EFFECT OF HUMAN BREATH ON MOSQUITO ATTRACTION TO MAN

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ABSTRACT. The effect of human breath on the attraction of *Aedes aegypti* to the human host is quantitated. The effect of several concentrations of carbon dioxide on mosquito attraction in the presence or absence of human breath is also studied. Mosquito attraction to man when breath is excluded is ca. 60 percent less than with breath included. Introducing additional CO_2 in the room from a compressed gas cylinder in the presence of breath did not raise mosquito attraction significantly. Also, excluding breath but raising the CO_2 concentrations to 1428 and 4104 ppm, did not bring attraction to the level obtained with breath alone (CO_2 494 ppm). The possibility of a substance in the breath responsible for greater attraction is discussed.

INTRODUCTION. The response of anopheline mosquitoes to odor from a host was demonstrated in olfactometer experiments by Laarman (1955, 1958) and that of *Anopheles quadrimaculatus* (Say) and *Aedes aegypti* (L.) by Willis (1947). The importance of odor in the attraction of *A. aegypti* to a host by quantitating different behavioral parameters such as approach, landing, probing etc., was established in several studies conducted in this laboratory (Khan *et al.*, 1966a, b, 1967).

Besides odor, carbon dioxide also plays an important role in mosquito orientation to the host. Carbon dioxide from the host is contributed mainly through exhaled breath and only partly from the skin. Breath and carbon dioxide, however, have often been used as interchangeable terms (Wright, 1962; Kellogg and Wright, 1962; Daykin *et al.*, 1965; Wright *et al.*, 1965 and others). This, however, may not be so and mosquito activation by breath may be of a different magnitude compared to activation by CO_2. The following study was undertaken to ascertain the difference.

METHODS. Experiments were conducted in a 158 x 95 cm and 240 cm high room. The room had two doors facing each other. The ceiling was of suspended acoustic tile. A 60 watt incandescent electric bulb concealed by a ground glass shade lighted the room. One door was completely sealed with masking tape. Several plastic tubes (0.5 cm ID) were run from the experimental room through the bottom of the door into an adjoining room. These consisted of the following:

1. A tube with its opening 30 cm below the ceiling attached at midpoint on one of the walls, ran down the height of the wall, came out from under the door and opened into the adjoining room. This latter opening is called end "a."
2. A second tube ran from the mid-bottom area of the same wall, came out from under the door and opened at end "b."
3. A third tube for air intake attached to a compressor pump could be connected to end "a" of the first tube or end "b" of the second.

The exit nipple of the compressor pump was connected through a flow meter to the inlet end of CO_2 infra-red analyser (Lira Model 200 MSA, Pittsburgh, Pa.). A plastic tube connected to the outlet of the infra-red analyser carried the gas sample after CO_2 analysis back to the experimental room by passing under the door. The flow of the gas for CO_2 sampling was maintained at a 2 L/min by regulating the compressor pump with a rheostat. A by-pass valve with a tube carried the excess flow back into the experimental room. In this way a circulating closed system was obtained at constant flow. Samples of air from the top or the bottom of the room were obtained simply by connecting tube-end "a" or "b" to the compression pump intake.

Twenty-five 6–8 days old *A. aegypti* females previously fed on 5 percent sugar solution only were placed in a 20 cm^3 cage made of steel wire frame and covered with 8 mesh/cm nylon netting on all six sides. The cage was attached to the center of the ceiling. A 5 cm diameter hole in the bottom of the cage was used for mosquito exit. This was covered with a cardboard flap hinged to the bottom with masking tape on one side and which could be pulled down with a long wire. A volunteer subject sat on the floor directly under the cage separated from the mosquitoes by 165 cm. When breath was intended to be excluded from the room the subject took one end of a plastic tube in his mouth and exhaled into the adjacent room through the other end, turned away from the door. To introduce extra CO_2 into the room another plastic tube attached to a compressed gas cylinder released the gas at floor-level under the cage close to the subject.

For each experiment the subject sat on the floor wearing a white shirt and trousers, exposed only on his arms, head and face. He opened the bottom exit hole of the cage gently with a long wire and counted the number of mosquitoes landing on him every 30 sec. for 5 min. During this time the CO_2 concentration, the temperature, and the relative humidity (hygrometer model #15–4050, Thermodynamics, Inc.) in the room were monitored. During the experiments, the doors were taped with the masking tape. The room was not completely air-tight and the suspended acoustic ceiling allowed the air to diffuse upwards slowly. This helped in that stable CO_2 concentrations were easily obtained for 5-min. periods and a quick and continuous build up of CO_2 did not occur. Eight different variations of the experiment were used as explained by letters A to H in Table 2. In experiment E, a cylinder (15 cm long and 8.5 cm diam.) filled with activated charcoal (370 gm) was used, through which the subject exhaled to filter odor from the breath. A male Caucasian, 42 years old, served as host throughout the experiments. All experiments were conducted at 27±1° C and 40 to 50 percent RH.

