_1	S_2	R_1	R_2	R_3
Fat content of head and thorax (mg./fly)	0·365	0·429	0·360	0·408	0·381
Fat content of fat body and abdominal cuticle (mg./fly)	0·120	0·182	0·095	0·097	0·185

strains, in *B* a constant difference between strains was assumed and in *C* two completely separate regression equations were calculated. The resulting residual sums of squares are given in Table XIV. The hypothesis that the rate of change of fat content with age is the same for the two strains, is tested by dividing the "improvement" mean square *B-C* by the residual mean square from analysis *C*. It will be seen that neither of the resulting "*F*" ratios is significant; thus there is no evidence that the difference between strains varies with age. The remaining step in the analysis is to divide the "improvement" mean square *A-B* by the residual mean square from analysis *B*. The resulting "*F*" ratio, tests the significance of the difference between strains corrected for age-difference using a common regression coefficient. It will be seen that a significant difference between strains is revealed in both sections. The nature of this difference is shown in Table XV, which gives the regression equations obtained in analysis *B*.

In Tables III and IV, conventional variance analysis methods are applicable, and the results are given in Table XIII of this Appendix.

Calculation of rates of absorption of γ-B.H.C. by S_1 and R_1 strain of flies.

Absorption of γ-B.H.C. through the fly's cuticle into the interior may be assumed to take place by virtue of the difference in partial saturation of γ-B.H.C. between exterior wax and internal fat. The magnitude of this difference is given by $W/W_1 - F/F_1$ where W_1 is the product of the total amount of exterior wax/20 flies and the solubility of γ-B.H.C. in fly wax, while F_1 is the corresponding quantity for fat. Assuming linearity we then have

$$dW/dt = -b(W/W_1 - F/F_1) \qquad (1)$$

giving

$$W = W_0 - b\int_0^t (W/W_1 - F/F_1)\,dt \qquad (2)$$

The validity of the assumption of linearity was checked graphically by plotting *W* against the integral in (2) obtained from the figures in Table X by the ordinary trapezoid method. It was found that, providing the 24-hour observations are omitted, there is no suggestion of systematic non-linearity. The omission of the 24-hour observations can be justified on the ground that the last step in evaluating the integral is particularly unreliable because of the large time interval.

Estimates of the limiting rate of absorption *b* as defined by (1), found by regression analysis were:

for R_1 flies $b = 0{\cdot}050 \pm 0{\cdot}005$ μg./h.
for S_1 flies $b = 0{\cdot}050 \pm 0{\cdot}025$ μg./h.

The limits quoted are 95 per cent confidence limits based on residual variation of *W* about its regression line on the integral term.

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STUDIES ON INSECTICIDE-RESISTANT ANOPHELINES.

III.* A preliminary report on lipoid content in some strains of *A. atroparvus.*

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INTRODUCTION.

It has been tried to explain D.D.T.-resistance by two main theories; one of them is Kearns' well known enzymatic dehydrochlorination theory (see *e.g.* Kearns, 1955). The other one is Wiesmann's "fat barrier" theory (Wiesmann and Reiff, 1956; Wiesmann, 1957), which is based on the findings of enhanced lipoid content in resistant insects (*Musca* and *Drosophila*).

In the framework of investigations carried out with our laboratory-developed strains of D.D.T.-resistant *A. atroparvus*, it was of interest to ascertain, which of both mechanisms of resistance, if any, is predominant in our resistant strains. Enzymatic dehydrochlorination in these strains is being investigated by Dr. N. Frontali of this laboratory. In the present work, on the other hand, preliminary results on lipoid content in two normal and two resistant strains have been summarized.

Besides an attempt to elucidate the mechanism of resistance, the following point of interest presented itself: It has been found by one of us that cetyl bromoacetate (CBA) is more toxic to resistant than to normal flies (Ascher, 1957:1958). This was ascribed (Ascher, 1958*a*) to the enhanced and different lipoid content found by Wiesmann and Reiff (1956) in resistant flies. It was, therefore, deemed

*Part I: Mosna, Rivosecchi and Ascher, *Bull. Wld. Hlth. Org.*, **19**, p. 297 (1958). Part II: Mosna, Palmieri, Ascher, Rivosecchi and Neri, *Bull. Wld. Hlth. Org.* (in press).

essential to accompany toxicity-tests on anophelines of compounds, to which resistance is negatively correlated, by an assay of the lipoid content in normal and resistant strains.

Wiesmann's work on the significance of total lipoids in insecticide resistance is founded on the earlier researches of Mer and Furmaska (1953) and Munson and co-workers (Munson, 1953; Munson and Gottlieb, 1953; Munson, Padilla and Weissmann, 1954). Investigations on epicuticular waxes, and their relation to the activity of D.D.T. in different insects, have been carried out by Hurst (1949), Böhm (1951) and by Indian workers (Pradhan, Nair and Krishnaswami, 1952; Pal, Sharma and Krishnamurthy, 1954). Recently, the subject of correlation between lipoids and resistance, which has been treated by now already in numerous publications, was reviewed by Tahori (1958).

As regards the connection between lipoid content and D.D.T.-resistance in mosquitoes, there exist the following papers and indications in the literature:

Starved fourth-instar larvæ of *A. quadrimaculatus* develop D.D.T.-poisoning symptoms and show " knock-down " much more rapidly than fed ones, according to J. C. Jones (cited by Munson, Padilla and Weissmann, 1954). This, however, can hardly be accepted as a strong argument in support of the importance of lipoids in defending insects from the toxic effects of D.D.T. The seasonal change of LC_{50}'s (D.D.T.) in *A. maculipennis* and *A. sacharovi*, in Iran, was ascribed by de Zulueta, Jolivet, Thymakis and Caprari (1957) to pre-hibernation fat body development, in accordance with Wiesmann's theory. Similar seasonal changes in D.D.T.-susceptibility were noted in larvæ of *A. sergenti* in Jordan (Singh and Gad cited by Brown, 1958, p. 40) and in other mosquitoes (Brown, 1958 *loc. cit.*). On the other hand, Doby, Deblock, and Gaeremynck (1956) found no correlation between different levels of lipoid content in larvæ of *Aedes ægypti* (obtained by various larval foods) and their susceptibility towards D.D.T.

EXPERIMENTAL.

(A) BIOLOGICAL MATERIAL.

The following normal and resistant strains of *A. atroparvus* were used in this investigation:

Susceptible reference strains.

SENS-Roma — an Italian susceptible strain originating from Ferrara, described already in Part I of this series.

SENS-Hamburg — a Northern-German highly susceptible strain obtained through the courtesy of Prof. F. Weyer, Hamburg.

Resistant strains.

(Described already in Part II, selected by D.D.T.-pressure from SENS-Roma).

RAFM — a D.D.T.-resistant strain obtained through selection by tarsal contact (Busvine and Nash method, 1953) of *adults, females* and *males* (AFM) during 32 generations.

RL — a D.D.T.-resistant strain obtained through D.D.T. selection pressure in the first and third *larval* (L) stage during 32 generations.

The mode of rearing has been described in detail in Part I. The larvæ were fed with a mixture of one part of fine bran and one part of " piscidin " (dried Daphnia powder). Great care was taken to rear the larvæ of all strains under identical conditions.

(*B*) DETERMINATION OF LIPOID CONTENT.

1. It was attempted first to obtain relative values of lipoid content using the staining method on filter paper with the stain " Fettschwarz-Geigy ", following closely the procedure of Wiesmann and Reiff (1956). In each experiment, 50 individuals of each strain were macerated in a mortar with acetone and anhydrous sodium sulphate. Experiments were carried out with fourth instar larvæ, with pupæ, and with adult females. The intensity of the stained drops on the filter papers was registered with the " Elettro-ferografo " -AKARI AK-REFO 3, a semi-automatic registering apparatus for paper electrophoresis, and the curves were evaluated planimetrically. Though a very great number of extractions was done, the method was found to be utterly unreliable as far as lipoids of mosquitoes are concerned and the results were irreproducible. Thus not only that differences in fat content found in one series of experiments could not be replicated in subsequent series, but there were also found completely different orders of the intensity of the stained drops among strains on replications of the assays. This could be seen even with the naked eye. Endeavours were made, therefore, to perfect the staining and thus to obtain better reproducibility of results. The staining and washing times and the washing procedure in 50 per cent alcohol were rigidly standardized. All of this was of no avail.

2. It was decided, therefore, to use instead the usual method of extraction with the Soxhlet following the procedure of Anselmi and Cesari (1950) for fat determination. For each determination, 2000 females of *A. atroparvus*, killed and stored in the deep freeze (—20°) after one day of life on glucose, were ground in a glass mortar with twice their weight of anhydrous sodium sulphate. The mixture was left for an hour in the covered mortar and then transferred quantitatively into a filter-paper package (Anselmi and Cesari, 1950) in the Soxhlet, upon which extraction commenced. Each strain was done in two modes of extraction:

(*a*) acetone (seven hours).

(*b*) ether (seven hours) followed by: (*b'*) the residue remaining after the ether extraction was re-extracted with acetone for three hours.

The fat extracted was dried in a vacuum exsiccator over solid paraffin wax until constant weight.

Experimental arrangement $b + b'$ served as an independent check on experiment *a*.

RESULTS AND DISCUSSION.

In Table I, the results of the extractions with acetone (*a*), and with ether followed by acetone ($b + b'$) are confronted with each other on one hand, and with

the results of Busvine-Nash (1953) assays (cited from Part II of this series) on the other hand.

TABLE I.

Results of the extraction with acetone (a), and with ether followed by acetone (b+b′) confronted with each other on one hand, and with the results of Busvine-Nash (1953) assays on the other hand.

Experiment Number	Strain	Mode of extraction	Fresh weight of 2000 ♀♀ (grams)	Weight of fat extracted (grams)			Percentage of fat extracted, calculated on fresh weight			Percentage of total fat extracted, calculated on fresh weight	LC_{50}, Busvine-Nash method, Percentage — Duration of contact	
				a	*b*	*b′*	*a*	*b*	*b′*		1 hour	24 hrs.
1	SENS-Hamburg	*a*	4·6477	0·2092			4·50			*4·50*	2·5	
2	SENS-Hamburg	*b+b′*	4·4325		0·1505	0·0403		3·39	0·91	*4·30*		
3	SENS-Roma	*a*	5·2094	0·3134			6·016			*6·016*	3·4	
4	SENS-Roma	*b+b′*	5·0806		0·2029	0·0495		3·994	0·994	*4·968*		
5	RL ..	*a*	4·7108	0·2970			6·30			*6·30*		2·4
6	RL ..	*b+b′*	4·3687		0·2121	0·0573		4·85	1·31	*6·16*		
7	RAFM ..	*a*	5·1031	0·3446			6·752			*6·752*		>4·0
8	RAFM ..	*b+b′*	4·2631		0·2569	0·0389		6·026	0·760	*6·786*		

The results in Table I indicate a tendency towards higher lipoid content in the resistant strains and perhaps even a certain correlation between height of resistance and lipoid content. In the strains SENS-Hamburg, RL and RAFM the results obtained by methods *a*, and *b+b′* were very similar; in strain SENS-Roma, on the other hand, the acetone extraction gave a much higher value than the ether extraction followed by acetone. Indeed, the acetone extraction value in this strain was nearly as high as those of the resistant strains. This unexpected result admonishes us to treat the problem with the utmost caution. Replications on these and other normal and resistant strains of anophelines have to be done, before anything final can be said about the correlation of total lipoid content and D.D.T.-resistance in anophelines.

It may be asked at this point, why tarsi, which are so important as sites of the penetration of D.D.T., were not extracted instead of whole mosquitoes. Indeed, in the early stages of this work, following Wiesmann's lead on houseflies, we extracted the tarsi of 1,000 mosquitoes, but the quantity of fat extracted was so minute, as not even to give any colour on staining drops of the acetone extract on filter paper with " Fettschwarz-Geigy ".

Trying to follow up the iodine numbers of our extracted fats proved to be perfunctory, too. Though all the fats extracted gave with Hanus' method (Anon., 1942;1950) iodine numbers of about 85, the values obtained must be considered illusory. All the fats, though stored under nitrogen, acquired rapidly the smell of rancid fats (probably due to auto-oxidation). This problem has thus to be reinvestigated with special precautionary methods (nitrogen!) during maceration, extraction, etc. The use of petrol ether as extracting solvent might be indicated, too.

Following up the rate of solubility of D.D.T. in the fats, as was done by Wiesmann and Reiff (1956) for house-flies, also proved impracticable due to the very slow rate and evidently much lower solubility of D.D.T. in our fats extracted from anophelines.

This work is being continued at present on strains RLAF and R. *Dieldrin* of *A. atroparvus* (*see* Part II) and on a normal and a D.D.T.-selected strain of *A. stephensi*.

SUMMARY.

After unsuccessful attempts to find differences in the lipoid content of normal and resistant strains of *A. atroparvus* by a staining method, extractions (Soxhlet) were done on females with acetone, and with ether followed by acetone. Results obtained on two normal strains (SENS-Hamburg and SENS-Roma) and two D.D.T.-resistant strains (RL and RAFM) seem to indicate a tendency towards higher lipoid content in the resistant strains. Due to certain inconsistencies, the assays have to be repeated on these and other strains, before a final statement can be made.

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COMPONENTS OF INSECTICIDE TOLERANCE.

BY

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[October 3, 1958.]

In a recent address I suggested, and gave some of my reasons for believing that there are primarily two types of resistance to insecticides. One I have called "multicomponent or generalized resistance", the other, "specific resistance". The first is considered to be characterized by the multiple adjustment of a number of nominally independent factors, the second by the presence of some specific mechanism, the most obvious and established of which is detoxification of the insecticide. The two types of resistance are not regarded as mutually exclusive categories and may, and it was suggested often do, co-exist, some of the knottiest problems in insecticide resistance being resolvable on these hypotheses (Spiller, 1958).

The literature on insecticides and resistance to insecticides contains a good deal of information which may be brought forward as support for these suggestions. Others, however, may interpret the same evidence in a different way. It is my view that the interpretation others give the information adduced will be dictated very largely by their conceptual image of the nature and make-up of the insecticide tolerance of susceptible, that is, non-resistant insects. This should be a central theme in resistance studies but, as far as I am aware, has been seldom discussed or commented upon. Therefore I would like to present my views and my thinking on the nature and make-up of the insecticide tolerance and to show how this leads, it seems to me inevitably, to the concept of multicomponent resistance.

All insecticides are site-specific. Some may act at more than one site, but no insecticide acts at all the multitudinous and varied sites that comprise the insect body. Conceivably the site may be common to every cell, and be relatively abundant there, but more often they will be less common, being restricted to a particular system or part of the system, as for example the nervous system. In some cases we know what the sites are but do not know where they are, in other cases we know where they are but do not know what they are. Organo-phosphorus insecticides inhibit cholinesterase and other esterases, but it is uncertain in what

structures and locations the inhibition is critical; D.D.T. induces repetitive discharge in sensory nerve, probably by increasing permeability, but the exact way in which the membrane is affected is unknown.

