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**OBSERVATIONS ON THE NIGHTTIME RESTING AND BITING  
HABITS OF ANOPHELINE MOSQUITOES IN DDT-TREATED  
AND -UNTREATED BUILDINGS <sup>1</sup>**

By CLARENCE M. TARZWELL, *Senior Assistant Sanitarian (R)* and FRANK W. FISK, *Sanitarian (R)*, *United States Public Health Service*

Laboratory cage tests and controlled experiments in houses have clearly demonstrated that residual-spray deposits of DDT are lethal to mosquitoes for considerable periods. Although these tests gave

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valuable information on the durability of DDT residual deposits, they did not give information on the mortality of malaria mosquitoes naturally entering treated dwellings in search of a blood meal. In order to secure a lethal dose of DDT from residual deposits, mosquitoes must actually touch the material and be exposed to it for a considerable period. This period has been shown to vary (1), depending on the temperature, age of treatment, density and distribution of the DDT crystals, and the resistance of the individual mosquitoes. Thus, the habits of the mosquitoes in question are of prime importance in determining the likelihood of their being exposed to DDT deposits for a sufficient time to produce death. If, after entering a treated house, mosquitoes spend all or most of their time flying around, or if they proceed directly to a host, feed, and leave immediately, it is obvious that they would not secure a lethal exposure to the DDT. While it has been known for some time that *Anopheles quadrimaculatus* mosquitoes spend most of their daytime hours resting quietly in dark, damp, cool, quiet places, no detailed information has been noted on their hour-to-hour activities in buildings during the night or on the length of time they rested on walls or ceilings before or after feeding. It was to gain some idea of these activities that the studies herein described were undertaken.

#### PROCEDURE

Observations on the nighttime behavior and resting habits of anopheline mosquitoes were conducted in rooms especially prepared for the study. The walls and ceilings of these rooms were marked off by means of chalk lines into rows of squares, each of which had an area of approximately 1 square yard. Each row was designated by a letter and each square by a number, so that they could be easily located. Scale drawings were made of the walls and ceilings, showing the squares and all surfaces upon which a mosquito might rest. These charts were used for plotting the exact location of all mosquitoes observed during the night studies. For rapidity of observation the room was divided into sections, and each observer was furnished with a drawing of the section assigned to him.

The rooms used in the study had one or more windows and doors which were left open so that mosquitoes could enter or leave at will. A cow, goat, or the observers themselves served as attractants for the mosquitoes.

Each night study was divided into observation periods which were spaced at intervals ranging from 15 minutes to over an hour, depending on the number of mosquitoes to be counted and their degree of restlessness. All observation periods were numbered consecutively throughout the night. These numbers were used as subscripts to the

symbols representing the mosquitoes, to indicate the periods when the mosquito was first and last seen. At each counting period, the observer used a flashlight to cover systematically the area corresponding to that appearing on the chart. He began at the same spot each time and plotted the location of all resting mosquitoes seen by placing a symbol on the chart at the proper point. A different symbol was used to indicate the type of mosquito seen (whether anopheline or culicine, engorged or unengorged). During each observation period, the locations of all mosquitoes were checked against the symbols on the chart, and if a mosquito corresponded with a point plotted for a preceding period, it was presumed to be the same mosquito. If a mosquito was not indicated by a symbol on the chart, it was judged to have just entered the building, and a new mosquito locus was plotted on the chart, and given a subscript indicating the number of the period. If there was no mosquito for a previously plotted symbol, the number of the period in which it was last seen was used as the second subscript number to indicate the length of the resting period. Thus, an analysis of the data from the charts of all observers on a given night enabled the computation of the average resting period for each type of mosquito noted, as well as the actual number of each type present at various times of the night. Since every mosquito which shifted resting positions during the night accounted for more than one mosquito locus, the total number of mosquito loci plotted throughout the night was always greater than the total number of mosquitoes actually entering the room. To reduce this error to a minimum, care was exercised in the use of the flashlights.

These overnight studies were carried out in two types of buildings, a small cow barn before and after treatment, and a test room 3 and 4 months after treatment.