RESULTS. Table 1 gives analysis of variance of the data and Table 2 the level of difference among means. With the exclusion of breath, mosquito attraction was reduced by ca 60 percent (cf. D and H, Table 2). When the subject was breathing inside the room the CO_2 concentration was ca 594 ppm. Increasing the CO_2 content of the room to 1917, 4317 or to 6786 ppm only slightly increased mosquito attraction but not significantly (cf. D with A, B & C). Breathing through the activated charcoal column reduced attraction somewhat, though insignificantly compared to the attraction obtained when the subject breathed in normally, or even when additional CO_2 was introduced in the room (cf. E with A–D). Excluding breath from the room but adding CO_2 to 4000 ppm did not bring mosquito attraction equal to that obtained by simply breathing in the room (cf. F and G with D).

DISCUSSION. Mosquito attraction to man when breath was excluded from the room was about 60 percent less (exp. H) compared to that obtained with breath included (exp. D). This could have been due to decreased CO_2 concentration in the air or due to the absence of any attractant factor(s) in addition to the CO_2 contributed by the breath. Decreased CO_2 concentration, however, was not found to be responsible. The background CO_2 concentration in the room air was ca 423 ppm. Breath from the subject in the room raised it to 594 ppm, an increment of 171 ppm. However, the mean attrac-

TABLE 1.—Analysis of variance of data on the effect of breath and CO_2 on mosquito attraction.

	df	SS	MS
Total	82	1242.7	
Tests	7	427.1	61.0*
Error	75	815.6	10.9

* $P < .005$.

TABLE 2.—Mean number of mosquitoes out of 25 landing and biting the subject in 5 minutes and the CO_2 and the temperature gradients in the room.*

Exp. ID	No. of Replicates	Experiment	No. of Mosquitoes landing & biting	CO_2 (ppm)		Temp ° C	
				Top	Bottom	Top	Bottom
A	10	Breathing in + 6786 ppm of CO_2	9.3a	6786	3826	26.5	25.0
B	16	Breathing in + 4317 ppm of CO_2	9.0a	4318	1439	26.5	25.5
C	9	Breathing in + 1917 ppm of CO_2	8.7a	1917	626	27.0	26.0
D	17	Breathing in + no extra CO_2	8.7a	594	492	27.0	26.5
E	4	Breathing in through activated charcoal column	7.4ab	526	418	27.0	26.5
F	8	Breathing out + 4104 ppm of CO_2	5.7bc	4101	2354	26.5	25.0
G	6	Breathing out + 1428 ppm of CO_2	5.2cd	1428	586	26.5	25.5
H	15	Breathing out	3.4d	475	398	27.0	26.0

* Means not followed by the same letters are significantly different at 5% level (Duncan's multiple range test using Studentized Ranges).

tion obtained with 1428 and 4104 ppm of CO_2 in the absence of breath was 5.2 and 5.7 mosquitoes respectively and (exp. G & F) significantly lower than 8.7 mosquitoes attracted in the presence of breath (exp. D). This suggests strongly that an attractant factor in breath is involved. Further, the attractant factor does not seem to be easily removed by activated charcoal, at least not by the quantity used, because mosquito attraction in experiment E was not significantly lower than that of D though it did decrease a little.

Smith *et al.* (1970) recently studied the effect of breath on mosquito attraction and found it less efficient in attracting mosquitoes than the hands. We have observed this consistently in our studies on factors attracting mosquitoes to human host. When tested sequentially even pure CO_2 combined with heat and moisture would attract as many mosquitoes as the palm (Khan *et al.*, 1966b) but compared side by side in a dual post system using a large cage, palm would pull almost all the mosquitoes away from the breath side (unpublished data). We, therefore, confirm the findings of Smith and co-workers. However, in our studies no attempt was made in isolating hands from the breath and Smith and co-workers did not compare hands or breath simultaneously nor did they add extra CO_2 to that of breath in their experiments. Besides, their studies were conducted in a dual post olfactometer and ours in a small room. It would not be valid, therefore, to compare the two studies in the same context. They attribute the attractiveness of the hands and the breath to the presence of L(+)-lactic acid. This may be true but we strongly feel that this is not the only chemical responsible for mosquito attraction. We agree more with their conclusion—viz "our data indicate the possible presence of other chemicals that may be true attractants for female *A. aegypti* or at least possess synergistic action."