It is not to be expected that apparently identical sites in different species will have identical sensitivity. A well established example is the very different sensitivities of bee-brain and housefly-brain acetylcholinesterases to organophosphorus compounds (Metcalf and March, 1950). Such species-specificity of sensitive site may account for much of the species-specificity of insecticide toxicity, but it does not account for all of it. Malathion and parathion are about equally potent inhibitors of locust brain AChE, yet parathion is about 20 times as toxic as malathion to locusts (Stringer and Fielding, 1956).

With at least one insecticide the sensitivity of the site is much affected by temperature, the threshold concentration of D.D.T., necessary to induce repetitive trains in cockroach nerve, increasing some 11-fold as the temperature increases from 16°C. to 30°C. (Yamasaki and Ishii, 1954). Without doubt, this change in the sensitivity of the site is largely responsible for the marked temperature dependence of the toxicity of D.D.T. to insects.

Possibly sensitivity of the site is also affected by the internal environment. Changes in hæmolymph concentration or constitution might be particularly important for the blood is in intimate contact with most tissues. Enzyme activity may be affected by ion concentration or by excess substrate; in the present context, cholin esterase is the obvious example (Smallman and Wolfe, 1954). Non-enzymatic sites may also be affected by hæmolymph composition. Immediately after feeding, *Rhodnius* are markedly but transiently more susceptible to D.D.T.; this may well be due to an increase in the sensitivity of the site brought about by the changed ionic and osmotic concentration of the hæmolymph, for the period of increased susceptibility is readily extended by preventing diuresis and is quickly terminated when regulation occurs (Spiller, 1955).

These, then, comprise the first series of components of insecticide tolerance: the innate sensitivity of the site of action and the various biological factors which affect this sensitivity. They may be referred to as the site components.

There are many barriers between the place where the insecticide is applied and the site at which it acts. Contact insecticides and fumigants must penetrate the cuticle, stomach poisons must enter through the gut wall. There is good evidence that the amount of insecticide, entering the insect, is not a constant proportion of the dose applied, the proportion diminishing as the dose increases (Hewlett, 1958). At the molecular level the attractive forces between site and insecticide can hardly extend beyond a few Ångstroms. Site finding must, therefore, be largely a hit and miss procedure, the proportion of effective molecules being almost vanishingly small compared with that required to maintain the molecular concentration necessary if sufficient molecules are to encounter sufficient sites to upset the normal physiology. Usually this molecular concentration is maintained or increased by more molecules penetrating from the exterior, but if the material applied is insecticidally inert and has to be converted to active material, the rate of conversion may be more critical than the rate at which penetration occurs. Concurrently, the insecticide may be inactivated by being bound or stored at sites where it is ineffective, as for example in fat globules and

storage lipoid, or by being detoxified. This relationship between the dose applied and the amount at the site of action, has an average value for each insecticide and each bioassay system which has been termed the availability coefficient (Stringer, 1948; Blackith and Gorringe, 1953). Obviously, the availability coefficient will vary from individual to individual in the population for it will be affected by all factors other than dose which determine the number of molecules reaching the sensitive site. Collectively, these may be designated the pre-site components.

If " to kill " is " to cause the death of " then insecticides kill insects. But the word that should be operative is " cause " rather than " death " for there may be a rather long and at first not very obvious chain of events, beginning with the observed biochemical or biophysical effects of an insecticide on some particular system and ending with death of the insect. There are, therefore, primary effects and secondary effects, the primary effects being on or at some particular site and being similar, if not identical, under different conditions and in different insects, while the secondary effects which follow, induced or brought about by the primary effects, may not be the same in different insects, either species or stage, and may differ in the same species and stage under different conditions of testing.

In *Rhodnius*, as in other insects, D.D.T.-poisoning induces first hyperactivity, then knockdown and finally death. Under dry conditions, D.D.T.-poisoned *Rhodnius* die of desiccation before their metabolic reserves are exhausted; under more moderate conditions death occurs while there is still an abundant supply of water. Knockdown occurs, not when hyperactivity reaches a certain fixed intensity, but at different hyperactivity levels depending upon the metabolic reserves of the insect. And knockdown can readily be reversed by raising the ambient temperature. And death of the poisoned insect is not inevitable, but can be circumvented in a variety of ways (Spiller, 1955).

Here, then, is another series of components of insecticide tolerance, the post-site components, the factors which determine how the insect will react after a certain quantity of insecticide has reached its site of action and induced the primary effects. A slightly larger metabolic reserve or a more rapid mobilisation of that reserve may enable an individual to get through the crisis of hyperactivity to which other insects succumb. It would have a higher insecticide tolerance than its less fortunate brethren even though there may be no differences in the sensitivity of the primary sites.

The insecticide tolerances, as experimentally determined, are not the true tolerances. They contain an extraneous component which increases or decreases each true tolerance by a variable amount, converting the true tolerance to an apparent tolerance. This extraneous component is supplied by the testing system employed for no system of applying insecticide, however standardized, can guarantee that each individual receives an identical amount of insecticide. If an insect walks on a film of insecticide, the deposit may not be uniformly available, and different individuals may vary in behaviour. Both factors may affect pick-up. If the insects are sprayed in tunnel or chamber or exposed to an aerosol or falling mist, the pick-up may be affected by whether the insect remains stationary or walks about, or, if it flies, for how long it is airborne and in which part of the chamber the flight occurs. In Peet Grady tests, the dose acquired by individual flies may vary more than six-fold, the average coefficient of variation of the dose

being 28·5 per cent. (Murray, 1940). The precision of topical application from microdrop applicator, either syringe, pipette or loop, is not very high, the uncontrolled variation, again measured as the coefficient of variation of the dose, being around 8 - 14 per cent (Buck, 1949); 5·4 per cent (Hopf, 1951) and 2·4-9·4 per cent (Hewlett, 1954) with different applicators and techniques. That this is rather low precision will be apparent when it is remembered that the extreme doses will be some ±3 times these values from the mean, and that the highest dose will be twice the lowest when the coefficient of variation is still only 11·1 per cent.

These variations of the dose acquired or applied do not affect the mean tolerance of the population, but the variance is increased by an amount which is readily computed if the variation in the dose is known. This increase is rather slight unless the variations in dose are rather large; then these can contribute as much as one third of the variance of the apparent tolerances (Finney, 1952; Hewlett, 1954:1958).

Even if the dose could be measured with precision, there are almost insuperable difficulties in guaranteeing that each dose is applied in precisely the same way so as to occupy a microscopically identical locus. Yet with some insecticides the kill obtained may differ markedly when the insecticide is applied to adjacent loci. Excellent examples are the observations of Fisher (1952) that the same dose of D.D.T. applied to different segments of the housefly tarsi, cause markedly different kills; and the observations by Hewlett (1954) that allethrin was about 1·5 times as lethal to *Tribolium* if applied at the junction of prothorax and mesothorax, instead of at the junction of head and prothorax. It is unknown whether such effects occur in dosage mortality experiments as usually performed. Possibly the insects are exposed for sufficiently long, or a large enough volume of solution is applied, to encompass all loci. Possibly the dose is so distributed that different loci do in fact get variable doses. Nor is it clear what effect this would have on the tolerance parameters but *a priori* an increase in variance might be expected, both because a new random variable is introduced and on the argument that applying identical doses to loci of different sensitivity, is likely to produce the same overall effects as applying variable doses to the same locus which is already known to increase the variance. If in the experiment already quoted from Hewlett (1954) the dose had been applied at either the anterior or posterior position without discrimination, the mean tolerance would have been midway between those found and the variance would have been increased approximately 37 per cent.

Thus the insecticide tolerance of an individual insect, if this could be measured, might be considered as made up from four sources. These are:—

(*a*) an error of variable size, converting the true tolerance to an apparent or found tolerance, the error arising solely from the difficulties of precise measurement and application of the insecticide;

(*b*) the pre-site components which determine how much of the applied dose reaches the actual site of action;

(*c*) the site components which determine what effect a given number of molecules will have at the sensitive sites, and

(*d*) the post-site components which determine how the insect will react to a certain intensity of effect produced at the site of action.

Thus tolerance is regarded as a composite attribute, the average expression of a number, perhaps a large number, of independent or largely independent components. Some of the known components have already been mentioned: thickness and structure of cuticle, amount and composition of lipoid, toxification and detoxification, innate sensitivity of the site, composition of the blood, metabolic reserves. It is not difficult to think of other components or possibilities and undoubtedly more will be discovered.

The contributions from each component may differ greatly in size. Some contributions may be rather small, some very large, and each component in its turn may make a different contribution to tolerance when tests are carried out under different environmental conditions and each may take new and unrelated values as the insect grows to maturity or senesces, or when the nutritional status is altered.

Thus an old individual with thick cuticle, much lipoid and a half digested meal, may survive a dose of insecticide which would kill a young and fully glutted animal, having a thinner cuticle and less lipoid. But the converse may well be true. It is not likely to be easily predicted. If only four components were involved in determining the insecticide tolerance and these were presented only as alternatives, young or old, thick or thin, much or little, glutted or starved, there would be 16 possible categories, the components in each category probably summating to different tolerances. If each of the four components had ten gradations in magnitude there would be 10,000 possible combinations. Although this is a large number, the assumptions are very moderate. Almost certainly there are not four but many components which affect insecticide tolerance. And in a population many of these components are represented, not by two or ten alternatives, but as effectively continuous variables. Hence the number of possible combinations is likely to be infinite, each uniquely determining a tolerance, although obviously many of the tolerances would be nominally identical.

Just as there is no information on how many components may be involved in the make-up of the tolerance, so there is little to suggest how the contributions of each component are integrated to become the whole tolerance. The simplest assumption is that the contributions are simply additive. If true, the tolerances of the individuals, comprising the population, would be normally distributed. However, tolerances are usually distributed log-normally rather than normally, suggesting that the contributions are *not* merely added together to make the whole tolerance. A more important thought is that an insecticide tolerance is not a uniquely determined entity. Numerically identical tolerances may be compounded in quite different ways in different individuals. At first sight this may seem to imply, almost as a corollary, that animals included within a narrow range of tolerances, may in fact be no less random in their tolerance make up than the whole population. However, detailed analysis soon shows that this is only true for those animals whose individual tolerances lie at the mean or median tolerance value or whose average tolerance is the same as that of the whole population. Individually there appear to be many exceptions, but when treated collectively those individuals whose apparent tolerance is above the population mean contain more of the high values of every component, those below the mean contain more of the low values of every component. This conclusion is not affected in any way

by whether the components considered are genetically determined or phenotypic or merely the uncontrolled variations of technique. Those individuals exhibiting high apparent tolerance will contain a larger proportion of the higher values of the gene-controlled component, of the phenotypic component and of the error component.

An oversimplified numerical example may help to establish the concept more clearly. Consider the insecticide tolerance of an individual, a housefly or a drosophila, to be made up, additively, from five components A, B, C, D, E, each of which has five equally probable magnitudes, 1, 2, 3, 4, 5. The fly with the smallest tolerance will have the following constitution

$$\underset{1}{A}+\underset{1}{B}+\underset{1}{C}+\underset{1}{D}+\underset{1}{E}=T \text{ (Tolerance) } 5$$

and the fly with the largest tolerance will have as its constitution

$$\underset{5}{A}+\underset{5}{B}+\underset{5}{C}+\underset{5}{D}+\underset{5}{E}=T\ 25.$$

These values, the maximum and minimum tolerances, can only be arrived at in one way. Obviously if we find flies with T25 then each component has made its maximum contribution, if a fly has T5 then each component has made a minimum contribution. Between these extremes, no tolerance is uniquely determined. There are five ways of arriving at T24 or T6; 121 ways of making T20; 185 ways of compounding T19, and so on.

In all there are 5^5 or 3125 combinations. The values of T19 to T25 inclusive can be made up in 432 different ways and these combinations will contain the five outcome levels the following numbers of times:—1(15); 2(35); 3(70); 4(122); 5(190). That is, 13·8 per cent $(\frac{432}{3125})$ of the population will contain 30·4 per cent $(\frac{190}{625})$ of the maximum contributions (5's), about 20 per cent of 4's, and about 11 per cent of 3's. These, too, are the proportions of the levels of magnitude in each and every one of the components. All values of each component will appear but their proportions will change, the higher values being increasingly represented in individuals with "greater than" tolerances, the lower values predominating if individuals with "less than" tolerances are selected.

From this follows the concept of multicomponent resistance. Selection of the higher tolerance values by graded doses of insecticide, followed by breeding from the survivors will, if repeated in each generation, gradually increase the average tolerance of the population, provided the magnitude of some of the components is in some way genetically determined. And this is exceedingly likely for, in the last analysis, the amount of random or truly non-genic variation is rather small. Further, if some evidence for inheritability must be produced, selection in pure lines does not lead to an increased tolerance (Merrell and Underhill, 1956) and there is the overwhelming certainty that unless some part of the tolerance was genetically determined it would not be possible to increase the tolerance by selection with insecticide.

It must be obvious that when multicomponent resistance is involved, the particular balance of contributions that a selection programme achieves will depend on many factors. One is the presence or absence of particular genes and their

frequency in the gene pool of the first selected population. Another might be selection pressure, for under too harsh a selection programme some genes, perhaps involved in maximising a major component of tolerance, may not have time to be distributed throughout the population before the specimens which carry them are eliminated. Likewise, some genes may be lost if the manifold effects of selection produce gene combinations independent of resistance but deleterious to the vigour or breeding of the population. What is to be expected is that, depending upon the ebb and flow of these various effects, the selected population will eventually achieve a genic balance which maximises the tolerance. But it is not to be expected that the same route will always be traversed, nor that the final gene pool of independently selected populations will be identical, nor that the genes present will be integrated in an identical manner (King, 1955), although every selection programme may achieve some degree of resistance.

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UNSPECIFIC RESISTANCE MECHANISMS IN THE HOUSE-FLY, *MUSCA DOMESTICA* L. *

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[November 14, 1958.]

INTRODUCTION.

THE phenomenon of resistance to synthetic organic insecticides of flies and other insects is known since about twelve years. Originally this resistance was generally considered to be specific as to the selecting agent. Yet there are still many workers, who think that specific defense mechanisms are responsible for the phenomenon of resistance. The purpose of this paper is to show the relativity of the specific mechanisms in relation to the whole resistance problem.

There is no doubt that specific resistance mechanisms have an important part in the resistance, as in particular is the case in the detoxication of D.D.T. by D.D.T.-dehydrochlorinase. There are, however, several observations, which point to aspecific mechanisms. So Blickle *et al.* (1948) state that flies, selected with B.H.C., show not only lindane-resistance, but also decreased susceptibility to D.D.T., pyrethrins and "Lethane". Wilson and Gahan (1948) established the fact that selection of house-flies with D.D.T., induces also resistance to chlordane, toxaphene, rotenon, "Thanite" and pyrethrins. Harrison (1950) obtained a D.D.T.-resistant strain of flies, which also proved to be resistant to pyrethrum, whereas a strain, selected with pyrethrum pressure developed resistance to D.D.T. De Pietro-Tonelli (1950) found in a D.D.T.-resistant strain a distinct decrease in susceptibility to lindane, aldrin and other chlorinated hydrocarbon insecticides. Mosna (1952) ascertained in a D.D.T.- and chlordane-resistant strain, apart from resistance to chlorinated hydrocarbons, also resistance to an organic sulphur compound and to pyrethrum, and concludes that resistance of the house-fly is not specific to chlorinated hydrocarbons.