Two all-night observations were conducted in the small cow barn before treatment, and a third observation was made after spraying. During these observations, a cow was tethered in the building as a bait animal. The pretreatment counts were made on the nights of September 1 and 5, 1944, and the posttreatment count on the night of September 25, 1944. The treatment consisted of 200 mg. of DDT per square foot. Studies in the test room were carried out in late September 1945, 4 months after a treatment at the rate of 117 mg. of DDT per square foot. A crated goat was used as a bait animal, in addition to the three observers who were present during the test. All windows were left open and unscreened, to allow free movement of the mosquitoes into and out of the room. Early in the evening, observations were made at hourly intervals, but these intervals were reduced to 15 minutes when it became apparent that the mosquitoes were all moving at least once during each period.

Five overnight studies were made on the biting habits and knock-down of mosquitoes in this same test room 3 months after spraying. During these studies, the investigator spent the night on a cot without a bed net, so that the mosquitoes which entered could feed on him at will. He noted the number of biting attempts and made gross observations throughout the night as to the number and kind of mosquitoes in the room. During the first three studies, an exit trap was placed in one window, while the other two windows were left open on the first night and provided with inlet cones on the second and third nights. The inlet windows were screened an hour before dawn to prevent the entrance of mosquitoes seeking daytime resting places. In the last two studies, no traps were used and the windows were not screened to prevent the entrance of mosquitoes just before dawn. All mosquitoes were collected from the test room and the exit trap at approximately 9 o'clock the following morning and classified as to species and condition. Precipitin tests were made on all fully engorged females for the determination of blood meals.

Two similar studies were made, with a cow as bait, in a barn which had been sprayed 11 months previously at the rate of 200 mg. of DDT per square foot. Sheets were spread on the floor during the night to catch the moribund mosquitoes. These were gathered up at 5:30 a. m. so as to retain all mosquitoes knocked down during the night, while eliminating those mosquitoes entering in search of a daytime resting place.

#### RESULTS AND DISCUSSION

The numbers of engorged and unengorged *A. quadrimaculatus* females noted in the cow barn during the two prespraying studies made in September 1944, are shown in table 1. In each of the studies engorged mosquitoes accounted for only about 14 percent of the total number observed, even though a cow was in the barn throughout

TABLE 1.—Results of nighttime counts of *Anopheles quadrimaculatus* mosquito loci in an untreated barn, with calculated average resting periods for unengorged and engorged females

| Date               | Unengorged females |                             |                    | Engorged females |                             |                    |
|--------------------|--------------------|-----------------------------|--------------------|------------------|-----------------------------|--------------------|
|                    | Number             | Resting period (in minutes) |                    | Number           | Resting period (in minutes) |                    |
|                    |                    | Average                     | Standard deviation |                  | Average                     | Standard deviation |
| Sept. 1-2.....     | 1,227              | 184±4                       | ±138               | 211              | 180±9                       | ±129               |
| Sept. 5-6.....     | 770                | 140±3                       | ±86                | 134              | 155±10                      | ±115               |
| <i>Total</i> ..... | 1,997              | 167±3                       | ±122               | 345              | 170±7                       | ±124               |

the night. Contrary to expectations, the observed resting period of the unengorged and engorged mosquitoes was not significantly different. For the two nights, the observed average resting period for the unengorged mosquitoes was 167 minutes, and for the engorged 170 minutes.

There was considerable variation in the observed resting period of the mosquitoes, some remaining in place for only a few minutes, others remaining in place for over 10 hours. It was evident that some mosquitoes moved due to the disturbing influence of the lights used in counting. Since the actual time that each mosquito rested on a particular spot was always greater than the observed resting period, the averages are somewhat low. Further, the calculation of these averages was complicated by the fact that over a third of the total number, or some 909 mosquitoes, did not move after alighting and were still in place at 7 a. m. and remained in the same position during most of the day. Because it was desired to obtain the average nighttime resting period, it was arbitrarily decided to include only the period up to 7 a. m. in the calculation of the average resting periods.

The posttreatment study was conducted later in September and on a somewhat cooler night, during which the temperature dropped to 62° F. As a result, a much smaller number of mosquitoes entered the barn. The number of mosquitoes observed resting in the barn and their average resting periods are shown in table 2.

