R57-10

Reprinted from ANNALS OF THE ENTOMOLOGICAL SOCIETY OF AMERICA,
Vol. 44, No. 1, March, 1951.

LOCI OF SENSORY END-ORGANS USED BY MOSQUITOES (AEDES AEGYPTI (L.) AND ANOPHELES QUADRIMACULATUS SAY) IN RECEIVING HOST STIMULI

LOUIS M. ROTH
Quartermaster General Laboratories,
2800 S. 20th Street, Philadelphia 45, Pa.

During the recent war intensive investigations on mosquito repellents were carried out in various countries. Much of this research involved testing large numbers of chemical compounds as possible repellents for protecting troops in mosquito-infested areas (Christophers, 1947; McCullogh and Waterhouse, 1947; Travis *et al.*, 1949). However, interest in the fundamental problem of the nature of the stimuli which attract mosquitoes to their hosts was revived (*e.g.*, Willis, 1947; Parker, 1948) and the results of these and other workers have shown that moisture, temperature, to some extent body odor, and carbon dioxide (for at least one species of *Anopheles;* van Thiel, 1947), are factors in attracting mosquitoes. Most of the evidence to date indicates that the olfactory organs of insects are located in the antennae (see reviews by Marshall, 1935, Wigglesworth, 1939, Dethier and Chadwick, 1948). Bates (1949) in reviewing the food habits of adult mosquitoes stated that the ability to orient to odors by means of the antennae ". . . is shared by so many different insects that it can safely be assumed to exist in mosquitoes even though in this particular case direct experimental evidence is lacking." The following work was done to add to our knowledge of mosquito behavior by determining the sensory end-organs of *A. aegypti* (L.) and *A. quadrimaculatus* Say which receive host stimuli.

EXPERIMENTAL METHODS

The following experiments involved the study of the behavior of female mosquitoes which had various sense organs removed. The mosquitoes were fed on water and honey solution and none had a blood meal prior to testing. Operations were performed with microdissecting scissors, on mosquitoes kept under carbon dioxide anesthesia in the manner described by Roth (1948). The individual operations are performed in only a few seconds but females can be kept under continuous anesthesia for as long as 60 minutes without affecting their feeding behavior after recovery. Two hours of continuous anesthesia may result in high mortality of the test insects. The ages of *A. aegypti* used ranged from 4 to 11 days and those of *A. quadrimaculatus* were 5 to 14 days old. Unless otherwise stated all experimental and control insects were placed in cages 11″ x 11″ x 15″ and at various intervals after their recovery from anesthesia their responses (*i.e.*, attraction, probing, feeding) to the human arm and hand, and temperature alone, were observed.

EXPERIMENTS ON AEDES AEGYPTI

Experiment 1. To determine if the eyes are necessary for the female to locate its host.

The eyes of 30 females were covered with a layer of shellac rendered opaque with carbon black. The shellac was applied with a micropipette. In most cases the shellac also, unavoidably, covered the pedicelli of the antennae and the palpi (often cementing the palpi to the base of the labial theca).

Generally these "blind" mosquitoes were not as active nor as readily disturbed as normals. However, in subsequent tests practically all were activated to flight, within 3 minutes, and readily found the arm when it was introduced into the cage. In several exposures, although many probed, only 4 were capable of taking blood. It was found that these 4 mosquitoes had little or no shellac at the base of the labium and their palpi were not cemented to the labium. On the contrary, practically all the females which probed but could not pierce the skin had their palpi cemented to the labium. This prevented the labium from being "kinked" during probing, so that the tips of the stylets could not be inserted into the skin. This was shown to be the case when shellac was applied to 13 other females, cementing their palpi to the base of the labium (eyes not covered). Though all probed readily, none were capable of taking blood. Shellac placed on the ventral surface of the labium near its base did not prevent feeding as long as the theca was not united to the base of the stylets and was capable of being bent under the head.

Conclusion: The eyes are not necessary, in a small area such as a cage, in locating the host.

Experiment 2. To determine the role of the antennae in locating the host and probing.

A. When the arm is placed in a large celluloid cylinder (20″ long x 6″ wide) with a cheesecloth barrier at one end, and the tube is introduced into a cage containing *A. aegypti* females, the mosquitoes are activated to flight, are attracted by the arm in the cylinder, and may fly down the tube to rest and probe through the cheesecloth barrier which is being held in place by the hand of the observer (Fig. 1). To determine if individuals without antennae would react in a similar manner, the following experiment was performed.

Twelve flagellar segments from both antennae were removed from 80 females. Twelve flagellar segments from one antenna from each of 80 females were removed (40 with the right and 40 with the left antenna missing to the first flagellar segment). Eighty controls with both antennae intact plus the 160 experimentals (a total of 240 mosquitoes) were placed in one cage. After 18 hours, 6 females lacking both antennae and 4 females with one antenna missing were dead or unable to fly, and these were discarded. The celluloid cylinder was then introduced to determine the response of the mosquitoes. Three 3-minute trials were made. None of the 74 females lacking both antennae were recovered in the cylinder; twenty-two (29%) of those lacking one antenna came down the tube (9 with the right and 13 with the left flagellar segments removed); seventy-one (89%) controls came down the cylinder to probe through the cheesecloth. The mosquitoes which came down the cylinder were removed from the cage.