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* [*Communication No.* 40 *of the Agrobiological Laboratory* "*Boekesteyn*" *'s-Graveland* (*The Netherlands*)].

An interesting investigation of Pimentel *et al.* (1955) shows that it is possible by selecting with one substance to induce resistance at the same time to other compounds. With D.D.T.-pressure these investigators obtained a strain, which developed resistance also to lindane, dieldrin and parathion. Also by selection with lindane, dieldrin or parathion the resulting strains appeared to develop resistance to the compounds which had not been used during selection.

These facts demonstrate that resistance is not always specific as to the selecting agent. It is difficult, however, to interpret the results obtained with different strains, as the exceptional genetic variability not only causes considerable geographic differences but also entails heterogeneous compositions in local populations. A representative sample of *Musca domestica* L. is therefore illusory. Moreover the way of selection — note that the breeding methods are also selecting! — is probably largely affecting the result.

METHODS AND MATERIALS.

The original normal laboratory strain is the offspring of a natural population, collected in 1952 in a dairy barn at 's-Graveland, where up to that time insecticides had never been used. The resistant strains were selected by treating 200-400 0-1 day old flies of each generation with a dose of insecticide, giving approximately 90 per cent kill. After the selection the survivors were treated in the usual manner.

With D.D.T., lindane, "Diazinon"* and N, N-dimethyl-phenylcarbaminate† further designated as S 17, four strains have been selected. Originally the selection was carried out by atomizing the insecticide by means of xylene-water emulsion in the breeding cage. Later on we had to change this selection method, because with increasing resistance, the high doses of xylene, needed to solve the insecticides, became too harmful. Therefore the flies were later exposed to contact with an insecticide deposit in glass jars.

The susceptibility of the successive generations was determined with the petri dish method by using petri dishes of 4″ diameter. The inside surface of the lid was treated with one ml. of an acetonic solution. During ten minutes the dishes remained open, in order to allow the acetone to vaporize. After that, the dishes were closed and provided with ten flies each. Usually seven concentrations were used for calculating the probit-regression curve, each with at least three replicates. The age of the flies used in the experiments, was 3-5 days. Control took place after four and twenty-one hours. The LC 50‡ and the LC 95‡ stated in the following pages are related to the control after twenty-one hours.

RESULTS.

Table I shows the results, obtained after selection for 15-40 generations. The development of the resistance in the successive generations of these strains has been

* Trade name of J. R. Geigy A. G., Basle, for O, O-diethyl-O-(2-isopropyl-4-methyl-6-pyrimidyl) phosphorothioate.

‡ LC 50 = concentration causing 50 per cent kill; LC 95 = concentration causing 95 per cent kill.

TABLE I.

LC50 and LC95 of several strains of Musca domestica *L. expressed in mg./l.*

Compound \ Strain	Normal strain		D.D.T. Pressure (RD)		Lindane-Pressure (RL)		S 17-Pressure (RS)		Diazinon Pressure (RP)	
	LC 50	LC 95	LC 50	LC 95	LC 50	LC 95	LC 50	LC 95	LC 50	LC 95
D.D.T.	250	1800	> 100,000	> 100,000	> 100,000	> 100,000	250	27,000	2000	> 100,000
Toxaphene	80	3000	> 100,000	> 100,000	> 100,000	> 100,000	> 100,000	> 100,000	> 100,000	> 100,000
Dieldrin	1·3	22·2	> 100,000	> 100,000	> 100,000	> 100,000	> 30,000	> 30,000	100	100,000
Aldrin	0·5	6·1	6400	> 100,000	30,000	> 100,000	450	61,000	33	4,000
Chlordane	6·7	33	10,000	> 100,000	19,000	> 100,000	190	2,320	250	90,000
Lindane	0·75	2·25	3·7	100	300	> 100,000	20	270	25	150
"Diazinon"	0·5	1·3	0·6	1·7	1·8	5·2	3·0	9·0	5·0	12·0
S 17	250	500	200	400	300	800	300	800	250	500

described before in detail (Meltzer, 1956). It is remarkable that the strains did not only acquire resistance to the insecticides, used in the selection procedure, but also to other compounds, even when belonging to entirely different types. It is surprising, e.g., that D.D.T. pressure induced a very high toxaphene and dieldrin resistance. Still more remarkable is the fact that selection with the carbaminate S 17 and with the phosphorus compound "Diazinon" gives rise to high resistance to chlorinated hydrocarbons.

In this connection the carbaminate and the phosphorus compound form a separate class, because the resistance induced by these substances is of a distinct low level as compared with the other insecticides. In view of the resistance levels it is obvious that in each strain the same sequence is found for the different insecticides; only the resistance to D.D.T. of the strains selected with the carbaminate (RS) and with the phosphorus compound (RP) makes an exception (*vide* Table II). Lindane, the phosphorus compound and the carbaminate have caused in the respective strains a much higher resistance to toxaphene, dieldrin, aldrin and chlordane as is the case to themselves.

TABLE II.

Resistance factors of four resistant strains, based on the LC50.

Insecticide	LC_{50} normal strain	Resistance factor*			
		RD	RL	RP	RS
D.D.T.	250	~	~	8	1
Toxaphene	30	~	~	~	~
Dieldrin	1·3	~	~	80	25,000
Aldrin	0·5	12,800	5,000	66	75
Chlordane	6·7	1,500	3,000	35	28
Lindane	0·75	5	400	33	27
"Diazinon"	0·5	1·2	3·5	10	6
S 17	250	0·8	1·2	1	1·2

* The symbol ~ stands for not measurable

This multi-resistance is induced simultaneously, as is proved by determining the susceptibility of each generation to the insecticides concerned in the phosphorus resistant strain RP. Graphs 1 and 2 show that building up is beginning with the F1 and that resistance is gradually increasing. This occurs with the selecting agent "Diazinon" and with lindane and D.D.T. as well. In spite of the clear multi-resistance, which is building up with "Diazinon" pressure, there is no development of resistance to the carbaminate S 17.

GRAPH 1

Development of multiresistance in the strain, selected with "Diazinon"—pressure (RP). Susceptibility of successive generations to "Diazinon" and lindane.

An experiment with allethrin on the D.D.T.-resistant strain (RD) and the lindane-resistant strain (RL) shows that these strains have also acquired a certain degree of resistance to this type of compounds (Table III). It is obvious that both the knockdown and the killing effect of allethrin is less on the resistant strains than on the normal strain.

GRAPH 2.

Development of multi-resistance in the strain, selected with "Diazinon"-pressure (RP). Susceptibility of successive generations to DDT and N, N-dimethyl-carbaminate (S 17).

DISCUSSION.

With regard to the chemical and physiological properties of the insecticides concerned in this investigation, four types may be distinguished:

(*a*) D.D.T.-type
(*b*) B.H.C.-type (toxaphene, dieldrin, aldrin, chlordane, lindane)
(*c*) Organophosphorus-type.
(*d*) Carbaminate-type.

These types are recognizable in Tables I and II as a result of the mode of reaction of the resistant strains. In this connection it is remarkable that a very high level of resistance is developed to D.D.T., if selection takes place with D.D.T. or lindane, and a much less resistance is caused by selection with the phosphorus compound or the carbaminate.

TABLE III.

Knockdown and kill of the susceptible strain (N), the RD-strain (D.D.T.-pressure) and the RL strain (lindane pressure) after continuous exposure to allethrin in petri dishes.

Strain	Conc. (mg/l)	Knockdown percentages after					Percentage kill (21 h)
		15′	30′	45′	60′	4h	
N ..	20	3	10	10	13	83	84
	60	10	30	40	37	90	90
	200	23	57	60	73	80	85
	600	43	90	97	100	97	92
RL ..	20	0	0	3	3	23	44
	60	0	0	3	7	40	60
	200	0	7	13	17	43	43
	600	7	67	73	73	73	60
RD ..	10	0	0	..	0	0	9
	100	3	13	..	17	37	39
	1000	43	100	..	100	93	83

Resistance to the phosphorus compound is in any case of a low order, whereas resistance to the carbaminate is negligible. Resistance to the B.H.C.-type of compounds is decreasing in the sequence toxaphene-dieldrin-aldrin-chlordane-lindane.

The effectiveness of the insecticides on the flies within any strain is ranged in the same order, independent of the selecting agent. Only D.D.T. forms an exception, the resistance in the strains RP and RS being low (Table II). The above mentioned sequence does not correspond at all with the activity of the compounds on normal flies. This phenomenon has been observed before by Busvine (1954).

Supposing that the insecticidal activity of the B.H.C.-type of compounds can be reduced to the activity of the pentagon structure of five chlorine atoms as toxaphore, which is found in all insecticides of this group, the differences in activity must be determined by other properties of the whole molecules. As possible factors are to be considered lipoid solvability, phase distribution, surface active properties. These physical factors are likely to be responsible for the divergent activities and the level of resistance in the resistant strains, so that a correlation between the activity on normal and resistant flies gets lost.

In any case there is no evidence for a specific resistance in the four resistant strains concerned, and therefore it does not seem likely that a specific detoxication mechanism like D.D.T.-dehydrochlorinase (Sternburg *et al.*, 1954), could be the

most important cause. For in this instance we might expect that selection pressure of one compound may induce resistance to insecticides of the same type but not to compounds of entirely different types. On the contrary we established the fact, that lindane pressure causes a higher level of resistance to D.D.T. than to lindane Selection with the carbaminate results in a strain, which is much more resistant to the other insecticides than to S 17.

Notwithstanding these facts, it is not pretended that enzymatic detoxication cannot contribute much in resistance. No doubt D.D.T.-dehydrochlorinase plays a major part in D.D.T.-resistance. The contribution of the composition of lindane to pentachlorocyclohexene (Sternburg and Kearns, 1956) and other substances (Bradbury and Standen, 1955: 1956: 1958) may be of similar interest with respect to lindane-resistance. But these specific mechanisms do not contribute to the explanation of multi-resistance. Therefore some more general, *i.e.*, unspecific mechanisms — unspecific with regard to the agent —, should be held responsible.

In spite of the established multi-resistance, the D.D.T.-resistant strain proved to be nonresistant to S 17 and the other strains develop only slight resistance to it. But as S 17 induces resistance to two types of chlorinated hydrocarbons and to a phosphorus compound, it seems likely that there is acting an unspecific mechanism. Moreover it is probable that the same or a similar unspecific mechanism is stimulated by selection with the other insecticides. Only in that case it is easily understood that the degree of resistance to the various insecticides shows each time the same sequence, independent of the type of the selecting agent.

S 17 is closely allied to the urethanes, which are known as narcotics. As these narcotics possess the property to be adsorbed to protein surfaces, it is not unlikely that S 17 has the same property. Our experiments with phenyl carbaminates on the cholinesterase activity have shown strong evidence for this hypothesis (Walop *et al.*, 1956). When roaches (*Periplaneta americana* L.) are exposed to contact with these phenylcarbaminates, their central nerve system shows a high increase in cholinesterase activity. After a longer lapse of time this activation turns into inhibition. The increase of cholinesterase activity can be understood, if we suppose that the bound cholinesterase is expelled from the protein by the carbaminate, so that *in vivo* much more cholinesterase molecules are liberated than normally is the case. In addition to the supposed adsorption to cell membranes, S 17 shows a strong penetrating power. This can be seen in the experiments, for symptoms of intoxication appear very soon after exposure of the flies to contact with S 17 in petri dishes.

It is highly improbable that the resistance induced by S 17, could be caused by stimulating decomposition mechanisms. If so, one has to suppose that the activity of a great number of enzymes had been increased at the same time. Moreover these detoxication mechanisms are in all strains not effectively acting on S 17. Much more likely seems the assumption that the permeability of the cells to the insecticides concerned is decreased. Also this mechanism is apparently little effective on S 17, because of the strong penetrating power of the phenylcarbaminates. A decrease of the cell permeability in general, however, would be a very important and effective protection against other insecticides.

The assumption that a decrease of the cell permeability for certain substances may play a part in resistant fly strains is supported in literature. Pratt and Babers (1953) stated that the exposed thoracial ganglia of resistant flies are stimulated only weakly by D.D.T.-emulsion as compared with normal flies. Wiesmann (1956) found the cholesterol content of the central nerve system and of the fly muscles of resistant flies two to three times as high as in normal flies. As cholesterol is an essential component of cell membranes, a change in the proportion might have some influence on the permeability. Indeed it appears from results with vital dyes that the permeability to these substances is decreased in the resistant flies (Wiesmann, 1956 *loc. cit.*).

It is true that there is up till now no proof for the assumption that the decrease in cell permeability is a cause of resistance. There are, however, several observations, which confirm that resistance is accompanied by a lowered permeability. Further it is clear that the central nerve system might be effectively protected against insecticides by a decrease in permeability. At last this mechanism is sufficiently unspecific to explain multi-resistance. Apart from this unspecific mechanism, specific enzymatic detoxifying mechanisms may play an important part. The latter, however, are not likely to be considered responsible for multi-resistance.

SUMMARY.

1. By selection of house-flies, *Musca domestica* L., with D.D.T., "Diazinon" and N, N-dimethyl-phenylcarbaminate (S 17), four resistant strains were obtained.

2. These four strains appeared to be resistant to all chlorinated hydrocarbon insecticides, which were used in this investigation. Resistance to "Diazinon" proved to be of a considerably lower degree; to S 17 no resistance or only a slight one (resistance factor 0·8-1·2) was developed.

3. Building up of multi-resistance during insecticide pressure appeared to be developed gradually already from the F1 generation.

4. The level of resistance to the various compounds is not correlated with the degree of toxicity to the normal strain, but shows for all strains the same sequence. So this sequence is not dependent on the selecting agent.

5. It is not likely that multi-resistance could be explained by specific detoxifying mechanisms. It is more likely that unspecific mechanisms will be the basis of multi-resistance.

6. It is supposed that great value has to be attached to a decrease of the permeability of cell membranes as unspecific cause of multi-resistance.

7. Though S 17 induces multiresistance, the mechanism of this resistance is not sufficiently effective to protect the insect against S 17, perhaps on account of the penetrating power of the latter.

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INFLUENCE OF RELATIVE HUMIDITY ON THE NORMAL TOLERANCE OF THE MOSQUITO, *CULEX FATIGANS* WIED., TO D.D.T.

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INTRODUCTION.

