THE CAUSE OF OCCASIONAL HIGH COUNTS OF *AN. GAMBIAE* IN MORNING PYRETHRUM SPRAY COLLECTIONS IN HUTS SPRAYED WITH FENITROTHION,[1] KISUMU, KENYA

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INTRODUCTION. The organo-phosphorous insecticide, OMS-43 (fenitrothion) was shown by entomological evaluation to be highly effective in reducing hut densities of the malaria vectors *An. gambiae* and *An. funestus* in a Stage VI trial[6] carried out near Kisumu, Kenya in 1969–1971. A 40 percent and a 50 percent water dispersible powder diluted to 5 percent concentration was applied as a residual spray to the interior mud wall and thatch ceiling of sleeping huts and other buildings in the family compounds in the trial area. The evaluation was based on four rounds of spraying from August 1969 to October 1970 each applied at a target dose of 2g/m^2 spaced 3 to 4 months apart.

The entomological evaluation consisted of fortnightly morning pyrethrum spray collections from sleeping huts in five entomological stations in the sprayed area and three stations in an unsprayed comparison area, (Fig. 1) together with exit-trap captures, night biting collections, bioassays of sprayed surfaces and air bioassays inside sprayed huts.

Throughout the trial, the average hut density recorded in the pyrethrum spray collections in the sprayed area seldom exceeded one *An. gambiae* per hut up to 6 months following the last spray round. A pronounced resurgence in the hut densities of the species was not observed until 8 months after the fourth round of spraying.

The reduction of *An. funestus* hut densities was even more dramatic as there was no evidence of a recovery up to the time the evaluation was concluded in May 1971, nearly 10 months after applying the fourth round of spray. The results of bioassay tests indicated that OMS-43 had a slow knock-down effect on *An. gambiae*.

Despite the excellent control achieved with OMS-43, there were occasions when morning pyrethrum spray collections exceeded one *An. gambiae* female per hut as early as 11 days after a spray round. For example, out of a total of 206 morning collections made from the five entomological stations in the sprayed area (A B C D E) Figure 1, there were 17 collections with counts exceeding one and up to as many as five *An. gambiae* females per hut. Of special significance was the high proportion of animal-fed *An. gambiae* in precipitin tests made of a sample of the engorged females in these collections.

Since it is the custom for people living in the trial area to keep their cattle penned up during the night in corral-type enclosures near their sleeping huts, the cause of the occasional high counts was attributed to unusual numbers of cattle-fed *An. gambiae* entering houses in the early morning hours. A similar feeding and resting behaviour had been reported by Smith (1958) in studies made in the Pare-Taveta area of East Africa. He found that a high proportion of *An. gambiae* that had fed on cattle outdoors were resting inside sleeping huts during the day and he noted further that the ingress of the mosquitoes occurred at dawn and early morning. Also, Smith and Wietz (1959) in the same area of East Africa, determined by precipitin tests the presence of

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[6] WHO Anopheles Control Research Unit II, Unpublished Monthly Reports.