Six hours later the celluloid cylinder was again introduced into the cage. However, this time a rabbit was placed against the cylinder outside the cage. Eight mosquitoes came down to feed and all of these were normal controls. Thus 99% of the controls were attracted to man and rabbit. Twenty-nine percent of the females with one antenna missing were attracted to man while no mosquitoes with both antennae missing to the first flagellar segment were attracted into the cylinder.

The operated mosquitoes were placed in 5″ x 6″ circular, wire-screen cages and their feeding response was tested on the arm. Again it was found that in general mosquitoes lacking 12 flagellar segments from both antennae were not attracted to man, but if the cage was placed so that

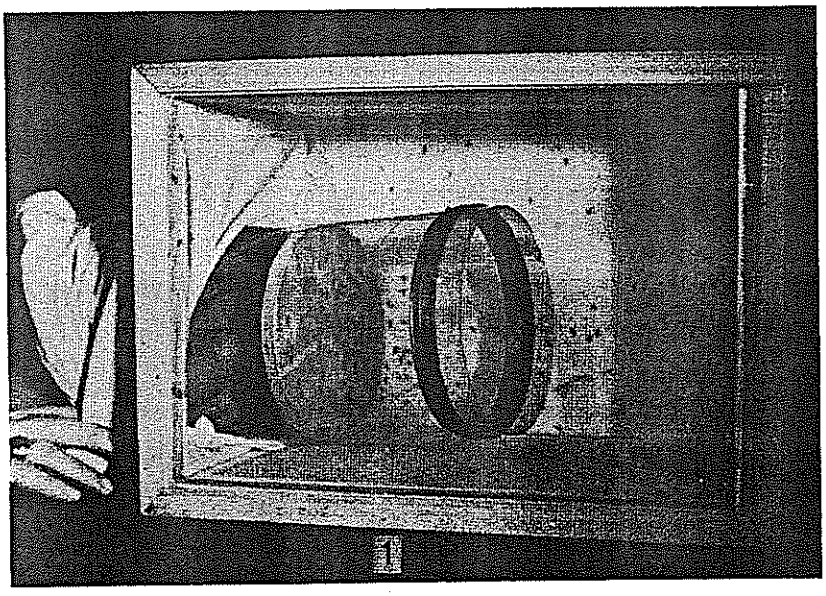


FIG. 1. *Aedes aegypti* females attracted to the human arm and hand. The celluloid cylinder was originally designed by Dr. Edwin R. Willis, Dr. Ralph C. Davidson, and Mr. Robert L. Peffley, of The Ohio State University, to isolate females which are attracted to man and presumably would take a blood meal.

the females rested directly above the skin (separated from it by the wire screen), some eventually probed and fed. If feeding was interrupted and the cage moved, the females probed haphazardly. If by chance they came to rest on the portion of the screen next to the arm, they would proceed to feed. Those females with one antenna missing were capable of finding the arm and feeding.

B. One of the most characteristic responses of *A. aegypti* females to man is obtained when the arm is introduced into a cage containing these insects. Almost immediately the females are activated to flight, quickly settle on the skin and feed (Fig. 2). The indifference of mosquitoes lacking the flagellar segments of the antennae was again clearly shown

in the following experiment. The flagellar segments from both antennae were removed from 50 females, one antenna from 50 others, and 50 unoperated insects were used as controls. These 3 groups were placed in 3 different cages. Twenty-two hours later the mosquitoes were tested by placing the arm within each cage for a period of 3 minutes; none of the 49 mosquitoes (one dead) lacking both antennae responded (Fig. 3); 45 out of 48 (two dead) females lacking the flagellar segments from one antenna landed and fed; 50 controls were attracted and fed. Both the controls and those lacking one antenna were discarded. The females lacking the flagellar segments from both antennae were kept and tested at various intervals with the following results:

After 30 hours the arm was introduced into the cage. During the 3-minute exposure 3 or 4 mosquitoes began to fly about. One landed on the arm but neither probed nor fed.

At the 44-hour trial, one landed on the finger and fed during a 3-minute exposure. There was no direct flight to the arm, the mosquito apparently landing by chance. Two or three mosquitoes flew aimlessly about, the others remaining quiescent.

At the 51-hour trial period several females flew about the cage, but none landed on the arm. Two females were seen to probe the cloth mesh of the cage. The arm was kept in the cage, and some of the mosquitoes were made to walk on the skin. In a one-half-hour exposure, 7 females fed to repletion, while at least that many remained indifferent, though resting on the skin. Further tests were made at 68, 79, 91, and 108-hour intervals. None of the mosquitoes were attracted to the arm. At the 91-hour trial, 2 females fed during a one-half-hour exposure, when placed directly on the skin. However, others remained indifferent even though they rested on the skin.

