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STUDIES ON THE ATTRACTION OF *AEDES AEGYPTI* (DIPTERA: CULICIDAE) TO MAN

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Abstract: A simple olfactometer was constructed for studying physical and chemical factors attracting *Aedes aegypti* (L.) to man. A stream of 1% CO_2 in air activated and attracted the mosquitoes. It was more attractive at 60% relative humidity (RH) than at the other RH tested (26%, 42%, 76% and 90%). Mosquitoes were deterred from high RH (76% and 90%), but thirsty mosquitoes were attracted to high RH (76%). Mosquitoes were attracted to convection heat (34°C) if the air was humid (40% RH) and to a warm air stream if the air was humid (60% RH), but not if the air was dry. The attraction of mosquitoes to lactic acid was confirmed. When passed over a human forearm, a 1% CO_2 stream was more attractive than a stream of air, though both attracted mosquitoes. One percent CO_2 at 34°C and 60% RH was as attractive as 1% CO_2 passing over the forearm. A 5% stream of CO_2 passed over glass beads upon which emanation of the forearm had been trapped attracted mosquitoes from 1–5 min., depending on how much emanation had been trapped. The causes for discrepancy in the results of various investigators on the responses of mosquitoes to CO_2 and humidity are discussed.

Studies have been made of parts of the human body and its products in search of specific chemical attractants to mosquitoes (Brown 1958, Khan et al. 1965, Maibach et al. 1966, Skinner et al. 1965). While certain attractive materials have been found [lysine and alanine (Brown & Carmichael 1961), sex hormones (Roessler 1960, 1961, Roessler & Brown 1964), carbamino compounds (Brown 1966), L-lactic acid (Acree et al. 1968, Smith et al. 1970), methionine (Ikeshoji 1967, Ikeshoji et al. 1963), unspecified blood constituents (Schaerffenberg & Kupka 1953), lysine, cadaverine and estradiol (Bos & Laarman 1975)], nothing has proved to be as attractive as man himself.

The purpose of this investigation was to develop a method of collecting emanations from the forearm for the purpose of determining the components which are attractive to female mosquitoes [*Aedes aegypti* (L.) var. *queenslandensis* Theo.]. For this purpose an olfactometer was built and various factors which possibly affect the attraction of mosquitoes to skin or skin emanations were studied.

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The following is a short review of the subjects studied.

Carbon dioxide. The effect of CO_2 as an attractant to mosquitoes is controversial although its activating effect on mosquitoes has been confirmed by all investigators. Carbon dioxide attracts *Ae. aegypti* (Brown et al. 1951, Willis & Roth 1952) and other mosquitoes (Van Thiel 1947, Van Thiel & Weurman 1947, Brown 1951, Snow 1970). Other investigators (Rudolfs 1922, Willis 1947, Laarman 1955, Kellogg & Wright 1962, Daykin et al. 1965, Khan & Maibach 1966) concluded that CO_2 merely activates rather than attracts mosquitoes.

Relative humidity and moisture. Some investigators claim that mosquitoes avoid high as well as low humidities (Rudolfs 1923, 1925, Muirhead-Thomson 1938, Roth & Willis 1952, Platt et al. 1957, Bar-Zeev 1960). Others have reported that mosquitoes are attracted to high humidity and moisture (Reuter 1936, Parker 1948, Brown et al. 1951) or are unaffected by it (Christophers 1947). Still other workers claim that thirsty mosquitoes (deprived of water for some time) are attracted to high humidity and moisture (de Meillon 1937, Bar-Zeev 1960, Khan & Maibach 1966, 1971).

Heat. Warmth is a factor in the attractiveness of surfaces to mosquitoes (Howlett 1910, Christophers 1947, 1960, Brown 1951). Some investigators found that the combined effect of heat and moisture is attractive (Parker 1948, Brown 1951, Kellogg & Wright 1962, Wright et al. 1965, Daykin et al. 1965, Hocking 1963, Khan & Maibach 1966, 1971).

Odors. The response of mosquitoes to host odors was reported by Laarman (1955, 1958), Willis (1947), Brouwer (1960), Rahm (1957, 1958), Clements (1963), Khan & Maibach (1966), Maibach et al. (1966), and Khan et al. (1967). Others (Wright & Kellogg 1962, Wright et al. 1965, Daykin et al. 1965, Wright 1975) discount the role of odor and claim that heat and moisture are the only factors which attract mosquitoes. Reuter (1936) and Brown (1958) assigned the role of attraction chiefly to temperature, humidity and CO_2.