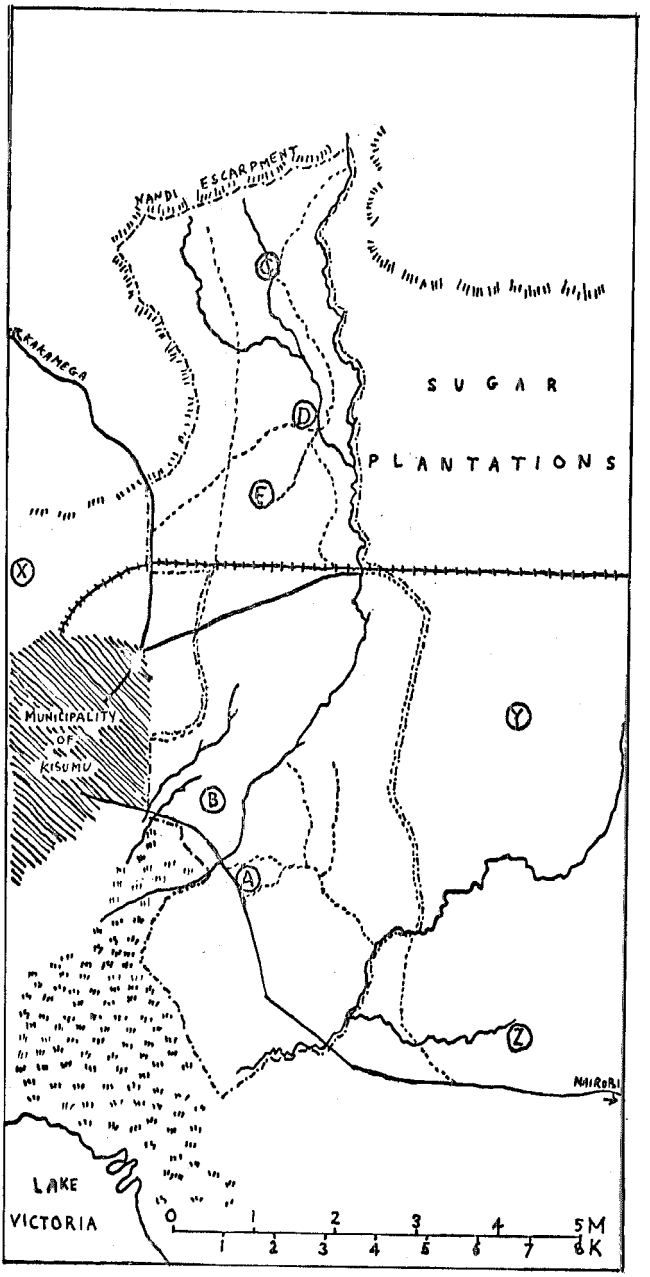

FIG. 1.—OMS43 Stage VI Trial Area. WHO/ACRU II, Kisumu, Kenya.

a large proportion of cattle-fed *An. gambiae* resting in huts during the day.

With such a pattern of feeding and resting behaviour prevailing in the *An. gambiae* population, it would be expected that a morning collection of surviving mosquitoes from sprayed huts would yield a higher proportion of cattle-fed *An. gambiae* than human-fed, which feed and rest indoors and, accordingly, would be subject to a much higher mortality rate due to longer insecticidal exposure.

To confirm whether the ingress of cattle feeding *An. gambiae* in the morning was the cause of the occasional high counts observed in the Kisumu Stage VI trial, a study was undertaken to determine:

(1) the difference in numbers of *An. gambiae* collected in early morning and late afternoon in sprayed huts compared with unsprayed huts.

(2) the proportion of engorged *An. gambiae* precipitin positive for human and animal blood in morning and afternoon collections.

The study was also augmented by bioassays to confirm the period of time required for knock-down of *An. gambiae* females in contact with surfaces sprayed with OMS-43 and also the airborne knock-down effect of the insecticide in sprayed huts. Since a slow knock-down of *An. gambiae* had been noted in bioassays of OMS-43 made in the Stage VI trial, it was felt that this might have contributed to the occasional high counts.

METHODS OF STUDY. A study site was selected in the Kano Plain area at a place called Wawidhi 'B' located 17 miles south-east of Kisumu (Fig. 2), where the local people live in family compounds called "Bomas."

A Boma typically consists of a group of 5 to 6 sleeping huts, several small animal shelters, grain storage huts, and one corral centrally located where cattle are penned overnight. The Bomas are usually surrounded by a dense hedge consisting of an admixture of shrubs, (cactus, euphorbia, thorns) sometimes interspersed with trees.

The study Boma contained five sleeping huts, a cattle corral and the usual animal and granary shelters (Fig. 2).

Two of the five sleeping huts were residually sprayed with a 40 percent w.d.p. formulation of OMS-43 at a calculated dose of 2g/m^2. These were designated huts S_1 and S_2. Two other huts designated C_1 and C_2 were left unsprayed to serve as checks or comparisons for the sprayed huts.

