MULTIPLICATION OF ARBOVIRUSES IN CELL LINES FROM *AEDES ALBOPICTUS* AND *AEDES AEGYPTI*

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A LIMITED degree of multiplication of mosquito and tick-borne viruses has been previously shown in primary mosquito and tick cell cultures or tissues maintained *in vitro*.[1-6] Suitor[7] reported the multiplication of Japanese encephalitis virus in Grace's continuous cell lines of *Antheræa eucalypti*, a moth, which however, is not a vector of arboviruses. But the recent development of mosquito cell lines[8] in this laboratory has made it possible to study the growth and behaviour of arboviruses extensively.

This communication reports the results of a successful study on the multiplication of some of the arboviruses in Singh's recently established[8] cell lines of *A. albopictus* and *A. ægypti*.

A. albopictus and *A. ægypti* cell lines from 22nd passage to 29th passage were used in this study. Cells were grown as stationary tube cultures for testing the multiplication of the arboviruses. Stock cultures of both the cell lines were maintained in bottles. The cells were harvested as described by Singh,[8] 1 : 10 and 1 : 3 dilutions of cell suspensions of *A. albopictus* and *A. ægypti* respectively were prepared and 0·5 ml. of the suspensions dispensed per tube. At 30° C. cells of *A. albopictus* grew into a complete monolayer in three days and those of *A. ægypti* in five days.

The following virus strains were used : Chikungunya (VRC No. 634029) and Sindbis (AR 339) of arbovirus group A ; Kyasanur Forest disease (VRC No. 616104–10), Japanese encephalitis (VRC No. P 20778), West Nile (E 101), dengue 1 (VRC No. 623996), dengue 2 (VRC No. 64421), dengue 3 (VRC No. 633798) and dengue 4 (VRC No. 624000) of arbovirus group B. All these virus strains have undergone many passages intracerebrally (i.c.) in infant or adult albino mice and are well adapted to these animals.

Virus titrations were made in VERO cells which were originally received from Yale Arbovirus Research Unit, New Haven, Connecticut, USA. Monolayer cultures of VERO cells were grown as stationary tube cultures in minimal essential medium (MEM) with 10% foetal bovine serum. Fully grown monolayers maintained in MEM with 2% fœtal bovine serum were used for viral assay.

Titrations of dengue viruses were carried out by inoculations of tenfold dilutions of the virus i.c. in 2-day-old infant mice, the inoculum being 0·02 ml. per mouse.

Fifty cultures of each cell line were inoculated with 0·1 ml. volume of virus diluted in MEM, so as to give 2·0 to 3·0 log LD_{50} or $TCID_{50}$ of each virus per culture.

The inoculum was titrated at the same time to find out the exact dose of virus. Titrations were carried out either in VERO cells or i.c. in infant mice. All inoculated mosquito and VERO cell cultures were washed twice, after two hours of incubation, with Rinaldini's Salt Solution and MEM/M 199 respectively and the fresh medium was added. Mosquito cell cultures were incubated at 30° C. and VERO cultures at 37° C.

Every day from 1st to 6th day and then on 8th, 10th and 15th days after inoculation, two tubes inoculated with each virus, other than dengue viruses from each cell line were stored at — 50° C. at least for 24 hours. In case of dengue viruses the tubes were stored on 1st, 3rd, 5th, 7th, 10th, 15th and 20th post-inoculation days. The tissue culture fluids from the two tubes of each day were rapidly thawed, pooled, centrifuged and titrated either in VERO cell cultures or i.c. in infant mice to assay the infective virus content in the tissue culture fluids on the day of storage. The infective virus titres were calculated as 50% tissue culture infective dose ($TCID_{50}$)/0·1 ml. for VERO cultures or 50% lethal dose (LD_{50})/0·02 ml. for mice, according to the method of Reed and Muench.[9]

The growth-patterns of the viruses which multiplied in *A. albopictus* and *A. ægypti* cell lines are represented in Figs. 1, 2 and 3.

