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MODIFIED RESPONSE OF *ANOPHELES ALBIMANUS* TO DDT RESIDUAL HOUSE SPRAYING IN PANAMA

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The Gorgas Memorial Laboratory, in cooperation with the former Tropical Disease Control Branch of the Office of the Surgeon General (U. S. Army) and the former Office of Scientific Research and Development, initiated experiments with DDT as a residual house treatment for the control of malaria in 1944. The area chosen for these experiments was a group of native villages on the middle Chagres River in Panama, where *Anopheles albimanus* is the malaria vector.

A description of the area, the climate and meteorology, the composition and habits of the mosquito population, and the results of the first fifteen months observations have been previously reported (Trapido, 1946). It was shown that following DDT residual spraying of dwellings the mosquitoes were affected in several ways. Within the houses three important effects were observed. First, there was a large reduction in the numbers of mosquitoes (of the order of 70 to as much as 99 per cent). Second, of those mosquitoes taken in treated houses there was a marked reduction in the percentage engorged with blood. Third, among such engorged mosquitoes as were taken in treated houses there was a very high 24 hour mortality rate. (Unengorged mosquitoes taken in treated houses survived for 24 hours in the same proportion as unengorged individuals from houses in untreated villages, thus demonstrating a selective killing of the mosquitoes potentially capable of transmitting malaria—the engorged individuals.) Apart from these striking effects noted in houses of a treated village there was also reported a "peripheral effect." That is, for a period of two or three weeks after treatment there was a large drop in the numbers of mosquitoes taken in a horse-baited stable trap in the center of the village, and even some transitory effect on a similar trap 300 feet from the village. There was no apparent effect in a trap 900 feet from the village.

A most complete malaria history of the villages in this area has been available for the twelve years before the introduction of DDT and the eight years since. For ten years thick blood films had been taken on all residents each month, and for the subsequent years thick film records on a bimonthly basis are available. Following the introduction of DDT residual spraying, there was a dramatic drop in the malaria incidence to a point where only an occasional positive was found. These positives in virtually all instances could be attributed to infections acquired away from the experimental villages, since these positive individuals were in almost all cases found to have been absent from the villages during several previous surveys. The history of the decline of malaria in these villages has been recorded by Dr. Herbert C. Clark in the annual reports of the Gorgas Memorial Laboratory (1945–51), and will not be considered further here.

With the decline of malaria in these villages the detailed semi-weekly house

captures of mosquitoes were discontinued at the end of 1946, although the routine DDT spraying was continued at approximately four month intervals. Despite the continued absence of any but occasional malaria positives the appearance of resistance to DDT and other chlorinated hydrocarbon insecticides in flies and certain culicine mosquitoes made it seem advisable once again to pick up the field observations to establish whether there had come about any change in the way *A. albimanus* in these villages responded to DDT residual house spraying after eight years of treatment of this sort. The present paper reports on the first four months of these renewed observations, January through April 1952.

FIELD OBSERVATIONS

General

A. albimanus is a species which enters dwellings at dusk and disappears from them at dawn. Just how much of the night is spent in houses has not been established. The diurnal resting places are out-of-doors. While it would be desirable to study the resting habits of these mosquitoes by means of window traps as has been done with certain anophelines in Africa, Iran and Southeast Asia, the house construction in Panama is such that this is not possible. As the writer has noted earlier (Trapido, 1946, p. 393) 90 per cent or more of the mosquitoes enter and leave the house in other ways than through the doors and windows. The houses of the experimental area are for the most part constructed with cane walls and palm thatch roofs. The bulk of the mosquitoes enter and leave either through spaces between the canes of the walls, or through the space between the tops of the walls and the overhanging eaves. The technique used for measuring mosquito abundance in the houses was therefore that described earlier (Trapido, 1946): a resident of each house in which collections were made was provided with a suction tube and a cage in which mosquitoes could be held alive. Collections were made for two and a half hours after dusk in the evening, and an hour before dawn in the morning. Mosquitoes collected by this means were held for twenty-four hours, when the dead individuals were removed from the holding cages, and the living ones subsequently chloroformed. Collections were made semi-weekly, on Monday and Thursday nights of each week. The results are thus comparable with those obtained during the 1944–1946 period.

