(1) removal of the aft cabin doors and construction of a tank whose ends will form the sides of the fuselage; (2) installation of a pitched bottom inside the tank which will provide for the flow of the granules and eliminate the requirement for the supporting frame; (3) installation of an indicator to show flow of the pellets. This might be a weight gauge mounted in or under the tank to show change in the total weight or an off-pressure switch mounted on the venturi which would indicate flow of pellets through the latter; (4) addition of a dust pan type spreader to the rear of the venturi box which would widen the ejection port from 24 to 50 inches and depress the present slot from 6 to 2 inches. The purpose of this spreader would be to widen the current effective swath of 35 feet to at least 50 feet.

Because of its high degree of maneuverability, its ruggedness, and load capacity, the L-20 "Beaver" was selected as a possible vehicle for the USAF Special Aerial Spray Flight. Development of professional insecticide dissemination kits specifically engineered for installation in this airplane increased its applicability.

Operational experience in the Air Force during the past two years has shown that addition of the L-20 has expanded substantially the operational scope of the Spray Flight and enhanced the value of this unit by insuring its ability to cover all aspects of the terrain.

As a result of effective utilization of the airplane and continuing improvement through modifications of the respective spray kits, the L-20 "Beaver" has come to be a most satisfactory supplementary dissemination vehicle and has indirectly become an integral unit in the over-all Air Force program for the aerial dissemination of insecticides.

THE ATTRACTIVENESS OF HUMAN SWEAT TO MOSQUITOES AND THE ROLE OF CARBON DIOXIDE

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Although human sweat has long been suspected of being attractive to mosquitoes, early investigations (Howlett, 1910; Crumb, 1922; Rudolfs, 1922) found little to substantiate this belief. However, more recent experiments (DeLong *et al.*, 1949; Willis, 1947; Parker, 1948) have yielded evidence that sweat can be attractive. Furthermore, field experiments with robots (Brown, 1951) showed that sweat-soaked clothing was almost twice as attractive to Canadian *Aedes* mosquitoes as equally moist clean clothing. Moreover, laboratory experiments with an olfactometer (Brown, Sarkaria and Thompson, 1951) showed that the vapour from an aqueous solution of human sebaceous sweat would bring approximately 40 per cent more females of *A. aegypti* to the experimental port than to the central port which carried only water vapour.

Human sweat is comparable to a weak solution of sodium chloride in water and contains, at least in traces, practically all of the non-colloidal constituents of blood plasma. It is produced in large part by the sudoriferous glands, which are simple tubular structures distributed over the entire body surface, being most abundant on the palms of the hands, the soles of the feet, and the forehead (Kuno, 1934).

This sudor is admixed in most regions with sebum, which is produced by the sebaceous glands; these are complex and alveolar in structure, opening most frequently into hair follicles. Sebaceous glands are most abundant in the armpits and inguinal areas, about the scalp and face and around body apertures, and are also distributed all over the body surface with the exception of the palms of the hands and the soles of the feet. Sebum is an oily material containing lower fatty acids, higher primary alcohol esters and cholesterol, in addition to albumins and inorganic salts. In the experiments reported in this paper, sweat from the armpits and sweat from the forehead were tested separately to determine whether they evoked the same response in mosquitoes. Both these sources of sweat are a mixture of sudor and sebum, armpit sweat containing more sebum and less sudor than forehead sweat. It was found impossible to obtain pure sudor from the palms of the hands in sufficient quantity for experiment.

MATERIAL AND METHODS. The stock of *Aedes aegypti* (L.) was maintained in a greenhouse at temperatures of 68–85° F. and relative humidity of 45–70 per cent. The larvae were reared in enamel pans on a diet of 1 part dried yeast and 2 parts dog biscuits. The adults were confined in a large, screened cage 10 ft. long by 4½ ft. wide by 8 ft. high, the population being maintained at 500–1500 females. They were fed on raisins and on blood secured from a live rabbit placed in the cage once or twice a week.

All experiments were performed in the large cage. The olfactometer employed has been fully described elsewhere (Brown, Sarkaria and Thompson, 1951) and consists essentially of two ports 3.5 ins. in diameter, suspended 16 ins. apart and each emitting an air stream at 5 litres per minute. The air leading to the ports was bubbled through gas-washing bottles containing the liquid to be tested.