MATERIALS AND METHODS

The olfactometer (FIG. 1) was made of an aquarium 31 × 31 × 62 cm standing on its smaller side on a wooden frame (a), 35 × 35 × 35 cm. The bottom (b), made of Plexiglas, had 2 round holes (8 cm in diam.) on each side of its center and 7 cm apart. Into each hole was inserted a glass tube (c) (22.5 cm long and 7.4 cm inner diam.) protruding 1 cm inside the olfactometer. The upper end of each tube had a funnel (d) 7.5 cm long. Its largest diameter fit the entrance to the tube (7.4 cm in diam.) and its smallest diameter was 2.0 cm. The funnel formed a trap for mosquitoes entering the tube. The lower end of each tube was tapered to 4.0 cm in diam. and closed with a rubber stopper (e) which had a hole in its center through which a tubing connector (f) was inserted. The latter was connected by means of a Tygon tube (g) to a cylinder of compressed air or 1% CO_2 in air. A flowmeter with adjustable knobs showed the gas flow rate (2.5 liters per min.). A metal plate (h) (26 × 10 cm), connected to a glass rod (i), which passed through the top of the olfactometer, enabled closure or opening of the top of the traps by moving the plate. The olfactometer top, made of Plexiglas, was removable, and had an opening 10 × 10 cm covered with a nylon net (j). The olfactometer top had a hole (2.5 cm in diam.) closed with a rubber stopper (k), for introducing mosquitoes. An electrical heating coil, in a screened metal tube (l) and connected to a variable voltage control, was placed inside the olfactometer to control the temperature. The front and back of the olfactometer were made of clear glass, and the left and right sides were opaque. Food was furnished to the mosquitoes by placing water and honey on the nylon net on top of the olfactometer. Dead mosquitoes were removed from the floor of the olfactometer by introducing a glass tube through the hole on top of the olfactometer, and sucking them out with a vacuum cleaner.

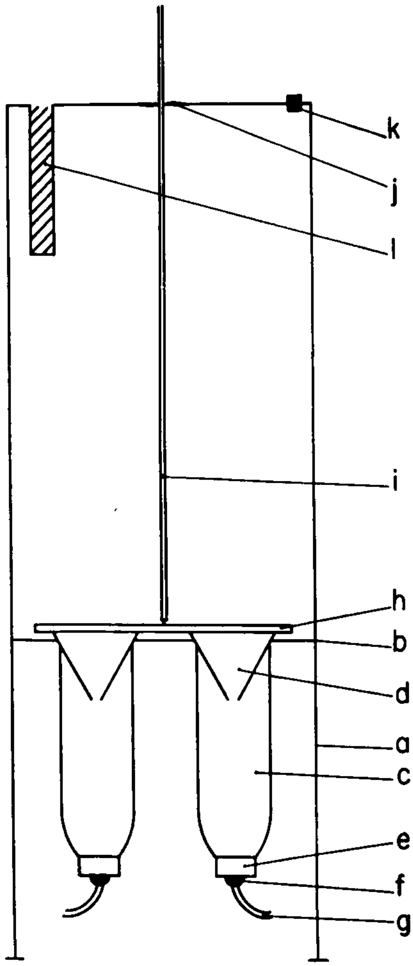

FIG. 1. Diagram of the olfactometer. a, wooden frame; b, bottom of olfactometer; c, glass tube; d, funnel; e, rubber stopper; f, tubing connector; g, Tygon tube; h, metal plate; i, glass rod; j, opening, covered with a nylon net; k, rubber stopper; l, electric heating coil.

In each experiment about 350 female *Aedes aegypti*, 1 to 3 weeks old, fed on honey and water only, were used. In each test the number of mosquitoes entering the traps in 3 min. (unless otherwise specified) was counted. After each test the mosquitoes were sucked out from the traps with a vacuum cleaner (at a reduced voltage) into an L-shaped tube and returned to the test population in the olfactometer. At each replicate the ports were flushed with air for 15 min. and interchanged, so that when a control was used on the right-hand side in one replicate, it was on the left-hand side in the next.

