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TECHNICAL MANUSCRIPT 400



# MULTIPLICATION OF YELLOW FEVER VIRUS IN INSECT TISSUE CELL CULTURES

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JULY 1967

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

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BIOLOGICAL SCIENCES LABORATORY

Project 1C522301A082

July 1967

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

## ABSTRACT

Yellow fever virus (Asibi strain) has been grown successfully in insect tissue culture cell lines from the moth Antheraea eucalypti and the mosquito Aedes aegypti. Increases in virus titer ($MICLD_{50}$) of two to three logs were evident in 24 to 72 hours at 26 C in a hemolymph-free medium. Virus-infected A. eucalypti cells remained healthy and in a state of reproduction throughout 12 weeks' incubation, but in A. aegypti cultures, cell lysis reduced the numbers to 10% of the original count within 120 hours.

# MULTIPLICATION OF YELLOW FEVER VIRUS IN INSECT TISSUE CELL CULTURES

Transmission of yellow fever by virus-infected mosquitoes was documented in 1901.[1] In 1937 Whitman[2] reported conclusive evidence that yellow fever virus actually multiplies in the infected mosquito. A study of yellow fever virus multiplication in insect tissue culture might contribute to the knowledge of reproduction of the virus and virus-vector relationships as well as to further knowledge of the epidemiology and control of yellow fever. The establishment of insect tissue cell lines from the moth Antheraea eucalypti and the mosquito Aedes aegypti by Grace[3,4] has made this study possible; in addition, the successful growth of Japanese encephalitis virus in the moth cell line by Suitor[5] indicated its feasibility. This report describes the multiplication of Asibi strain yellow fever virus in both the A. eucalypti and A. aegypti cell lines.

A. eucalypti tissue cell cultures were grown and maintained in a hemolymph-free medium previously described.[6] Suspension cultures of approximately 1 x $10^6$ cells per ml in 250-ml Falcon plastic flasks* (25 ml of medium per flask) were inoculated with approximately 1 x $10^7$ mouse intracerebral $LD_{50}$ ($MICLD_{50}$) per ml doses of monkey serum seed virus, incubated for 1 hour at 26 C at pH 6.5, and washed, and the medium was replaced. Virus titers ($MICLD_{50}$) were determined at 24-hour intervals by the method of Reed and Muench during 8 days of incubation at 26 C with shaking on a New Brunswick rotary shaker at 60 rpm on both culture supernatant and supernatant plus cells. The initial pH of the cultures (6.5) remained at this level throughout the incubation. A. aegypti cell cultures were maintained and evaluated in a similar manner, but in a modification of the above medium (without asparagine, acetate, pyruvate, or malic, succinic, fumaric, and ascorbic acids; glucose was the only sugar; nystatin concentration was reduced by one-half).

The data in Figure 1 show two- to three-log increases in virus titer in A. eucalypti cell cultures between 24 to 72 hours following inoculation. Lower titers were obtained subsequently. Virus in medium without cells exhibited an exponential decrease in titer. The infected moth cells maintained a healthy appearance and increased in numbers throughout the incubation period.

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* Falcon Plastics, Los Angeles, California.

