an N—P bond with a charged nitrogen, a species which would be expected to be an active phosphorylating agent. The validity of this hypothesis is being tested chemically, as well as biologically.

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Dengue Type 2 Virus in Naturally Infected Aedes albopictus Mosquitoes in Singapore

Abstract. *A strain of dengue type 2 virus has been isolated from* Aedes albopictus *collected in Singapore. This is the first report of a natural isolation of dengue virus from this species, which has long been suspected as a vector in nature.*

Mosquito-borne hemorrhagic fever is a severe clinical syndrome etiologically associated with strains of dengue virus and newly recognized in southeastern Asia and India (*1–3*). During an investigation of the first recognized outbreak of the disease in Singapore in 1960–61 (*4, 5*), strains of dengue virus were isolated from the serums of two patients (*5*), five pools of *Aedes aegypti* mosquitoes, and one pool of *A. albopictus* mosquitoes. Of the two viruses isolated from patients, one was identified as type 1 dengue and the other as type 2 dengue (*5*). All of the virus isolates from mosquitoes proved to be type 2. This is the first report of an isolation of dengue virus from naturally infected *A. albopictus*, a proven efficient experimental vector of dengue (*6*) and long suspected as a vector in nature on epidemiological grounds (*7–9*).

Although *A. aegypti* has been incriminated as the principal vector of dengue for many years, it was not until 1960 that the first isolation of the virus from naturally infected mosquitoes was reported (*1*). At that time, isolations were made of dengue type 3 virus from *A. aegypti* collected in the Philippines in 1956 and of dengue type 2 from *A. aegypti* collected in Bangkok in 1958 during hemorrhagic fever epidemics. Subsequently, additional strains of virus have been isolated from *A. aegypti* collected in India (*10*), Thailand (*11*), and South Vietnam (*3*). These various isolations include all four types of dengue virus. No isolations were made from *A. albopictus*, collected and processed under similar circumstances.

In November 1960, following the peak of the epidemic in Singapore, and while cases were still occurring, a mosquito survey was initiated. Adult mosquitoes were taken routinely on a weekly basis from urban and rural houses, in diurnal and nocturnal biting collections, and from animal bait. A total of 12,505 mosquitoes, representing more than 40 species of eight genera, was collected in a 3-month period. Of these, more than 6000 female mosquitoes were processed for virus isolation.

Collected mosquitoes were held alive for a minimum of 24 hours before being processed, in order to allow for digestion of any freshly engorged blood. They were then lightly anesthetized with chloroform and accurately identified as to species. Female mosquitoes were stored at −70°C. Frozen mosquitoes were pooled by species, date collected, and location. Generally, not more than 50 mosquitoes were included in each pool. The pooled frozen mosquitoes were suspended in 3 ml of diluent (33 percent normal inactivated rabbit serum in beef heart infusion broth) and centrifuged at 10,000 rev/min for 20 minutes. A portion of the supernatant was treated with a penicillin-streptomycin mixture in the cold, and subsequently inoculated intracerebrally (0.01 ml) and intraperitoneally (0.03 ml) into two litters of 1- to 2-day-old mice. The mice were observed for signs of illness for 21 days. Brains were harvested from all sick mice, and suspended and inoculated into new groups of mice. Where no illness occurred between the 5th and 8th days after inoculation, the brains were harvested from two mice and stored.

From 4 to 5 weeks after inoculation, the surviving mice were challenged with doses of approximately 100 LD_{50} of adult-mouse–adapted dengue type 2 (Trinidad 1751) virus. Where dengue-challenge resistance occurred, the stored mouse brains were prepared as 20-percent suspensions, then diluted to 10 percent, and inoculated into mice. Portions of all mosquito suspensions and mouse brain suspensions were stored at −70°C. Part of the laboratory work was done in Singapore, where the mice were held in a mosquito-proof room. The remainder of the work was done in San Francisco, a dengue-free area.

Five strains of dengue virus were isolated from *A. aegypti* pools and one strain was isolated from a pool of *A. albopictus*. The *A. albopictus* strain (SM-18) was from a pool of 49 females that were collected in November 1960 in urban Singapore, while they were attempting to feed on human bait. The dengue virus infection rates were 0.8 per 1000 for *A. albopictus* compared to 18.6 per 1000 for *A. aegypti* (*12*).

Strain SM-18 caused illness in infant mice 10 days after inoculation with the mosquito suspension, but it adapted to mice with difficulty. Ten serial brain passages in infant mice were required before the incubation period was reduced to 7 days and a regular pattern of illness and death appeared. Of the

Table 1. Neutralization of dengue strain SM-18 virus from *Aedes albopictus* by dengue types 1, 2, 3, and 4 hyperimmune serums prepared in mice and rabbits.