Chikungunya, Sindbis, Japanese encephalitis and West Nile, all mosquito-borne viruses, grew well in *A. albopictus* cell cultures (Fig. 1). Approximately 10,000 to 100,000-fold increase from the original inoculum was observed with these viruses. Dengue 1, dengue 2, dengue 3 and dengue 4 viruses, which are also transmitted by mosquitoes, showed multiplication in *A. albopictus* cell cultures but the

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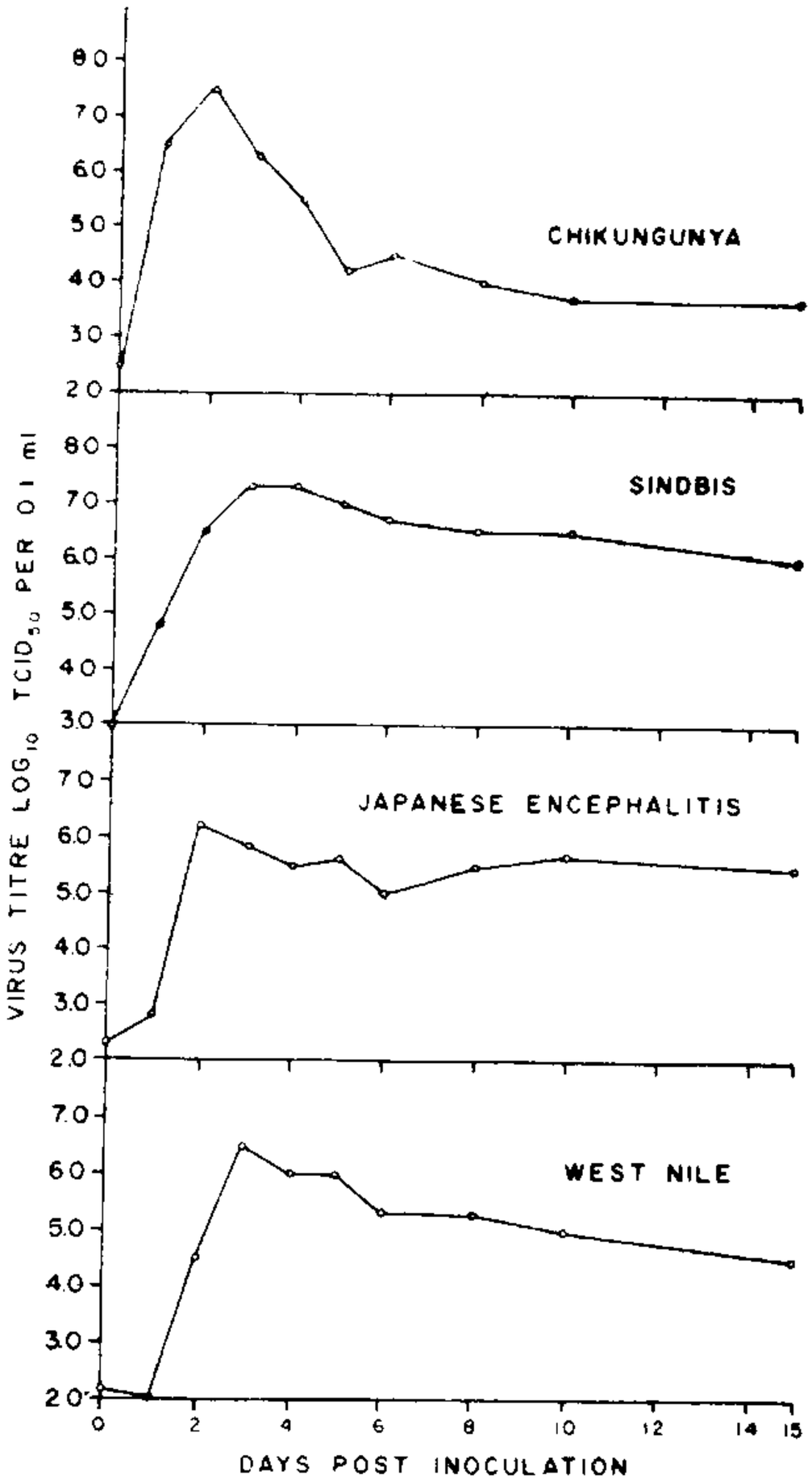

FIG. 1. Multiplication of chikungunya, Sindbis, Japanese encephalitis and West Nile viruses in *A. albopictus* cell cultures.

maximum titres reached did not exceed 2·0 to 3·0 log 10 LD_{50} (Fig. 2).