Observers at each port counted the number of approaches made by mosquitoes within 0.5 in. of its surface. When either observer had counted 50 approaches, the count was stopped and both figures were recorded. A set of 8 such counts constituted a test. Position error between the two ports and counting error between the two observers, neither of which ever exceeded 10 per cent, were compensated by appropriate interchanges of ports and observers (Brown, Sarkaria and Thompson, 1951).

ATTRACTIVENESS OF ARMPIT SWEAT. The vapour from armpit sweat at various concentrations was compared with moist air for its attractiveness to the mosquitoes. The sweat was obtained by placing filter papers under the armpits of several young Caucasian males who had taken strenuous exercise. The samples thus obtained from a group of subjects were then placed in a known volume of distilled water. The average sample of sweat on a filter paper weighing 186 mg., and the aliquots of water chosen per filter paper being 6, 60 and 600 cc., the aqueous concentrations thus obtained were 31, 3.1 and 0.31 mg. per cc. These solutions were refrigerated until testing, at which time they were added to one of the gas-washing bottles leading to the olfactometer, distilled water being used in the other bottle as a control. Tests with the 3 concentrations were performed under environmental conditions of 65–85 per cent R.H. and 74–92° F. The ratio between the experimental figures and the control figures has been termed the Attractiveness Ratio.

The tests showed that the sweat vapour attracted no less, and often considerably more, mosquitoes than did the moist air stream. The variance involved in the 8 counts of each test was statistically analyzed, and the value of Student's t was obtained to determine the probability of the figures indicating a significant difference between the presence and absence of the sweat. At the highest concentration, 1 of the 3 tests showed statistical significance at the 5 per cent level; at the intermediate concentration, 2 of the 4 tests were significant; and at the lowest concentrate, none of the tests showed that a significant difference existed. When

the data for all the tests are considered together at each concentration, a significant difference at the 5 per cent level is to be found at 31 mg. per cc. and at 3.1 mg. per cc., but not at 0.31 mg. per cc. Thus it may be concluded that at adequate concentrations armpit sweat vapour is significantly attractive, drawing on the average (in this experiment) 25 per cent more adult *Aedes aegypti* than water vapour alone. The variance is such that certain tests may show no difference in the numbers attracted, while others may show 50 per cent more mosquitoes approaching the source of the sweat.

A second experiment was carried out in which cloth-covered spheres were employed instead of the olfactometer. A single layer of black broadcloth was wrapped around black plastic billiard balls mounted on vertical rods and held 16 ins. apart. Tests of 8 counts each were conducted in which a cloth-covered sphere soaked in armpit sweat was compared with one soaked in distilled water. The counting procedure was identical to that used in the olfactometer experiments, except that the mosquitoes were required to touch the surface of the cloth in order to be counted. A series of 3 tests were carried out at each of the concentrations previously described. The results gave average attractiveness ratios of 1.01, 1.02 and 0.93 for sweat concentrations of 31, 3.1 and 0.3 mg./cc. respectively. It may be therefore concluded, without statistical analysis, that human armpit sweat presented on clothing exerts no attraction for *Aedes aegypti* under these laboratory conditions.

ATTRACTIVENESS OF FOREHEAD SWEAT. A series of olfactometer experiments was performed with sweat collected from the foreheads of white Caucasian males. The aqueous elutions from filter papers were diluted to give concentrations of 29, 2.9 and 0.3 mg. of sweat per cc. Three tests of 8 counts each were carried out for each concentration of sweat, employing moist air as the control, at 43–63 per cent R.H. and 82–96° F.

It appears from the results that the sweat vapour at all three concentrations was slightly less attractive than moist air. Statistical analysis however, shows the t values (for 23 degrees of freedom) to be only 1.21, 0.88 and 0.32 for 29, 2.9 and 0.3 mg./cc. respectively and thus insufficient for significance. It may therefore be concluded that the difference in attractiveness between forehead sweat and moist air is negligible.