PYRETHRUM SPRAY COLLECTIONS. At fortnightly intervals, pyrethrum spray collections were made from the sprayed hut S_1 and the unsprayed C_1 at 0700 hours. On the same day an afternoon collection was made at 1400 hours from huts S_2 and C_2. Four days later the collections were repeated in reverse order with collections from huts S_2 and C_2 taken in the morning and S_1 and C_1 in the afternoon.

This collection routine was followed for 3 months from June to September 1971 inclusive. The mosquitoes captured were recorded by date, hour and number of *An. gambiae* females. The engorged females were prepared for precipitin tests to determine the proportion positive for human and animal blood. About one-half of the fed specimens were tested.

BIOASSAY. Bioassays of mud and thatch surfaces sprayed with OMS-43 at 2 g/m^2 were made to establish the length of the exposure period required to obtain a 100 percent knock-down of *An. gambiae* fed females. As shown in Figure 1 the test hut was located at Nyagbongo in the same entomological station where bioassays had been made previously in the Stage VI trial.

The earlier results had indicated a slow knock-down action of the OMS-43 even on surfaces sprayed three and four times at intervals of 2 and 3 months apart. The test huts used for the present bioassays were previously sprayed four times, the last spraying having been applied 10 months before the fifth application of 2 g/m^2 on which this bioassay was made. Just prior to the 5th application bioassays of the ten-month-old residues showed a

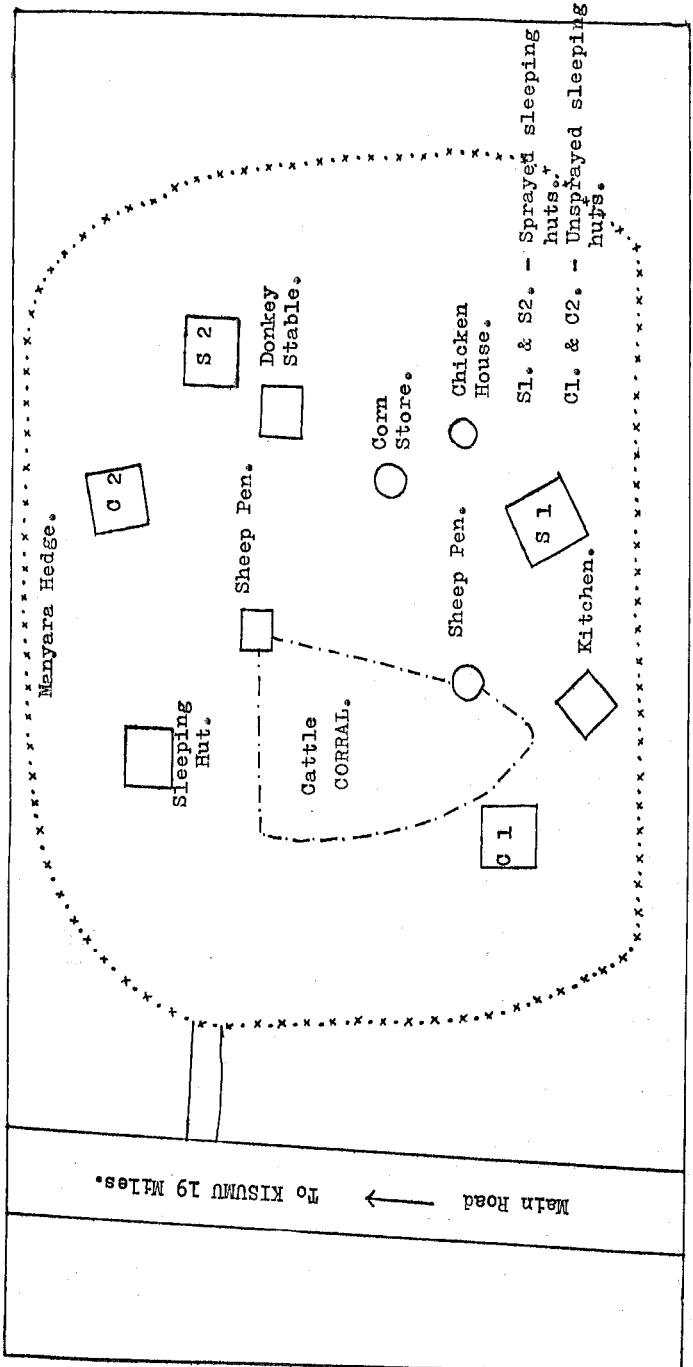

FIG. 2.—Family compound (BOMA) used as site of morning and afternoon pyrethrum spray collection. Wawidhi 'B', Kisumu, Kenya. 1971.

continuing insecticidal action (80 percent mortality on mud surfaces and 35 percent on thatch).