The *A. ægypti* cell line could support the multiplication of chikungunya and West Nile viruses only (Fig. 3). The multiplication of chikungunya virus was rapid and the maximum virus titre of $10^{4.0}$ $TCID_{50}$ was reached by the 8th day. The growth of West Nile virus was slow and the maximum virus titre of $10^{5.0}$ $TCID_{50}$ was observed on the 15th PI day. Results on the multiplication of Sindbis virus in this cell line were erratic. Virus growth was detected only on 6th and 15th PI day, when the virus titre observed was $10^{4.3}$ and $10^{4.5}$ $TCID_{50}$ respectively.

Neither of the cell lines supported the growth of Kyasanur Forest disease virus which is mainly transmitted by ticks.

Cytopathic effect was observed with Japanese encephalitis, West Nile, dengue 1, dengue 2, and dengue 4 viruses in *A. albopictus*

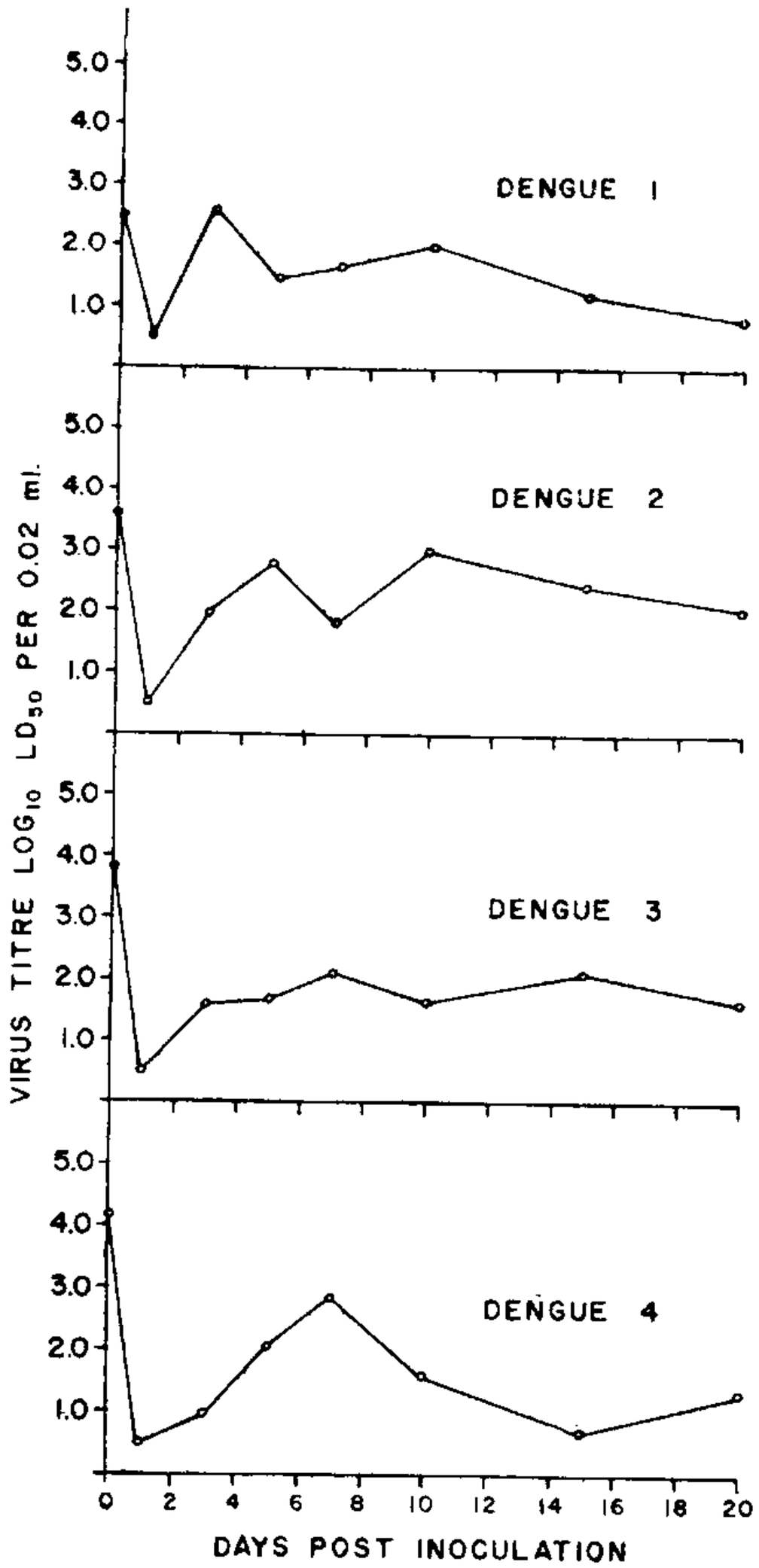

FIG. 2. Multiplication of dengue viruses in *A. albopictus* cell cultures.