C. The flagellar segments of both antennae from 64 females were removed, and the remaining stumps were covered with shellac (applied with a micropipette). It was assumed that this operation completely eliminated the functioning of the antennae. In covering the antennal remains, parts of the eyes were unavoidably covered with shellac.

The arm and hand was exposed to the mosquitoes for six 10-minute trials at 16, 24, 42, 48, 70, and 90 hours after the operations. During these exposures only 5 females landed on the arm and fed. There was no direct flight to the arm. During some of the trials several mosquitoes would land on the arm and rest without feeding, or fly away after a short time.

The flagellar segments of both antennae from 50 females were removed as close to the pedicelli as possible, usually leaving only small stumps of the first flagellar segments protruding slightly beyond the margins of the pedicelli. At various intervals the arm was introduced into the cage (usually one-half-hour exposures), and the mosquitoes were disturbed and made to land on the skin to test their feeding response.

At 17 hours (after the operations) 1 fed; 20-hour trial, 7 fed; 34-hour trial, 10 fed; 39-hours, 7 fed; 60 hours (1 dead), 7 fed; 116 hours, 4 fed. Of a total of 48 (2 dead at the first trial) experimental females, 36 (75%) fed when made to land or placed directly on the skin. Six tests were made, the mosquitoes being exposed to the arm for a total of 3 hours. Eleven females failed to feed. Generally, those females that fed often

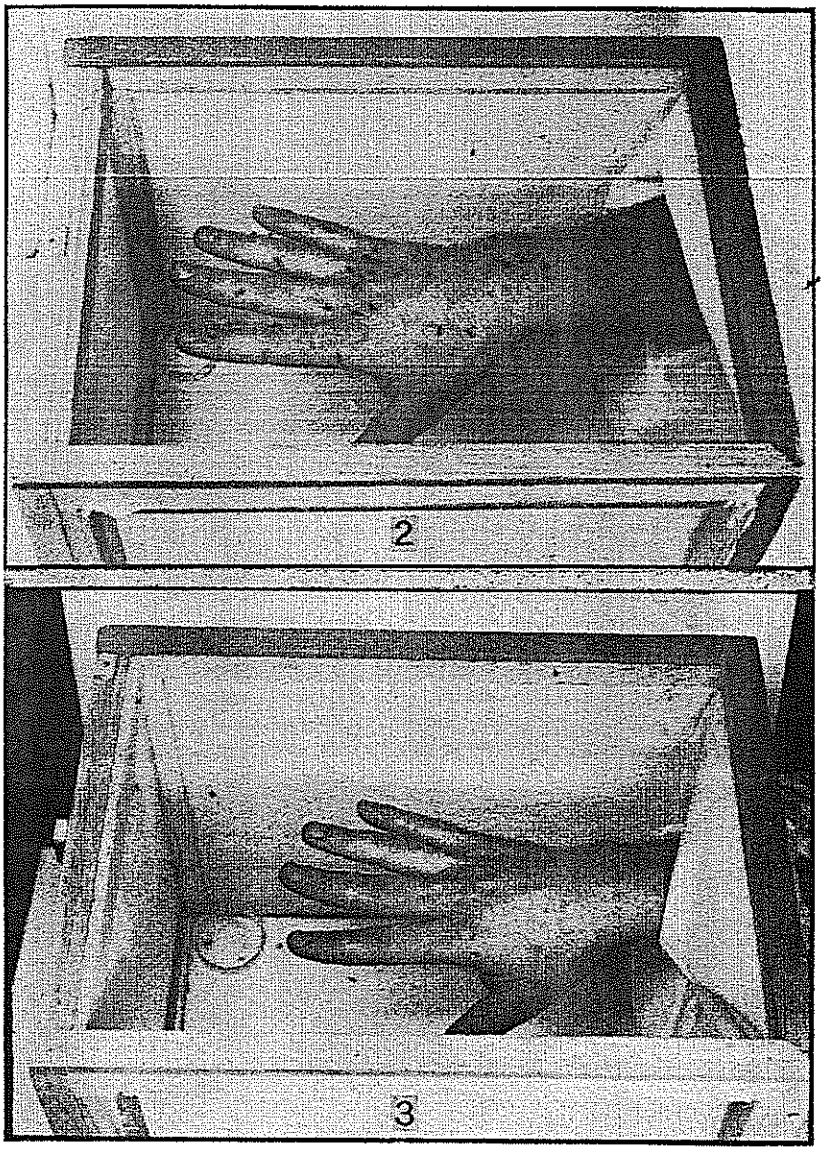


FIG. 2. Cage containing 100 normal females of *Aedes aegypti* showing the 100 percent feeding response elicited within three minutes after the hand is introduced into the cage (some females are feeding on the undersurface of the arm and hand; females resting on the sides of the cage have already fed).

FIG. 3. Cage containing 50 antennectomized females of *Aedes aegypti* showing the lack of response to the hand.

probed a few minutes after being placed on the skin. However, the probing time was variable and some took longer than others.