In conducting the test 3 W.H.O. bioassay exposure chambers each containing ten *An. gambiae* insectary bred, fed females were placed in contact with the sprayed mud walls and thatched ceiling. The time taken to obtain complete knock-down of all the test mosquitoes was recorded. Nine such tests were made at a fortnightly interval, beginning 7 July, 1971, 12 days following the application of the insecticide and were concluded 22 September 1971, 89 days later.

AIR-BIOASSAY. At another site at Wathorego located about six miles north of Kisumu (Fig. 1), air bioassays tests were routinely made to determine the airborne effectiveness of OMS-43. These tests were made in five sprayed huts used as entomological station in the Stage VI trial as described for the surface bioassays. In this test ten *An. gambiae* insectary bred females were placed in each of five 6" x 4" x 4" wire mesh cages hung overnight for 12 hours, 50 cm. away from the sprayed wall. After an exposure of 12 hours, the mosquitoes were removed and placed in paper cups and the numbers knocked down recorded. To determine the survival rate they were held for a period of 24 hours and the mortality noted.

RESULTS

MORNING AND AFTERNOON COLLECTIONS. The number and average hut densities of *An. gambiae* females recorded in morning and afternoon pyrethrum spray collections in the sprayed and unsprayed huts at the Wawidhi 'B' study site are shown in Table 1. The data indicate that morning collections in the sprayed huts yielded a much higher number of *An. gambiae* females than afternoon collections. This was a consistent observation in all of the 14 collections made during the 90-day period of the study. The collections averaged 13.4 *An. gambiae* females per hut in the morning compared with only 2.4 per hut in the afternoon.

In contrast, high numbers were recovered in both the morning and afternoon collections in the unsprayed comparison huts which averaged 70.9 and 68.4 *An. gambiae* females per hut respectively.

PRECIPITIN TESTS. The data from precipitin tests made of the engorged *An. gambiae* in the morning and afternoon collections in the sprayed and unsprayed huts are shown in Table 2.

The morning collections in the sprayed huts yielded a high proportion of animal-fed females averaging 78.3 percent of the total number tested (120) in the 14 collections. But in the unsprayed comparison huts the animal fed specimens represented only 32.4 percent of 207 engorged females tested.

In the afternoon collections the number of *An. gambiae* engorged females taken in the sprayed huts was very low totaling only 15 mosquitoes in 14 collections out of which only one was positive for human blood (6.7 percent) and 14 for animal blood (93.3 percent). The number of fed females (252) in 14 afternoon collections in the unsprayed comparison hut was also high, but there was a substantial increase in the percentage animal-fed (51.6 percent) over the morning collections.

The results of the precipitin tests of fed females (252) taken in 14 afternoon collections in the unsprayed comparison hut showed a striking increase in the proportion animal-fed (51.6 percent) over the morning collections (32.4 percent). Proportionally the human-fed declined from 67.6 percent to 48.4 percent during the corresponding period.

BIOASSAY TESTS. The results of bioassay tests made to determine the knock-down of *An. gambiae* in minutes by days after spray on mud and thatch surfaces are given in Table 3. The data show no knock-down in the first 30 minutes exposure period on mud and thatch surfaces throughout the period of the trial from 12 days to 89 days after application of the spray.