While the area in which this experiment has been carried out is a tropical one in which mosquito breeding continues throughout the year, there are, nevertheless, fluctuations during the year in the abundance of the various species of mosquitoes, depending on variations in rainfall, humidity, the condition of the vegetation in the lacustrine situation in which the larvae develop, and other factors. These variations in climatic factors vary in some degree from year to year, as well as within a particular year. The absolute number of mosquitoes from year to year reflects these differences, but, in general, the abundance curves for different years are similar. The comparison of the mosquito populations of the present four month period of study have therefore been made with the same four month period for which data are available from the original experiment.

The routine of spray treatment maintained at the experimental villages over the period of years was the same as that employed when the experiment was initiated in 1944. The material used was five per cent technical grade DDT in kerosene. In the case of cane-walled houses the outside of the walls as well as the inside was sprayed since many mosquitoes alight on the outer walls and enter through crevices between the canes. The overhanging eaves of all houses were sprayed whether the roof was of palm thatch or galvanized iron since many mosquitoes also alight there on entering and leaving the houses. Inside the houses, all walls and furnishing were sprayed as well as the undersides of the roofs.

The present studies were made at two of the villages used in the earlier work, Gatuncillo and Santa Rosa. During the experiment previously reported, Santa Rosa had been used as a control and was not sprayed until it was sacrificed as a control in September 1947. The spraying of these villages on the 17th and 18th of January 1952 represented the thirteenth treatment for Santa Rosa in 52 months, and the twentieth treatment for Gatuncillo in 86 months. There is probably no village anywhere in the tropics which has been sprayed with DDT with such frequency over so long a period of time as Gatuncillo. It would therefore appear that if any resistance or behavior change were to develop in a natural population of anophelines, Gatuncillo would be a most favorable place to look for it.

Numbers of A. albimanus in DDT treated dwellings

When Gatuncillo was first sprayed with DDT at the beginning of November 1944 the effect on the gross numbers of mosquitoes in houses was immediate and dramatic. During the first month after this spraying the reduction in numbers of anophelines was from 81 to 100 per cent. (Trapido, 1944). The overall reduction in anophelines in houses during the four month period after the first spraying (Nov. '44–Feb. '45) was 88.3 per cent, after the second spraying (Mar.–June '45), 90.9 per cent, and after the third spraying (July–Oct. 1945), 96.8 per cent.

In 1952 control villages for comparison were no longer available, but we are able to make direct comparisons of the numbers of *A. albimanus* caught in houses of Gatuncillo itself during the first eighteen weeks (January to April) of 1945 and 1946, the first two years of treatment, with those taken during the same weeks of 1952. This comparison is made in Table 1. The average number of *albimanus* per night during the first eighteen weeks of 1945 was 4.6, that for 1946 was 1.4, and the average for the two years was 3.0. In striking contrast there were 21.7 *albimanus* per house per night during the comparable period of 1952, after twenty DDT treatments. (Data for the first three weeks of 1952 are lacking since the routine of house catching was not established until the fourth week of the year, immediately following the 20th spraying.) It is at once evident that there are many more *albimanus* entering treated dwellings than was the case during the first two years of the DDT spraying routine.

We may also examine with profit the data from Santa Rosa given in Table 2. This had been an unsprayed control village during the early work at Gatuncillo.