Those females which fed were removed and checked under a dissecting microscope to see how much of the first flagellar antennal segment remained. Although the length varied somewhat with the individuals, usually all had only small stumps protruding a little beyond the margins of the pedicelli. Sixteen of these females were cleared in KOH, mounted in chloral gum and examined under the high power of a compound microscope. The remaining stumps of the first flagellar segments bore only minute spicules, a few scales and sometimes a few small setae. None of the specimens had any of the sensilla which are restricted to about the apical two-thirds of the first flagellar segment, and numerous over the surface of the other flagellar segments.

The flagellar antennal segments from 35 females were removed close to the pedicelli and the remaining portions (scapes, pedicelli and stumps of the first flagellar segments) were covered with shellac. The arm was introduced into the cage 8 different times at 24, 30, 43, 48, 52, 69, 78, and 93 hours after the operations. Most of the exposures lasted 30 minutes with a total exposure time of about 3½ hours. The mosquitoes were disturbed and made to land on the skin. A total of 22 (63%) mosquitoes fed.

Conclusions: The removal of the flagellar segments from one or both antennae leaving only the scapes, pedicelli and small stumps of the first flagellar segments does not affect the flight of the mosquito, although those with the flagellar segments of both antennae removed are not as active as normal females; the bilaterally flagellectomized females tend to rest on the sides of the cage and usually fly only when disturbed.

Mosquitoes with the segments from one antenna removed to the first flagellar segment are capable of locating the human arm and feeding. However, from the small number of females, as compared to normals, coming down the cylinder, it is probable that they have some difficulty orienting themselves when a barrier (*i.e.*, celluloid cylinder) is introduced.

Females, the antennae of which have been completely removed, remain more or less quiescent and are not attracted from a distance by stimuli (from the arm and hand) which activate normal individuals. However, these females may feed when placed directly on the skin, indicating that another organ(s) is involved in receiving the stimuli which induce probing.

Experiment 3. To determine the role of the mouthparts in probing.

Since in the previous experiments antennectomized females were not attracted to the arm, it is evident that the antennae alone receive host stimuli from a distance. The following experiments were performed to determine the role of the mouthparts in the induction of the probing response.

A. *Palpi:* The terminal palpal segments (or more) from both palpi of 50 females were removed. The palpal sense organs, somewhat similar (externally) to the sensilla on the antennae, are found on the fourth or last segments in this species.

After 9 hours the arm was introduced into the cage for 3 minutes. Thirty females fed. Thirty-three out of 35 normal controls fed. After 11½ hours, the arm was again introduced into the experimental cage

and 12 more mosquitoes fed in 3 minutes. A total of 42 of the 50 females lacking at least the terminal palpal segments were attracted to the arm and took a blood meal. Seven failed to feed while one died during the experiment.

B. *Labellum:* By cutting just above the base of the labellum this structure was removed from 50 females. The tips of the stylets were also removed in this operation. The arm was introduced into the cage at 3 different intervals after the operation, for 3-minute exposures. During the 11-hour trial, 24 females landed and probed (2, 3-minute exposures); after 22 hours, 9 came to the skin and probed. A total of 33 (66%) females lacking the labellum probed but were unable to pierce the skin.

C. *Stylets (labrum, mandibles, hypopharynx, and maxillae):* When mosquitoes are anesthetized with carbon dioxide, some individuals will have the labium kinked or bent so that the compact bundle of stylets is exposed near the clypeus. It is a simple matter to sever these structures at the point of exposure. The stylets may then be removed by grasping the free ends with forceps and pulling out the bundle so that it slides through the labial gutter, or the severed bundle (now non-functional) may be left in the labial gutter.

The above operation was performed on 25 females. After 5 hours the arm was introduced into the cage. Eighteen females landed and probed during the 3-minute exposure, the proboscis merely bending when pressed into the skin. The arm was again introduced into the cage and the remaining 7 mosquitoes disturbed. Five more landed and probed. A total of 23 (92%) females with the stylets removed or severed, probed the skin.

D. *Labium:* The labium from each of the 25 females used in C (already lacking the stylets) was removed, the cut being made in the region under the palpi.

The arm was introduced into the cage for a 5-minute exposure, 15 hours after the operation. At this time 3 females had died. Thirteen females, having only antennae and palpi landed and probed. The probing response of a female lacking the proboscis consisted of walking over the skin surface and stopping now and then to move the head up and down. Some pushed the head against the skin. The remainder of the mosquitoes were disturbed, and 7 more landed and probed. A total of 20 (91%) females probed; two failed to probe.

E. *Proboscis and Palpi:* Twenty females from Experiment D, which already lacked stylets and labium, had both their palpi and what remained of the base of the labium removed close to the clypeus. The proboscides and palpi were also removed from 19 females used in Experiment B (already lacking the labellum) giving a total of 39 females having only the antennal appendages remaining on the head.

The arm was introduced into the cage at 6½, 12, and 22 hours after the removal of proboscides and palpi. A total of 25 females landed and probed; six failed to probe and 8 died during the experimental period.

Conclusions: Females lacking the terminal palpal segments which bear sensilla organs, are attracted to and will feed on man. The removal of the labellum, stylets, labium, proboscis, and palpi, does not eliminate the probing response.

