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ISOLATION OF CHIKUNGUNYA VIRUS FROM *AEDES AEGYPTI* FED ON NATURALLY INFECTED HUMANS IN CALCUTTA

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EPIDEMIOLOGICAL evidence and laboratory transmission studies have implicated *Aedes ægypti* as the principal vector of chikungunya virus in the recent epidemics in Calcutta.[1-4] It has been of interest therefore to determine whether humans circulating the virus could infect this species. For this purpose a colony of *Aedes ægypti* was established at the Calcutta School of Tropical Medicine from larvæ and pupæ collected from Calcutta City and nearby Howrah.

Unfortunately it was difficult to persuade people to allow themselves to be fed on by the mosquitoes. However, five individuals with fever and clinical symptoms similar to those associated with chikungunya virus infection gave permission. Since most arboviruses circulate during the earliest phase of the illness, and generally only for a relatively short period, it was necessary to feed the mosquitoes before the diagnosis was definitely established. In the present trials, virus was isolated from the blood of only three of the patients.

The first patient was an entomological technician who was presumably infected while collecting mosquitoes in localities where cases of chikungunya virus infection were occurring. He became ill on July 27, 1964. On July 29th, he was exposed to 40 *Aedes ægypti* and thirty-eight of them engorged. Chikungunya virus was isolated from a blood sample obtained at the time the mosquitoes were feeding, the titer of which was approximately 10^4 LD_{50}/0·02 ml. The mosquitoes were kept at room temperature until August 5th when they were allowed to feed on normal infant mice from the laboratory colony before shipment to the Poona headquarters of the Virus Research Centre.

During this interval 16 of the mosquitoes died but all of the remaining mosquitoes were successfully transported by air in closed wooden containers. After arriving in Poona, the mosquitoes were maintained in an environmental room at about 30° C. and 80% relative humidity. On August 11th, 13 days after feeding, each of a group of eight of the mosquitoes was allowed to feed on a separate two-day-old mouse but none of the mice became sick. Individual two-day-old mice were again exposed to the bite of several mosquitoes on August 17th and one of these became ill on the 4th day after the feeding. An agent was isolated from its brain which was identified as chikungunya virus by complement fixation test with known normal and hyperimmune sera. A third feeding was

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made on August 25th of a group of 14 mosquitoes but none of the mice became ill. All of the mosquitoes found dead in the cage after arrival in Poona and those remaining alive after the August 25th feeding were stored at — 50° C. Most of these mosquitoes were tested individually, or in small groups, for the presence of virus. The mosquitoes were ground in a mortar and suspended in 1·5 ml. of bovine albumin phosphate saline containing 1,000 units of penicillin and 1 mg. of streptomycin per ml. Following light centrifugation to throw down the larger particles, the supernatant fluid of each suspension was inoculated in 0·02 ml. volumes intracerebrally into two groups of two-day-old mice. Chikungunya virus was isolated from a pool of two mosquitoes, one of which presumably fed on the mouse from which virus was isolated.

On September 24th, 1964, a hospital sweeper became ill and was admitted to the Carmichael Hospital of the School of Tropical Medicine, Calcutta. His temperature was 100·5° F. and he complained of severe bodyaches and joint pains. No rash was seen at that time. The patient was exposed to a group of 50 mosquitoes but only 20 became engorged. The titer of chikungunya virus in the blood of the patient at the time the mosquitoes fed was $10^{2\cdot8}$ LD_{50}/0·02 ml. Three mosquitoes died in Calcutta and were discarded. The engorged mosquitoes were transported in Barraud boxes in wooden containers to Poona by train and arrived on October 2, 1964. On arrival, 14 mosquitoes were found dead. These were ground in one pool and inoculated intracerebrally into infant mice. Chikungunya virus was isolated from this group of mice. On October 9th each of the remaining three mosquitoes was fed on a different two-day-old mouse. The mice did not become sick. A pool of the three mosquitoes was tested for the presence of virus by intracerebral inoculation of a suspension prepared from them into infant mice, but no virus was isolated.

Sixty-eight *Aedes ægypti* engorged on a third patient from whom chikungunya virus was isolated. In this case the blood sample obtained at the time the mosquitoes engorged had a titer of $10^{3\cdot5}$ LD_{50}/0·02 ml. The mortality among this group of mosquitoes was high and only 16 survived the trip to Poona. Unfortunately, the mosquitoes dead on arrival were not tested for virus. No virus was isolated from any of the remaining mosquitoes fed on this patient.

It has therefore been possible to demonstrate the infection of *Aedes ægypti* following engorgement on the blood of humans during the period of viremia. Although only two mosquitoes were shown to become infected, the titers of virus in the blood of these patients at the time the mosquitoes fed was not very high. The stage of the infection during which the titer of virus is highest is not known in chikungunya virus infection in man but much higher titers have been found in other patients during the course of the clinically apparent illness and one would expect that with higher titers a larger proportion of the mosquitoes would become infected after feeding.[5,6]

These results provide further evidence for the role of *Aedes ægypti* as an important vector of chikungunya virus and suggest that this virus might become established in a human population by person-to-person transfer by bites of *Aedes ægypti*. A city the size of Calcutta might very well be able to provide a sufficiently large susceptible population at all times to maintain chikungunya virus as an endemic infection.

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