It is now accepted that in a strain of an insect species the stage of development, age within the stage, sex and nutrition generally influence the normal tolerance to an insecticide. Differences in such tolerance are also known to occur from strain to strain and from place to place within the same species. According to Holborn (1956) several strains of the rust-red flour beetle, *Tribolium castaneum* Herbst., bred in the laboratory, showed differences in their susceptibility to gamma-B.H.C. and pyrethrins which he thought were due to the differences in vigour. Brown (1950) pointed out that a strain of an insect, continuously bred under laboratory conditions, may show greater susceptibility than a strain of the same species bred under natural field conditions and that this may be due to the fact that in the laboratory the weaker individuals are not eliminated. Hoskin and Gordon (1956) stated that "a strain developed by breeding only from those individuals which survive exposure to such diverse stresses as extremes of temperature, lack of moisture, abnormal food, will have an altered ability to withstand many kinds of stresses including exposure to chemicals". They called this change from the normal tolerance as "vigour tolerance".

There is also increasing evidence in literature to show that normal tolerance in an insect species can be affected by the environmental conditions under which the insects are bred and tested against insecticides. While the effect of temperature on the susceptibility of different insects to insecticides has been widely investigated, *inter alia*, by Potter and Gillham (1946), Pradhan (1949*a*), Brown (1951), Vinson and Kearns (1952), Hafliger (1954), Hadaway and Barlow (1957) and Pradhan and Mundkur (1957), there is paucity of information on the influence of humidity.

David (1945) observed that humidity does not influence the susceptibility of *Aedes ægypti* L. when a solution of pyrethrins in kerosene oil is applied in the

form of a mist in a testing chamber. Laug (1946) found that the addition of moisture to a film of D.D.T. on glass increased the toxicity of the film to house-flies. According to Ramakrishnan *et al.* (1948), higher humidity increased the toxicity of D.D.T. applied on mud surfaces to several species of *Anopheles* mosquitoes in field trials. Pradhan (1949*b*) observed that high humidity, during the continuous exposure period, increased the toxicity of D.D.T. to *T. castaneum* but decreased the toxicity to the larvæ of *Plutella maculipennis*. He further observed that higher humidity, after the exposure, increased the susceptibility of *T. castaneum*. Collins and King (1953), on the other hand, reported that the level of humidity, after treatment, had no effect on the susceptibility of *T. castaneum* to D.D.T. applied on filter papers. Burnett (1956) and Bordas and Navarro (1955) have further shown in their field experiments that with Dieldrin and B.H.C. applied on mud walls, higher humidity effected higher mortality of certain species of *Anopheles*.

Barlow and Hadaway (1956) have discussed the results of Burnett (1956 *loc. cit.*) and Bordas and Navarro (1955 *loc. cit.*) and have come to the conclusion that the apparent correlation between relative humidity and toxicity of deposits on mud walls is due to the effect which humidity has on the availability of the insecticide in the deposit. Working with *A. ægypti* they have reported that changes in relative humidity do not affect the kills of the insect when the insecticides, including D.D.T., are topically applied.

The work described in the present paper was started in September, 1949, in these laboratories with the object of ascertaining the causes of differences in the susceptibility levels of the laboratory strain of *Culex fatigans* Wied. to D.D.T. when tested from time to time. The results reported in the paper* emphasize unmistakably the positive role of humidity in causing the changes in the susceptibility of the insect.

MATERIAL AND METHODS.

Susceptibility tests were carried out at fortnightly intervals over a period of eight years commencing from September, 1949. The test insects were drawn from a colony of mosquito *C. fatigans* which was raised from a single egg raft brought from the field on September 17, 1945 (Newman *et al.*, 1949). Adult female mosquitoes, 40 ± 5 hour old, fed on moist raisins, were used. Filter papers Whatman No. 1, 11 cm. diameter were impregnated with solutions of pure *pp'*-D.D.T. in liquid paraffin in varying concentrations. The insects were confined on the treated papers under glass funnels, 7·0 cm. in diameter. In the controls, insects were exposed to filter papers which had been impregnated with liquid paraffin only. In each exposure, 20 mosquitoes were used and there were two replicates for each concentration. The exposure period was one hour. After the exposure, the insects were transferred to glass bottles three-inch diameter × 5-inch. Observations on the mortality of insects were recorded after 24 hours.

The breeding of the insect and assessment of variations in the susceptibility of the insect to D.D.T., were carried out in a partially conditioned room in which variations in temperature and relative humidity, during any one day or succeeding days, were not appreciable. The variations in humidity were, however, appreciable in different months or seasons of the year. Over the period of eight years

*A short note appeared in *Nature*, **181**, p. 199 (1958).

ending September 1957, the temperature and relative humidity ranged between 80 and 92°F., and 48 and 92 per cent respectively.

Controlled experiments on the effect of humidity were also carried out. Two different levels of humidity 49±6 per cent and 88±4 per cent were adjusted in separate humidity cabinets maintained at 84±2°F. Pupæ of *C. fatigans* (from the laboratory colony), taken in glass dishes 3-inch dia. ×2-inches, containing water, were then placed in mosquito netting cages, 9×9×9 inches. These cages were then placed in the humidity cabinets. The adults on emergence were allowed to remain in the respective humidity cabinets and were fed on moist raisins. Females, 40±5 hour old, were collected and exposed on filter papers, impregnated with varying concentrations of *pp'*-D.D.T. in liquid paraffin in the respective cabinets. The method of treatment of filter papers, exposure of insects, replicates and number of insects in each replicate were as described earlier. After exposure, the insects were allowed to remain in the cabinet for another 24 hours when observations on mortality were recorded.

RESULTS.

The data obtained were subjected to probit analysis. The median lethal concentrations as well as the regression equations of log concentration-probit kill were determined. In Table I are presented the regression equations, values of LD 50 (MLD), temperature and relative humidity for the fortnightly tests carried out over a period of eight years. For convenience, the results have been graphically shown in Chart 1 where additionally data on the rainfall have been given. The results obtained at the controlled relative humidity levels have been graphically presented in Chart 2.

While in the fortnightly tests there was no mortality in controls, there was some mortality in controls in the experiments carried out at the two controlled humidity levels. Allowance has been made for this by applying the correction factor for the natural mortality (Finney, 1944).

DISCUSSION.

The results given in Table I and Chart 1 show that the values of LD 50 are higher during August to October than the values during November to February. This picture of rise and fall in the values of LD 50 has been maintained during the years and there are not wide differences in the values of LD 50 for the same period from year to year. The variations in temperature, during the course of these experiments, are not so wide as to possibly account for the variations in the values of LD 50. Variations in relative humidity, on the other hand, are very wide and appear to have a positive role. This is spectacularly brought out in Chart 1. With higher humidities, particularly during the period of August-October in any year, greater values of LD 50 have been obtained. During November-February when the relative humidity is lower, the values of LD 50 are also lower. The results shown in Chart 2 confirm the above observations. The value of LD 50 at 49±6 per cent relative humidity is 102·3 mg./sq. ft., while at 88±4 per cent relative humidity the value is 216·3 mg./sq. ft. It is clear that humidity bears an inverse relation to the mortality effected. The differences in the value of LD 50, due to relative humidity, are so wide that it appears essential not to confuse this effect with vigour tolerance.

TABLE

Normal tolerance of C. fatigans to *pp'-D.D.T. — Values*

Date of exposure		1949-1950			
		Temperature °F	Relative humidity (Percent)	Regression equation	LD. 50 in mg./sq. ft.
..	First fortnight				
September ..	Second „	85	92	Y=4·87+9·42 (×—2·15)	146·2
	First „	83	88	Y=4·89+10·76 (×—2·15)	144·5
October	Second „	82	75	Y=5·47+13·75 (×—2·12)	121·6
	First „	85	67	Y=5·07+ 7·08 (×—2·08)	117·5
November ..	Second „	84	69	Y=5·28+ 3·48 (×—2·08)	100·0
	First „	83	66	Y=5·09+ 7·01 (×—2·05)	108·4
December ..	Second „	80	68	Y=4·94+ 6·32 (×—2·06)	117·5
	First „	80	72	Y=5·12+11·27 (×—2·05)	109·1
January . ..	Second „	84	84	Y=5·17+ 6·78 (×—2·04)	103·5
	First „	82	69	Y=4·73+ 7·04 (×—2·06)	125·9
February	Second „	84	62	Y=5·36+ 9·13 (×—2·10)	115·3
	First „	84	62	Y=5·30+ 7·14 (×—2·15)	131·8
March	Second „	84	62	Y=5·08+ 6·94 (×—2·15)	139·6
	First „	84	62	Y=4·92+ 7·89 (×—2·15)	144·5
April	Second „	84	62	Y=5·12+11·55 (×—2·15)	138·0
	First „	85	66	Y=5·32+ 8·56 (×—2·14)	141·3
May	Second „	..	..	..	
	First „	..	..	..	
June	Second „	..	..	..	
	First „	..	..	..	
July	Second „	..	..	..	
	First „	86	92	Y=5·06+12·84 (×—2·15)	139·6
August	Second „	85	92	Y=5·00+11·34 (×—2·15)	139·6

—Exposure was not carried out.

I.

of LD 50 at fortnightly intervals.

1950-1951				1951-1952			
Temperature °F	Relative humidity (Percent)	Regression equation	LD. 50 in mg./sq.ft.	Temperature °F	Relative humidity (Percent)	Regression equation	LD. 50 in mg./sq. ft.
86	92	Y=5·15+11·13 (×—2·15)	136·5	86	88	Y=4 94+10·67 (×—2·16)	146·2
85	88	Y=5·06+11·45 (×—2·15)	139·6	84	80	Y=5 10+ 9·79 (×—2·15)	137·4
84	66	Y=5·15+12·84 (×—2·14)	134·9	85	81	Y=4·89+16·67 (×—2·16)	146·6
84	66	Y=5·11+10·76 (×—2·15)	137·4	82	61	Y=5·22+13·69 (×—2·14)	133·7
84	66	Y=4·56+ 7·79 (×—2·16)	160·3	83	66	Y=5·45+ 9·50 (×—2·12)	118·3
84	60	Y=5·01+13·23 (×—2·15)	141·3	..	..	..	
..	..	..			..	..	
84	62	Y=5·03+12·11 (×—2·15)	140·6	..	..	..	
80	55	Y=5·21+12·13 (×—2·15)	136·5	84	56	Y=4·79+ 6·70 (×—2·11)	138·7
80	61	Y=5·55+ 9·81 (×—2·14)	122·7	80	72	Y=5·18+ 7·60 (×—2·10)	118·9
81	61	Y=4·98+10·29 (×—2·15)	112·7	85	57	Y=5·49+ 8·48 (×—2·09)	107·2
84	66	Y=5·10+ 8·91 (×—2·15)	140·6	..	..	..	
86	63	Y=5·45+10·89 (×—2·12)	120·2	86	53	Y=5·07+ 6·88 (×—2·05)	109·1
86	47	Y=5·29+10·63 (×—2·14)	128·8	86	57	Y=5·19+ 4·28 (×—2·07)	106·9
84	62	Y=5·19+14·46 (×—2·14)	132·4	88	57	Y=5·11+10·40 (×—2·15)	138·0
83	66	Y=5·05+14·90 (×—2·19)	153·8	86	66	Y=5·02+ 6·49 (×—2·13)	133·4
85	66	Y=4·82+11·57 (×—2·16)	149·8	86	62	Y=5·13+ 9·56 (×—2·15)	137·4
87	77	Y=5·11+ 8·46 (×—2·15)	137·4	88	74	Y=5·11+ 9·12 (×—2·15)	136·5
87	76	Y=5·10+13·79 (×—2·15)	139·3	88	70	Y=5·23+ 9·60 (×—2·15)	133·7
90	81	Y=5·28+13·34 (×—2·14)	132·7	88	85	Y=5·11+11·67 (×—2·15)	140·0
88	85	Y=4·78+14·59 (×—2·16)	149·3	86	84	Y=4·86+ 8·30 (×—2·16)	133·4
90	81	Y=5·07+10·43 (×—2·15)	139·3	87	88	Y=4·89+ 9·81 (×—2·17)	152·1
87	88	Y=5·02+11·10 (×—2·15)	142·9	86	84	Y=4·92+10·90 (×—2·15)	142·9
87	88	Y=5·04+ 9·77 (×—2·15)	140·0	87	88	Y=5·10+12·23 (×—2·15)	139·0

TABLE

Date of Exposure		1952–1953			
		Temperature °F	Relative humidity (Percent)	Regression equation	L.D.50 in mg./sq. ft.
	First fortnight	87	81	Y=4·93+ 7·65 (×—2·13)	138·0
September ..	Second „	86	82	Y=5·15+ 9·90 (×—2·15)	136·8
	First „	86	76	Y=4·81+ 7·61 (×—2·13)	142·9
October	Second „	84	73	Y=5·08+10·76 (×—2·15)	138·7
	First „	..	..	..	
November ..	Second „	82	55	Y=5·15+10·37 (×—2·15)	137·4
	First „	83	59	Y=4·95+ 8·68 (×—2·11)	134·9
December ..	Second „	84	62	Y=5·06+ 6·98 (×—2·10)	123·0
	First „	82	61	Y=5·21+ 7·35 (×—2·10)	118·9
January	Second „	82	48	Y=5·28+ 7·02 (×—2·10)	114·8
	First „	84	63	Y=5·09+ 8·26 (×—2·12)	128·2
February	Second „	85	56	Y=5·15+ 9·93 (×—2·15)	136·5
	First „	85	64	Y=5·03+ 8·23 (×—2·14)	127·1
March	Second „	85	63	Y=4·92+ 7·92 (×—2·13)	145·2
	First „	81	61	Y=4·98+ 7·40 (×—2·17)	149·8
April	Second „	82	69	Y=4·96+12·08 (×—2·19)	156·7
	First „	86	70	Y=5·12+10·29 (×—2·16)	140·6
May	Second „	86	66	Y=5·13+ 6·99 (×—2·12)	126·5
	First „	87	67	Y=5·11+ 8·16 (×—2·10)	121·9
June	Second „	88	92	Y=5·04+ 6·40 (×—2·14)	131·1
	First „	87	92	Y=5·19+ 8·24 (×—2·15)	134·9
July	Second „	88	85	Y=4·79+11·55 (×—2·18)	158·1
	First „	85	84	Y=4·95+10·29 (×—2·21)	166·0
August	Second „	87	88	Y=5·21+16·26 (×—2·20)	152·8

—Exposure was not carried out.

I.—(*Contd.*).