Experiment 4. To determine the combined role of both antennae and palpi in the induction of probing.

The flagellar segments from both antennae and the terminal palpal segments were removed from 15 females. The mosquitoes were exposed to the arm at 6 different intervals (10, 35, 43, 67, 95, and 106 hours after the operations). Exposures ranged from 10 to 30 minutes with the total exposure amounting to about 2 hours. Only 2 females fed. These were checked closely under high power and one was found to have one entire first antennal flagellar segment. In the other specimen, the first flagellar antennal segments were cut close to the pedicelli and did not include the sensilla found on the apical two-thirds of the segment. In both females the palpi were cut close to the clypeus so that the terminal segments plus their sensilla were lacking.

The experiment was repeated using 40 females. The arm was placed in the cage at 21, 47, 68, 77, and 90 hours after the operation, with exposures ranging from 5 to 30 minutes (total exposure 1½ hours). Only 4 females fed, and upon examination all of these had one or both first antennal flagellar segments intact (but terminal palpal segments missing).

To insure the complete elimination of the antennae and palpi, the experiment was again repeated using 39 females, with 20 of these individuals having the remains of the antennae and palpi covered with shellac; the remaining 19 had the antennae removed and the stumps covered with shellac and with their palpi left intact but covered with shellac. Their response was tested 8 different times at 22, 46, 51, 70, 77, 96, 104, and 122 hours after the operation. Exposures usually lasted about 30 minutes with a total exposure of 3½ hours. Four mosquitoes probed, one of these taking a blood meal; (one probed a warm glass rod; one was seen to probe the small glass water container, and when placed on the hand continued to probe). These mosquitoes were checked under high power and the stumps of both palpi and antennae were well covered with shellac.

In all of these tests the females were placed directly on the skin since, without the antennae, they were not attracted to the arm. The total exposure time for these, previous, and later experiments where the antennae and mouthparts were removed does not mean that each mosquito rested on the skin of the observer that length of time. It represents the length of time spent by the experimenter in trying to induce the mosquitoes to probe.

When placed on the skin of the observer or when a warm glass rod was brought nearby, some of these females showed slight vibratory motions of the proboscis and slight up and down movements of the head, with the proboscis extended forward. In true probing the proboscis is generally brought at right angles to the surface and is pressed into the skin. Some females rubbed their hind legs together, rubbed the wings with their hind legs, or rubbed the proboscis with their front legs when stimulated by the hand or a warm glass rod.

Conclusion: Antennectomy plus palpectomy greatly inhibits the probing response in females and will in almost all cases abolish it completely.

The Temperature Response of Females. It is well known that female

mosquitoes can be attracted and induced to probe by temperature alone (Howlett, 1910; Marchand, 1918; Crumb, 1922). The following experiments were undertaken to determine what structures are involved in the reception of temperature stimuli.

Experiment 5. To determine if antennectomized females will be attracted by temperature alone.

A thermos flask (doublewalled, silvered) with its tip broken and containing water heated to 56°–57° C. was used as the attracting stimulus. With the vacuum broken enough heat escapes through the walls to attract normal females. An empty thermos kept at room temperature does not attract mosquitoes. The thermos had a layer of cheesecloth wrapped around it to give the attracted females a surface on which to land and probe. The flask was placed in a cage of normal females before and after being used in a cage of experimental mosquitoes, in order to check its attractiveness.

Fifty-nine females had their antennae removed close to the pedicelli and the remaining stumps covered with shellac. The thermos was introduced for 10-minute exposures at 25, 43, 48, 71, and 95 hours after the operations. Only 2 females landed on the thermos and probed. Two others landed but failed to probe. In a control experiment using 30 normal females, all were attracted to and probed the thermos during four 10-minute trials.

The experiment was repeated with 28 antennectomized females but the insects were given a choice of two thermos flasks, one filled with hot water and the other empty (control). Six 10-minute trials were made. A total of 6 females landed on the warm thermos (3 probed) and 6 landed on the control flask (none probed). The small number of insects which landed on the warm thermos and the fact that an equal number of insects landed on the control, shows that the antennectomized females were not attracted by temperature.

Experiments were performed to determine the effect of the removal of various numbers of flagellar segments on the attraction of females by temperature. Groups of females lacking different numbers of antennal segments were placed in the same cage and exposed to the warm thermos. Those that landed and probed during the 10-minute trial periods were removed from the cage (with an aspirator) and examined under a binocular microscope to determine the number of flagellar segments remaining on the antennae. In the first experiment 4 groups of mosquitoes were used; these groups and the results of 4 trials are shown below:

Number of Females Used	Number of Antennal Flagellar Segments Present on Each Antenna	Total Number and Percent of Females Landing and Probing During Four Trials
29	3	5 (17%)
28	6	25 (89%)
31	9	31 (100%)
29	13 (control)	29 (100%)

This experiment was repeated using similar groups as above but including females with 0 and 1 flagellar segments. The results were similar and showed that females with 3 or less flagellar segments were little or not at all attracted by temperature while a high percentage of females with 6 or more flagellar segments were attracted by temperature.

