Plaque Formation by an Arbovirus in a Mosquito Cell Line

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Established lines of insect cells have been obtained only recently and their use in arbovirus studies is only beginning (Suitor, 1966; Converse & Nagle, 1967; Rehacek, 1968; Singh & Paul, 1968a, b; Banerjee & Singh, 1968; Yunker & Cory, 1968). Most arbovirus growth reported in insect cell lines does not cause concomitant cytopathic effect (CPE) and plaque formation has not been reported before.

The cell line employed in this study originated from tissues of the mosquito Aedes albopictus (Singh, 1967). The cells were cultured in a manner identical to that described by Singh (1967) and complete monolayers were obtained about 5 days after subculture. Because this line contains cell types of various sizes, a single cloning was made in order to isolate the smallest cell type. Small coverslips were placed in Petri dishes and dilutions of cell suspensions added. After incubation, coverslips containing single colonies were transferred to tubes in order to increase the cell population. Although this method still led to cell types of different morphologies, one such 'clone' was used for these initial tests of plaque formation.

Singh & Paul (1968a) reported appearance of CPE when cells of Aedes albopictus were infected with a mouse-brain-adapted strain of Japanese encephalitis virus. In the present work, the OCT-541 (35-24) strain of Japanese encephalitis virus (Rohitayodhin & Hammon, 1962) adapted to growth at 24° was used. It was obtained from the American Type Culture Collection in its 39th tissue culture passage and from this was prepared a virus pool in primary hamster kidney cells cultured at 28°. The pool had a TCD50 of 10° for both hamster kidney and Aedes albopictus cells when titrated under liquid medium.

The following method was used for plaque formation. Monolayers in T-15 glass flasks of the cloned Aedes albopictus cells at about 6 days old were washed once with phosphate buffered saline, pH 7.4, containing 0.75% bovine plasma albumin. Virus dilutions, made in the same solution, were added (0.2 ml. volume for T-15 glass flasks), and the virus allowed to absorb while the flasks were rocked slowly for 2 hr at 28°. The flasks were then rinsed twice in phosphate buffered saline + bovine albumin and the monolayers overlayered with 2 ml. overlay medium at 43°. The overlay consisted of equal volumes of 2% Agarose (Seakem) in distilled water and double-strength Mitsuhashi & Maramorosch (M & M) medium, prepared as described by Singh (1967) except that only 8% foetal bovine serum (FBS) was used: the final concentrations were 1% Agarose, 4% F.B.S, in single-strength M & M medium.

After incubation for 1 week at 28°, 2 ml. of a second over layer containing 1/30,000 neutral red was added. Plaques were evident within another 24 hr, but were clearer at 72 hr. The plaques had irregular outlines, but were of similar size (about 4 mm. diameter: Plate 1). Plaque titres were about 10 times less than the TCD50 in hamster kidney or Aedes albopictus cells in liquid medium, but were very reproducible. The plaque content was directly proportional to the virus concentration over the range showing clearly countable plaques. The plaque number was reduced when the virus
Monolayers of *Aedes albopictus* cells in T-60 flasks showing plaques due to Japanese encephalitis virus, strain OC-541 (35-24) on left. Uninfected, control cells on right.
was neutralized by incubation in ascitic fluid from mice immune to Japanese encephalitis virus (1 hr at room temperature) before inoculation of the cells.

The fact that the cell line used can be cultured continuously and that the plaques formed in the present system can be easily counted makes it a potentially powerful new tool for quantitative assay of arboviruses in insect cells.

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Naval Medical Research Institute
National Naval Medical Center
Bethesda, Maryland 20014, U.S.A.

REFERENCES


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