Whenever emanation from the forearm was tested or collected, the forearm was placed in a glass tube 36.5 cm long and 9.0 cm inner diam. The tube was closed at one end; on the open end a rubber tube (cut from a surgeon's glove) was placed so that when the forearm was introduced inside the tube, the rubber tightened around the upper end of the forearm. Compressed air, or 1% CO_2 in air, was introduced from a cylinder into the tube containing the forearm and then into the olfactometer or a collecting container. The tube containing the forearm had 2 small glass tubes on each end and was connected to Tygon tubes for the ingress or egress of the gas. The rate of flow was 2.5 liters per min., giving a speed of 7.9 m/min. at the entrance of the trap. This speed was selected after testing various flow rates.

RESULTS

In the tables and text, unless otherwise specified, "average number of mosquitoes" means average number of mosquitoes entering the traps in 3 min. "One percent CO_2" means 1% CO_2 in air.

Response to air and 1% CO_2 in air passed over the forearm

The response to compressed air as well as 1% CO_2 in air passed over the forearm was tested (TABLE 1). The control side consisted of a similar stream of air or 1% CO_2 brought to 60% relative humidity (RH), somewhat similar to that obtained with air passed over the forearm (see TABLE 4).

Air as well as 1% CO_2 passed over the forearm was more attractive than air or 1% CO_2 at 60% RH. These results reconfirm that forearm emanations were attractive to the mosquitoes. The attraction was enhanced in the presence of 1% CO_2. There was practically no difference in the temperature at the entrance to the 2 traps. The amount of CO_2 emanating from the forearm is infinitesimal (Rahm 1956, Brouwer 1960, Frame et al. 1972).

Decontamination of the traps. The ports of the olfactometer were interchanged after each replicate. When a test on forearm emanation was performed, the olfactometer traps had to be flushed with air before the next test. This was done by passing an air stream of 1.3 liters per min. into the 2 traps. The following experiments were performed to determine how long such an air stream should flush the traps for decontamination. For this purpose a stream of 1% CO_2 was passed over the forearm into 1 trap of the olfactometer for 3 min. The mosquitoes that entered the traps were removed, and a stream of air was flushed into the 2 traps for varying periods. At the end of the flushing period, a stream of 1% CO_2 was passed into each of the 2 ports, and the number of mosquitoes entering the 2 traps was compared (TABLE 2).

Ten minutes was not enough to remove all the emanation of the skin adsorbed on the walls of the trap. Fifteen minutes of flushing was necessary to decontaminate the traps.

TABLE 1. Response of adults of *Aedes aegypti* to air and 1% CO_2 passed over the forearm.

TREATMENT	NO. OF REPLICATES	AVG NO. OF MOSQUITOES ± SD†
Air passed over forearm	6	**7.1 ± 1.4
Air at 60% RH (control)	6	1.6 ± 0.5
1% CO_2 passed over forearm	5	*54.6 ± 26.6
1% CO_2 at 60% RH (control)	5	12.8 ± 7.5

*Significant at the 5% level.
**Significant at the 1% level.
†Average number of mosquitoes entering the traps in 3 min. ± standard deviation.

TABLE 2. Results of decontamination of the olfactometer traps by air-flushing.

AVG NO. OF MOSQUITOES IN CONTAMINATED TRAP†	AVG NO. OF MOSQUITOES IN NONCONTAMINATED TRAP†
10 min. flushing (8 replicates)	
**25.3 ± 11.1	11.6 ± 10.6
15 min. flushing (8 replicates)	
8.1 ± 4.4	6.6 ± 3.7
30 min. flushing (5 replicates)	
8.2 ± 3.4	9.6 ± 7.4

**Significant at the 1% level.
†Average number of mosquitoes entering the traps in 3 min. ± standard deviation.

Response to 1% CO_2

The response to dry 1% CO_2 versus dry air was compared. Cylinders of compressed air or 1% CO_2 in air were used. These gases had less than 1.6% RH. Since it was thought that possible contamination of odor inside the olfactometer might affect the results of the attraction to 1% CO_2, we used the following procedures. In experiment A, a stream of dry compressed air at 2.5 liters per min. was passed for 4 hr prior to the experiment through the olfactometer via the 2 traps and out through the nylon net on its top. In experiment B, room air was similarly driven for 16 hr through charcoal (to absorb any odors) and then through the olfactometer by an aquarium pump (1.5 liters per min.). The average number of mosquitoes that entered the traps in which dry 1% CO_2 and dry air passed was as follows: experiment A, 14.8 ± 4.7 and 2.3 ± 1.5, respectively ($P = <0.01$); experiment B, 26.3 ± 14.2 and 3.8 ± 3.4, respectively ($P = <0.01$). Dry 1% CO_2 was attractive compared to dry air. Whenever a stream of 1% CO_2 was passed into the olfactometer, the activity of the mosquitoes immediately increased.