Conclusions: Antennectomized females are not attracted by temperature. A high percentage of attraction is obtained from females with 6 to 13 flagellar segments; this percentage is markedly reduced when 10 or more segments are removed.

Experiment 6. To determine if temperature alone will induce antennectomized females to probe.

The previous experiment showed that antennectomized females would not be attracted to a warm thermos, but it was indicated that a female would probe if she happened to land on the thermos. In the following experiment the stimulus consisted of a test tube containing warm water, brought close to the resting female. The tube containing warm water was tested on normal females first to see if it would induce probing, before being used on the antennectomized females. Fifteen females had their flagellar segments removed and their remaining stumps covered with shellac. They were tested at 75, 99, 106, 128, and 147 hours after the operation. Ten (67%) of the females were induced to probe. A total of 1 hour was spent in trying to induce probing.

Conclusion: Temperature alone may induce antennectomized females to probe.

Experiment 7. To determine if temperature alone will induce probing in females lacking antennae and the terminal palpal segments.

Thirty-three females had their antennae removed and the stumps covered with shellac. The terminal palpal segments, or more, were also cut off. The females were tested in the same manner as in the previous experiment. Five trials were made at 14, 24, 55, 64, and 75 hours after the operations. Only one female was induced to probe. This female probed in spite of the absence of the fourth palpal segments (checked under a microscope). A total of 2 hours was spent in trying to induce these females to probe.

Conclusion: Antennectomy plus removal of the terminal palpal segments in practically all cases eliminates the probing response to temperature.

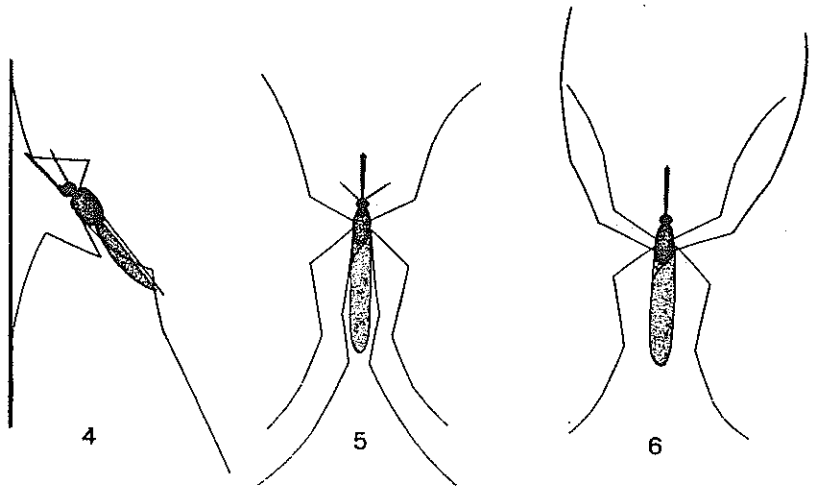
EXPERIMENTS ON ANOPHELES QUADRIMACULATUS

Experiment 8. To determine the role of the antennae in locating the host and probing.

The 13 flagellar segments of both antennae from 50 females were removed close to the pedicelli and the remaining stumps were covered with shellac. The arm was introduced into the cage at 8, 20, 24, 25, 30, and 42 hours after the operations. Exposures ranged from 15 to 45 minutes with a total exposure of 2½ hours. In general the mosquitoes were rather easily disturbed in spite of the absence of antennae. However, they showed no directional response and in most cases were forced to land on the skin, by placing the hand between them and the side of the

cage, where they fed or probed readily. Forty-six of the 50 antennectomized females probed or fed (30 fed; 16 probed) and 4 died during the experiment. Of 20 normal controls, 18 fed in a 2-minute exposure, 7 hours after the start of the experiment.

It was found that some of the females which probed but which did not feed had the base of their palpi cemented for a short distance to the base of the proboscis, by the shellac. In normal probing the palpi are raised (cf. Robinson, 1939), project upward and forward, and vibrate rapidly as the stylets are pushed into the skin. When the palpi are cemented to the proboscis, near its base, they cannot be extended during probing and therefore push against the skin thus preventing the stylets from piercing. It is purely a mechanical barrier,



Diagrams of females *of Anopheles quadrimaculatus* at rest, showing positions of the legs. FIG. 4. Lateral view; normal female. FIG. 5. Dorsal view; normal female. FIG. 6. Dorsal view; antennectomized female. Note the position of the hind legs when the finger of the observer is placed in front of the mosquito.

since females lacking palpi feed readily. Some females appear highly "excited" and, though they probe vigorously, do not feed, although their palpi are free and capable of being raised. Antennectomized females may probe a warm glass rod when brought near the screen on which they are resting.