TABLE 2.—Numbers of *Anopheles quadrimaculatus* females resting in a DDT-sprayed barn, with average resting periods

| Date             | Unengorged females |                             |                    | Engorged females |                             |                    |
|------------------|--------------------|-----------------------------|--------------------|------------------|-----------------------------|--------------------|
|                  | Number             | Resting period (in minutes) |                    | Number           | Resting period (in minutes) |                    |
|                  |                    | Average                     | Standard deviation |                  | Average                     | Standard deviation |
| Sept. 24-25..... | 59                 | 40±3                        | ±24                | 27               | 33±4                        | ±20                |

As indicated, the resting period after treatment was greatly shortened, possibly due to the irritating effect of the DDT deposit. Differences in the resting periods before and after treatment and the percentage of the total mosquitoes resting for stated periods are shown graphically in figure 1. Although the average resting period of *A. quadrimaculatus* on an untreated surface is considered adequate for obtaining a lethal dose of DDT under most conditions, it is apparent that the normal resting periods do not prevail after treatment

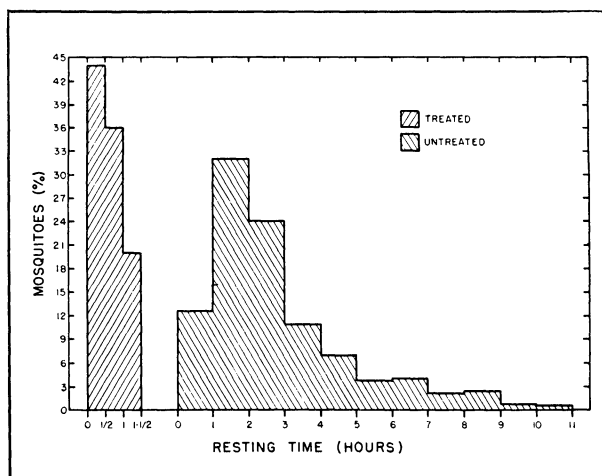


FIGURE 1.—Nighttime resting periods of *Anopheles quadrimaculatus* mosquitoes in a cow barn before and after treatment with 200 mg. of DDT per square foot as indicated by the percentages resting for stated intervals.

and cannot be used in determining exposure to sprayed surfaces. However, it is indicated that, in general, a large percentage of the mosquitoes will rest on treated surfaces for a period sufficient to insure a lethal dose. The fact that before treatment only about 14 percent of the observed *A. quadrimaculatus* females were engorged, even though a cow was continually present in the barn, indicates that many of those naturally entering buildings for the purpose of feeding rest on walls for some time before feeding, as well as after they have fed. In the treated barn about 31 percent of the resting females were engorged. This greater percentage of engorged females in the sprayed barn may indicate that many mosquitoes which entered and rested on the walls temporarily before biting, were so irritated that they left without biting, thus increasing the ratio of fed to unfed individuals. This may be the explanation for the lack of mosquito annoyance experienced by occupants of treated houses immediately after spraying, who often report freedom from bites for the first week or two. Following this there is a period of several weeks during which the annoyance gradually increases, even though a high percentage of the biting mosquitoes are subsequently killed by exposure to sprayed surfaces.

At dusk a considerable number of mosquitoes entered the barn in a few minutes; this occurred both before and after treatment. Before treatment the number of mosquitoes resting in the barn increased progressively throughout the night (fig. 2). The average number of mosquitoes present each hour and the increase in number each hour

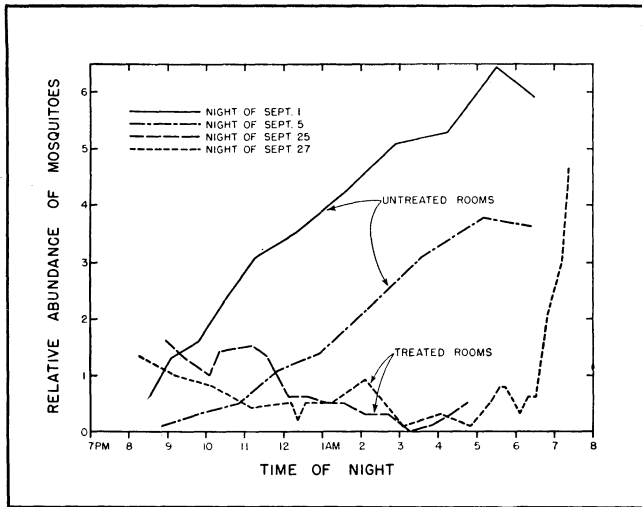


FIGURE 2.—A comparison of the behavior and trends in abundance of nighttime resting *Anopheles quadrimaculatus* mosquitoes in untreated and treated buildings. Each unit on the vertical scale represents 100 mosquitoes for the untreated rooms and 10 mosquitoes for the treated rooms.

are shown in table 3. The number of unengorged mosquitoes increased throughout the night, with the greatest increase occurring between 1:30 and 2:30 a. m. The engorged mosquitoes decreased steadily after 11:30 p. m.

