PARABIOTIC TWINNING OF MOSQUITOES[1]

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Parabiotic twin mosquitoes are employed in attempts to determine the type of immune mechanism in mosquito species that are refractory to malaria infections. It has been shown (1) that the factors governing susceptibility of mosquitoes to malaria are systemic rather than localized in certain fixed tissues such as the stomach wall; therefore when it became necessary to determine whether immunity of one species was due to the lack of required metabolites or was due to antagonistic factors that destroyed the parasites, parabiotic techniques seemed likely to provide means of exploration. The study is in progress but results, although indicating the latter thesis (2), are not complete. The technique of parabiotic twinning of the mosquitoes seems, however, to warrant reporting at this time.

Aedes aegypti, highly susceptible to *Plasmodium gallinaceum* and *Culex pipiens,* refractory to the parasite are fed simultaneously on a chick with a high gametocyte count. The two species are then joined by means of a tiny glass capillary tube about 100μ in diameter so that they share common haemolymph. The mosquitoes are anesthetized with CO_2 or immobilized on a cold microscope stage (3), and the capillary tube is inserted into one mosquito in the membranous area posterior to the mesothoracic spiracle. When haemolymph fills the capillary tube, the other end is then inserted into the other mosquito, taking care not to trap a bubble of air in the tube. The capillary is held by special tongs fabricated from 20 gauge Pitkin needles (Figure 1) or by negative forceps. The parabiotic twins are held on the tongs for 15–20 minutes to allow haemolymph to harden around the area of the inserted capillary (Figure 2) and then are transferred to holding cages and maintained on 4 percent sugar water.

The twins are able to walk and feed but rarely fly except very short hops. One parabiotic pair survived for 22 days and several pairs have lived 14–18 days. Experimental pairs in the malaria susceptibility studies are required to remain together for 8 days, for the development of the parasite from gametocyte fertilization to sporozoite. Seventeen pairs have survived this period of time. Many pairs survive from 2 to 4 days but frequently separation of the pairs occurs during the first 12 hours or one of the individuals will die before the experiment is completed. Short term survival might be of value in physiological, insecticidal, or other investigations but are of limited value in the susceptibility experiments. The survival of the parasites in each host is challenged by inoculating each mosquito into susceptible chicks.

Exchange of haemolymph has been demonstrated by means of dyes and in-

FIG. 1.—Special tongs for inserting glass capillary in parabiotic mosquito. A modified, spring loaded, Pitkin needle.

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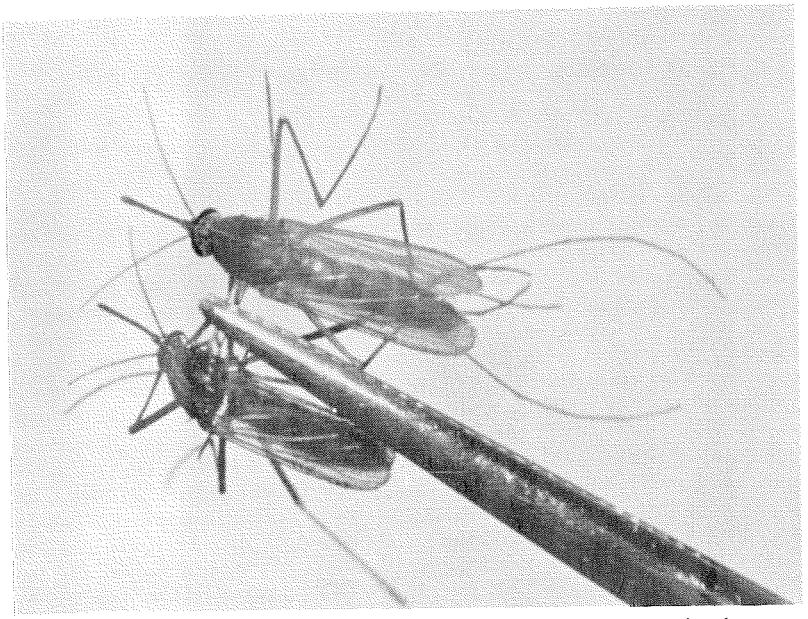

FIG. 2.—Parabiotic twin *Aedes aegypti* and *Culex pipiens,* sharing common haemolymph.

secticides. When dye is injected into one mosquito it can be seen to flow to the other mosquito. Later it may be seen returning to the injected species. Minute amounts of DDT, malathion, or chlordane, applied topically to the outward side of one mosquito results in apparent death of this mosquito in 15–20 minutes. The twin, to which insecticide has not been applied, dies 45 minutes to one hour later, depending on the concentration of insecticide. This technique shows promise in studies relating to insecticide resistance as well as in experiments on susceptibility of insects to pathogenic agents.

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