1953-1954				1954-1955			
Temperature °F	Relative humidity (per-cent)	Regression equation	L.D. 50 in mg./sq. ft.	Temperature °F	Relative humidity (Per-cent)	Regression equation	L.D. 50 in mg./sq. ft.
87	92	Y=4·83+ 8·87 (x—2·19)	163·7	86	92	Y=4·81+ 8·74 (x—2·23)	179·5
86	85	Y=4·95+ 11·61 (x—2·19)	157·4	86	92	Y=4·87+11·49 (x—2·23)	176·2
82	84	Y=4·61+10·10 (x—2·14)	150·0	84	79	Y=4·89+ 9·28 (x—2·22)	171·8
82	76	Y=5·19+10·25 (x—2·14)	134·0	85	70	Y=5·15+ 7·87 (x—2·15)	134·0
82	75	Y=4·90+ 9·85 (x—2·16)	146·2	85	74	Y=5·23+ 8·65 (x—2·15)	133·0
83	62	Y=5·11+ 7·13 (x—2·07)	113·8	83	73	Y=5·20+ 9·37 (x—2·10)	119·7
82	65	Y=5·10+ 5·70 (x—2·08)	114·8	84	69	Y=4·91+ 8·38 (x—2·11)	132·4
84	73	Y=4·90+ 9·14 (x—2·09)	126·2	82	66	Y=4·96+ 5·91 (x—2·08)	121·9
81	76	Y=5·21+ 7·71 (x—2·04)	102·3	78	71	Y=5·06+11·25 (x—2·15)	139·6
81	76	Y=5·08+ 8·61 (x—2·07)	114·8	82	75	Y=5·05+ 9·18 (x—2·13)	133·7
83	76	Y=4·95+ 5·93 (x—2·08)	123·0	81	73	Y=5·08+ 4·76 (x—2·07)	113·2
81	76	Y=5·07+ 5·64 (x—2·13)	129·4	85	66	Y=5·07+ 6·64 (x—2·15)	136·8
84	66	Y=5·06+ 7·04 (x—2·10)	124·2	82	75	Y=5·07+ 7·27 (x—2·13)	130·6
85	74	Y=4·98+ 9·95 (x—2·11)	130·3	84	76	Y=4·97+10·28 (x—2·15)	141·9
83	76	Y=4·95+10·87 (x—2·15)	143·9	82	73	Y=4·91+ 8·52 (x—2·13)	138·7
85	77	Y=4·89+ 7·29 (x—2·13)	141·3	82	73	Y=5·02+ 8·80 (x—2·15)	139·6
85	77	Y=4·87+ 7·63 (x—2·17)	156·0	85	80	Y=4·94+ 6·21 (x—2·15)	145·2
86	84	Y=5·19+12·28 (x—2·19)	148·6	86	77	Y=5·08+ 6·79 (x—2·19)	150·7
89	85	Y=5·04+10·50 (x—2·15)	139·6	89	85	Y=5·12+10·87 (x—2·15)	137·4
87	88	Y=5·26+11·83 (x—2·14)	131·5	88	92	Y=4·90+6·61 (x—2·24)	180·3
87	88	Y=5·18+ 8·77 (x—2·15)	133·0	86	84	Y=5·01+ 8·67 (x—2·15)	141·3
87	88	Y=4·75+10·17 (x—2·16)	152·8	86	84	Y=5·01+ 9·68 (x—2·17)	148·6
86	84	Y=4·92+10·46 (x—2·16)	145·9	85	92	Y=4·99+10·56 (x—2·21)	162·9
86	88	Y=4·91+9·51 (x—2·19)	158·5	88	86	*	

* The highest concentration of *pp'*-DDT which could be prepared in liquid paraffin, did not produce high mortality of insects, to enable the regression line being drawn.

TABLE

Date of exposure		1955–1956			
		Temperature °F	Relative humidity (Percent)	Regression equation	L.D 50 in mg/sq. ft.
September ..	First fortnight	86	92	Y=4·93+ 9·67 (x—2·26)	182·8
	Second „	85	92	Y=5·20+10·27 (x—2·34)	204·2
October . ..	First „	84	92	Y=4·98+ 6·05 (x—2·26)	181·1
	Second „	83	76	Y=5·24+ 8·15 (x—2·27)	171·8
November ..	First „	83	76	Y=5·18+11·13 (x—2·15)	134·9
	Second „	83	76	Y=4·98+ 8·65 (x—2·15)	142·9
December ..	First „	83	76	Y=5·05+ 7·40 (x—2·18)	147·9
	Second „	82	76	Y=5·08+ 6·41 (x—2·10)	121·6
January	First „	82	76	Y=5·07+ 7·17 (x—2·15)	138·0
	Second „	83	76	Y=5·24+11·84 (x—2·08)	113·8
February	First „	82	69	Y=5·06+ 5·64 (x—2·07)	115·3
	Second „	85	63	Y=5·08+ 6·34 (x—2·10)	123·9
March	First „	84	69	Y=5·08+ 4·99 (x—2·14)	134·3
	Second „	85	70	Y=4·90+ 5·67 (x—2·11)	133·4
April	First „	87	74	Y=5·06+ 4·78 (x—2·10)	121·6
	Second „	82	76	Y=5·05+ 5·56 (x—2·10)	122·7
May	First „	90	65	Y=5·25+ 8·08 (x—2·10)	117·2
	Second „	90	71	Y=5·16+ 4·56 (x—2·05)	102·6
June	First „	89	78	Y=4·93+ 3·80 (x—2·15)	146·2
	Second „	90	85	Y=5·30+ 6·82 (x—2·15)	126·2
July	First „	87	92	Y=5·08+ 7·00 (x—2·17)	149·5
	Second „	87	92	Y=4·78+ 6·21 (x—2·24)	188·8
August	First „	88	90	Y=5·24+ 6·83 (x—2·27)	171·0
	Second „	85	92	Y=4·99+ 8·11 (x—2·25)	179·6

—Exposure was not carried out.

1.—(*Concld.*).

1956–1957				1957–1958			
Temperature °F	Relative humidity (Percent)	Regression equation	LD. 50 in mg./sq. ft.	Temperature °F	Relative humidity (Percent)	Regression equation	LD.50 in mg./sq. ft.
87	92	Y=4·78+ 7·89 (x—2·22)	176·6	84	84	Y=5·02+ 6·88 (x—2·27)	186·6
86	88	Y=4·94+10·10 (x—2·21)	165·2	84	76	Y=5·00+ 7·25 (x—2·30)	198·3
84	92	Y=4·78+ 7·43 (x—2·22)	177·1				
84	84	Y=5·06+ 9·05 (x—2·23)	167·0				
84	69	Y=5·11+ 7·85 (x—2·11)	124·3				
84	69	Y=5·29+ 6·87 (x—2·15)	127·1				
84	73	Y=5·09+ 4·95 (x—2·07)	113·6				
84	69	Y=4·93+ 6·75 (x—2·13)	138·4				
82	65	Y=5·12+ 5·41 (x—2·02)	99·1				
81	69	Y=5·21+ 6·96 (x—2·10)	117·1				
84	66	Y=5·25+ 7·64 (x—2·10)	116·4				
81	61	Y=5·24+ 5·02 (x—2·04)	99·2				
84	69	Y=5·24+ 5·32 (x—2·08)	107·5				
85	57	Y=5·28+ 8·08 (x—2·10)	115·1				
82	64	Y=5·12+ 4·14 (x—2·05)	104·8				
..	..	..					
86	63	Y=5·26+ 7·03 (x—2·08)	109·7				
88	64	Y=5·27+ 7·06 (x—2·08)	109·7				
88	72	Y=5·20+12·71 (x—2·17)	141·9				*
90	71	Y=5·14+ 7·93 (x—2·15)	135·5				
90	85	Y=5·19+ 4·82 (x—2·05)	101·6				
84	92	*					
88	90	Y=5·28+ 8·04 (x—2·29)	181·1				
88	85	*					

*The highest concentration of *pp'*-DDT which could be prepared in liquid paraffin, did not produce high mortality in insects to enable the regression line being drawn.

CHART 1.

Laboratory variations in mortality of the mosquito, Culex fatigans *Wied. exposed to DDT at fortnightly intervals.*
A : LD_{50} mg./sq. ft., B : Temperature °F, C : Relative Humidity, D : Rainfall (Denotes traces).

CHART 2.

LOG (CONCENTRATION IN MG./SQ. FT.)

Contact toxicity of pp'-DDT in liquid paraffin to Culex fatigans *Wied.*

(A) At 29±1°C and 49±6% R.H., (B) At 29±1°C and 88±4% R.H.)

The results discussed above are at variance with those obtained by Burnett (1956 *loc. cit.*) and Bordas and Navarro (1955 *loc. cit.*). The difference may be due to the influence of the relative humidity on the availability of the insecticide on the surface of the substrate or on the physiological activity of the insects. It appears that, even as pointed out by Barlow and Hadaway (1956 *loc. cit.*), the results of the above authors have been more influenced by the effect of the humidity on the availability of the insecticide on a porous surface such as mud. In the present experiments, where the substrate is filter paper and the solvent for the insecticide is 'non-volatile' liquid paraffin, it is reasonable to expect that the relative humidity has not influenced the availability of the insecticide on the surface but only the physiological activity of the insect. Barlow and Hadaway (1956 *loc. cit.*) have, however, reported that when D.D.T., γ-B.H.C., dieldrin or diazinon are *topically* applied on *A. ægypti* the toxicity is not influenced by relative humidity. Further work is called for to explain whether these differences, in results, are due to the variations in insect species (Pradhan, 1949*b*) or method of application or the nature of substrate.

SUMMARY.

The normal tolerance of an insect to an insecticide as distinct from vigour tolerance, and the factors reported in literature as governing it, have been described. The variations in the normal tolerance of laboratory-bred *C. fatigans*

exposed to D.D.T. at fortnightly intervals, over a period of eight years, have been described and it is shown that humidity plays an important role in influencing the susceptibility of the insect. Higher humidity increases the normal tolerance in insects. Confirmatory evidence on influence of humidity on the tolerance of the insect has been provided. The need for keeping the effects, due to relative humidity and vigour tolerance, separate is emphasized.

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SUSCEPTIBILITY AND SEASONAL TOLERANCE OF LOCAL ADULT ANOPHELES TO LONG-TIME USE OF D.D.T. AND B.H.C.*

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THE occurrence of resistance in the *M. domestica* species (1946, Sweden), as reported after a year's use of hydrochlorinated insecticides, is nowadays as well known as the appearance of the same phenomenon, a few years later, in the *Anopheles sacharovi* species (1951, Greece). The progressive increase of the number of species, which had acquired resistance, was followed by an increase in the number of insecticide resisting malaria vectors. The appearance of resistance in the Anopheles did not seem to be a surprising fact for the above mentioned insects are precisely the ones against which the insecticides have been used on a large scale for many years.

The information concerning this problem, as received and disseminated by the World Health Organization, was serious warning especially for the States which have used chlorinated hydrocarbon insecticides for a great number of years. At the same time, they have made attempts to perform such researches, the results of which could be tested by standard test methods before making a public statement concerning the existence of resistance. The elaboration of a precise definition has contributed a great deal to a perfect understanding of the phenomenon as well as to its delimitation.

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* The data included in this note were presented at the Third Regional Malaria Conference for South-East European countries in Bucarest (June 23-30, 1958).

Research work in the Danube Plain, the Dobrudja and Transylvania was carried out by M. Duport, M. Sandulescu and collaborators (A. Cristescu, I. Combiescu, I. Sandescu, A. Atanasiu, St. Marotinescu) while in Moldavia and the Danube Delta by Prof. E. Ungureanu and collaborators (A. Teodorescu, V. Crismaru, C. Teodorescu).

The following six Anopheles species: *A. gambiæ* (French West Africa, Liberia, Nigeria), *A. quadrimaculatus* (The United States, Mississippi), *A. sacharovi* (Greece), *A. stephensi* (Iran, India, Iraq, Saudi Arabia), *A. subpictus* (Indonesia), *A. sundaicus* (Burma, Indonesia), constitute instances of confirmed physiological resistance in some areas of the above mentioned countries.

The progressive extension of the phenomenon considered from a geographic viewpoint, as well as its extension to an important number of different varieties of insects, and the use of residual insecticides for many years in the countries where this method is still necessary, concerning either the antimalaria control measures or the eradication programmes of malaria, require a severe carrying out of the control of the sensibility of local vectors as well as thorough researches in the resistance problem. The technical conference on insect-resistance to consider an international collaboration programme of research (July 25-31, 1957) acknowledging " the nature, amplitude and urgency of the resistant-insects problem ", outlined the main features of the problem and indicated the main lines of research to be carried out in order to satisfy the present necessities.

MALARIA CONTROL METHOD IN R.P.R. ROUMANIA, 1947-1958.

Among the malaria-countries which presented up to ten years ago mezo and holoendemic areas, Roumania is to be found too. After the epidemic wave from 1945-47, the constantly applied malaria control measures for the elimination of parasites, have been mainly imagocidal, combined with radical case treatment--systematical schizonto-gametocide treatment of cases, suppressive treatment and chemoprophylaxis.

A period of experimental laboratory and field trials in the demonstration station of TIC..., from 1947 to 1948, has been followed since 1949 by the use of residual insecticides on a large scale.

The considerable fall in the incidence of the malaria disease all over the country — from 338,198 cases in 1948 to 903 in 1954—decided the Ministry of Health to go over directly, in 1955, to the " attack phase " of the eradication programme. The same serious attention was paid to the problem of susceptibility to insecticides of the vectors.

ANOPHELES VECTORS.

Favourable conditions for the development of Anopheles are to be found over a surface of approximately 30 per cent of the entire territory of Roumania, with the following distribution of species: *A.m. typicus* characteristic on the Moldavian plateau and the hilly areas of the country, does not transmit the disease in the climatic conditions of areas above 300-350 m. *A.m. messeae* is prevalent in the areas liable to flooding near the Danube and other big rivers, as well as in the rice-fields; it accounts for having maintained the disease and its rather serious character, in these above mentioned areas. *A. labranchiæ atroparvus* is to be found under larval stage in the breeding places of stagnant brackish waters both in the coastal areas of the Black Sea and in the interior areas. *A. sacharovi*, a highly anthropophilic species in Roumania, is to be found on a limited narrow belt of territory of about 10 to 15 km., which begins at the southern frontier of Dobrudja

and continues along the seashore and the coast of the Razelm Lake up to the Delta of the Danube.

The Roumanian Anopheles vectors are all hibernants with the exception of *A. labranchiæ atroparvus* which is a half-hibernant insect. Annually, during the active period, from spring till autumn, they give rise to a succession of five to seven generations.

The period of transmission lasts in different areas four to five months, depending on climatic conditions.

The characteristics of intra and peridomesticity of the Anopheles vectors required in the first stage of systematic malaria control 1949-50, the use on a large scale of the integral insecticide method with residual insecticides combined with radical case treatment.

IMAGICIDE METHOD.

The anti-imaginal insecticides used were D.D.T. solution or emulsion (approximately at D.D.T. 2 g./m²) and B.H.C. solution or emulsion (approximately at 0·25 - 0·37 g. isomer gamma/m²). There were used, B.H.C. crystals at 0,0025 - 0,0030 g./m² isomer gamma and 0,005 - 0,010 g./m² isomer gamma for the anti-larval fight. Delarvisations within limited areas were carried out in the building yards of Giurgiu (1952-53) and Cara-Su Valley (1954-55).

In our country, the date of the beginning of the anti-imaginal operations, depending on phenological data, is between April 20 and May 5, that is to say, ten or fifteen days before the appearance of the first generation of adult Anopheles.