TABLE 3.—Observed numbers of *Anopheles quadrimaculatus* mosquitoes resting in an untreated barn each hour of the night (average of 2 studies), and the increase each hour

| Time        | Unengorged females | Increase | Engorged females | Increase | Total females | Total increase |
|-------------|--------------------|----------|------------------|----------|---------------|----------------|
| 8:30 p. m.  | 22                 | 22       | 12               | 12       | 34            | 34             |
| 9:30 p. m.  | 60                 | 38       | 25               | 13       | 85            | 51             |
| 10:30 p. m. | 93                 | 33       | 41               | 16       | 134           | 49             |
| 11:30 p. m. | 129                | 36       | 63               | 22       | 192           | 58             |
| 12:30 a. m. | 185                | 56       | 61               | -2       | 246           | 54             |
| 1:30 a. m.  | 239                | 54       | 58               | -3       | 297           | 53             |
| 2:30 a. m.  | 307                | 68       | 55               | -3       | 362           | 75             |
| 3:30 a. m.  | 365                | 56       | 48               | -7       | 411           | 49             |
| 4:30 a. m.  | 408                | 45       | 44               | -4       | 452           | 41             |
| 5:30 a. m.  | 465                | 57       | 43               | -1       | 508           | 46             |
| 6:30 a. m.  | 440                | -25      | 46               | 3        | 486           | -22            |

After treatment conditions were reversed. Following the rapid influx at dusk (fig. 2), the number of mosquitoes in the barn did not increase, and after midnight decreased. The number counted at each observation period is shown in table 4. Differences in the number of mosquitoes present during each hour of the night before and after treatment are shown graphically in figure 2. The reduction of the number of resting mosquitoes in a treated building, as indicated in figure 2, might well be one reason for the protection afforded by DDT residual sprays.



TABLE 4.—Numbers of *Anopheles quadrimaculatus* mosquitoes resting in a DDT-treated barn at various times during the night and the increase or decrease between periods

| Time             | Unengorged females | Increase | Engorged females | Increase | Total females | Total increase |
|------------------|--------------------|----------|------------------|----------|---------------|----------------|
| 9 p. m. ....     | 14                 | 14       | 2                | 2        | 16            | 16             |
| 9:30 p. m. ....  | 11                 | -3       | 2                | 0        | 13            | -3             |
| 10 p. m. ....    | 6                  | -8       | 4                | 2        | 10            | -3             |
| 10:30 p. m. .... | 10                 | 2        | 4                | 0        | 14            | 1              |
| 11 p. m. ....    | 12                 | 2        | 3                | -1       | 15            | 1              |
| 11:30 p. m. .... | 11                 | -1       | 2                | -1       | 13            | -2             |
| 12 m. ....       | 3                  | -8       | 3                | 1        | 6             | -7             |
| 12:30 a. m. .... | 3                  | 0        | 3                | 0        | 6             | 0              |
| 1 a. m. ....     | 3                  | 0        | 2                | -1       | 5             | -1             |
| 1:30 a. m. ....  | 3                  | 0        | 2                | 0        | 5             | 0              |
| 2 a. m. ....     | 2                  | -1       | 1                | -1       | 3             | -2             |
| 2:30 a. m. ....  | 2                  | 0        | 1                | 0        | 3             | 0              |
| 3:15 a. m. ....  | 0                  | -2       | 0                | -1       | 0             | -3             |
| 3:45 a. m. ....  | 1                  | 1        | 0                | 0        | 1             | 1              |
| 4:15 a. m. ....  | 2                  | 1        | 1                | 1        | 3             | 2              |
| 4:45 a. m. ....  | 2                  | 0        | 3                | 2        | 5             | 2              |

Observations in a treated room indicated much the same conditions as those observed in the barn after treatment. Following the rapid influx of mosquitoes at dusk, the mosquitoes rested only a short period and left. This condition existed until about 6:30 a. m., when there was an influx of mosquitoes in search of daytime resting places (fig. 2). The number of mosquitoes observed at each counting period is shown in table 5.