Response to RH

The response to various RH was tested by comparing the attraction of the mosquitoes to a flow of 1% CO_2 at various RH. The RH was recorded at the entrance to the trap by an electrical humidity recorder (Hygrodynamics Inc., Silver Spring, Md., U.S.A.). A variety of RH was obtained by mixing wet 1% CO_2 (bubbled through water) with dry CO_2 (taken directly from the cylinder) at varying ratios. The 76% RH was obtained by bubbling the 1% CO_2 in water and the 90% RH by bubbling 1% CO_2 in warm water (85°C) (TABLE 3).

There was no significant difference between dry CO_2 and CO_2 at 26% RH. There was a significant preference for 42% RH and 60% RH compared to

TABLE 3. Comparison of attraction of *Aedes aegypti* adults to dry 1% CO_2 and to 1% CO_2 at various RH.

1% CO_2 at various humidities†	Dry 1% CO_2†	No. of replicates
26% RH		
13.8 ± 3.8	14.0 ± 9.9	10
42% RH		
**30.2 ± 11.0	13.2 ± 5.5	10
60% RH		
*54.6 ± 26.6	12.8 ± 7.5	5
76% RH		
6.4 ± 3.0	**20.2 ± 7.3	5
90% RH		
0.7 ± 0.7	**21.4 ± 12.4	7

*Significant at the 5% level.
**Significant at the 1% level.
†Average number of mosquitoes entering the traps in 3 min. ± standard deviation.

dry CO_2. The mosquitoes preferred a low RH to very high RH of 76% and 90%.

Comparison of the 2 preferred RH, 60% and 42%, in a similar way (7 replicates) showed that the mosquitoes distinctly preferred the higher humidity (17.4 ± 4.9 and 2.6 ± 1.5, respectively; $P = <0.01$).

In the above experiments it was assumed that the mosquitoes were satiated since they had water ad libitum. It was interesting to determine the response of thirsty mosquitoes. For this purpose the mosquitoes were deprived of water for 13–16 hr and the effect of 76% RH versus dry CO_2 tested (7 replicates) as previously. The average number of mosquitoes was 32.8 ± 7.4 and 14.8 ± 8.5, respectively ($P = <0.01$). Thirsty mosquitoes, thus, distinctly preferred the high humidity to dry CO_2, as opposed to satiated mosquitoes, which preferred the lower humidities (TABLE 3).

RH *obtained at the entrance to the trap when 1% CO_2 was passed over the forearm.* In tests on forearm emanation, the forearm, as previously described, was enclosed in a glass tube. The gas passing over it picked up the humidity emanating from the forearm. The buildup of humidity as a function of time was tested at the trap entrance (TABLE 4).

The RH increased rapidly with time and reached 72% at the end of 3 min., which was the standard experimental period.

Response to temperature

The response to 34°C (temperature of the human skin) was tested in the olfactometer under various conditions. This temperature was obtained at the entrance to the trap by wrapping an electrical heating coil around the outside of the trap. The heat was regulated by a voltage-variable regulator. A thermistor was placed at the entrance to the trap, so that the temperature was continuously recorded in an electrical temperature recorder (telethermometer).

Response to convection heat. The response to convection heat (without a stream of air) was tested. One trap was heated so that the temperature at its entrance was 34°C; the other trap was at room temperature (26°C). The RH was that of the room air (about 40%). The average number of mosquitoes was 15.7 ± 16.4 and 0.7 ± 1.7, respectively (10 replicates), with the convection current of heated air (at a room RH of 40%) significantly more attractive ($P = <0.01$).

Response to a dry air stream at 34°C. A dry air stream was passed through one trap at a temperature of 34°C and a similar dry, unheated air stream (26°C) was passed through the other trap. The average number of mosquitoes was 0.4 ± 0.7 and 0.3 ± 0.4, respectively (8 replicates). Thus, when the air was dry, the mosquitoes did not respond to the high temperature.

