Studies on Dengue in Vellore, South India *

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Studies of dengue at the Vellore Field Station of the Virus Research Centre, Poona, during the past several years led to the detection, up to December 1963, of 137 group B arthropod-borne virus infections in humans. From the acute-phase sera of these patients 104 strains of dengue virus were isolated in infant mice. These comprised 60 type 1 dengue strains, 31 type 2 and 13 type 4. During 1961 and 1962 type 1 isolates predominated. Type 2 virus reappeared in 1963, having last been isolated in 1959. Type 4 was recovered in 1960, 1962 and 1963 from human sera. Types 1 and 4 were isolated from *Aedes aegypti* in 1961.

Most viruses were recovered from ill staff and students at the Christian Medical College and Hospital, Vellore. Their illnesses predominantly resembled classical dengue. Haemorrhagic disease associated with dengue was not recognized.

Subclinical dengue infection was rare in a group of student nurses studied in 1961.

A bimodal yearly distribution of cases and virus isolations with peaks in March and September was observed. These peaks correlated well with periods of rising and falling ambient temperatures. When the mean minimum temperature dropped below 20°C, cases were no longer seen, nor were isolations made.

Dengue virus was isolated both from acute-phase sera containing complement-fixing and/or haemagglutination-inhibiting antibody and from sera without detectable antibody. Antibody responses were generally very broad. Almost half of the patients yielding an identified dengue virus developed their highest CF or HI antibody titres for related group B viruses.

Factors felt to be of importance in the isolation of dengue virus included the inoculation of very young mice with human serum obtained early in the illness, the careful daily observation of each inoculated mouse, and the passage of mouse brain to additional litters at the first indication of mouse illness.

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Isolation of Dengue Viruses from Haemorrhagic Fever and Dengue Patients in Singapore *

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Acute phase sera obtained from haemorrhagic fever and dengue patients in Singapore were routinely inoculated into 1-day-old mice for virus isolation. In 1963, 12 viruses were isolated, including two strains of dengue virus type 1, five strains of dengue type 2, and two strains of dengue type 3. The dengue type 3 virus strains were the first isolations in Singapore since the first appearance of dengue viruses in 1960.

In 1964 up to August, 21 viruses were isolated, of which 12 were dengue virus type 1 and four were dengue type 2. These dengue viruses were isolated in the first passage in infant mice showing sickness, paralysis or death. The incubation period of these viruses at the first-passage level varied from four to 14 days. These viruses were adapted to

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2- to 3-day-old mice after 6-10 mouse-brain passages. Most of these viruses were isolated from blood specimens obtained from patients on the second day of illness. No viruses were recovered from autopsy material obtained from six patients who died.

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Dengue Viruses Isolated from Haemorrhagic Fever Cases in Malaysia *

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Mosquito-borne haemorrhagic fever caused by dengue virus was first recognized in Penang, Malaysia, in November 1962. From then until April 1964, 61 cases were confirmed in Penang with a sporadic seasonal incidence. Prominent features of the disease in Penang were fever, vomiting, abdominal pain, haemorrhagic signs, marked thrombocytopenia, appearance of Tuerk cells, and circulatory collapse. The median age was 7.5 years, the case-fatality rate 8.2%. An additional three relatively mild cases were diagnosed in adults in Kuala Lumpur from December 1963 to February 1964.

Fourteen strains of dengue virus were isolated from the acute-phase sera of Penang patients, and an additional two strains from the mild cases in Kuala Lumpur. For virus isolation, one- to two-day-old Swiss white mice were inoculated intracerebrally and intraperitoneally with a 1 : 4 dilution of patient's serum. Brains were harvested from sick mice for serial passage. Where no frank illness occurred, brains were " blind-harvested " from two mice about 10 days after inoculation. About five weeks after inoculation, surviving mice were challenged with 100 LD_{50} of dengue virus to demonstrate whether or not dengue immunity had developed. If dengue challenge was resisted, " blind-harvested " brains were passed serially until virus was demonstrated by illness and death in the mice.

Most of the virus isolates required less than three blind passages before illness appeared in the mice, although additional passages were necessary before a consistent pattern of illness and death developed. Five required more than three blind passages. Incubation periods in infant mice ranged from 9 to 18 days when illness was first observed and from 4 to 7 days after 10 serial passages. All the Penang strains were isolated from children 10 years of age or younger. The two Kuala Lumpur strains were from adults who had illnesses more like classical dengue than like haemorrhagic fever. These strains required no blind passage and produced frank illness in mice in a well-defined incubation period. All isolations were made from patients' sera taken not later than the fourth day of disease. Isolations were made throughout the year. The validity of all the virus isolations was established either by reisolation or by demonstration of dengue challenge resistance in mice inoculated with the original serum specimen from which the virus was first isolated. Three of the Penang strains and one Kuala Lumpur strain have been tentatively identified as dengue type 2 on the basis of cross-neutralization tests in mice. The remaining strains have not yet been typed.

Where paired serum specimens were available, haemagglutination-inhibiting and neutralizing antibodies rose rapidly to high titres. It was interesting to observe that dengue virus could be isolated from serum containing significant amounts of dengue-neutralizing antibody. Of the 47 Penang patients from whom virus was not isolated, 14 showed significant rises in dengue antibody and the remaining 33 had significantly high levels of dengue antibody.

Chikungunya virus did not appear to be involved, as evidenced by negative serology and failure to isolate the virus.

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