TABLE 5.—Observed numbers of *Anopheles quadrimaculatus* mosquitoes resting in a DDT-treated room at various times during the night

| Time             | <i>Anopheles quadrimaculatus</i> females |          |       | Culicines |
|------------------|--|----------|-------|-----------|
|                  | Unengorged                               | Engorged | Total | Total     |
| 8:20 p. m. ....  | 13                                       | 0        | 13    | 22        |
| 9:05 p. m. ....  | 10                                       | 0        | 10    | 15        |
| 10:05 p. m. .... | 7  | 1        | 8     | 0         |
| 11:05 p. m. .... | 3  | 1        | 4     | 1         |
| 12:10 a. m. .... | 4  | 1        | 5     | 1         |
| 12:20 a. m. .... | 2  | 0        | 2     | 1         |
| 12:35 a. m. .... | 5  | 0        | 5     | 2         |
| 12:50 a. m. .... | 5  | 0        | 5     | 2         |
| 1:15 a. m. ....  | 5  | 0        | 5     | 4         |
| 2:05 a. m. ....  | 8  | 1        | 9     | 3         |
| 3:05 a. m. ....  | 1  | 0        | 1     | 1         |
| 4:05 a. m. ....  | 3  | 0        | 3     | 4         |
| 4:50 a. m. ....  | 1  | 0        | 1     | 0         |
| 5:20 a. m. ....  | 3  | 2        | 5     | 6         |
| 5:35 a. m. ....  | 7  | 1        | 8     | 3         |
| 5:50 a. m. ....  | 8  | 0        | 8     | 2         |
| 6:05 a. m. ....  | 3  | 0        | 3     | 2         |
| 6:20 a. m. ....  | 6  | 0        | 6     | 0         |
| 6:35 a. m. ....  | 6  | 0        | 6     | 2         |
| 6:50 a. m. ....  | 17                                       | 4        | 21    | 3         |
| 7:05 a. m. ....  | 17                                       | 18       | 30    | 5         |
| 7:20 a. m. ....  | 24                                       | 23       | 47    | 3         |

These results also indicate that in treated buildings the mosquitoes quickly become irritated, so that their resting period is reduced to a matter of minutes. Since no anopheline mosquitoes and only one of the culicines remained beyond the 15-minute intervals, it is assumed

that the maximum resting period is less than 15 minutes. Engorged mosquitoes comprised 23 percent of the observed mosquitoes.

The results of the five overnight biting and knock-down studies which were conducted in the test room 4 months after treatment at the rate of 117 mg. of DDT per square foot are summarized in table 6. In the first three studies the entrance windows were screened and

TABLE 6.—Numbers of naturally entering mosquitoes, active and knocked-down, recovered from a test room which had been sprayed 4 months previously with DDT, and the source of their blood meals as indicated by precipitin tests

| Group                        | <i>Anopheles quadrimaculatus</i> |                       |       | Culicines         |            |       |
|------------------------------|----------------------------------|-----------------------|-------|-------------------|------------|-------|
|                              | Engorged                         | Unengorged            |       | Engorged          | Unengorged |       |
|                              | Females                          | Females               | Males | Females           | Females    | Males |
| <b>GROUP 1</b>               |                                  |                       |       |                   |            |       |
| (3 studies)                  |                                  |                       |       |                   |            |       |
| (Windows closed before dawn) |                                  |                       |       |                   |            |       |
| Down on floor.....           | 5 H, <sup>1</sup> 1 U.....       | 41<br><i>A. cr.</i> 2 | 1     | 1 H, 4 N.....     | 129        |       |
| Alive in room.....           |                                  |                       |       |                   | 1          |       |
| Down in traps.....           | 3 N, 2 U.....                    | 15                    |       |                   | 14         |       |
| Alive in traps.....          | 1 H, 1 N.....                    | 10                    |       |                   | 3          |       |
| <b>GROUP 2</b>               |                                  |                       |       |                   |            |       |
| (2 studies)                  |                                  |                       |       |                   |            |       |
| (Windows not closed)         |                                  |                       |       |                   |            |       |
| Down on floor.....           | 1 H, 7 E, 30 B,<br>7 N, 9 U..... | *56                   | 1     | 3 H, 1 N, 1 U.... | 11         | 1     |
| Alive in room.....           | 4 H, 2 E, 35 B,<br>6 N, 6 U..... | *45                   |       |                   | 2          | 1     |

<sup>1</sup> Supplementary key:  
 H = Human.  
 U = Unsatisfactory for test.  
 N = No reaction.  
*A. cr.* = *Anopheles crucians*.  
 E = Equine.  
 B = Bovine.  
 \* = Many were partly engorged.