Response to a humid air stream at 34°C. A humid air stream (60% RH) warmed to 34°C was passed through one trap and a similar nonwarmed (26°C), humid air stream was passed through the other. The average number of mosquitoes was 6.6 ± 5.4 and 0.2 ± 0.4, respectively (10 replicates), with the humid air stream at 34°C significantly more attractive ($P = <0.01$) compared to a humid air stream at 26°C.

Response to dry and humid streams of 1% CO_2 at 34°C. Similar results were obtained with 1% CO_2 instead of air as follows. In experiments in which dry 1% CO_2 at 34°C was compared with dry 1% CO_2 at 26°C, the average number of mosquitoes was 5.8 ± 3.3 and 4.0 ± 2.5, respectively (10 replicates). In experiments in which 1% CO_2 at 60% RH and 34°C

TABLE 4. RH obtained at the entrance to the olfactometer trap when a stream of 1% CO_2 was passed over the forearm.

Time from start of experiment (seconds)	% RH
10	<1.6
25	55
30	59
90	63
110	64
130	66
145	68
160	69
180	72
215	77
230	78

was compared with 1% CO_2 at 60% RH and 26°C, the average number of mosquitoes was 16.5 ± 7.8 and 2.4 ± 2.5, respectively (10 replicates, P = <0.01).

Attraction of emanations of the forearm versus heat. It was interesting to compare attraction to 34°C and skin emanations. (The RH in both cases was about 60%.) For this purpose 1% CO_2 was passed over the forearm into one port of the olfactometer (the average RH was about 60%, see TABLE 4) and 1% CO_2 at 60% RH and 34°C was passed into the other port. The average number of mosquitoes was 20.8 ± 10.8 and 21.6 ± 10.6, respectively (10 replicates). This shows that attraction to forearm emanations was counterbalanced by attraction to 34°C.

Experiments in collecting emanations from the forearm

Compressed air was passed over the forearm into a 500-ml Erlenmeyer flask and out into the room in the same manner as used previously. The Erlenmeyer flask was filled with glass beads to increase the inner surface area and immersed in bits of ice to which salt had been added. The temperature inside the flask was −15°C. Glass beads were chosen because it was shown (TABLE 2) that the trap made primarily of glass adsorbed emanations of the skin efficiently. The collection was continued for 30 min., at which time the Erlenmeyer flask was brought to room temperature. A stream of 1% CO_2 was then introduced into the flask and from there to one port of the olfactometer. Another stream of 1% CO_2 at 60% RH was introduced into the control port. In 1 experiment the flask was filled with glass beads 3 mm in diam., and in another with glass beads 1.5 cm in diam. The total number of mosquitoes entering the trap into which 1% CO_2 was passed into the flask (containing glass beads 3 mm in diam.) in 5 min., versus the total number entering the trap into which 1% CO_2 at 60% RH was passed in 5 min. was 138 and 134, respectively. In another experiment in which the Erlenmeyer flasks had glass beads of 1.5-cm diam., the number of mosquitoes entering the trap in 5 min. was 47 and 40, respectively. There was no difference in the number of mosquitoes that entered the 2 traps.

In another experiment, a stream of 1% CO_2 was passed over the forearm; from there it bubbled through water and then into one port of the olfactometer. Another stream of 1% CO_2 bubbled through water and from there to the other port (control). The average number of mosquitoes was 1.2 ± 0.9 and 2.7 ± 0.5, respectively (5 replicates). This shows that emanations of the forearm did not attract the mosquitoes after bubbling in water and suggests that the emanations were dissolved or absorbed by the water.

Effect of dehydrating agents. Studies were made to find the effect of dehydrating the forearm emanations. For this purpose 1% CO_2 was passed over the forearm, then through a dehydrating agent and into one port of the olfactometer. Another stream of dry 1% CO_2 was passed through the other port (control). The following dehydrating agents were tested: Drierite, KOH and molecular sieves, types 3A and 5A (manufactured by Union Carbide, U.S.A.). These sieves are natural zeolites (aluminosilicate) having pores which normally contain water of hydration. The dehydrating agents absorbed the attractive compounds of the emanations (TABLE 5).

