THE TRANSMISSION OF DENGUE BY *AEDES POLYNESIENSIS* MARKS

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Numerous epidemics of dengue have been reported from Melanesia and Polynesia south of the equator since early in the 19th century (Hirsch, 1883). Prior to World War II *Aedes aegypti* (Linn.) was the only proven vector of this virus known to occur in these areas. During World War II epidemiologic observations indicated that vectors other than *A. aegypti* might be responsible for the transmission of dengue in certain parts of Melanesia (Daggy, 1944 and Mackerras, 1946). As a result of these observations, experimental work was undertaken which demonstrated that *Aedes scutellaris* (Walker), a species widely distributed in Melanesia, could transmit the infection.

In the course of studies on the epidemiology of human filariasis on Tahiti from 1950 to 1952 the senior author noted that the local distribution of *A. aegypti* did not seem extensive enough to account for the widespread epidemic of dengue which had occurred on the island in 1944. Two species of mosquitoes, *Culex quinquefasciatus* Say and *Aedes polynesiensis* Marks, are widespread and abundant on Tahiti. The former species has been shown to be incapable of the biological transmission of dengue (Simmons *et al.*, 1931). The latter species, which is the most important vector of human filariasis (*Wuchereria bancrofti*) in Polynesia, has only recently been recognized as distinct (Marks, 1951) from the closely related mosquito, *Aedes pseudoscutellaris* (Theobald).

In 1953, an opportunity was afforded in the United States to test the ability of *A. polynesiensis* to transmit dengue using monkeys as vertebrate hosts. Since dengue infections in monkeys cannot be recognized by clinical manifestations, serological methods were employed to establish their occurrence.

MATERIALS AND METHODS

Virus. One ml. of human serum containing the Hawaiian strain of dengue virus which had never been passed in mice was inoculated intraperitoneally into

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The work in Baltimore was supported in part by a grant from the office of Naval Research, Department of the Navy.

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The work performed in Cincinnati was carried out under the auspices of the Commission on Virus and Rickettsial Diseases of the Armed Forces Epidemiological Board and was supported by funds provided under contract AF 18 (600)-548 with the U.S.A.F. School of Aviation Medicine, Randolph Field, Texas.

the monkey which served as the donor for all mosquito feedings. Prior to its use this serum had been stored in the frozen state for 5 weeks in a box containing solid CO_2. Titration in suckling mice indicated that the serum had at least $10^{5.5}$ infective doses of virus per ml.

Monkeys. Two rhesus monkeys (*Macaca mulatta*), Nos. 1 and 2, and two cynomolgus monkeys (*Macaca irus* group) from the Philippine Islands, Nos. 488 and 490, were used in these experiments. Monkey No. 490 was used as the donor. These animals were housed in a mosquito-free area in a building separate from that in which the mosquito colonies were maintained. Prior to their use, the sera of these monkeys were tested for the two immunologic types of dengue antibody and none was found.

Mosquitoes. The *A. polynesiensis* used in these experiments were from a laboratory colony which was started with specimens from the Samoa Islands and which is maintained at the School of Hygiene and Public Health, Johns Hopkins University. Before specimens were selected for these experiments the colony was carefully checked to be sure that it was not contaminated with *A. aegypti* or any other species of mosquito. In addition, each mosquito used was identified at the time that it fed on the donor monkey and also at the time that it fed on the recipient monkey.

The mosquitoes fed readily when the shaved leg of an anesthetized monkey was inserted into the cage in which they were held. The engorged specimens were placed in cylindrical, pint-sized ice cream cartons, the tops and bottoms of which had been replaced by bobbinet. These cartons were kept in a constant temperature incubator in which the dry bulb temperature was maintained at 79° to 80°F. A high relative humidity was assured by placing the cartons on pads of moist cotton. Except for the experimental blood meals, the mosquitoes were fed only on apple slices.

Serological procedures. Serial dilutions of sera obtained from each monkey prior to and subsequent to the experimental feedings of mosquitoes were tested for the presence of hemagglutination-inhibition antibodies against 16 units of dengue hemagglutinin of the Hawaiian type. The recovery and characteristics of hemagglutinins from the two types of dengue virus have recently been reported elsewhere (Sweet *et al.*, 1953).