The first knock-down of any mosquito did not occur until after 1 hour of ex-

TABLE 1.—Comparison of hut densities of *An. gambiae* females collected in morning and afternoon pyrethrum collections in sprayed and unsprayed huts near Kisumu, Kenya 1971

Date 1971	Days after spray	Collection at 0700 hours				Collection at 1500 hours			
		Sprayed hut		Unsprayed hut		Sprayed hut		Unsprayed hut	
		No.	No. per hut	No.	No. per hut	No.	No. per hut	No.	No. per hut
22–23/6	3–4	70	35.0	551	275.5	1	0.5	369	184.5
6–7/7	17–18	43	21.5	116	58.0	2	1.0	183	91.5
20–23/7	31–34	13	6.5	68	34.0	2	1.0	69	34.5
3–6/8	45–48	26	13.0	89	44.5	4	2.0	153	76.5
17–20/8	59–62	12	6.0	74	37.0	7	3.5	68	34.0
31/8–3/9	73–76	8	4.0	17	8.5	1	0.5	58	29.0
14–17/9	87–90	15	7.5	77	38.5	16	8.0	57	28.5
Total		187	13.4 (Avg.)	992	70.9 (Avg.)	33	2.4 (Avg.)	957	68.4 (Avg.)

TABLE 2.—Comparison of precipitin tests of *An. gambiae* females taken in morning and afternoon pyrethrum spray collections in sprayed and unsprayed huts near Kisumu, Kenya 1971

Date 1971	Days after spray	Collection at 0700 hours						Collection at 1500 hours					
		Sprayed			Unsprayed			Sprayed			Unsprayed		
		No. tested	% positive		No. tested	% positive		No. tested	% positive [1]		No. tested	% positive	
			Human	Animal		Human	Animal		Human	Animal		Human	Animal
22–23/6	3–4	32	15.6	84.4	50	78.0	22.0	1	0/1	1/1	50	60.0	40.0
6–7/7	17–18	35	14.3	85.7	39	82.0	18.0	0	....	....	40	62.5	37.5
20–23/7	31–34	8	25.0	75.4	28	71.4	28.6	1	0/1	1/1	32	31.3	68.7
3–23/	45–48	15	26.7	73.3	40	35.0	65.0	3	0/3	3/3	55	34.5	65.3
17–20/8	59–62	10	10.0	90.0	26	96.2	3.8	2	0/2	2/2	24	25.0	75.0
31/8–8/9	73–76	7	57.1	42.9	11	27.3	72.7	0	....	....	30	76.7	23.3
14–17/9	87–90	13	38.5	61.5	13	53.8	46.2	8	12.5	87.5	21	42.9	57.1
Total		120	21.7	78.3	207	67.6	32.4	15	6.7	93.3	252	48.4	51.6

[1] Percentage not calculated with numbers fewer than five.

TABLE 3.—Time required for 'knock-down' of insectary bred *An. gambiae* females in bioassays of mudwall and thatch roof of a hut sprayed with fenitrothion at 2 g/m² near Kisumu,[1] Kenya 1971

