COMPARATIVE GROWTH OF DENGUE VIRUSES IN *AEDES AEGYPTI* AND *AEDES ALBOPICTUS* AFTER PARENTERAL INFECTION[1]

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ABSTRACT. Growth of dengue 1, 2, 3, and 4 viruses was studied in *Aedes aegypti* and *Aedes albopictus* after parenteral infection. Growth curves for all four dengue viruses were similar in both mosquito species. Virus titers peaked on days 4 to 6 postinfection at over 10^7 MID_{50} per mosquito.

Recent studies by Gubler and Rosen (1977) have described the quantitative aspects of dengue virus replication in *Aedes albopictus*. Although this species has been shown to be a highly competent vector of these viruses in the laboratory (Simmons et al. 1931, Snijders et al. 1931, Gubler and Rosen 1976), another species, *Ae. aegypti*, is the principal vector of dengue/dengue hemorrhagic fever in Asia. Little is known of the quantitative aspects of dengue virus infection in this latter species. This report describes the comparative growth characteristics of all 4 dengue serotypes in both *Ae. aegypti* and *Ae. albopictus* female mosquitoes.

MATERIALS AND METHODS

The mosquitoes employed were *Ae. aegypti* from a colony established in 1975 with specimens collected in Jakarta, Indonesia. The *Ae. albopictus* were from a colony established in 1971 with specimens from Honolulu, Hawaii. Eggs from this colony were taken to Indonesia in 1975 and recolonized. Mosquitoes were reared in an insectary maintained at ambient temperature, humidity and photo-period, which in Jakarta, Indonesia, was approximately 12 hr of daylight and 12 hr of darkness. Larvae were given a diet of Purina® rabbit chow pellets, and adult mosquitoes were provided with 10% sucrose both before and after infection with dengue viruses.

VIRUSES. The viruses employed were the 4 prototype strains of dengue (Hawaiian, New Guinea C., H87 and H241). None had been passed in mice or cell cultures, but all had been passed several times in mosquitoes. Stock pools of virus were prepared by intrathoracic inoculation of *Ae. aegypti* (Rosen and Gubler 1974). After 7 days incubation at 32°C., mosquitoes were killed by freezing and triturated in phosphate buffered saline (PBS), pH 7.4, containing 30% heat inactivated (56°C for 30 minutes) calf serum. After centrifugation at 1,575 × g for 30 min at 4°C, the supernatant fluid was dispensed in 0.3 ml aliquots into screwcap vials and rapidly frozen by immersion in a mixture of alcohol and solid carbon dioxide.

INFECTION AND ASSAY OF MOSQUITOES. Both species of mosquitoes were infected parenterally on the same day with a single virus serotype by the method of Rosen and Gubler (1974). After infection, mosquitoes were placed in 0.5 liter cardboard containers covered at each end with fine mesh nylon and held in the same incubator at 32°C and approximately 50 to 60% relative humidity. Mosquitoes were provided with a

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maintenance diet of 10% sucrose. At daily intervals through day 4, then at 2-day intervals through day 14, and on days 18, 25, and 35 postinfection, 5 mosquitoes of each species were killed by freezing and individually assayed for virus content by the method of Rosen and Gubler (1974). Mosquitoes were disintegrated by sonic energy in a conical centrifuge tube containing 0.5 ml of PBS with 30% heat inactivated calf serum. After centrifugation at 1,575 × g for 30 min at 4°C, serial dilutions of the supernatant fluid were made using PBS with 5% heated calf serum and inoculated into groups of uninfected *Ae. aegypti*. These mosquitoes were held from 10 to 14 days at 32°C and examined for the presence or absence of viral antigen in the brain tissue by the direct fluorescent antibody test (Kuberski and Rosen 1977). Generally, at least 5 mosquitoes inoculated with each dilution were tested and the mosquito infectious dose 50 (MID_{50}) calculated by the method of Reed and Muench (1938).

RESULTS AND DISCUSSION

Comparative growth curves for the 4 prototype dengue viruses in *Ae. aegypti* and *Ae. albopictus* are shown in Figure 1. Mosquitoes were infected with 10^3 MID_{50} of dengue 1, 3, and 4 viruses and with 10^2 MID_{50} of dengue 2 virus. Most points, except those marked with an asterisk, represent the geometric mean of 5 mosquitoes. In both species and with all 4 dengue serotypes, replication generally peaked by days 4 to 6. Maximum mean virus titers in *Ae. aegypti* with dengue 1, 2, and 4 were over $10^{7.5}$, whereas mean dengue 3 titers attained a maximum about fivefold higher at 10^8 MID_{50} per mosquito. In *Ae. albopictus*, virus titers for all viruses were approximately $10^{7.5}$ MID_{50} per mosquito. In both species, virus titers slowly declined to about 10^6 MID_{50} on days 25 and 35 postinfection.