RESULTS

One lot of *A. polynesiensis* was fed on the donor monkey 4 days after inoculation of the dengue virus, another lot was fed 6 days afterwards, and a third lot was fed 8 days afterwards. These three lots of mosquitoes, after being held for extrinsic incubation periods of 16 days, 14 days, and 12 days respectively, were subsequently fed on three recipient monkeys. The number of specimens of each lot which fed on each of the recipient monkeys is given in Table 1.

The hemagglutination-inhibition titers of the sera of the donor and recipient monkeys are given in Table 2. It will be noted that all monkeys developed antibody indicative of dengue infection.

TABLE 1

The number of A. polynesiensis *of each lot which fed on the three recipient monkeys*

Mosquito Lot No.	Days After Inoculation of Virus Fed on Donor Monkey	Extrinsic Incubation Period	No. of Mosquitoes Fed on Indicated Recipient Monkey		
			Cynomolgus 488	Rhesus 1	Rhesus 2
1	4	16	23	35	31
2	6	14	33	50	40
3	8	12	26	25	30
Total mosquitoes fed on each monkey.....			82	110	101

TABLE 2

Hemagglutination-inhibition titers of sera of the donor and recipient monkeys against 16 units of Hawaiian dengue hemagglutinin

Monkey No.	Mode of Infection	Titer of Antibody at Indicated Time		
		Before use	36 days	59 days
Cynomolgus 490....	Human serum intraperitoneally	<10	—	80*
Cynomolgus 488....	Mosquitoes	<10	20	20
Rhesus 1.....	Mosquitoes	<10	80	—
Rhesus 2.....	Mosquitoes	<10	>1280	640

* This specimen was obtained at 56 days.

DISCUSSION

The demonstration that *A. polynesiensis* is capable of transmitting dengue is not surprising in view of the taxonomic relationship of this species to the other proven vectors, *A. aegypti*, *Aedes albopictus* (Skuse), and *A. scutellaris*. All are members of the subgenus Stegomyia. In addition, *A. polynesiensis* belongs to the *scutellaris* group of this subgenus as does *A. scutellaris*. Inasmuch as the *scutellaris* complex, which now includes 17 species distributed throughout the Australian Region and the eastern part of the Oriental Region, is a very homogenous group, it is not unlikely that others of its members are also capable of transmitting this virus.

Both rhesus and cynomolgus macaques have previously been shown to be susceptible to infection with dengue (Blanc *et al.*, 1929; Simmons *et al.*, 1931; Findlay, 1932). Since these primates occur in endemic areas of the human infection, it has been suggested (Simmons *et al.*, 1931) that they might have a role in the epidemiology of the infection similar to that subsequently shown for the lower primates in the epidemiology of yellow fever. If such is the case, mosquito vectors which occur both in the bush and around human habitations, such as *A. albopictus* and members of the *scutellaris* group, would be especially important in the maintenance of the transmission chain.

In addition to the observations for Tahiti, there are data which suggest that *A. polynesiensis* is an important vector of dengue in other widely separated parts of Polynesia. An extensive epidemic of dengue was reported from Manua Islands in American Samoa (Hargrave, 1931). A recent mosquito survey of these islands showed that the only species of *Aedes* present is *A. polynesiensis* (Jachowski, 1953). Similarly, a widespread epidemic of dengue occurred in the Marquesas Islands (Mumford and Adamson, 1944). Again, a recent survey by the senior author (Stone and Rosen, 1953) indicated that the only species of *Aedes* present is *A. polynesiensis*. While it is possible that *A. aegypti* existed in these areas at the time of the dengue epidemics and subsequently disappeared spontaneously, such an event is unlikely. Finally, it was reported (Amos, 1947) that the predominant mosquito in the neighborhood of each outbreak of dengue in Suva, Fiji Islands, in 1943 was a member of the *scutellaris* group (the three species of this group including *A. polynesiensis*, now known to occur in the Fiji Islands, were not recognized as distinct at that time). *A. aegypti* was present in the area but was very rare.

The biology and control of *A. polynesiensis* is similar to that of *A. scutellaris* described by Mackerras (1946). Both species are more difficult to control than *A. aegypti*.

SUMMARY

Epidemiologic observations in Polynesia suggested that *Aedes polynesiensis* Marks served as a natural vector of dengue in that area. This mosquito species was shown to be capable of transmitting dengue from monkey to monkey in the laboratory. A hemagglutination-inhibition test was used to demonstrate the occurrence of the infection in the experimental monkeys.

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