Further experiments in collecting emanations from the forearm. It was found that when emanations of the forearm were collected in a cold container, water vapor emanating from the forearm (which was also collected) absorbed or dissolved the attracting compounds. Collection of the emanations was, therefore, performed in the same manner, but the glass beads were kept at room temperature, preventing water condensation. These experiments were continued by the senior author at the Israel Institute for Biological Research, Ness-Ziona, Israel. Five percent CO_2 in air was used instead of 1% CO_2 because the former was readily available. The

TABLE 5. The effect on *Aedes aegypti* adults of removal of water from skin emanations using dehydrating agents.

Dehydrating agent	No. of replicates	Avg. no. of mosquitoes† — 1% CO_2 passed over forearm, then through dehydrating agent	Avg. no. of mosquitoes† — Dry 1% CO_2
Drierite	5	19.6 ± 14.3	19.8 ± 4.9
KOH	6	30.6 ± 14.3	22.6 ± 13.5
Molecular sieves			
Type 3A	4	12.3 ± 6.5	19.0 ± 3.6
Type 5A	4	33.5 ± 3.4	31.5 ± 5.2

†Average number of mosquitoes entering the traps in 3 min. ± standard deviation.

TABLE 6. Attraction of *Aedes aegypti* adults to 5% CO_2 stream passed over glass beads on which emanations from the forearm had been collected.

Time air passed over forearm to glass beads (min.)	No. of replicates	Time 5% CO_2 passed over glass beads attracted mosquitoes (min.)	Average no. of mosquitoes trapped in the 1st min. ± SD	
			Treated glass beads	Control (5% CO_2)
3	10	1	37.2 ± 11.5	1.7 ± 2.3
6	10	1–2	34.5 ± 7.9	0.2 ± 0.6
12	8	2–4	38.1 ± 9.9	0.4 ± 0.5
24	7	2–5	35.2 ± 13.8	0.3 ± 0.7
48	5	2–5	34.4 ± 13.9	0.8 ± 1.6

latter percentage was obtained by mixing compressed air with 5% CO_2. Preliminary experiments showed no difference in attraction between 5% CO_2 and 1% CO_2.

The strain of *Aedes aegypti* used, called the Ness-Ziona strain, has been reared at this Institute for 25 years. Tests with the olfactometer showed this strain to be less responsive to emanations from the skin, as well as to CO_2, than the var. *queenslandensis* reared in San Francisco. It was also observed that the Ness-Ziona strain is more reluctant to pass through the opening of the trap (2.0 cm in diam.), and the entrance was increased accordingly to 5 cm in diam. This change greatly increased (8- to 10-fold) the number of mosquitoes entering the traps. Therefore, in the following experiments, the number of mosquitoes entering the traps in 1 min. (instead of 3 min.) was counted.

Procedure. A 1-liter Erlenmeyer flask was filled with glass beads, 5 mm in diam. A stream of compressed air was passed over the forearm for a certain period of time, as previously described, and from there into the Erlenmeyer flask containing the glass beads (which was kept at room temperature, 28°C), and out into the room. A stream of 5% CO_2 at 2.5 liters per min. was passed for 1 min. into the Erlenmeyer flask and from there into one port, and another stream of 5% CO_2 (control) passed into the other port. The tests were continued as previously described (the ports being exchanged after each test) as long as attraction was maintained. It was possible to determine the length of time the glass beads attracted the mosquitoes in relation to the time that emanations of the forearm had passed over the glass beads. Whenever attraction to the treated glass beads occurred, the number of mosquitoes entering the trap was 20 to 50 per min. as compared to 0 to 3 for the control. A stream of 5% CO_2 passing over the forearm attracted an average of 30.0 ± 10.0 mosquitoes in 1 min., as compared to 0.4 ± 0.7 for the control (8 replicates) (TABLE 6).

In spite of variations in the time of attraction (1–5 min.), the general trend in the attractiveness of the treated glass beads correlated with the length of exposure time of the glass beads to emanations of the forearm, up to 24 min.

Effect of lactic acid

Since lactic acid was shown to be attractive to mosquitoes (Acree et al. 1968, Smith et al. 1970), we evaluated its effect under our experimental conditions. Fifty µg of L(+) lactic acid in acetone was dripped onto a filter paper. The paper dried and was placed in a 1-liter Erlenmeyer flask. Five percent CO_2 was passed into the flask and from there to one port of the olfactometer, and 5% CO_2 into the other port. The average number of mosquitoes entering the trap in 1 min. was 20.0 ± 4.3 and 4.0 ± 2.8, respectively (4 replicates, $P = <0.01$). Lactic acid was attractive to the mosquitoes and it may be assumed that it plays a role in this mosquito's attraction to man.