CARBON DIOXIDE IN SWEAT. A series of three experiments were performed to investigate the possibility of CO_2 being responsible for the attractiveness shown by armpit sweat. Here the olfactometer was employed with the usual procedure, at 40–65 per cent R.H. and 77–94° F., the materials being added to the gas-washing bottles. In the first experiment, a solution of armpit sweat at 3.1 mg. per cc. was freed of CO_2 by precipitating it as barium carbonate by the addition of 10 per cent $BaCl_2$ solution in the ratio of 1 cc. per 1 gm. of sweat; this CO_2-free sweat solution was compared with normal sweat solution at the same concentration. In the second experiment, a similar sweat solution was saturated with CO_2 vapour added through a bubbler; it was then compared with the normal sweat solution. In the third experiment a solution of CO_2 in water was prepared, having the same concentration as that which occurs in armpit sweat. This solution was made by adding 382 mg. of $NaHCO_3$ and 61 cc. (at N.T.P.) of CO_2 to 100 cc. of distilled water. The solution therefore contained 0.20 per cent bound and 0.11 per cent free CO_2, which were the average figures obtained when fresh armpit sweat was analyzed for its content of CO_2 (Am. Publ. Health Assoc., 1946). This solution was then tested against distilled water, in order that its vapour might be compared with moist air.

The average attractiveness ratios obtained in the first two experiments indicate that there is no difference in either case between the experimental and the control sweat solutions. The attractiveness ratio of 0.87 in the third experiment might have been taken to indicate that the experimental solution was slightly less at-

tractive than the control, but statistical analysis indicates that the difference is due to the high variance and thus is not significant ($t = 0.74$). It may therefore be concluded that CO_2 neither increases nor decreases the attractiveness of armpit sweat or aqueous solutions.

CARBON DIOXIDE IN CLOTHING. In field experiments with robots, Brown (1951) had found sweat-soaked clothing to be considerably attractive to species of Canadian *Aedes* but clothing soaked in water saturated with CO_2 to be no more attractive than clothing soaked in adulterated water. It was decided to repeat the latter experiment under laboratory conditions with *Aedes aegypti*, utilizing the cloth-covered spheres. A series of 5 tests, each with 8 counts, was performed with the CO_2 saturated water as the test solution and distilled water as the control, at 53–74 per cent R.H. and 77–84° F.

The results, not tabulated here, indicated that the black broadcloth soaked in CO_2-saturated water gained attractiveness ratios between 1.12 and 1.38, being on the average 24 per cent more attractive than the cloth soaked in distilled water. Five similar tests were performed using white cheesecloth as the covering; here the average attractiveness ratio was 1.33 with the values ranging between 0.93 and 1.98. Statistical analysis revealed that the t value, for all counts taken with the black broadcloth was 4.38, and with the white cheesecloth 4.44, both of which indicate highly significant differences at the 0.1 per cent level. It may therefore be concluded that the addition of CO_2 to wet clothing significantly increases its attractiveness to *Aedes aegypti* under laboratory conditions.

VOLATILE ACIDS AND BASES. Of the fatty acids which might be expected to occur in sebaceous sweat, oleic, valeric and lactic acid have been found unattractive to *Aedes sollicitans* (Rudolfs, 1922). Lactic and propionic acid have proved slightly attractive, and by comparison formic and acetic acid slightly repellent, to *A. aegypti* (DeLong *et al.*, 1949). Acetic and lactic acids were found to be indifferent at low concentrations and comparatively repellent at high (Brown, Sarkaria and Thompson, 1951) when tested in the olfactometer. Since the addition of CO_2 to water-soaked cloth-covered spheres had proved attractive (see above), the effect of adding 0.1 per cent acetic acid to these surfaces was investigated. Four tests of 8 counts each were performed at 70–85 per cent R.H. and 78–80° F. In every test the surface with acetic acid was less attractive, the attractiveness ratios falling between 0.61 and 0.70.

In a second series of 8 tests with 8 counts each, performed with the olfactometer, the volatile acids which were present in a solution of armpit sweat (3.1 mg. per cc.) at the normal pH of 6.5 were first released from ester linkages by acidification to a pH of 1.9 with dilute H_2SO_4. When air was then bubbled through this solution and the vapour emitted through the olfactometer port, the attractiveness ratios as compared with the vapour from a similar but untreated sweat solution were between 0.64 and 0.97, with a mean of 0.82. Statistical analysis of all 64 paired counts revealed a t value of 2.90, which indicates a significant difference at the 1 per cent level. The individual test with the highest attractiveness ratio showed its difference to be significant at the 5 per cent level.

