cation was 2½ miles directly windward N.W.) of a large breeding area and 4 iles from another situated directly to the st. These midges may have come from ther or both of these sources. About 70 rcent of the breeding areas on St. John cur on the eastern half of the island and e adult *Culicoides* are carried along with e prevailing winds over the mountains ,100–1,200 feet), spilling over onto the est end of the island. It would appear at many of them may travel more than miles with the wind.

SUMMARY. On the island of St. John, . S. Virgin Islands, the midge, *Culicoides rens,* breeds in tidal mangrove swamps d in areas of vegetation at the edges of ackish and nearly fresh water ponds. mergence begins within one week after rched mud is submerged by rising pond vel due to heavy rainfall. As water level lls, due to evaporation, emergence is imulated. The emergence of these flies om wet mud follows the receding water d emergence will continue from any ven sq. ft. area for a period of 1 week. dults may be carried by prevailing winds for more than 4 miles over mountains 1,200 ft. high.

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COLLECTION OF ORAL SECRETION FROM MOSQUITOES[1]

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INTRODUCTION. The reactions of humans d experimental animals to mosquito tes are generally agreed to be allergic in ture (McKiel and West, 1961). The lergens are introduced into the host as e mosquito probes and feeds. The aller- ns appear to be associated with the saliva the mosquito; this is substantiated by the work of Hudson *et al.* (1960), who showed that the bites of mosquitoes whose salivary ducts had been severed caused no skin reactions in sensitized subjects.

In previous studies of the bite reactions in our laboratory, the materials used for experimental sensitizations and/or skin-sensitivity reactions have been extracted from whole mosquitoes, from abdomen or head-and-thorax portions, or from dissected salivary glands. For refinement of these studies it was necessary to attempt collection of the saliva which contains the components active in the allergic reactions.

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It is well-known that female mosquitoes of many species may be induced to feed on blood through various membranes. A comprehensive survey of such work is given by Tarshis (1956). In the method we have used to collect oral secretion of mosquitoes, female mosquitoes have been induced to feed on distilled water through Silverlight [2] membranes, and in the process of feeding they apparently inject oral secretion into the water. After a period of feeding, the oral secretion in the remaining fluid has been concentrated and stored. Initial studies were much encouraged by the report of a similar method used by Benjamini *et al.* (1960), working with fleas. Following the lead of Benjamini and his co-workers, we have referred to the product as oral secretion since as yet we have no proof that the saliva is uncontaminated.

METHOD. A petri dish containing 10 ml. of distilled water was covered with Silverlight membrane which was held taut and in place by two elastic bands. The distilled water used, as measured in a conductivity cell, showed conductivity values equivalent to 0.5–0.8 p.p.m. NaCl. The dish was warmed in an oven at 40° C. for ten minutes before being placed, membrane-side down, on the brass-screening roof of a cage containing 3,000 to 4,000 mosquitoes. Females were attracted to the warm membrane and would imbibe distilled water if a sufficient temperature differential was maintained between the membrane and the surrounding environment. The appropriate temperature of the water and membrane was maintained during the 45-minute feeding periods by placing a small oven over the inverted dish. Each oven was constructed from the lid of a ½ lb. tobacco tin. Inside this was coiled an electrically-heated tape whose temperature was controlled by a variable transformer. The temperature at the membrane surface was measured by a calibrated copper-constantan thermocou Usually, the feeding dishes and ovens w set up in pairs.

After the 45-minute feeding period, a oral secretion contained in the remaini water was concentrated by lyophilizati The products of two feeding dishes w transferred to a 50 ml. flask, and, followi rapid shell-freezing, the flask was attac to a 12-port Virtis freeze-drying unit. T freezing mixture in the unit was ma tained at a temperature of —60° C., a the whole system was evacuated to a pr sure of less than 0.1 mm. Hg. After d ing was completed, flasks were sealed under vacuum, using a cross-fire burn The vacuum in the flasks was tested af 24 hours by means of a high freque coil. The flasks were stored in a de freeze unit.

Mosquitoes used in these experiments cluded laboratory-reared *Aedes aegy* (L.) and *Anopheles quadrimaculatus* S *A. aegypti* were reared by the method McKiel (1957). *An. quadrimacula* were tray-reared in the larval stages. A we have used *Aedes stimulans* (Walk and *Aedes vexans* (Meigen) which w field-collected, and identified as lar Adult mosquitoes were held in cages 20 20" x 20" during the experiments.

RESULTS. *A. aegypti* females fed on distilled water when the membrane t perature was held between 29° and 32° with an ambient temperature of 22° and a relative humidity of 70 perc The optimum membrane temperature der these conditions appeared to be ab 30.5° C. If the mosquitoes were star (deprived of sucrose solution) for 24 ho before the membrane feeding, appr mately 30 percent of the females took distilled water during the 45-minute riod; this figure was determined by vi ing obvious abdominal distension, as s under a dissecting microscope. The s vation procedure caused considerable m tality, and for standard practice, mak collections over a period of several mon it was not used. If no starvation occur before membrane feeding, it was shown

[2] Silverlight membranes were obtained from Julius Schmid Inc., 423–429 West 55th Street, New York 19, N. Y. They are now no longer being manufactured.

-tracer experiments that an average of ercent of the females in a cage contain- 3,500 to 4,000 mosquitoes would take the labelled feeding fluid. The *A. aepti* used in these experiments were all least 72 hours old.

For *Anopheles quadrimaculatus,* the mbrane temperature requirements were nd to be more exacting. With an amnt temperature of 22° C. and 70 per- t relative humidity, the optimum mmne temperature was approximately 33° although females would probe and d at membrane temperatures between ° and 35° C. This same temperature ge was found suitable for the fieldght mosquitoes.

The lyophilized material from *A. aepti* has been used in sensitization experints. Twenty-four guinea pigs received radermal or subcutaneous injections; ht controls received material derived m "membrane feedings" in which no squitoes were present in the cage; the naining 16 pigs received the lyophilized l secretion. The freeze-dried material s dissolved in small volumes of physiical saline for injection. Each test ani- l became sensitized to mosquito bite stance as shown by a positive skin reion to a mosquito bite received after sensitization course. No control anils became sensitized. Sensitivity was o tested in all animals by intradermal ection of test and control materials; the extent of reactions was measured with the aid of intravenous injections of Evans Blue dye. Test animals showed positive reactions in response to injections of the *A. aegypti* oral secretion; control animals showed no significant response. None of the animals showed sensitivity in response to injections of control material, showing that no sensitizing materials were derived from the membrane. Rabbits have also been used successfully in sensitization trials.

CONCLUSIONS. It appears that at least some of the reactive principles responsible for the allergic state of bite-sensitivity have been collected from *A. aegypti,* and, insofar as this is true, mosquito saliva has been collected. Further tests on the oral secretion from *A. aegypti* and on the materials collected from other species are at present incomplete.

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