DISCUSSION

As seen in FIG. 1, the traps of the olfactometer were placed at the bottom of the olfactometer, the reason being that mosquitoes tend to stand on the sidewalls and ceiling of a cage and seldom on the bottom. Few mosquitoes go down the open traps unless they are attracted or are particularly active.

The role of CO_2 as an attractant for *Ae. aegypti* and other mosquitoes has been debated. This may be due to the following factors. (a) The method used. CO_2 is not a strong attractant, but a rather medium or weak attractant. In any one test if less than 10% of a population responds [as in the large-cage olfactometer used by Willis & Roth (1952), and as in our experiments], then attraction to CO_2 may be overlooked if a small number of mosquitoes is used. The space available to the mosquitoes in an olfactometer appears to affect their response. We believe that studies on flying insects should not be performed in a small space, as this may interfere with their natural reaction, particularly when the stimuli studied are not of a very strong nature. Willis & Roth (1952) obtained different reactions of *Ae. aegypti* to CO_2 in a small-cage olfactometer as compared to a large one. Only in the latter case was CO_2 shown to be attractive. (b) The strain or variety of *Ae. aegypti* used. There may be large differences in the response to stimuli of different strains or varieties. As pointed out earlier in this paper, the Ness-Ziona strain was less responsive to CO_2 and much more reluctant to enter the opening of a trap (2.0 cm in diam.) than the var. *queenslandensis* used in San Francisco. It would be of much interest to compare responses of different varieties of *Ae. aegypti* to different stimuli. (c) The intensity of response of the mosquitoes at the time of the test. If the stimulus is strong, such as when an arm is introduced into a cage of unfed *Ae. aegypti*, the mosquitoes respond and feed within a short period at any time of the day or night. In the case of weak stimuli, there may be large variations in response. Such variations have been observed by Khan et al. (1969) and others, as well as in our experiments, as can be seen from the standard deviations. It may be assumed that if a series of experiments is carried out at a time when the response is low, attraction to CO_2 may not be detected.

It appears that variations in the intensity of response are due to variations in the physiological states of the mosquitoes, as well as to various external stimuli. It has been observed that removal of the mosquitoes from the traps of the olfactometer by means of a vacuum cleaner (at a reduced voltage) caused increased activity of the mosquitoes in the olfactometer for a few minutes, due to air current. (We waited at least 30 min. from one test to the next.) It has also been observed that after a number of replicates were carried out on the same day, the mosquitoes tended to be less responsive in the last replicates as compared to the first ones. This might be due to fatigue, especially since the mosquitoes removed from the traps were reintroduced into the olfactometer.

Our results on RH are in agreement with those of some investigators and at variance with those of others. One reason for this discrepancy may be the water balance of the mosquitoes at the time of the tests. If the mosquitoes are deprived of water for a number of hours before a test, they may show attraction to high humidity and moisture. The same is true if they are gravid (Bar-Zeev 1960). If a test is run with a mixed population of different physiological states (Brown et al. 1951) or in the field (Brown 1951), the response to humidity may differ from that obtained in the laboratory with mosquitoes in a defined physiological state.

Most investigators agree that odor is an important factor in attracting mosquitoes. Our results confirm these findings by showing that odors emanating from the forearm attract mosquitoes. The amount of CO_2 emanated from the forearm is insignificant (Rahm 1956, Brouwer 1960, Frame et al. 1972). It has been shown above that the attraction of the mosquitoes to 1% CO_2 passing over the forearm was equal to the attraction of 1% CO_2 at 60% RH and 34°C. In this experiment, emanations of the forearm counterbalanced the attraction to 34°C. It should be noted, however, that the emanations were from a limited skin surface (forearm). A higher concentration of emanations might overcome the effect of heat.

The variations in the length of time that treated glass beads attracted the mosquitoes do not appear to be due mainly to variations in the response of the mosquitoes because the response to the treated glass beads was generally an all-or-none reaction (either 20–50 mosquitoes entered the trap in 1 min. or only 0–3 mosquitoes). The differences in response can be attributed to the different amount of attractive materials emanating from the forearm at different times. This method allows an effective trapping of volatile attractants, and offers a system to enable identification of attractive compounds.

The attraction of lactic acid was confirmed. It may be assumed that at least part of the attraction to emanations from the skin is due to lactic acid.

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