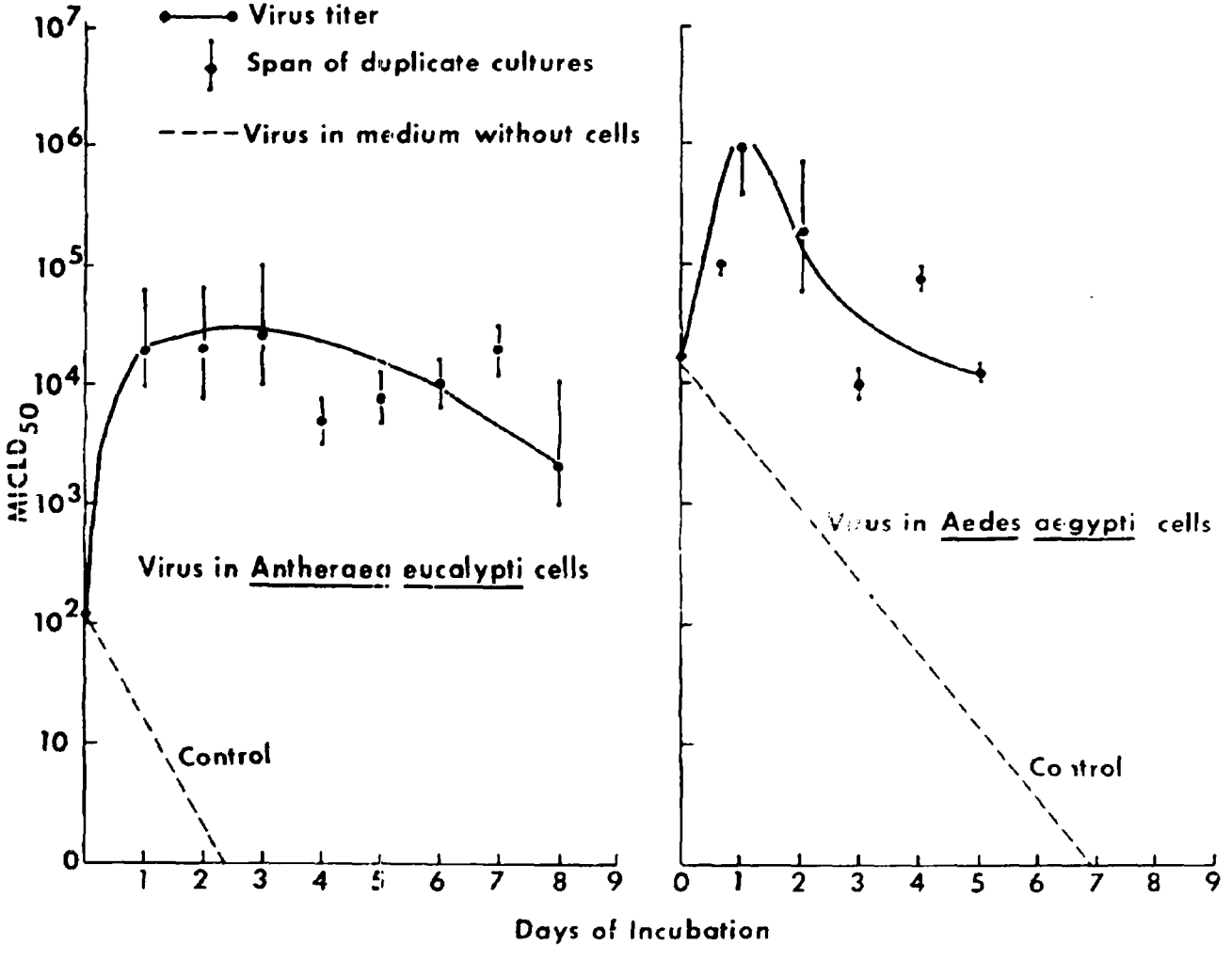


Figure 1. Replication of Yellow Fever Virus in Insect Tissue Culture. A. Virus in Antheraea eucalypti cells; B. virus in Aedes aegypti cells.

As shown also in Figure 1, virus in A. aegypti cells exhibited a two-log increase in titer within 24 hours, followed by lowered titers on subsequent days. In contrast to the A. eucalypti cells, the infected A. aegypti cells decreased in numbers to approximately 50% of the original count at 24 hours; 25% at 48 hours; and 10% at 96 hours. As before, virus in medium without cells exhibited an exponential decrease in titer.

Because the inocula for all cultures were approximately equal ($1 \times 10^{6.7}$ to $1 \times 10^{7.0}$ $MICLD_{50}$ per ml), the two-log difference in the virus titers at zero time in A. eucalypti and A. aegypti cultures was probably a reflection of more efficient virus adsorption by A. aegypti cells during the 1-hour incubation of inoculum and cells.

Continued incubation of the A. eucalypti cell cultures gradually increased virus titer from $1 \times 10^{3.3}$ $MICLD_{50}$ per ml at 8 days to $1 \times 10^{4.3}$ at 14 days, $1 \times 10^{4.5}$ at 21 days, and an additional peak of $1 \times 10^{5.5}$ at 5 weeks. Throughout this period, the medium was replaced and cell populations were reduced approximately 50% ($0.5 \times 10^{6}$ per ml) at 14-day intervals.

Because cell lysis in the A. aegypti cultures reduced cell populations to approximately 10% of the original counts after 5 days' incubation, virus determinations were terminated in these cultures at that time.

This study is thought to be the first report of multiplication of yellow fever virus in insect tissue culture and the first report of multiplication of any virus in Grace's A. aegypti cell line.