However the same routine of collecting data on the numbers of mosquitoes in houses was followed here for comparison with the then treated Gatuncillo. The figures given for the first eighteen weeks of 1945 and 1946 represent the normal prevalence of *albimanus* in houses of this village in the absence of DDT treatment. As mentioned above, this village was sacrificed as a control in September 1947, and on the 18th of January 1952 it experienced its thirteenth DDT treatment. The figures given for the second and third weeks of 1952 show the average numbers of *albimanus* per house per night for the two weeks before the thirteenth

TABLE 1

Average numbers of Anopheles albimanus *per house per night at Gatuncillo in the first and second years, and in the eighth year of DDT treatment*

(January through April of each year)

WEEK OF THE YEAR	1ST YEAR (1945)	2ND YEAR (1946)	AVERAGE OF 1ST AND 2ND YEARS	8TH YEAR (1952)
1st	5.8	3.5	4.7	—
2nd	4.0	2.8	3.4	—
3rd	3.3	1.3	2.3	—
4th	4.3	1.1	2.7	5.0
5th	4.3	2.0	3.2	6.0
6th	13.5	1.5	7.5	11.2
7th	4.0	2.0	3.0	14.2
8th	7.3	0.0	3.7	14.5
9th	7.3	4.0	5.7	18.2
10th	3.5	1.0	2.3	28.2
11th	2.3	2.0	2.2	22.8
12th	3.0	0.8	1.9	25.2
13th	1.3	0.6	1.0	21.7
14th	1.0	0.0	0.5	30.0
15th	6.8	0.0	3.4	55.7
16th	4.0	0.0	2.0	22.3
17th	4.5	0.3	2.4	32.2
18th	3.0	2.6	2.8	18.5
Average	4.6	1.4	3.0	21.7

treatment. It will be seen that they are essentially the same as the figures for the comparable weeks of 1945 and 1946 *when DDT had not been applied*. Of even greater significance are the figures for the subsequent weeks of 1952 immediately following the thirteenth spraying. It will be seen that the numbers of *albimanus* per house per night closely approximate the average numbers of this species in houses during chronologically comparable weeks of 1945 and 1946, prior to any treatment. The very close similarity in the numbers of *albimanus* in houses before the use of DDT and in the fifth year of its use may be summed up in the average figure for the first eighteen weeks of 1945 and 1946, 31.6, and the comparable figure for 1952, 34.6.

There can be no doubt from the data presented in these two tables that there

has been a very great change in the response of *A. albimanus* to DDT residual spraying of dwelling. These data appear plainly to demonstrate that DDT residual spraying no longer reduces the numbers of these mosquitoes in houses.

Engorgement of A. albimanus in DDT treated dwellings

While the killing of anophelines or otherwise reducing the numbers which enter dwellings is a desirable end in itself, this is only one of the ways by which the interruption of the transmission of malaria may be accomplished. The same

TABLE 2

Average numbers of Anopheles albimanus *per house per night at Santa Rosa, during two years prior to DDT treatment and in the fifth year of DDT treatment*

(January through April of each year)

WEEK OF THE YEAR	YEARS PRIOR TO TREATMENT			FIFTH YEAR OF TREATMENT (1952)
	1945	1946	Average of 2 years	
1st	20.8	10.3	15.6	—
2nd	28.2	30.5	29.4	20.2
3rd	36.0	17.5	26.8	41.2
4th	27.2	47.3	37.3	27.0
5th	58.3	43.7	51.0	67.5
6th	35.0	48.2	41.6	42.8
7th	47.0	36.0	41.5	58.2
8th	41.3	57.7	49.5	27.0
9th	18.5	53.0	35.8	38.2
10th	30.7	19.3	25.0	27.5
11th	42.7	29.7	36.2	35.8
12th	49.3	14.2	31.8	40.2
13th	34.8	10.7	22.8	36.2
14th	45.2	15.3	30.3	33.7
15th	50.5	3.8	27.2	27.8
16th	52.8	7.5	30.2	15.2
17th	29.5	5.3	17.4	19.3
18th	24.3	16.0	20.2	30.9
Average	37.3	25.9	31.6	34.6

end may be brought about by modifying the behavior of the anopheline so that it does not readily feed on humans. This effect on the behavior of mosquitoes entering DDT treated dwellings was observed following the first treatment at Gatuncillo in 1944 when the author wrote: "—of 135 mosquitoes collected in dwellings since the spraying of the village only four, or 2.9% have contained blood. It is indicated that mosquitoes entering the treated dwellings and contacting the DDT residue become so activated that they do not feed." (Trapido, 1944).