It will be noted that the growth curves for all 4 dengue viruses were very similar in the 2 mosquito species, with only small

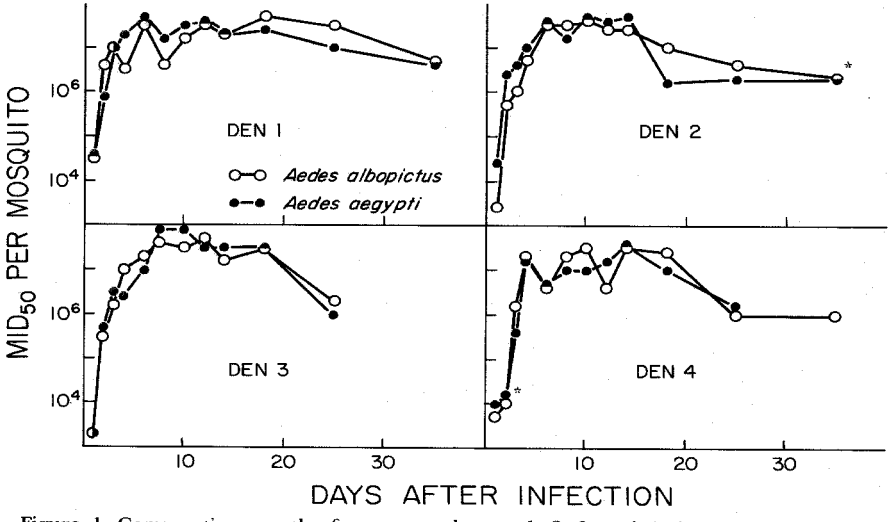

Figure 1. Comparative growth of prototype dengue 1, 2, 3, and 4 viruses in female *Aedes aegypti* and *Aedes albopictus* after parental infection. Points represent geometric means of 5 mosquitoes. Those followed by asterisks represent only 1 mosquito.

differences in the rate of replication or in virus content. The virus titers observed in both *Ae. aegypti* and *Ae. albopictus* were comparable to those observed previously by Gubler and Rosen (1977) in *Ae. albopictus* and considerably higher than those observed by Whitehead et al. (1971). With the exception of dengue 3 in *Ae. aegytpi* in the present study, all viruses grew to approximately $10^{7.5}$ MID_{50} in both species of mosquito. Titers of dengue 3 in *Ae. aegypti* were 10^{8} MID_{50} on days 8 and 10 postinfection, but it should be noted that there was fivefold or more variation in titer observed between days with the same virus and mosquito. On the other hand, the Jakarta *Ae. aegypti* showed a higher susceptibility to oral infection with dengue 3 than the other serotypes (Gubler et al. 1979a), an observation which is consistent with the higher virus content per mosquito observed in the present study.

It has been shown that geographic strains of both *Ae. aegypti* and *Ae. albopictus* vary in susceptibility to oral infection with dengue viruses (Gubler and Rosen 1976, Gubler et al. 1979a). In both studies, however, it was observed that virus replication in the mosquito was not related to oral susceptibility. Thus, once the virus passed the gut barrier into the hemocoel, virus replication was as extensive in resistant mosquito strains as in susceptible strains. Furthermore, no variation in virus content per mosquito was observed between different geographic mosquito strains. Although only single geographic strains of *Ae. aegypti* and *Ae. albopictus* were used in the present study, the findings suggest that both species are equally competent in supporting dengue virus replication after infection by the parenteral route. This conclusion is supported by results of vector competence studies with strains of *Ae. aegypti* and *Ae. albopictus* from Bantul, Central Java, Indonesia. Both species showed comparable oral infection rates, virus replication and transmission rates with the Bantul strain of dengue 3 virus (Jumali et al. 1979). All results support the conclusion that *Ae. albopictus* may play an important role in transmission of rural epidemics of dengue/dengue hemorrhagic fever which have been occurring more frequently in recent years in Indonesia (Sulianti Saroso 1978, Gubler et al. 1979b).

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