grandifolia) treeholes. *Orthopodomyia alba* larvae have been collected from early May through mid-September (7,14,28 May, 19 June, 16 July, 30 July, 11 August, 11 September, 1976), while *O. signifera* have been collected only from mid-July into mid-September (16,30 July, 11 August, 11 September, 1976). Collections were made over a similar time period in 1975 also.

A distinct difference in water conditions has been noticed for the larval habitat of each species. *Orthopodomyia alba* has been collected only from beech treeholes in which very dark coffee-colored water rich in organic matter occurs, while *O. signifera* has been collected only from beech treeholes in which the water is considerably clearer and darkened only by suspended dirt particles. Other species associated with *O. alba* in collections were *Aedes bendersoni*, and *Anopheles barberi; Aedes triseriatus* and *Anopheles barberi* were found in association with *O. signifera.* The significance of this differential larval habitat and species association in beech treeholes is under investigation.

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A SPECIAL STUDY TO SHOW LARVAL DENSITIES IN RELATION TO WATER DEPTH

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We studied species and species densities in relation to water depth to show our *Aedes* problem to our staff. We firmly believe that most of our mosquito problem is produced in areas that are dry much of the time and wet some of the time. Thus, the thought was that we would find the heaviest densities of *Aedes* in fairly shallow water.

In the 15 sites studied 14 had heaviest densities in the 1 to 6 in. depths. In the 13 in. depth or deeper, densities were very light or nil. Even in shallow sites breeding larvae of other genera the heaviest larval densities were in 1 to 6 in. of water.

We realize the chance of "spooking" immatures when wading in deep water is good. We tried to minimize this effect in this study. We concluded that the greatest numbers of any species were in 1 to 6 in. of water. These 15 sites were studied between May 27 and June 8. Species found were: *Culiseta inornata* and *Ae. vexans* which were predominant, some *Cx. territans, restuans, tarsalis* as well as some *Ae. sticticus* and *Ae. canadensis.* If this study had been done earlier in the year our univoltine *Aedes* might have shown us a different picture.

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AN *IN VITRO* FEEDING TECHNIQUE FOR ARTIFICIALLY DEMONSTRATING VIRUS TRANSMISSION BY MOSQUITOES[1]

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Certain viruses that are possibly transmitted by insects do not lend themselves readily to experimental insect transmission studies for lack of suitable laboratory recipient hosts. The available animal hosts do not produce apparent infections following injection of virus by a peripheral route (such as provided by a feeding mosquito). They may not produce viremia or even antibodies after various methods of virus exposure. Moreover it is not always possible to achieve a favorable feeding response even when a satisfactory host animal is available. Therefore several *in vitro* techniques were explored to overcome this difficulty. One method, utilizing *Aedes aegypti*, which proved successful is described here.

Glass capillary tubing (7 cm long and ± 1 mm outer diameter) is drawn to a fine point in a tiny flame. The capillary orifice should be capable of easily receiving the mosquito's proboscis. Feeding of unconfined mosquitoes is accomplished by inserting the *entire* proboscis into the finely-drawn capillary containing 0.005 ml of heat-inactivated defibrinated chicken blood and per-

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mitting the mosquito to dangle free. (*Note*: fetal calf serum with or without sucrose has been used in place of the blood meal.)

Using sharply-pointed jeweler's forceps and a stereoscopic microscope, a lightly-anesthetized (chloroform or CO_2) mosquito is firmly grasped at the base of the folded wings. By shifting the closed forceps to the left hand and taking a blood-charged capillary in the right hand, the capillary is quickly inserted over the proboscis. Capillary and mosquito are held close together for a few seconds and then, very gently, the forceps are relaxed from the wings and the capillary with dangling mosquito is transferred to a small Styrotex rack containing a series of holes into one of which the capillary tube is slowly introduced and the mosquito allowed to feed (Figs. 1, 2). The serum quickly thickens around the capillary orifice—thus holding the proboscis in position. Care must be taken to remove the first and second pairs of legs to prevent the female from using them as levers to free herself. Occasionally a female becomes detached but she can be repositioned easily.