It is therefore of considerable importance to examine the present status of this phenomenon in order to determine whether DDT residual house treatment has any further value as a measure for the reduction of the malaria transmission

potential. In summarizing the results following the first three DDT treatments of Gatuncillo in 1944–45 it was shown that only 7.1 per cent of the *Anopheles* taken in treated dwellings were engorged compared with 47.4 per cent in untreated houses (Trapido, 1946, Table 4). During the period from February through April 1952, for which similar data are now available from Gatuncillo, we found 7.6 per cent of the *A. albimanus* to be engorged. It is therefore apparent that there has been no change in the effectiveness of DDT in reducing the proportion of anophelines which feed in houses at Gatuncillo.

We are also able to examine this effect of DDT at Santa Rosa. There we are able to compare the percentages of engorged *albimanus* from houses during the first four months of 1945 and 1946, before this village was sprayed, with the figures obtained during the period February through April 1952, following the thirteenth spraying of the village. These data are given in Table 3. We see that the summary figure of 4.5 per cent engorged for 1952 represents a considerable

TABLE 3

Per cent of Anopheles albimanus *engorged at Santa Rosa, during two years prior to DDT treatment and in the fifth year after DDT treatment*

	YEARS PRIOR TO TREATMENT		FIFTH YEAR OF TREATMENT (1952)
	1945	1946	
January	52.8	47.4	—
February	33.5	34.5	1.2
March	37.0	28.9	5.7
April	42.6	41.7	6.9
Average	42.1	39.2	4.5

drop from the 42.1 per cent in 1945 and 39.2 per cent in 1946. These results are quite similar to those obtained at Gatuncillo, and confirm the observation made there that DDT residual spraying over a period of years continues to reduce the proportion of *albimanus* which feed in dwellings just as it did initially.

Selective mortality of engorged A. albimanus in DDT treated dwellings

The third effect observed on the mosquitoes of treated dwellings at the Chagres River villages in the early work during 1944 to 1946 was the high mortality rate of engorged mosquitoes. During the four month periods following the first three DDT treatments of Gatuncillo in 1945 the average survival of engorged anophelines was only 4.2 per cent, while 38.3 per cent of the engorged individuals from the then unsprayed villages of Santa Rosa and Guayabalito survived for a similar period. On the other hand, there was no material difference in the 24 hour survival rates of unengorged anophelines from houses in treated and untreated villages at that time. At Santa Rosa and Guayabalito, which were then untreated, the 24 hour survival rate for unengorged anophelines was 36.7 per cent, while at Gatuncillo, which was treated, the survival rate was 41.7 per cent (Trapido,

1946, Tables 5 & 6). In 1952, at Gatuncillo, there was no longer a marked difference in the 24 hour survival rate of engorged and unengorged *Anopheles albimanus*, the rate for engorged individuals being 35.2 per cent and for unengorged 30.7 per cent.

The writer's interpretation of the high mortality among engorged mosquitoes during the early work at the Chagres River villages was that since they carried the weight of a blood meal, the engorged mosquitoes tended to rest longer and were thus more likely to accumulate a fatal dose of DDT. This selective killing of engorged anophelines was considered an important factor in lowering the malaria transmission potential. The survival rates derived from the data obtained during the first four months of 1952 would indicate that for reasons which are not superficially apparent the selective killing of engorged *albimanus* in DDT treated houses at Gatuncillo no longer occurs.

DISCUSSION

It was unfortunately not possible to re-establish horse-baited stable traps at the experimental villages until some weeks after the January 1952 spraying had been completed. Thus it is not possible at this time to report whether or not the transitory reduction of mosquitoes in treated village areas, an effect observed in 1944 and 1945, is still apparent. The routine semiweekly catches of mosquitoes in stable traps at Santa Rosa were begun in the ninth week, and at Gatuncillo in the twelfth week of 1952. The catches from these traps are sufficient to indicate that while the general level of anopheline abundance was somewhat higher than that recorded for the same time of year in the 1945 to 1947 period, the increase was not sufficient to account for the relatively large numbers of anophelines in houses of treated villages.