Date 1971	Days after spray	No. tested	Percent knock-down in minutes															
			30	60	90	120	150	180	210	240	270	300	330	360	390	420	450	480
Mudwall																		
7/7	12	10	0	0	30.0	50.0	70.0	100	....	....	....	....	....	....	....	....	....	...
14/7	19	30	0	10.0	76.7	86.7	96.7	100	....	....	....	....	....	....	....	....	....	...
21/7	26	30	0	20.0	53.3	90.0	100	....	....	....	....	....	....	....	....	....	....	...
28/7	33	30	0	3.3	70.0	100	....	....	....	....	....	....	....	....	....	....	....	...
4/8	40	30	0	0	43.3	73.3	93.3	100	....	....	....	....	....	....	....	....	....	...
11/8	47	29	0	27.6	76.3	100	....	....	....	....	....	....	....	....	....	....	....	...
25/8	61	30	0	3.3	43.3	80.0	96.7	100	....	....	....	....	....	....	....	....	....	...
8/9	75	30	0	0	0	0	16.7	33.3	63.3	83.3	93.3	96.7	96.7	100	....	....	....	...
22/9	89	30	0	0	0	0	3.3	33.3	56.7	53.3	90.0	96.7	100	....	....	....	....	...
Thatch roof																		
7/7	12	28	0	3.6	25.0	32.1	50.0	78.6	100	....	....	....	....	....	....	....	....	...
14/7	19	30	0	0	20.0	56.7	86.7	96.7	96.7	100	....	....	....	....	....	....	....	...
21/7	26	29	0	0	3.4	6.9	20.7	55.2	86.2	93.1	96.5	96.5	100	....	....	....	....	...
28/7	33	29	0	0	6.9	31.0	69.0	93.1	100	....	....	....	....	....	....	....	....	...
4/8	40	28	0	0	0	7.1	21.4	39.3	78.6	92.9	92.9	96.4	100	....	....	....	....	...
11/8	47	29	0	0	3.4	34.3	72.4	82.8	86.2	93.1	96.6	100	....	....	....	....	....	...
25/8	61	28	0	0	3.6	7.1	25.0	60.7	82.1	92.9	96.1	100	....	....	....	....	....	...
8/9	75	30	0	0	0	0	3.3	6.7	10.0	13.3	33.3	56.7	70.0	76.7	90.0	90.0	93.3	100
22/9	89	27	0	0	0	0	7.4	14.8	29.6	48.1	81.5	85.2	96.3	100	....	....	....	...

[1] Nyagbongo (Entomology Station C).

posure on mud walls and 1½ hours on thatch and continued at this slow rate up to 60 days after spraying. During the concurrent period total knock-down took 2 to 3 hours on mudwalls and from 3½ to 6 hours on thatch.

From post-treatment days 75 to 90, the exposure period required for the first knock-down on mud walls and thatch extended to 2½ hours. For complete knock-down, the exposure period was prolonged from 5½ to 6 hours on mud walls and from 6 to 8 hours on thatch.

AIRBORNE EFFECT OF OMS-43. Data on the airborne effect of OMS-43 in sprayed huts are presented in Table 4. With a 12 hour exposure, the percentage of knock-down of caged insectary bred *An. gambiae* fed females is seen to decrease progressively in time after spraying. However, the percentage knock-down remained at a high level (above 80 percent) through the 43rd day after spraying. Thereafter the mortality was less than 50 percent.

DISCUSSION AND CONCLUSIONS. The results of this study support the observation of Smith (1961) that *An. gambiae* feeding outdoors on cattle and other domestic animals at night do enter huts in the early morning for day-time resting. This was confirmed in this study by the considerable numbers of animal-fed *An. gambiae* found in the morning pyrethrum-spray collections in both the sprayed and unsprayed comparison huts. The fact that the proportion of animal-fed *An. gambiae* to human-fed was much higher in the sprayed huts and the reverse in the unsprayed comparison huts is attributed to longer insecticidal exposure of the human-fed component of the population while resting and feeding indoors at night. The presence of a small number of human-fed females taken in the morning collections in the sprayed huts probably represents a segment of the indoor feeding population which entered the huts to feed and rest just before dawn. Such pre-dawn ingress and feeding of *An. gambiae* in Kenya was reported by Haddow (1954),

TABLE 4.—Airborne effect of fenitrothion on caged insectary bred *An. gambiae* females exposed overnight (12 hours) in sprayed huts near Kisumu,[1] Kenya 1971

Date and month 1971	Days after spray	Sprayed (5 huts) No. tested	Sprayed (5 huts) % knocked down in 12 hours exposure	Sprayed (5 huts) % 24-hour mortality	Unsprayed (1 hut) No. tested	Unsprayed (1 hut) % knocked down in 12 hours exposure	Unsprayed (1 hut) % 24-hour mortality
18/6	1	49	100	100	10	0	0
2/7	15	50	98.0	100	10	0	0
16/7	29	50	98.0	100	10	0	0
30/7	43	50	84.0	100	10	0	0
13/8	57	49	36.7	87.8	10	0	0
27/8	71	50	44.4[2]	66.7[2]	10	10.0	10.0
10/9	85	50	10.0	20.0[2]	10	0	10.0

[1] Wathorego (Entomology Station D).
[2] Corrected mortality, according to Abbott's formula.