When an *Anopheles* female is at rest, her proboscis and abdomen are in more or less a straight line. The front and middle pairs of legs touch the surface on which she is resting while her hind legs are extended posteriorly. When not in motion, the femora of the hind legs extend backward with their apices at about the level of, or slightly above the wings; the apices of the tibiae point postero-ventrally beyond and below the level of the abdomen; the tarsi extend laterally and their tips are widely separated away from the mid-body line. Both the hind femora and tibiae are held close to the body (Figs. 4, 5). The hind legs may be held motionless in the above position but more often

they show a definite movement, particularly when the mosquitoes are disturbed. The hind legs may be moved in unison, or as is more often the case, alternately. This motion is more or less a circular one and when the legs move in unison reminds one of a swimmer doing the breast-stroke. The femur is brought upward then forward and back in a rotary motion with the tips of the tarsi often reaching to or beyond the front legs. There may also be short movements with the tips of the tarsi describing an ellipse or a small circle. While feeding, the hind legs may rest on the skin or be held away from the surface. Sometimes, particularly if the female has difficulty piercing, the hind legs will be waved about, repeatedly touching the skin surface.

During the antennal experiments the following interesting observation was made. If the hand or a finger is placed in front of antennectomized females, the hind legs are brought forward and touch the skin (Fig. 6). Some of these females then either probe the area around them or walk to the finger and probe. If the hand is placed behind the female just far enough so that the tips of her hind tarsi can reach the skin, the legs will be extended to their full length to touch the skin surface; the legs may undergo typical rotary motions briefly touching the skin and the mosquito may then probe or walk away. It thus appears that a tactile sense in the hind legs may be involved in inducing probing in this species.

Conclusions: Antennectomized females cannot locate the host, but feed or probe more or less readily when they are placed on or near the skin.

Experiment 9. To determine the role of the mouthparts in probing.

A. *Labellum:* The labellum was removed from each of 15 females. The stylets were first freed from the theca and the labellum was removed by cutting just above its base. After recovery from anesthesia the stylets were re-ensheathed by the front legs of the mosquitoes.

In subsequent tests practically all landed and probed, but only 3 pierced the skin and fed. These were checked under a microscope; in all cases the labellum was completely lacking, and the tips of the stylets protruded from the cut end of the labium.

B. *Palpi:* The palpi were removed close to the clypeus from 20 females. Tested after 11 hours, all landed and probed, 16 feeding within 3 minutes. The remaining 4 females fed during a 3-minute exposure at a second 18-hour trial. These mosquitoes will probe when a warm glass rod is brought near them.

3. *Proboscis and Palpi:* The proboscides and palpi of 30 females were removed close to the clypeus, leaving the antennae as the only remaining head appendages. Two trials were made at 12 and 18 hours after the operations. Most of the females came to the arm readily and probed wildly, pushing the head against the skin.

Conclusions: Females will probe after the removal of the labellum, palpi, and proboscis and palpi.

Experiment 10. To determine the combined role of antennae and palpi in the induction of probing.

The flagellar segments were removed close to the pedicelli and the palpi were cut off close to the clypeus in 50 females. To insure the complete elimination of the function of both antennae and palpi,

the remaining stumps were covered with shellac. In most cases while covering the palpal remains, shellac was also unavoidably applied to the base of the labium, thus preventing feeding, since the theca could not be bent away from the stylets during probing. However, the mosquitoes were only tested for probing and were removed from the cage as soon as they responded. Both the hand and a warm glass rod were used to induce probing, either from the outside (brought up against the screen on which the mosquitoes were resting) or within the cage, the mosquitoes being made to land on the skin or the glass rod brought close to the female.

Three trials were made at 14, 22, and 26 hours after the operations, each exposure lasting 30 minutes. Forty-eight (96%) of the females probed (29 probed when stimulated by the hand and 19 probed a warm glass rod). Many probed after the finger or hand was brought near enough so that the hind legs, in their waving motion, could touch the skin. Some waved the legs and touched the skin several times and then either climbed on to the skin and probed, or probed through the cloth on which they were resting. The warm glass rod induced probing when brought close to the cloth of the cage on which the females were resting. Once induced to probe, some females continued to do so for a short time after the stimulus was removed. These antennectomized and palpectomized females could also be induced to probe by a current of warm air. All mosquitoes were examined after probing to check the completeness of the covering of the antennal and palpal stumps with shellac.

Conclusions: Females will probe readily after antennectomy and palpectomy.

Experiment 11. To determine the behavior of females after the elimination of all the sense organs on the head.

The flagellar segments of both antennae from 24 females were removed close to the pedicelli and their proboscides and palpi were severed close to the clypeus. Two tests were run at 7 and 9 hours after the operation. Five were dead at the first trial and, of the remaining 19, 17 gave the probing response which consisted of distinctly moving the body up and down and pushing the head against the skin or against the screen where the head was held. Two mosquitoes probed after being stimulated by a current of warm air. Most of the others were made to probe by permitting their hind tarsi to touch the skin.

The same operation was performed on 23 females, but in addition the eyes were covered with a thick coat of blackened shellac. The shellac, when applied with a micropipette, formed a large blob and covered not only the eyes, but almost the entire head, reaching to the back margin of the eyes and over the occipital region. The mosquitoes were tested only once, 7 hours after the operation. At this time 9 were dead or unable to walk or fly. Of the others, 8 gave a probing response (2 to a warm glass rod; 6 when stimulated by the hand). Six failed to probe. Though the probing response was distinct, it was usually of short duration.