Volatile bases such as ammonia and trimethylamine have been found to be unattractive to *A. aegypti* (Brown, Sarkaria and Thompson, 1951). In an attempt to discover if any of the volatile bases released on addition of alkali to sweat were attractive, a solution of armpit sweat at 3.1 mg. per cc. was made alkaline with Na_2CO_3 to a pH of 9.4. When air was bubbled through this solution and simultaneously through a similar but untreated solution, and the vapours compared in the olfactometer, the attractiveness ratios obtained in 3 tests were 0.97, 1.02 and 1.11, indicating that the treatment did not affect the attractiveness.

It may therefore be concluded that the

attractiveness shown by armpit sweat is decreased by the release of volatile acids present in the sebaceous portion and normally held by esterification, whereas the release of any volatile bases present has essentially no effect on the attractiveness.

CONCLUSIONS. Sweat from the armpits of Caucasian males has proved significantly attractive to adult females of *Aedes aegypti* when tested in the olfactometer at sufficient concentration. Sweat collected from the forehead was not attractive in the olfactometer. The attractiveness of moist clothing was not increased by the addition of armpit sweat.

It is unlikely that carbon dioxide is responsible for the attractiveness of human sweat in the olfactometer, since its addition to or removal from armpit sweat did not alter its attractiveness, nor did the addition of CO_2 at the level found in sweat increase the attractiveness of distilled water. However, the addition of CO_2 to water-soaked cloth significantly increased its attractiveness under laboratory conditions.

The volatile acids freed from sweat by acidification have a repellent effect on it, whereas the bases freed by rendering it alkaline do not alter its attractiveness.

Literature Cited

AMERICAN PUBLIC HEALTH ASSOCIATION. 1946. Standard Methods for the Examination of Water and Sewage. 9th Ed., pp. 32–37.

BROWN, A. W. A. 1951. Studies of the responses of the female *Aedes* mosquito. Part IV. Field experiments on Canadian species. Bull. Ent. Res. 42:575–582.

BROWN, A. W. A., SARKARIA, D. S., and THOMPSON, R. P. 1951. Studies of the responses of the female *Aedes* mosquito. Part I. The search for attractant vapours. Bull. Ent. Res. 42:105–114.

CRUMB, S. E. 1922. A mosquito attractant. Science 426:446–447.

DELONG, D. M., *et al.* 1949. Insect behaviour: mosquito attraction and repellency. Final Summary Report, Project 272, Office of the Quartermaster-General, Washington. March 31, 1949.

HOWLETT, F. M. 1910. The influence of temperature upon the biting of mosquitoes. Parasitology 3:479–484.

KUNO, Y. 1934. The Physiology of Human Perspiration. J. & A. Churchill, London. 268 pp.

PARKER, A. H. 1948. Stimuli involved in the attraction of *Aedes aegypti* (L.) to man. Bull. Ent. Res. 39:387–397.

RUDOLFS, W. 1922. Chemotropism of mosquitoes. Bull. N. J. Agric. Exp. Sta. No. 367, 23 pp.

WILLIS, E. R. 1947. The olfactory responses of female mosquitoes. Jour. Econ. Ent. 40:769–778.

WILLIS, E. R., and ROTH, L. M. 1952. Reactions of *Aedes aegypti* (L.) to CO_2. Jour. Exp. Zool. 121:149–179.

CULEX THRIAMBUS DYAR FROM NEW MEXICO. Three larvae of *Culex thriambus* Dyar were collected from residual pools in an irrigation canal at Virden, Hidalgo County, New Mexico, September 17, 1953. The larvae were found in association with *Culex tarsalis, Anopheles franciscanus,* and *Psorophora signipennis.* This collection was taken in connection with mosquito studies conducted by the Logan Field Station Section (formerly Water Projects Section), CDC, U. S. Public Health Service, and constitutes a new mosquito record for New Mexico.—Charles S. Richards, Comm. Dis. Center, Pub. Hlth. Service, U. S. Dept. of Health, Education and Welfare, Logan, Utah.