A feeding mosquito is removed from the blood meal before completing engorgement and, if infected with a virus, may be tested immediately or stored at an appropriate temperature for subsequent pathogen assay. Meanwhile the residual blood meal is ejected into a glass tube from the capillary by syringe and needle equipped with a tiny rubber gasket. The blood meal volume is increased by the addition of 0.03 ml of 2.5% fetal calf serum and the specimen is then tested for virus. This can be done by inoculating it into a suitable tissue culture system, or preferably (as antibiotics and fungicide are not needed) by following the technique of Rosen and Gubler (1974), in which the specimen is inoculated intrathoracically into a batch of uninfected mosquitoes to permit any virus present time to multiply sufficiently for easy recovery. After an extrinsic incubation period of 7–10 days at 26.7°C, these inoculated mosquitoes are harvested; they are triturated, centrifuged and the resulting suspension is inoculated intracerebrally into suckling mice, tissue culture or other appropriate system for eventual virus recovery and identification.

Initially it may take 15–20 min. to position properly a mosquito in the capillary but with practice the time can be reduced to 4–5 min. In one experiment 80% of 49 exposed mosquitoes engorged on the blood meal. No enhancement of feeding was noted when mosquitoes were exposed to blood meals over a 37°C water bath [this experience may be contrasted with that of Gatehouse (see Gibbs et al. 1973) who found temperature and relative humidity critical to the feeding sequence of *Stomoxys calcitrans*].

A possible drawback to the capillary feeder is that some virus ejected in the saliva may be taken up again by the feeding mosquito as there is no provision for blood circulation. Nevertheless, with this technique, low passage dengue 2 virus and Orungo virus were recovered from capillary blood meals which had been exposed to feeding infected mosquitoes, thus demonstrating *in vitro* transmission of virus.

COMMENTS: The idea of an artificial feeding technique for demonstrating pathogen transmission by haematophagous arthropods is not new. Perhaps one of the first attempts in this direction was that of Hertig and Hertig (1927) who conceived an ingenious device for artificially feeding phlebotomine midges. It was their belief that feeding would not occur unless the fly's labium was forcibly folded back (as occurs in nature) so that only the stylets penetrated the blood meal source. Accordingly the Hertigs produced exceedingly fine glass capillaries to achieve this end. They were likewise successful in developing a functional feeding capillary for mosquitoes. Subsequently M. Hertig achieved great success with this technique during his long-term studies of phlebotomines and leishmaniasis in Panama (Hertig and McConnell 1963).

Haas and Ewing (1945) successfully fed infected *Ae. aegypti* on exposed blood vessels of incubating hen's eggs and subsequently recovered

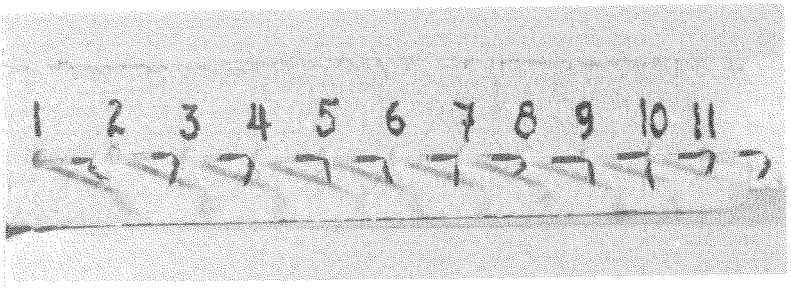

Fig. 1. Feeding mosquitoes positioned on capillary holding rack.

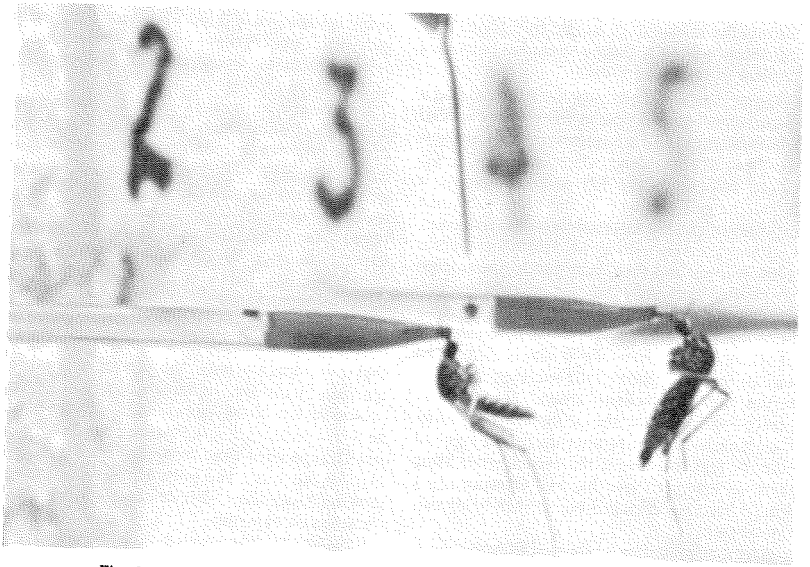

Fig. 2. Feeding detail. Dark spot on capillary indicates original blood level.