hour before dawn to shut out those mosquitoes in search of daytime resting places. When this was done, no live *A. quadrimaculatus* mosquitoes and only one live culicine mosquito were found in the room at the 9 a. m. inspection. A total of 34 dead and 15 live mosquitoes were taken in the exit trap. The live mosquitoes were killed for the determination of their blood meals. A total of 18 engorged females, only one of which was alive at the time of inspection, was recovered from the three studies. Of these only six gave positive blood reactions and all of these were for human blood, presumably that of the observer. A total of 215 unengorged mosquitoes was taken. Thus, the engorged mosquitoes comprised only about 7 percent of the total taken. These results indicate considerable protection against biting.

In the last two studies all windows were entirely open and no attempt was made to keep out the predawn flight of *A. quadrimaculatus*. At the 9 a. m. inspection only about half of the mosquitoes had been knocked down, which would seem to indicate that many mosquitoes had entered in search of daytime resting places and sufficient time had not yet elapsed for them to be knocked down. (The  $KD_{60}$  for daytime releases of mosquitoes in the room was 120 minutes at that time.) Of the total active and morbid *A. quadrimaculatus* mosquitoes recovered from the room, 41 percent were engorged sufficiently to permit precipitin tests, which showed a number of blood sources, as indicated in table 6. Since bovine blood predominated, it is evident that many entered to rest rather than to feed. However, four of those which were still alive had fed on human blood, whereas only one which was down on the floor had fed on human blood. Over half of the recovered anopheline mosquitoes were engorged, and approximately 10 percent of these had fed on human blood.

In every test the operator reported several times more bites than the number of mosquitoes fully engorged with human blood which were recovered. This was due in part to the escape of the engorged mosquitoes, as indicated by the numbers taken in the exit traps when these traps were in place, and perhaps to several attacks by the same mosquito in becoming fully engorged.

The results of the two overnight biting and knock-down studies in a cow barn sprayed 11 months previously are summarized in table 7.

TABLE 7.—Number of mosquitoes knocked down in a small barn sprayed 11 months previously with 200 mg. DDT per square foot with the source of blood meals indicated (totals from 2 nights' studies)

| Species                         | Engorged females   | Unengorged females | Males |
|---------------------------------|--|--------------------|-------|
| <i>A. quadrimaculatus</i> ..... | 110 bovine, 13 negative, 2 unsatisfactory .....          | 10                 | 1     |
| <i>Culicines</i> .....          | 24 bovine, 1 avian, 14 negative, 14 unsatisfactory ..... | 21                 | 1     |

These results show that over 11 months after treatment a considerable number of mosquitoes are knocked down before they are able to leave. As all the engorged mosquitoes satisfactory for precipitin tests had been feeding on the cow, it is probable that they had fed in the barn. Among the mosquitoes which had been knocked down and recovered from the barn floor, the engorged ones outnumbered the unengorged by about  $2\frac{1}{2}$  to 1.

#### SUMMARY

*Anopheles quadrimaculatus* mosquitoes which enter buildings to feed rest on walls or ceilings for considerable periods before as well as after feeding.

The observed nighttime resting period of unengorged and engorged *A. quadrimaculatus* females in an untreated building was not significantly different. The resting period varied greatly, ranging from a few minutes to over 11 hours.

In treated buildings the observed resting period was much shorter than that for untreated buildings, and the range was much less, varying from a few to 90 minutes. The average observed resting period for unengorged and engorged *A. quadrimaculatus* females was not significantly different, being  $40 \pm 3$  minutes for the former and  $33 \pm 4$  minutes for the latter.

After treatment, the percentage of engorged females resting on the walls increased from 14 to 31 percent, perhaps indicating that many of the unengorged mosquitoes are irritated by the DDT and leave before they attempt to bite. Immediately after spraying, irritation is produced in such a short time that considerable protection against biting is afforded.

In the untreated building, the number of *A. quadrimaculatus* females increased throughout the night, reaching a maximum about an hour before daylight, whereas after treatment, the largest number was present just after the influx at dusk, and only a small number of mosquitoes were present at any time during the remainder of the night.

#### ACKNOWLEDGMENT

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#### REFERENCE

- (1) Tarzwell, C. M., and Stierli, H.: The evaluation of DDT residual sprays for the control of anopheline mosquitoes in dwellings. Pub. Health Rep. Supplement No. 186, pp. 35-48 (1945).