The integral spraying includes the inside spraying of the dwellings and stables, the ceilings, the walls from the ceiling downwards to a line ranging 1 m. above the floor, along the corner line of the walls, entirely, as well as the ouside spraying of the windows and doors, of their surrounding surfaces and the house eaves too.

In reference to the delarvisation, the operations were repeated in accordance with the entomological indications: before the appearance of the IVth advanced stage larvæ and of the pupæ, both of them less susceptible to insecticides.

The anti-imaginal sprayed surfaces grew up between 1949 and 1951, from 57,790,615 m² to 81,626,275 m².

In 1953, the sprayings in a certain number of annually integrally treated localities, where morbidity due to malaria had reached a very low level, were interrupted.

The anopheline protection of these areas is carried out by the spraying of the foci and by barrier sprayings. Yet, there are some areas where the integral anti-imaginal method has been used for eight to ten years.

EVALUATION OF ANTI-IMAGINAL MEASURES.

In order to assess the effectiveness of measures, there have been employed: the measurement of anopheline density, biological test (wall-cage) and epidemiological test. The duration of residual toxicity under the local conditions of our

country are: in average, six months for D.D.T. at 2 g./m² and 2 1/2 to 3 months for B.H.C. at 0·25 - 0·30 g. isomer gamma/m².

From 1956, the Busvine-Nash test, standard method used by all countries investigating susceptibility and resistance of Anopheles to insecticides within the World Health Organization co-ordinated research programme, has been added to the annually carried out controls.

There have been performed microtests too, by applying microdoses of insecticide on the thorax of the insects.

The anopheline density is ascertained, in general, by the entomologists of the Malaria Stations by estimating the number of Anopheles in 10 - 12 dwellings situated at various distances from the breeding-places. The density in the studied localities is recorded before and after the spraying.

In certain localities the seasonal evolution is studied by means of a parallel, periodical recording of the anophelism in the localities (*a*) under continuous treatment, (*b*) those not subject to treatment, and (*c*) those, where measures had been interrupted for the last two to three years.

The control in the sprayed localities carried out in 1956, has generally shown a good efficacy of the insecticides.

The following data were recorded in the periodically controlled localities:

On the coast at Ag. and near the lake Razelm and Ens., insecticide-sprayed localities for a number of seven and five years, and unsprayed for the last three years (1955-1957), the appearance of the first generation and the succession of the following ones led to an increase of the anophelism.

Its highest level was recorded in July in average 106 Anopheles per dwelling at Ag. and 229 at Ens. (1957). In two other localities situated in the neighbourhood of the above mentioned ones, respectively Ef. and Zeb., treated with insecticides for nine and ten running years, the recorded anophelism was 0 from May to September with the exception of the locality Zeb., where, during August and September there were recorded 0·5 to 1·1 Anopheles per sprayed dwelling.

THE BUSVINE-NASH METHOD.

From 1956, the standard Busvine-Nash method has been added to the tests and investigations carried out.

In 1956, researches were carried out on a number of 22,569 Anopheles in all (including controls) captured in 36 different localities situated in the Danube plain, Dobrudja, Moldavia and Transylvania. The result (*World Health Organization*—Mal. 190/August 8, 1957) noted the absence of any acquired resistance of Anopheles to the insecticides used. Seasonal variations in susceptibility and an increase in tolerance of insecticides during the prehibernation period were noted. In 1957, a number of 40,738 Anopheles in all (including controls), captured in 24 localities situated within the same areas, were examined.

The reduced number of tested Anopheles, caught in the treated localities were captured, almost exclusively, in dwellings not subject to treatment.

During 1957, the field tests were carried out—with a few exceptions—on engorged or empty female Anopheles captured in dwellings or stables. They

were exposed to the insecticide in test-tubes which were also covered with a disc of filter paper, impregnated with the insecticide (a cork stopper was not used). The Anopheles thus treated, as well as the controls were kept under observation for 24 hours on the spot (in the stables), in cylindrical cages of perforated paper with a cellophane base (designed by E. Ungureanu). Mortality rates and knock-down rates of the Anopheles were calculated. After applying the Abbott formula, the CL_{50} (Swaroop, 1956) was calculated graphically. The majority of tests were carried out on Anopheles captured in localities where spraying had been interrupted for several years. A limited number of Anopheles from localities that had been subject to uninterrupted treatment, were captured in new dwellings or in dwellings which had not been treated.

The results of the tests carried out in 1957 confirm those for 1956 and provide some additional data on the variation in Anopheline susceptibility under certain conditions. The field tests carried out in 1957 — D.D.T., gamma-B.H.C. and dieldrin — show much the same mortality and CL_{50} in the case of Anopheles captured in neighbouring localities — that is to say:

(*a*) those under continuous treatment for a number of years;

(*b*) those localities where measures had been interrupted in the last few years;

(*c*) those not subject to treatment.

Thus, for D.D.T. in the case of Anopheles from Sar. and Pr. in the Danube plain (*A. labranchiæ atroparvus:* 69·5 - 75·9 per cent) in July and August, the results were: CL_{50} 1·8 per cent and 1·9 per cent at Sar. which had been treated for nine years, and 1·3 per cent and 1·9 per cent at Pr., treated for four years (1949-52).

For gamma-B.H.C. the CL_{50} was irregular in the case of Anopheles captured at Mur. and Car. (*A.m. messeæ* 100 per cent); this does not make it possible to confirm that variation in susceptibility was caused by the use of insecticides; for example, at Mur....., which was treated for 11 years, the CL_{50} varied in July and at the beginning and end of September as follows: 0·021, 0·02 and 0·06 per cent, while at Car..... (treated for five years, 1950-54): 0·13, 0·033 and 0·050 per cent.

The very low density at the height of the season in the treated localities and the absence of malaria cases confirm the results obtained by the Busvine-Nash method at Sar....., Ef...., Mur.... and Ciul....

In 1956, both in Iran (Zulueta *et al.*, 1957) and in Roumania*, increased anopheline tolerance of insecticides was noted in the *maculipennis* group at the beginning of the hibernation period. The same observation was made in 1957 for D.D.T., B.H.C. and dieldrin. Anopheles in the Danube plain (Cr.L....., Pr.....), showed a greater susceptibility — a CL_{50} to D.D.T. of 1·3 per cent to 1·9 per cent was noted in July and August; in October the CL_{50} was 3·2 per cent at Cr.-L..... In Northern Moldavia, where *A. maculipennis typicus* is the most widespread species, and at Pod.....-Iasi, susceptibility increased in June and July — CL_{50} of 1·8 per cent and 1·9 per cent reaching 3·4 per cent in August.

On the coast at Ag..... and Ens..... the period of maximum susceptibility to D.D.T. for Anopheles was August and September — CL_{50} of 1·2 to 1·6 per cent.,

* Unpublished working document World Health Organization/Mal./190 (1957).

and in October 3 to 3·6 per cent. Anopheles at Vi..... re-acted differently to D.D.T.: CL_{50} of 1·2 to 1·5 per cent from July to October. There are no more breeding-places in this locality which is from 3 - 4 kilometers from Ens..... where the adult anopheline fauna is similar — *A. sacharovi* being prevalent.

A. sacharovi seems moreover to be the most susceptible to a series of factors: injury in handling, temperature, insecticides etc. The following example is illustrative: on the coast at Ag..... in 1955 it was noted, on examination of the eggs

TABLE I.

Seasonal variation of anopheline susceptibility to D.D.T., gamma-B.H.C. and dieldrin (Busvine and Nash method).

Locality	Ag.				Pol.			
Species	*A. labr. atroparvus* 68·7 per cent *A. sacharovi* 27·1 „ „ *A. m. typicus* 1·6 „ „ *A. m. messeae* 1·9 „ „				*A. m. typicus* 96·9 per cent *A. labr. atroparvus* 3·0 „ „			
Date of test	August 31, 1957		October 17, 1957		July 9-11, 1957		September 25-27, 1957	
Temperatures during exposure	24°C.		16-17°C.		27°C.		24°C.	
Temperature during the following 24 hours: minimum and maximum	17-25°C.		13-16·5°C.		23-27°C.		21-24°C.	
Anopheles tested	Number tested	Uncorrected mortality (percent)	Number tested	Uncorrected mortality (percent)	Number tested	Uncorrected mortality (percent)	Number tested	Uncorrected mortality (percent)
D.D.T. 0·5 per cent ..	60	11·6	20	0	..	..	..	..
D.D.T. 1·0 per cent ..	60	16·6	20	0	144	19·4	..	..
D.D.T. 2·0 per cent ..	60	63·3	20	10·0	146	60·9	102	24·5
D.D.T. 4·0 per cent ..	60	100	21	57·1	..	..	100	69·9
CL_{50}		1·2		3·5		1·9		3·2
gamma-B.H.C. 0·01 per cent	50	18·0	27	0	..	..	..	..
gamma-B.H.C. 0·02 per cent	50	70·0	27	0	145	44·8	65	6·1
gamma-B.H.C. 0·04 per cent	50	96·0	27	0	147	90·4	105	13·3
gamma-B.H.C. 0·08 per cent	50	100	22	4·5	..	..	..	..
CL_{50}		0·018		> 0·08		0·021		> 0·08
Dieldrin 0·2 per cent ..	..	..	..	..	144	70·1	60	1·6
Dieldrin 0·4 per cent ..	..	..	..	..	142	100	51	9·8
CL_{50}						0·18		> 0·8
Controls	120	1·6	20	0	84	7·1	..	..

TABLE II.

Variations in susceptibility as related to temperature during exposure to insecticide (Busvine and Nash method).

Locality	Date of trials	Temperature: During exposure	Temperature: During following 24 hours	Number of Anopheles tested: Concentration of D.D.T. in Risella oil (per cent) 0·5	1	2	4	Controls	Uncorrected mortality, percentage: Concentration of D.D.T. in Risella oil (per cent) 0·5	1	2	4	Controls	CL_{50} (percent)
Saf.*	August 14, 1957	29 – 35·5 C.	23 – 31·5 C.	114	114	100	123	113	14·0	28·9	59·0	93·8	8·8	1·7
Saf.†	,,	26 – 30°C.	23 – 31·5°C.	116	114	110	108	101	12·0	21·9	44·6	81·5	6·9	2·3
Cr.-L.*	August 9, 1957	24 – 31°C.	23·5 – 29°C.	111	120	100	100	108	9·9	34·2	73·0	100	5·5	1·2
Cr.-L.†	,,	24 – 25°C.	23·5 – 29°C.	111	100	100	103	107	9·5	18·0	48·0	92·2	4·7	1·8

* Out of doors. † In stables.

from dissected dead and moribund Anopheles (M. Sandulesco and St. Marotinesco, 1957) that *A. sacharovi* constituted 79·5 per cent of the anopheline population, whereas it was only 33 per cent when eggs from normal batches were examined.

Tolerance of gamma-B.H.C. and dieldrin increased during the pre-hibernation period (Table I); Ag..... CL_{50} for gamma-B.H.C. from 0·018 per cent (August) > 0·08 per cent (October 17); at Pod....-Iasi, from 0·021 per cent (July 9-11) > 0·08 per cent (September 25-27); CL_{50} for dieldrin varied between 0·018 and 0·36 per cent (July-September) and at the end of September became > 0·8 per cent.

Experiments carried out at Saf..... and Cr.-L..... showed that the temperature during the time the Anopheles were exposed to insecticides, had a certain effect on their susceptibility; in the hot month of August the groups of Anopheles subjected to testing in stables and out-of-doors where the temperature is different, showed varying mortality rates and CL_{50} indicating higher susceptibility at higher temperatures (Table II).

MICROTEST METHOD.

The results of microtests on 7774 Anopheles, captured in the field, applying minute doses on the thorax, also show persistence of anopheline susceptibility to insecticides in localities that have been treated over a great many years, as well as an increase in tolerance during the pre-hibernation period.

SUSCEPTIBILITY OF LABORATORY BRED ANOPHELES.

The Busvine-Nash method has been used to test 3784 Anopheles (including controls) and the microtest for 704 Anopheles. The species were *A. maculipennis* (first generation) and *A. labranchiæ atroparvus* in successive generations.

Although certain features in laboratory Anopheles — the higher susceptibility of males than of females — as compared with those captured in the field, have been brought out, the experiments do not give grounds for drawing any definite conclusions. The groups tested were not large and the conditions under which they were bred (Bucarest) were not constant; the variable temperature and humidity in the Bucarest breeding rooms may have influenced the development of successive generations and may explain certain of the variations in the CL_{50} for D.D.T.

CONCLUSIONS.

In Roumania, from 1948-49 there have been used in the malaria control programme besides the radical treatment of the cases, residual insecticides against the malaria vectors—*A.m. typicus*, *A.m. messeæ*, *A. labr. atroparvus* and *A. sacharovi*. Since then, progressive large-scale use of D.D.T. and B.H.C. insecticides is being made particularly for anti-imaginal measures, the use of which has been continued in certain areas even during the "eradication programme begun in 1955". Evaluation of the method used is made by means of biological test using a mural cage measurement of anopheline density, epidemiological test, standard Busvine-Nash method (1956) and microtests.

Results of observations of tests carried out (1957) on Anopheles captured in 24 localities in the Danube plain, Northern Moldavia, on the coast and in Transylvania, brought out the following facts:

(*a*) Vectors in localities that have been treated for 8 to 11 years, have acquired no physiological resistance to insecticides; this observation is confirmed by the Busvine-Nash test, by marked reduction in anophelism after the localities have been treated and by the absence of malaria cases.

(*b*) Variations in anopheline susceptibility in treated and non-treated localities showed the existence of other determining factors: sex, age, seasonal physiological changes, variable temperature at the time the Anopheles were exposed to the insecticide (Busvine-Nash method).

(*c*) An increased tolerance of insecticides, during pre-hibernation, occurs in *A. maculipennis* (*typicus and messeæ*), *A. labranchiæ atroparvus* and *A. sacharovi* areas.

This increase was evident in the pre-hibernation period when tolerance was doubled in the case of D.D.T. and much higher for gamma-B.H.C. and Dieldrin.

The persistence of anopheline susceptibility to residual insecticides is such that anti-imaginal measures can continue to be applied in the Roumanian eradication programme.

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RESISTANCE TO INSECTICIDES IS A DANGER IN THE STRUGGLE AGAINST DISEASES TRANSMITTED BY INSECTS, IN VIEW OF THE RAPIDITY OF ITS ACQUISITION AND ITS IMPREDICTIBILITY.*

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[September 10, 1958.]

THE facts of resistance to insecticides are published every day in large numbers. Yet, despite the information given in 1954 by the Experts of the World Health Organization, one had to wait till 1956, that is, two years still, to read in the Chronicle of the World Health Organization, this sentence: "The salutary sentiment that the era of the efficacy of insecticides in the struggle against the vectors is coming to a close, forces its way through in the spheres of Public Health".

Why this long searching of conscience in the spheres of Public Health when the facts are so evident?