There appear to be two possible explanations of what may have happened to modify the response of *A. albimanus* to DDT treated houses at the Chagres River villages. The one is that these mosquitoes may have become resistant; the other is that through a selective killing off of those least irritated by DDT, the population now largely consists of individuals with an enhanced predisposition to irritability on minimal contact with DDT, which results in their having only fleeting sublethal contacts with DDT treated surfaces. It may also be that both these factors are involved, the one complementing the other. In this view, the present population would have developed both hyperirritability and some resistance: the hyperirritability would ensure that only minimal contacts were had with DDT treated surfaces, while the resistance, however slight, would raise the threshold of the time of contact for the accumulation of a lethal dose of DDT to a point where more individuals survived.

One or another or both of these factors operating in conjunction would explain the fact that the numbers of *albimanus* in houses are no longer reduced as they were in the first years of the use of DDT. There is no way to measure resistance on the basis of the field observations considered in the present paper, but certain of the observations strongly support the interpretation that an enhanced irritability to DDT has appeared. In the 1944 to 1946 period a preponderance

of the *albimanus* taken in houses would be found on walls, furniture, and on cross beams throughout the interior of the houses. In the course of making the night collections during the present study in 1952, it was observed that while there were many mosquitoes in the treated houses they were largely restlessly moving about in the air. The native collectors are adept with suction collection tubes and are able to collect these mosquitoes out of the air. Yet another behavior difference noted on the part of *albimanus* in treated houses in the present study has been their greater positive phototropism. In the native houses at the Chagres River villages the usual source of light at night is either a conventional kerosene lamp or an open flare-like lamp consisting of a wick made of a corn cob or similar absorbent material stuck in a bottle of kerosene. There is always a considerable accumulation of mosquitoes hovering about this light, and even flying through it, so that many mosquitoes are singed and found dead or moribund on the table around the foot of the lamp. Laboratory studies to measure quantitatively a possible resistance and enhanced irritability and response to light of *albimanus* from these treated villages, against a colony which has had no contact with DDT, are now in progress and will be reported in a separate paper.

The second effect of DDT, that of reducing the feeding of *albimanus* has apparently not been modified over the period of years, and seems to be at present the principal factor in reducing possible malaria transmission in DDT treated dwellings.

The ultimate success of a method such as that of DDT residual spraying of dwellings is measured by the malaria rates. As yet we have no evidence that malaria is returning to these villages. However, with the malaria seed bed reduced virtually to nil, it is to be expected that there would be some lag in time before fresh infections contracted elsewhere were introduced to infect the mosquitoes and again start the chain of transmission. A variable other than the mosquito numbers and behavior which might have affected the malaria rates was introduced at the experimental villages in 1946 when the drug routinely administered to persons found positive for malaria on the bimonthly blood thick film surveys was changed from atabrine to chloroquine.

SUMMARY

From observations conducted in 1944 to 1946 in a group of native villages on the middle Chagres River in Panama it had been concluded that the DDT residual spraying of dwellings initially reduced the malaria transmission by *Anopheles albimanus* in houses in three ways. During the first four months of 1952, following the twentieth spraying of one of these villages and the thirteenth spraying of another, it was found that two of these effects are no longer apparent. The gross numbers of *A. albimanus* in dwellings are no longer drastically reduced as was formerly the case, and the selective killing of engorged *albimanus* is no longer evident. A large reduction in the percentage of *albimanus* which successfully engorge with blood in DDT treated houses is still present to about the same degree as at the time of the initial observations in 1944 to 1946. This last appears to be the principal way in which *albimanus* is affected by DDT to modify the malaria transmission potential at the present time.

A possible explanation is given of how this change in the way *albimanus* responds to DDT residues may have resulted from the selection of a population which has become resistant and/or hyperirritable to DDT. Laboratory experiments to measure the contribution of each of these factors quantitatively are in progress.

The modification in the response of *A. albimanus* to DDT residual house spraying has not as yet been reflected in any rise in malaria rates at these villages.

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