Our data also indicate that the mosquito response in different concentration of CO_2 was not a result of the difference in CO_2 gradient (Table 2). The maximum gradient was found in experiment A and comparatively much smaller in experiment D when the subject was breathing inside with no extra CO_2

added; yet the attraction to the host was the same. Table 2 shows that there was greater concentration of CO_2 toward the ceiling than at the floor level though the gas is heavier than the air. This resulted perhaps partly from the strong convection currents set up by the host and partly by the by-pass valve-tube which carried the excess air-flow from the compression pump back to the experimental room at the floor level pushing the gas upwards.

Carbon dioxide has been used by several workers in mosquito traps for population sampling (Headlee, 1933; Reeves, 1951, 1953; Newhouse, *et al.*, 1966; Carestia and Savage, 1967 and others). Headlee (1934) found his mosquito collection increased 4 to 5 times with CO_2. Reeves (1953) felt that CO_2 chemotropism of mosquitoes may be the principal factor in host selection and that different mosquito species react differently to different CO_2 concentrations in the environment. Our own studies with *A. aegypti* suggest that CO_2 plays a dual role—a primary role of activation of mosquitoes and a secondary role of inducing landing and probing in the presence of heat and moisture (Khan and Maibach, 1966).

However, the role of breath except as a source of CO_2 has not been critically examined. While this study was in progress, Snow (1970) published his findings in Gambia, West Africa, demonstrating that significantly fewer mosquitoes approached the subject when CO_2 output was reduced. His subjects wore breathing apparatus containing soda lime which reduced CO_2 from exhaled breath by 95.5 percent. This is the only study to our knowledge which examined critically the effect of breath on mosquito attraction without using olfactometers. Our data show that when breath is excluded from the room, the mosquito attraction dropped to ⅓ and is in agreement with Snow's findings. However, we further demonstrate that excluding breath but introducing CO_2 in quantities greater than contributed by the breath does not restore the host attraction to mosquitoes to the level found in the presence of breath. This suggests that breath contains some added factor or factors which perhaps synergize the effect of CO_2. When breath was excluded from the environment, factors like moisture and the convective heat of the breath were also excluded. Although no drop in the temperature or the relative humidity was registered when breath was excluded from the room, some deficit in the total moisture and heat budget of the room must have occurred. How important this deficit was for mosquito attraction was not ascertained. In the field conditions, however, this cannot be envisaged as material factors except in the immediate neighborhood of the subject. Gillies (1969) using the formula of Bossert and Wilson (1963) illustrated the relative importance of different factors eliciting mosquito response to animal host. In the illustration given by him the long range odor zone is more effective in the orientation of certain mosquito species than the middle range CO_2 zone. It would be interesting to know, how exclusion of breath without corresponding decrease in CO_2 concentration will affect the effective range of the odor zone.

Among other constituents breath also carries L(+)-lactic acid which has been demonstrated to be attractive to mosquitoes (Smith *et al.*, 1970). This, however, may not be the only chemical responsible for attraction. Whatever may be the chemical nature of "odor" there is much evidence now in favor of chemical stimuli attracting *A. aegypti* females. The recent studies of Garcia and Laing (1970), who were looking into the findings by Harris and Cook (1969), and Harris *et al.* (1969) reporting insect larvae as host for *A. aegypti* females, further point in this direction. Garcia and Laing found that the attractiveness of eight species of caterpillars to mosquitoes was due to contamination by human odor. They also found that after handling these caterpillars were more attractive to *A. aegypti* female in the presence of breath, compared to CO_2 alone.

An interesting aspect of the study is

that attractiveness of the host to *A. aegypti* did not increase significantly with increasing CO_2 concentration. This is at variance with the results of Harden, *et al.* (1970) who caught more *A. aegypti* on a subject with CO_2 than without. The total number of *A. aegypti* they collected with or without CO_2, however, was small. They caught six mosquitoes in the presence of CO_2 and only one in its absence in five replicates. The discrepancy might also be due to different experimental conditions. Our results cannot be validly extrapolated to field conditions. The effect of human breath on the attraction of *A. aegypti* and other mosquito species in the field needs more investigation and better elucidation.

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