The fact is that not much time has elapsed since this resistance to insecticides has been known to be massive. It was discovered in Anopheles as late as 1952, and in Stegomyia still later in 1954; the only exception being *Musca domestica* where it was known since 1947. Thus the knowledge of the resistance to insecticides in the transmitters of malaria and yellow fever, is relatively recent.

This resistance, although it develops itself, has, all the same, fortunately remained in small portions, so much so that it does not prohibit to this day, the imagocide struggle in most of the regions. Besides, this resistance appears,

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according to the experiences chiefly in laboratory, to be essentially variable: sometimes pre-existing to the contact with the insecticide, sometimes most often undoubtedly, acquired. It seems worthwhile that the selection plays its role and that a genetical element is definitely at its basis.

It must be said here that this resistance recognized by the entomologists, defeated the purpose of the plans which had been established on the sole action of residual insecticides. Moreover large economic interests being in play, and at the same time a certain dogma of infallibility, it is not then surprising that vested interests and self respect, active jointly, induced many Public Health authorities not to admit, *a priori*, graciously and quickly, the new facts of resistance which were likely to make undurable the results which they had promised to their governments.

This notion, to which is added that of the exophilic (*see* particularly the authors' works), known since long, but recently in lime light, has a great importance for the future of the direction to be given to the struggle against the big tropical endemics transmitted by insects.

That is why it does not appear useless to us to insist once more on the creation of resistance or its "revelation" if we admit the existence of pre-existing genesis bestowed with this character.

As a matter of fact, in practice, we must prove that it is, almost certainly, the bad application of the residual insecticides, or at the very least their untimely and uncontrolled use, which is the basis of the creation of resistant races, involving one or the other insect.

Certainly, certain strains have always a degree of identical resistance (in the same conditions of course), but they are rare; more frequently, however, they are endowed with plasticity of behaviour.

It is thus that in our laboratory, with R. Nicoli, J. Aldighieri and R. Aldighieri, we have discovered, during investigations for the World Health Organization, the existence of one and only strain of *Pediculus humanus* resistant to D.D.T. in the South-East of France in 1954, and 18 appearing still surviving after 24 hours out of 95 strains tested; then in 1958, we again found eight strains out of ten surviving after 24 hours. Therefore it seems that a rapid resistance to D.D.T. has developed in the lice of this region, undoubtedly under the influence of ill-controlled, intermittent and partial use of more or less fresh powder with residual insecticides.

But these are especially the results in laboratory with different strains of *Aedes aegypti*, which have enabled us, like many other authors, to discover the plasticity of behaviour of these insects. These, it must be noted, were formerly reputed to be uniformly ultra-susceptible to D.D.T., which otherwise remains true in the majority of cases.

First of all there is an important point to note; it is that the resistance of larvæ in the fourth-stage, is, in our opinion, comparable to that of the adults, even if it is less in absolute value. It is, however, rather difficult to compare the two resistances, because technically it is much more easy to know the nature of the exact contact of adults than the larvæ; however, we think, all things being equal in other respects,

then the study of resistance of larvæ in the fourth-stage or of adults is as good in one case as in the other for measuring the importance.

If, as J. R. Busvine and W. Z. Coker have remarked, the preservation of resistant strains of *Aedes aegypti* without contact of insecticide, does not bring about any important change in the resistance, even after a number of months (indicating in this way the existance of a well-defined threshold of resistance), it has not appeared to us to be the same if the breedings are made in increased contact with the insecticide.

It is thus that a strain from HAITI has acquired, for its larvæ in the fourth-stage, in four generations a very rapidly increasing resistance, mortality descending from 95·7 per cent to 30 per cent at 26 - 29 °C.

This resistance of larvæ is transmitted to a certain number of adults, which by selection can create resistant strains. The quantity of antilarvian product does not appear to play any appreciable role, because in our experiences the doses of D.D.T. put into contact with the larvæ have been very much varied, yet we have not observed a different tendency to resistance in the surviving Stegomyia, whatever the dose. It is true that a massive action, by killing a maximum number of larvae, avoids the hatching of a great number of resistant adults, but it does not play an essential role in the development of resistance, considering the irrregularity of distribution of the artilarval D.D.T. in the practice of vector eradication.

This acquisition of resistance is very variable according to the strains and if Shidravi has been able to observe a strain which having been put for 15 generations in contact with the insecticide, had no resistance, and we ourselves have made similar discoveries, it is however not less true that one of our strains has found its resistance increased in an extremely rapid manner, after its first exposition of larvæ to the insecticide. This brings us to conclude that *the acquisition of resistance is not easily predictible, a priori, and increases very differently according to the strains. However, those which have a resistance, even very feeble in the beginning, appear pre-disposed to a rapid increase of this character.* This, in our opinion, renders necessary the experimental study of a number as large as possible of strains, before and during the vector eradication with residual insecticides, so that the results so obtained could be used effectively in the fight against the insects.

CONCLUSION.

Four strains of *Aedes aegypti*, Bobo-Dioulasso, Trinidad, Haiti and Tahiti, have shown, in laboratory, a very variable susceptibility to insecticides: 2 susceptible, 2 resistant.

In regard to the strains which are most (but not totally) resistant, it appears to us necessary, for determining a lethal state, to put the adult insect in contact with a pellicle (film) of D.D.T., as recent as possible. This is the reason perhaps why multiple pulverisations are needed in the genes of resistant strains while they are not needed in genes where they are susceptible.

Taking into consideration the creation, sometimes rapid, of an important resistance in strains having already a tendency to resistance through the

intermediary of larvæ, it is better, as far as possible, to avoid antilarvian struggle with residual insecticides in the zones where these strains exist; the existence of strains which do not seem to acquire resistance does not permit, except under prolonged study in the laboratory, to modify this imperative.

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A COMMON INFORMATION SOURCE FOR ALL RESISTANCE PROBLEMS IN BIOLOGY IS URGENTLY NEEDED IN VIEW OF THE PAUCITY OF EFFECTIVE COUNTERMEASURES TO INSECTICIDE-RESISTANCE !

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[September 15, 1958.]

INSECTICIDE-RESISTANCE in insects of medical importance towards the modern residual contact insecticides, described first by Speich and Wiesmann in *Musca domestica* in Sweden, and by Mosna (*Culex molestus*) and Saccà (*Musca domestica*) in Italy (Wiesmann, 1947; Mosna, 1947; Saccà, 1947), has become a source of major worry in recent years. The magnitude of the problem may be appreciated from Brown's (1958) exhaustive monograph on the subject. It is, therefore, that a few aspects of some of the attempted countermeasures against resistance, on the research of which the author was actively engaged, will be dealt with here. The lines of research analyzed in this paper still imply the use of *tarsal contact agents.*

1. D.D.T.-SYNERGISTS.

The author's colleagues (Davidovici, Levinson and Reuter, 1950) and former team (Ascher, Reuter and Levinson, 1951) have been among the first to come forth with potent D.D.T.-synergists (lanolin, benzyl benzoate) and have also shown in their 1951 paper that a (synergized) reconstituted D.D.T., consisting of five per cent or ten per cent *p-p'* D.D.T. and 90 to 95 per cent of a liquid mixture of D.D.T. by-products (*o-p'* D.D.T., *m-p'* D.D.T., etc.), acts much better and has a much longer residual effect than *p-p'* D.D.T. on both normal and (as we know today, moderately) D.D.T.-resistant housefly strains*. Many of these observations (see also Ascher and Reuter, 1953) have to find their explanation in physical synergism (Sumerford, 1954). It became evident, however, already in 1952, from the careful work of March, Metcalf and Lewallen (1952) that D.D.T.-synergist combinations are not immune to resistance and do not constitute, therefore, an answer to D.D.T.-resistance. Moreover, when the author re-tested in 1955 the "reconstituted"

*This phenomenon has been rediscovered independently some years later (1956) by Yasutomi.

D.D.T. mentioned above on the extremely D.D.T.- and polyvalent resistant K_1 (Geigy-Basle) strain of *Musca domestica*, results obtained on tarsal contact were much less spectacular and much less satisfactory than those obtained with the moderately resistant strains used in the 1951 work (Table I).

TABLE I.

Residual action of p-p' D.D.T. and of "reconstituted" D.D.T. on Swiss strains K_1 (highly resistant) and T_1 (susceptible), of Musca domestica.

(Females, three days old, fed water and sugar only; T=27°C.; Residues from acetone solutions, on glass; Continuous contact of two hours' duration; *Results expressed in* Σ % *k.d.* (i.e. sum of per cent k.d. observed after 10, 20, 30, 60, 90 and 120 minutes — for method see Kocher, Ascher, Roth and Treboux, 1954; Ascher, 1957).

Resistant flies (K_1)

g/sq. m.	Formulation.	Σ % K.D. Age of deposit in days 1	14	28	56	84
0·3	*p-p'* D.D.T. ..	0	0	0	0	0
0·075	"reconstituted" D.D.T. ..	10	44	12	34	10
0·15	" " ..	21	53	23	34	41
0·3	" " ..	159	161	156	169	166
	Susceptible flies (T_1)					
0·3	*p-p'* D.D.T. ..	341	288	243	219	198
0·075	"reconstituted" D.D.T. ..	310	262	154	99	74
0·15	" " ..	336	304	281	297	264
0·3	" " ..	585	419	394	366	364

Kocher (cited by Buxtorf and Spindler, 1954) found that combinations of D.D.T. with D.M.C. and a great number of other D.D.T.-synergists mentioned in the literature, were inactive against the K_1 strain. The same negative results were obtained in tarsal contact assays by Reuter and Ascher (1956) with the newly discovered (Tahori, 1955) D.D.T.-synergist di-(*p*-chlorophenyl) trifluoromethylcarbinol.

It has thus to be admitted that, though study of D.D.T.-synergists may help to throw some light on the biochemistry of D.D.T.-resistance, they will never restore to D.D.T. its former (pre-resistance era) activity, nor do they constitute a practical answer to the resistance problem.

2. COMPOUNDS TO WHICH RESISTANCE IS NEGATIVELY CORRELATED — AS INSECTICIDES AGAINST RESISTANT INSECTS.

It has been shown already four years ago (Ascher and Kocher, 1954) that resistance in houseflies, e.g., D.D.T.-resistance, may, paradoxically enough, also induce in certain cases an *enhanced susceptibility* to some specific chemicals. The name "resistance-induced enhanced susceptibility" (R.I.E.S.) has been recently suggested for this phenomenon (Ascher, 1958). These possibilities have been exposed by different workers (Ascher and Kocher, 1954; Mitlin, Babers and Barthel, 1956; Ascher, 1957, 1958*a*, 1958*b*; Ogita, 1957, 1958, 1958*a* and Ogita cited by Anon., 1958, 1958*a* — for an attempt at a theoretical explanation, see Ascher, 1958). The author considers this line of research a hopeful opening, but would like to advance some words of caution: Though the basis, underlying the approach, is perhaps a healthy and sound one, it should be considered always with the following reservations in mind:—

(*a*) So far no practical powerful insecticide of this type has been announced. While, e. g., Ogita's phenylthiourea (PTU) is a larvicide only and even as such weaker than thiourea, cetyl bromoacetate (CBA) is a very weak contact adulticide for the housefly and practically inactive against *Anopheles* adults and against housefly larvae (Ascher, 1958*d*). Moreover, R. I. E. S. for CBA in the adult housefly could be demonstrated only by *k. d. during continuous contact* (2 hours, see Kocher, Roth and Treboux, 1953). Indeed it has been found (Ascher, unpublished data 1955) and it has been stressed explicitly in various publications on CBA (Ascher, 1958*a*, 1958*b*) and the other alkyl and aryl bromoacetates (Ascher, 1958*d*, *cf.* p. 152, footnote) that there was *no* difference in 24 hours' LC_{50}'s of susceptible and resistant housefly strains subjected to *short time tarsal contact* and transferred thereafter to observation containers. There remained another quite baffling problem as regards CBA: The resistant housefly strains investigated (Ascher, 1958*b*) were K_t (extremely DDT- and polyresistant strain), R-Sard. (highly chlordane-resistant), P_2 (moderately diazinon-resistant with some cross resistance to DDT) and finally Ac-R (selected with chloroacetic acid for 80 generations, but developing practically no resistance towards this compound), - see Bettini and Boccacci, 1955, 1956 - and also no cross-resistance to DDT). It was, therefore, expected that R. I. E. S., as evidenced from 2 hours continuous tarsal contact, should follow the following pattern: $K_t >$ R-Sard. $\gg P_2 >$ Ac-R = normal reference strains, i. e., CBA susceptibility should be proportional to resistance level (e. g., in this case resistance level to chlorinated hydrocarbons). No such gradation was ever observed, all *selected* strains showing about the same enhanced response to CBA in continuous tarsal contact.

(*b*) It is hardly imaginable that the approach would be applicable to *all* resistant insects and indeed even to all resistant strains of a species, whose type of resistance may be dependent on different mechanisms, and bound to the mode of selection (as, e.g., larval or adult selection). Thus, e.g., in Danish field-collected resistant housefly strains, R.I.E.S. for CBA could not be confirmed (Keiding, 1958).

(c) Though these agents may act better on resistant strains, development of resistance to them is not excluded *a priori* in any way — though at least in the haloacetates there may be some hope of only slow and slight development of resistance, if the interesting findings of Bettini and Boccacci (1955) on chloroacetic acid will hold true also for the *derivatives of haloacetic acids.** This approach could and should be pursued, at any rate.

2a. COMPOUNDS TO WHICH RESISTANCE IS NEGATIVELY CORRELATED — AS "RESENSIBILIZING" AGENTS.

The idea to "resensibilize" resistant insects in the field — by the elimination of resistant individuals with the help of compounds to which resistance is negatively correlated — has gained impetus through Kearns' (cited by Anon. 1957) recent announcement. He was reported as having had accomplished "resensibilization" towards D.D.T. of a D.D.T.-resistant strain of houseflies containing five per cent of susceptibles, within three generations of pressure with an impure preparation of 1, 2, 2, 2,-tetrachloroethyl phosphoric acid (the compound found by Mitlin, Babers and Barthel, 1956). The author is of the opinion, however, that such a possibility is hardly realizable, especially in the field. The objections in this case are the same as advanced under paragraph 2 above. Moreover it is to be feared that in numerous field populations, and especially in housefly populations in urban areas in many parts of the world, the susceptible individuals have been practically eliminated.