Plasmodium gallinaceum from the hatching chicks. Ferris and Hanson (1952) modified the above technique to permit captive mosquitoes, stable flies, and horn flies to feed through the exposed air sac membrane of the egg; and Burgdorfer and Pickens (1954) successfully used the latter technique both to infect *Ornithodoros* ticks as well as effect pathogen transmission to chick embryos. I explored all of these techniques in the course of the present study but discarded them as being either too cumbersome or the feeding too unpredictable. Later Burgdorfer (1957) described a glass capillary feeding technique for infecting ixodid ticks with pathogens but then relied on a vertebrate host for the actual transmission experiment. In view of our results, those same capillaries might well be used to demonstrate *in vitro* tick transmission of pathogens. Mason et al. (1965) found that *Ae aegypti* could transmit Sindbis virus to chick embryo cell cultures in membrane-covered petri dishes when adenosine triphosphate was employed as a feeding stimulant. And Muangman et al. (1969), while investigating the anal discharge of Sindbis virus by infected *Ae. aegypti*, used capillary feeding techniques somewhat resembling those presently described but these were incidental to the main study.

A somewhat similar technique but involving a blood droplet rather than a "blooded" capillary is that of Gubler and Rosen (1976), in which a mosquito is confined in a cotton-plugged tube covered at the other end with fine-mesh *synthetic* netting on which is placed a measured drop of the blood meal. The mosquito is permitted to feed partially on the blood droplet after which it and the blood meal are treated as described above. In our hands this latter technique has met with only moderate success as many times a tube-confined mosquito will not engorge on the blood droplet. However, if that same recalcitrant mosquito subsequently has its proboscis introduced into a blooded capillary tube, it usually will feed. Thus, by using both techniques, a high degree of test feeding usually can be achieved. The present success with capillary feeding demonstrates that the bending or forcing back of the mosquito's labium is not necessary to initiate the feeding mechanism as suggested by the Hertigs. In conclusion, Hurlbut (1966) has measured salivation and virus (St. Louis) discharge into a mineral oil system by *Culex pipiens*. He noted that salivation may occur for periods as long as one hour at irregular rates with occasional brief interruptions. Hurlbut found that samples taken later in the test period contained more virus than earlier ones; there was also increased virus recovery when mosquitoes were held at 37°C as contrasted with 25–30°C.

With such artificial methods for demonstrating pathogen transmission by feeding mosquitoes, it becomes possible to conduct studies relatively easily with viruses for which no suitable small laboratory host animals are known.

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TRANSPORTING INSECTICIDES IN HALF-TON PICKUP TRUCKS

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American Cyanamid's "Abate" and Exxon's "Flit" have, in recent years, largely replaced the old stand-bys, diesel oil and New Jersey Larvicide, in the day-by-day spray operations of the Morris County Mosquito Commission. These newer materials are normally applied at 4–7 gal. per acre. The dosage rates of these newer materials, far lower than those of the older larvicides, allow treatment of typical nuisance areas with relatively small amounts of spray. Therefore, our district inspectors, whose primary task is the surveillance of potential breeding sites, seldom have occasion to spray more than 50 gal. each day with their portable compressed air sprayers and/or small gasoline engine pressure pumps. When necessary, additional tankloads of Abate can be prepared by using available water sources in the field. Backup crews are available to spray the larger nuisance areas with high-pressure truck-mounted sprayers, mist blowers, swamp buggies or helicopters.

Tanks, suitable for carrying 15–40 gal. of insecticides (well within the capacity of a half-ton pickup) can be obtained from agricultural, industrial, marine and surplus dealers. Some are sold as specialized units and others are available as spare parts for spray units or as non-returnable shipping containers. Custom-built units can be constructed in steel, aluminum, PVC, or fiberglas.

A careful review of all options was made, and 2 tanks were selected on the basis of adaptability, corrosion resistance, and economy. These were a 30-gal. galvanized "Tank for Water Pumps" and a 19½-gal. military surplus smokeless powder can. Together they occupy only a small portion of the area available in the beds of "wide-bodied" (Fleetside, Styleside, etc.) pickups. (Fig. 1) The remaining portions can be used for the mounting of a fog generator and concentrate reservoir or other equipment. The combined savings in bulk and weight gained in the adoption of low volume larvicides and adulticides has enabled the Morris