Conclusion: Probing may be induced in females lacking all of the sense organs found on the head.

DISCUSSION

The present results summarized in Table I show that the antennae of *Aedes aegypti* and *Anopheles quadrimaculatus* possess sense organs which function as receptors for receiving host stimuli from a distance. The results with *A. aegypti* using temperature alone as the stimulus, parallel closely those obtained using the arm and hand to attract females and induce probing. Since in using the arm as a stimulus one is dealing not only with chemical but thermal stimuli as well, it is not possible to conclude that the antennae function as distance chemoreceptors, although this is highly probable. The temperature experiments show that the antennae of *A. aegypti* do function as directional

TABLE I

SUMMARY OF THE BEHAVIOR OF FEMALES OF *Aedes aegypti* AND *Anopheles quadrimaculatus* AFTER THE REMOVAL OF VARIOUS SENSE ORGANS

CONDITION OF FEMALE	BEHAVIOR TO MAN			
	Aedes aegypti		*Anopheles quadrimaculatus*	
	Attraction	Probing	Attraction	Probing
1. *Antennae (mouthparts intact)*				
a. One antenna missing to first flagellar segment	Attracted	Probe	*	
b. Bilateral antennectomy	Not Attracted	Probe	Not Attracted	Probe
2. *Mouthparts (antennae intact)*				
a. Palpectomy	Attracted	Probe	Attracted	Probe
b. Labellectomy	Attracted	Probe	Attracted	Probe
c. Styletectomy	Attracted	Probe	*	
d. Proboscectomy	Attracted	Probe	*	
e. Combined proboscectomy and palpectomy	Attracted	Probe	Attracted	Probe
3. *Mouthparts, antennae*				
a. Combined antennectomy and palpectomy	Not Attracted	Probing abolished in almost all cases	Not Attracted	Probe

* Experiment not performed.

distance thermoreceptors. Combined antennectomy and palpectomy will in practically all cases abolish the probing response in *A. aegypti*. Thus the antennae and palpi are the chief organs used by this mosquito in locating its host and receiving the stimuli which induce probing. The antennae perform both functions while the palpi act as non-directional receptors receiving stimuli when the insect is on or near the skin of the host.

In the present experiments it was shown that the eyes of *A. aegypti* are not necessary for locating the host. Further evidence for this

is found in the fact that mosquitoes will orient, in a darkened chamber of an olfactometer, to an air stream bearing human body odor (Willis, 1947). It is also known that mosquitoes are capable of finding man in total darkness. However, Kennedy (1939) in a study of the visual responses of *A. aegypti* showed that vision plays an important role in upwind orientation and he believes that this combined with the activating effect of host scent results, in nature, in the insect flying towards the source of stimulation.

The probing response of *A. quadrimaculatus* is much more difficult to eliminate, by removal of various sense organs, than the response of *A. aegypti*. This is interesting in light of the fact that as a rule the effectiveness of a given repellent is lower against this anopheline than the culicine. These two species may react differently to the same repellent. Based on the variation in behavior of the above species DeLong et al (1945) classified various repellents into two major groups, namely, "tactile and olfactory." These workers suggested the possibility that the difference in behavior of the two species to a "tactile-type" repellent might be due to a difference in the sensory end-organs involved in receiving host stimuli. The elimination of palpi and antennae in *A. quadrimaculatus* does not eliminate the probing response. In fact elimination of all of the sensory organs on the head in this species does not necessarily abolish probing. The behavior of *A. quadrimaculatus* indicates that the hind legs of the female may function as sense organs. Whether or not these legs serve as tactile organs (*i.e.*, detect air currents, or convection currents set up by the heat from the arm) or function in some other manner has yet to be determined. Wesenberg-Lund (1921) has suggested that the long outstretched hind legs of *A. maculipennis* ". . . are used as organs of feeling." It is possible that the few cases of probing obtained from *A. aegypti* after removal of the palpi and antennae are also due to some sensory structures in the hind legs. If so it is apparent that this sense is less developed than in *A. quadrimaculatus*.

SUMMARY

1) The antennae and palpi are the chief organs used by *Aedes aegypti* females in locating the host and receiving the stimuli which induce probing. In cages 11″ x 11″ x 15″ the eyes are not necessary to locate the host. The antennae function as directional distance thermoreceptors, and probably chemoreceptors as well. The palpi receive stimuli when the insect is on or near the skin of the host. Temperature receptors in *A. aegypti* females are also found on the palpi. Antennectomized females are not attracted to man, and combined antennectomy and palpectomy in almost all cases will abolish probing.

2) The antennae of *Anopheles quadrimaculatus* females also receive directional host stimuli from a distance. In this species, a high percentage of females probe even after combined antennectomy and palpectomy. The behavior of *A. quadrimaculatus* indicates that the hind legs may function as sense organs (when near the host) possibly in detecting air currents, or convection currents resulting from the warmth of the host.