The author has carried out a study on "resensibilization" in 1955. He incorporated various materials into the larval breeding medium of the highly D.D.T.- and polyvalent-resistant K_r strain of houseflies at such a rate as to still allow the hatch of about 20 per cent adults†, whose D.D.T.-resistance was followed up. The materials chosen were either chelating or heavy metal precipitating agents (with the scope in mind to inactivate D.D.T.-dehydrochlorinase, should it contain a central metal atom), heavy metal salts (to compete with the central metal, if any), strong enzyme inhibitors (in order to inhibit D.D.T.-dehydrochlorinase or any other enzyme playing a vital part in D.D.T.-resistance), alkali and alkaline earth chlorides (in an attempt to inhibit the dehydrochlorination of D.D.T., by addition of chloride to: $D.D.T. \rightleftarrows D.D.E. + Cl'$), alkali bromides (bromides being compounds to which resistance is negatively correlated in the K_r strain, according to Ascher and Kocher, 1954) and various other substances, as in the following list:

*Interest in the insecticidal properties of esters of chloro-, bromo- and iodoacetic acid, a subject first broached in numerous, mainly American publications and patents in the 20's and 30's (see for reference, Boccacci and Bettini, 1956), has been revived since 1952 (Bettini and Boccacci, 1952) by a series of papers by Bettini and co-workers. Also the Indian workers Tiwari and Tripathi (1956, 1957) have contributed to our knowledge of insecticidal activity of esters of chloroacetic acid. In 1958, the gamut of esters of bromoacetic acid has been widened considerably by the work of Shiek and Gertler (1958) on the fungicidal activity of these esters.

†In cases in which such a kill could be obtained.

K-ferrocyanide K-ferricyanide Na-oxalate Borax Thiourea Ammonium molybdate	Cupferron Dithizon Versene 8-Hydroxyquinoline Bromoacetic acid Chloroacetic acid Iodoacetic acid Hexyl bromoacetate	KBr NaBr Cetyl bromide Choline bromide KCNS KNJ NaF
$CuSO_4$ $CoCl_2$	KCl NaCl $MgCl_2$ $CaCl_2$	Talc powder

However, there was no decrease in the D.D.T.-resistance of the adults, except in the case of NaCl, which when added in the massive dose of five per cent (!–non toxic) to the wet larval breeding medium, caused a small, but well reproducible reduction in the adults' resistance without impairing the vitality, longevity and wellbeing of the flies in any other way. In the same manner, feeding adults with NaCl solutions for some days, caused a certain reduction in D.D.T.-resistance (Tables II and II*a*).

TABLE II.

Effect of incorporation of chlorides into breeding media—on the height of D.D.T.-resistance in adults of strain K_1.

(Females, fed sugar and water only, 3 days old, were exposed to well crystallized residues of 1g/sq.m. D.D.T. in Petri dishes; Continuous contact of 2 hours' duration; T=27°C.)

	Per cent k.d. on D.D.T.-residues.							
	Incorporation in breeding medium of strain K_1							
Observation time (Minutes)	2 per cent NaCl	3 per cent NaCl	4 per cent NaCl	5 per cent NaCl	3 per cent $CaCl_2$	3 per cent KCl	Control, none	Strain T_1 (susceptible)
10	—	—	—	—	—	—	—	46
20	—	2	—	—	2	—	—	100
30	5	—	—	—	—	—	—	100
60	22	22	30	25	7	12	—	100
90	30	37	47	50	22	35	—	100
120	45	57	55	52	22	40	—	100
Σ% k.d.	102	118	132	127	53	87	0	546

Note:—No k.d. in controls in untreated Petri dishes, done on every strain.

TABLE IIa.

The effect of the adults of strain K_1 ingesting chloride solutions—on height of their D.D.T.-resistance.

(Females, fed during first three days of life on water and sugar, were offered for another two days chloride solutions instead of water, and were then exposed to well crystallized residues of 1g/sq.m. D.D.T. in Petri dishes; T=27°C.)

	PER CENT K.D. ON D.D.T.-RESIDUES, 5 DAYS OLD FLIES.							
	Salt solution offered.							
Observation time (Minutes)	3 per cent NaCl	4 per cent NaCl	5 per cent NaCl	10 per cent NaCl	3 per cent $CaCl_2$	3 per cent KCl	Control, water	Strain T_1 5 days old.
10	—	—	—	2	—	—	—	76
20	—	—	—	2	—	—	2	100
30	2	2	2	5	—	—	7	100
60	7	7	22	22	5	12	7	100
90	15	17	37	40	7	15	10	100
120	17	22	55	55	25	32	17	100
Σ% K.D.	41*	48*	116	126	37*	69	43*	576

*Effect of age (5 days) of flies.
Note:-No k. d. in controls in untreated Petri dishes, done on every strain.

But this reduction of D.D.T.-resistance is of no practical value or extent (Ascher, unpublished data 1955).

In 1957, the author applied decyl, lauryl, myristyl, cetyl and stearyl bromoacetate to K_1 larvæ in the same way, without obtaining any reduction of D.D.T.-resistance in the adults.

Recently, we fed adult houseflies of the K_1 strain for the first three days of life on sugar, and water containing various concentrations of H_2O_2 (up to ten per cent). This was done with the purpose in mind of accelerating the metabolism and thus causing a quicker using up of lipoid reserves. Lipoids play an important rôle in D.D.T.-resistance in the housefly, according to Wiesmann (Wiesmann and Reiff, 1956; Wiesmann, 1957). By feeding H_2O_2 no noticeable reduction in D.D.T.-resistance was obtained.

3. OVIPOSITION-INHIBITING TARSAL CONTACT AGENTS ("O.I.T.C.-AGENTS").

This new approach has been developed recently (Ascher, 1957*a*: 1957*b*: 1958*c*), but here again we have to consider that (*a*) though this principle has never been tested in the field, but we may say already now, that it will not be of a general

applicability; (*b*) though it has been demonstrated first in D.D.T.-resistant houseflies, it is not completely unlikeable that " resistance " may develop also towards agents having this property. Surely the older method of Bushland and Hopkins (1951:1953), which is based on a completely different principle but has the same scope (inhibition of oviposition of fertilized eggs in the female by release of radiation-sterilized males) and which has been employed successfully in screw-worm control on the island of Curacao (Baumhover, Graham, Bitter, Hopkins, New, Dudley and Bushland, 1955), does not run the risk of resistance. This risk may be not too serious either in mitotic poisons, which inhibit ovarian growth in the housefly upon ingestion. This approach (Mitlin, Butt and Shortino, 1957; *see* also Mitlin and Baroody, 1958), which has been developed independently of and simultaneously with our " O.I.T.C.-agents ", probably offers also a new possibility of insect control. The author considers that, for practical use the first main difficulty encountered will be the attraction of sufficiently high numbers of insects towards the baits containing the mitotic poisons.

4. PHYSICAL AGENTS—ABRASIVE DUSTS, EXSICCATING AGENTS, STICKING AGENTS.

Research on these lines has been pushed aside by the advent of D.D.T. With the experience gained since 1942 in the art of formulation and application of insecticides, many of the physical agents could now be improved and advanced to a higher state of perfection than that reached before the Second World War. The perseverance of Wigglesworth, and of Kalmus on research on abrasives* during the D.D.T. era, as well as the work of Wigglesworth and of Richards and their co-workers on detergents as exsiccating agents (see, *e.g.*, Wigglesworth, 1945; Beament, 1945; Richards and Korda, 1948, 1948*a*), though mainly of theoretical nature, could well serve as an interesting new starting point in this field. As regards sticking agents, it is not only that we should try to develop for the housefly sector an improved version of the fly paper, formulated with better attractants, but we should also search for new application forms of the sticking principle. Indeed, the author was quite impressed with results of preliminary experiments done in 1955 on the sticking properties for insects of residual layers of clove oil (mainly eugenol) and other volatile oils from plants. Such agents should be thought of not only as killing agents (i.e. killing by sticking up the insects on treated surfaces) but may perhaps act, instead of this, by gluing up the sensory organs of insects, by preventing copulation, oviposition at oviposition sites or by impairing other vital functions.

* * * *

*Extensive field tests on silica gels as desiccants against cockroaches in apartments are being conducted by various research groups in the U.S.A. (for particulars see Anon., 1958*b*).

This short passing in review of a few attempted countermeasures against resistance makes us well realise, how remote we are from any real solution of the problem. It would, therefore, seem to be most important to have immediate notice of any new work, idea or suggestion, which could constitute an opening for solving the resistance problem. Fortunately Prof. A. W. A. Brown, formerly of the Division of Environmental Sanitation, World Health Organization, has rendered an excellent service to research on insecticide-resistance by launching in June 1956 the "*Information Circular on the Resistance Problem*" which has proved highly useful to workers in this field.

The resistance problem is, however, a general one embracing practically all disciplines of applied biology. Unfortunately, the average insect toxicologist knows only little about resistance problems and countermeasures in the fungicide, herbicide, bactericide sector etc., nor does the pharmacologist and medical research worker interest himself as a rule in insecticide resistance. Indeed, resistance problems arising in various fields are highly different, since completely different organisms are encountered. There is no doubt, however, that much of the experience gained in one sector could be used to good profit in another one. A very useful beginning in this direction has been made at the symposium on "Origins of resistance to toxic agents" held in 1954 at Washington, D.C. and summed up in book form by Sevag, Reid and Reynolds (1955). This volume, which united contributions on resistance in insects, bacteria, protozoa, plants towards herbicides, tumor immunity, etc., in a single volume and even touched such distant fields as alcoholism, has been criticized as throwing together completely unrelated subjects*. There is no doubt, however, that notwithstanding the critics, the Washington symposium signifies the breaking up of new paths, as recognized also in a recent review (Wolf, 1958) in *Arzneimittel-Forschung*. A similar trend has been developed most fortunately by *Advances in Pest Control Research*, though this series deals naturally only in part with resistance problems.

It is not suggested here that we should have a new journal on resistance, but rather an abstract collating information on resistance in every field of biology. Such an abstract should include, under appropriate headings, not only the most recent informations on resistance towards insecticides, fungicides, antibiotics, etc., but could bring to the knowledge of the specialized worker studies such as, *e.g.*, those of the group of H. Erlenmeyer on prevention of onset of resistance towards I.N.H. in *Mycobacterium tuberculosis*, if Cu^{++} ions are added to the medium (Roth, Carrara and Erlenmeyer, 1953; Erlenmeyer, Fallab, Roth and Prijs, 1954; Roth and Erlenmeyer, 1954; Roth, Prijs and Erlenmeyer, 1955),—and on the other hand, those of Fox (1957), who showed that a DNA extract (70–90% "high molecular weight" DNA) from streptomycin-resistant pneumococci may induce resistance in streptomycin-sensitive bacteria (for other work on "transformed" bacteria see Lerman and Tolmach, 1957; and related studies with *Hemophilus influenzae* by Goodgal and Herriot, 1956, 1957). Of interest to the resistance worker should prove the selection of resistance in cancer cells towards carcinoclastic drugs (see, *e.g.*, Lettré and Kramer, 1952, 1953; Lettré, 1956; Schmähl and Riesenberg, 1956 and the recent researches of Yoshida,

*Adams (1956) starts his review in *Science* by stating: "This is a remarkable book in that it seems to be lacking in any unifying principle".

Hirono, Jackson, Law and others), the development of cancer cells refractory to X-rays (see, *e.g.*, Schubert, 1954), tumor isoimmunity (see, *e.g.*, Hirsch, 1958), field resistance of *Trypanosoma congolense* and *T. vivax* to "Antrycide" (Wilson, 1949), to cite just a few subjects. The cross-resistance studies of plasmodia towards paludrine, daraprim and sulfadiazine and related compounds, which have appeared mainly already during 1949-1950 (see summary in Thurston, 1953), could well have forewarned insect toxicologists that the endeavours to overcome D.D.T.-resistance by the successive application of the other chlorinated hydrocarbons, gamma-B.H.C., chlordane and dieldrin would be forlorn hopes. The unexpected cross-resistance of "Antrycide"-resistant trypanosome strains towards diamidines and of Melarsen-resistant strains towards totally unrelated As-free compounds, demonstrated already in 1949 (Wilson, 1949; Ormerod, 1952), could have made us predict incipient cross-resistance between phosphoric acid esters and chlorinated hydrocarbons *before* the use of phosphorus compounds in the field. On comparing penicillin-resistant and penicillin-sensitive strains of *Staph. aureus*, Markov, Saev, Klein and Monov (1958) noted that in resistant strains adapted to penicillin *in vitro* there was a strong reduction of phosphatase formation, while in resistant strains isolated from pathological processes there was hardly any such tendency, or indeed loss of any of the other signs of pathogenicity (Huggins and Talalay, 1945). To this we may have a somewhat remote parallelism in the biochemical differences found between resistant and normal strains of insects, which may be very clear in laboratory selected strains, while they often cannot be confirmed in resistant field strains. The problem of behaviouristic resistance, a term the validity of which has been disputed* recently (Brown, 1958, 1958*a*; Morrison, 1950), should be examined together with the sometimes reported acquired (?) loathing of rodents to pick up or to touch poison baits.

Perhaps the World Health Organization and the Food and Agriculture Organization would see their way to undertake the regular issue of such an abstract as a joint enterprise. This would greatly help the research of the subject and might even be instrumental in reaching some solution. It would establish "*Resistance*" as a clear cut topic in biology and would finally put an end to the "splendid isolation" of each of its branches.

*The author deems that perhaps the following concept might be useful as regards the discussion on the very existence of the phenomenon of behaviouristic resistance. He suggested some years ago (Ascher, 1956) that, "it may be assumed that, insects exert two complementary modes of resistance (behaviouristic and physiological) in coping with the new contact insecticides, and that it will depend on the nature of the insect, which mode of resistance is the first practically perceivable by us, the more easily available to the insect and which of the two will become the predominant one". He would now like to add that, it is quite possible —and can be supported from numerous findings in the literature—that in many cases of adults of *Anopheles* (Georgopoulos, 1951; Hadjinicolaou, 1954; Belios cited by Hadjinicolaou, 1954; Belios, 1954; Issaris and Sundararaman, 1954; Reid and Wharton, 1956) and perhaps also *Musca* (Madwar and Zahar, 1951), behaviouristic resistance was a first step towards true biochemical resistance. A part of an insect population can thus save itself from extermination, by picking up sublethal doses till an adequate height of biochemical resistance has been established. As long as biochemical resistance is still moderate, it might be possible sometimes to observe both modes of resistance concurrently (see e.g. Missiroli, Mosna and Alessandrini, 1948; Bruce and Decker, 1950; Silverman and Mer, 1952; Stoker cited by Anon., 1955, and for a recent, very instructive example—Kilpatrick and Schoof, 1957). Only when biochemical resistance has attained a very high level, behaviouristic resistance will disappear. It will thus not necessarily be found again, if a field strain, once said to exhibit behaviouristic resistance, is re-examined for it after the elapse of some years. Only in species, in which biochemical resistance cannot be developed, or in which it has not developed due to special environmental conditions, behaviouristic resistance may remain a constant feature accompanying contact-insecticide pressure (Trapido, 1952, 1954; Brown, 1958*b*).

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SELECTED REFERENCES ON RESISTANCE OF MOSQUITOES TO INSECTICIDES, 1954 THROUGH SEPTEMBER 1958.

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THE following references include some of the more important papers dealing with mosquito resistance to insecticides during this period. All articles do not concern proven cases of resistance. In fact, a number deal with suspected or behaviouristic resistance. Others concern susceptibility tests, methods of detecting resistance as well as physiological and genetical research. The heading entitled "Techniques", concerns references to methods of testing insect resistance.

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