Serums	Neutralizing antibody as represented by the $\log_{10}$ neutralization index with hyperimmune serums to:				
	D1 (Hawaiian)	D2 (New Guinea "C")	D3 (H-87)	D4 (H-241)	SM-18
Mouse	<1.2	2.2	<1.2	1.4	2.7
Rabbit	1.3	3.9	1.2	2.6	

52 mice that survived inoculation with SM-18 in the first several passages, 43 survived subsequent dengue challenge, which shows the acquisition of immunity, which, in turn, demonstrates the presence of dengue virus in the original inoculum. Virus strain SM-18 was reisolated successfully in mice from a stored sample of the original mosquito suspension.

The results of intracerebral neutralization tests in mice (Table 1) showed that SM-18 is a strain of dengue type 2 virus. Hyperimmune serums were prepared in adult mice by a series of five intraperitoneal inoculations with live virus, and in rabbits by a series of four intramuscular inoculations with live virus. Typing by the microprecipitin agar gel diffusion technique (*13*) confirmed the results of the neutralization tests. With SM-18 antigen (a 20-percent infant mouse brain suspension in borate-saline diluent at *p*H 9.0), precipitation occurred only with dengue type 2 and not with dengue types 1, 3, or 4, or with Japanese encephalitis hyperimmune mouse serum. No nonspecific precipitation occurred with normal mouse serum.

It has been established that *A. aegypti* is the primary vector of dengue-caused hemorrhagic fever in southeastern Asia, based on epidemiological evidence and numerous virus isolations. The significance of the single isolation of dengue virus from *A. albopictus* cannot be evaluated without further investigation, although epidemiological evidence suggests that this species is an important vector of endemic dengue in southeastern Asia (*8*).

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Enrichment of Serine-Acceptor Soluble RNA by Nucleic Acid Gels

Abstract. *Nucleic acid gels prepared by ultraviolet irradiation of polyuridylic acid on plastic beads can absorb a species of soluble RNA with increased serine-acceptor activity.*

Naturally occurring deoxyribonucleic acids can be immobilized on solid supports for the isolation of complementary strands of nucleic acid (*1*). Synthetic polynucleotides have been chemically coupled to solid supports and used (*2*) for the isolation of oligo- and polynucleotides on the basis of complementary hydrogen bonding. I now report further application of this technique for the isolation of a specific soluble RNA from mixtures with a nucleic acid gel.

Polyuridylic acid was made into insoluble gels by ultraviolet irradiations of polyuridylic acid (polyU) (*3*) on polyvinyl chloride beads (*4*) according to Britten's procedure (*5*), with the following modification. Ten grams of Geon 101 beads were coated with a solution containing 60 mg of polyU and dried with a heat lamp in a silicon-treated dish. The resulting powder was thinly spread in a large glass dish and irradiated with two 9-watt germicidal lamps (*6*). Six 30-second exposures were made at a distance of 7.5 cm, with stirring between exposures. About 100 mg of the polyU remained associated with 45 g of the beads after they were extensively washed with a warm citrate buffer (0.1*M* sodium chloride, 0.01*M* sodium citrate, *p*H 7.4) in a water-jacketed column (0.9 by 103 cm; void volume, 32 ml; bed volume, 65 ml). Soluble RNA from *Escherichia coli* (*7*) was purified with diethylaminoethyl cellulose chromatography (*8*), and a solution containing about 20 mg in the citrate buffer was recirculated through the column for 2 days at 0°C with a Sigmamotor T-8 pump. The unbound sRNA was flushed out from the column, at 0°C, with the citrate buffer until optical density (O.D.) readings of the collected fractions reached background. Then the column temperature was raised gradually to 45°C to displace material bound at the lower temperature (Fig. 1). An amount of solvent equal to one bed volume was allowed for elution at each temperature, and the elution was complete at 40°C with a midpoint around 20°C. No retention of sRNA was observed in a similar experiment with a column packed with only polyvinyl beads. To assure quick and complete displacement of the bound material, the elution temperature of 40°C was used in a typical experiment with the polyU gel column (Fig. 2).

The retained fractions accepted six times as much serine as either the original mixture or the fractions eluted at 0°C did. Although the

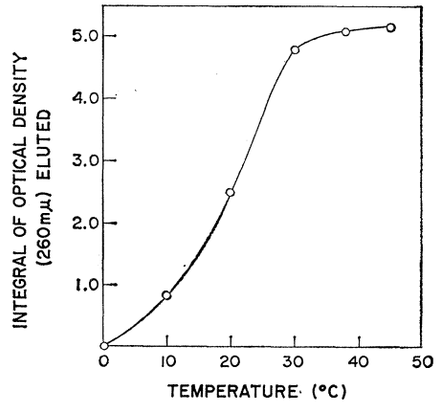

Fig. 1. Integral of the quantity of sRNA eluted from immobilized polyU gel as a function of temperature.