Van Someren *et al.* (1958), Teesdale (1959) and Smith (1961).

Another reason for the considerable numbers of *An. gambiae* taken in the morning collections in the sprayed huts is due to the slow knock-down effect of OMS-43 which was shown in bioassays to take 3 and 4 hours for complete knock-down on sprayed mud surfaces and 4 hours on thatch even with fresh insecticide deposits only 12 days old. As a result the *An. gambiae* entering the sprayed huts early in the morning to rest were still alive at the time of the morning collection at 0700 hours. Presumably a faster acting insecticide with a knock-down of 1 hour or less would have reduced the morning collection to a fraction of the numbers observed.

Of special interest was the marked increase in the proportion of animal-fed *An. gambiae* to human-fed in the afternoon collection in the unsprayed huts. This change suggested day time ingress of animal-fed *An. gambiae* after the 7 a.m. collection, also observed by Van Someren *et al.* in studies of *An. gambiae* behaviour made near the Kenya Coast.

The greatly reduced numbers of *An. gambiae* taken in the afternoon collection from the sprayed huts compared with the morning collections are attributed to progressive mortality of the daytime resting population from insecticidal action of OMS-43 rather than from natural causes. This is clear from the collection data from the unsprayed huts which showed no appreciable difference in the average numbers taken between the morning and afternoon collections, thus indicating a low natural mortality of daytime resting *An. gambiae*.

Although OMS-43 exhibited an airborne effect on *An. gambiae,* the knock-down action was not considered rapid enough to have reduced appreciably the number of animal-fed *An. gambiae* entering the sprayed huts at dawn an hour or so before the morning collections at 0700 hours.

Smith *(ibid.)* in studies at Pare-Taveta, Tanzania, observed a higher hut ingress rate of animal-fed *An. gambiae* in places with little vegetation. The study site at Wawidhi was also sparsely vegetated around the Boma. Under the circumstances it appears that some of the animal-fed *An. gambiae* which had remained outdoors later in the morning were induced to enter nearby huts due to the increasing intensity of sunlight and rising temperature with the advance of the day. This additional ingress augmented the animal-fed population of *An. gambiae* resting indoors, thus increasing the proportion of animal fed to human fed specimens in the afternoon collections.

It is concluded from this study that the early morning pyrethrum spray collection does not accurately reflect the effectiveness of a slow knock-down residual insecticide such as OMS-43. This is particularly true in the Kisumu, Kenya area where the man and his domestic animals live in close proximity with indiscriminate feeding on both hosts and day-time resting in huts. A more accurate assessment of the insecticidal effect should be obtained by delaying the collections until late afternoon, after the full impact of the insecticidal action on the day-time resting population had occurred.

SUMMARY

An entomological evaluation of the effectiveness of fenitrothion (OMS-43) as a residual spray in houses for the control of *An. gambiae* in a field trial near Kisumu, Kenya, showed on occasions higher than expected house densities of *An. gambiae* in morning pyrethrum spray collections as early as 11 days after spraying.

These findings suggested insecticidal failure but this was shown not to be the case according to the results of this special study in 1971. It was established that the occasional higher house resting densities were mainly due to animal-fed *An. gambiae* which had entered the sprayed houses at dawn for day-time resting after having fed outdoors at night on cattle and other domestic animals penned near the houses.

Due to the short period of insecticidal exposure between the time of house entry at dawn and the time of collection at 0700 hours there were still many survivors. When the collections were delayed until the afternoon there were few survivors, averaging less than one per house.

Bioassay tests made of fenitrothion residues on mud walls and thatch surfaces in this study confirmed a slow knock-down rate for the insecticide, taking 3 to 4 hours to produce 100 percent kill on relatively fresh deposits. It was concluded therefore, that evaluation of the insecticidal effect on resting *An. gambiae* would be more accurately determined by afternoon collections in an area such as Kisumu, Kenya where a proportion of *An. gambiae* population feed on domestic animals outdoors at night and enter houses at dawn for day time resting.

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