cell cultures only. The cultures inoculated with these viruses showed an increase in the granularity of the cells and a proportion of the cells came off the glass wall, as evidenced by the gradual denudation of the glass surface and a number of floating cells in the supernatant medium. At later stages, the cells were seen to clump together in large cellular masses (Figs. 4 and 5).

It is evident from the results of this study that the cell line derived from the larvæ of *A. albopictus* is more suitable for the multiplication of arboviruses than that from *A. ægypti*. Differences in the cell types of two cell lines, as described earlier by Singh,[8] might have something to do with the differences in the viral susceptibility of the two cell lines.

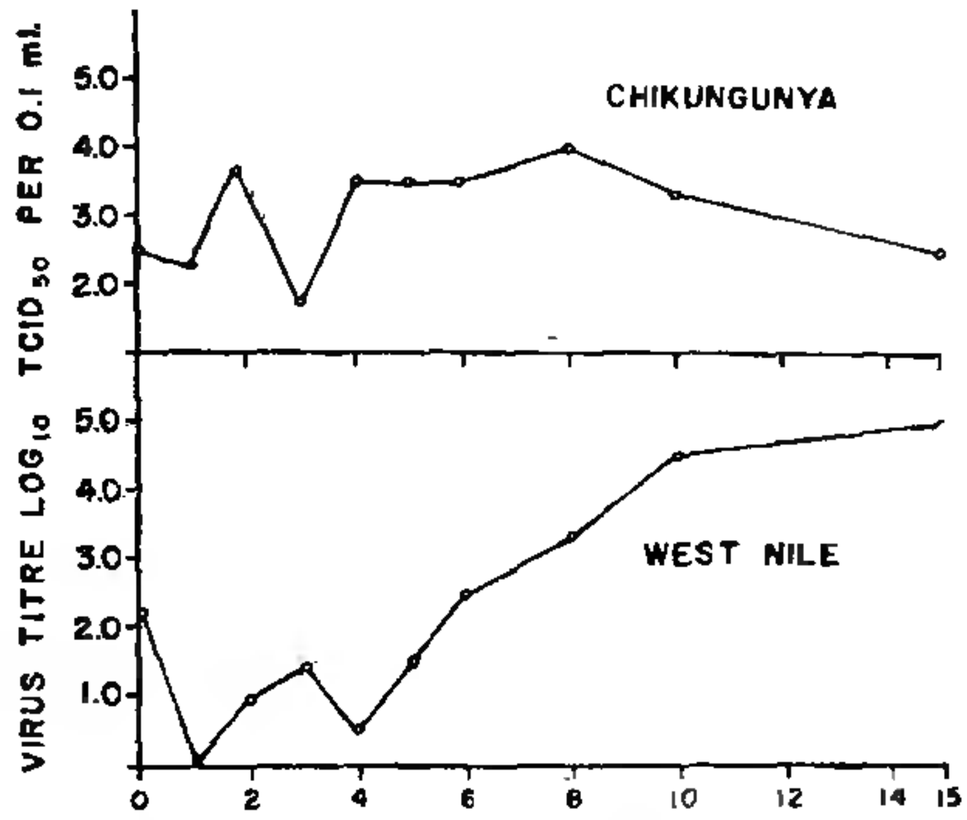

FIG. 3. Multiplication of chikungunya and West Nile viruses in *A. aegypti* cell cultures.

All of the mosquito-borne viruses tested, multiplied at least in one of the two cell lines whereas neither of the cell lines supported the growth of the tick-borne virus. However, Rehacek[6] has shown that the primary tick tissue culture can support the growth of some mosquito-borne viruses in addition to the tick-borne viruses.

Cytopathic effect reported here in insect cell culture with arboviruses has been observed for the first time. The detailed study on the nature of the cytopathic effect produced by these viruses in insect cell culture will be reported separately. Susceptibility of these cell lines for some of the other arboviruses isolated from India and for a few representative viruses from other groups is now being tested.

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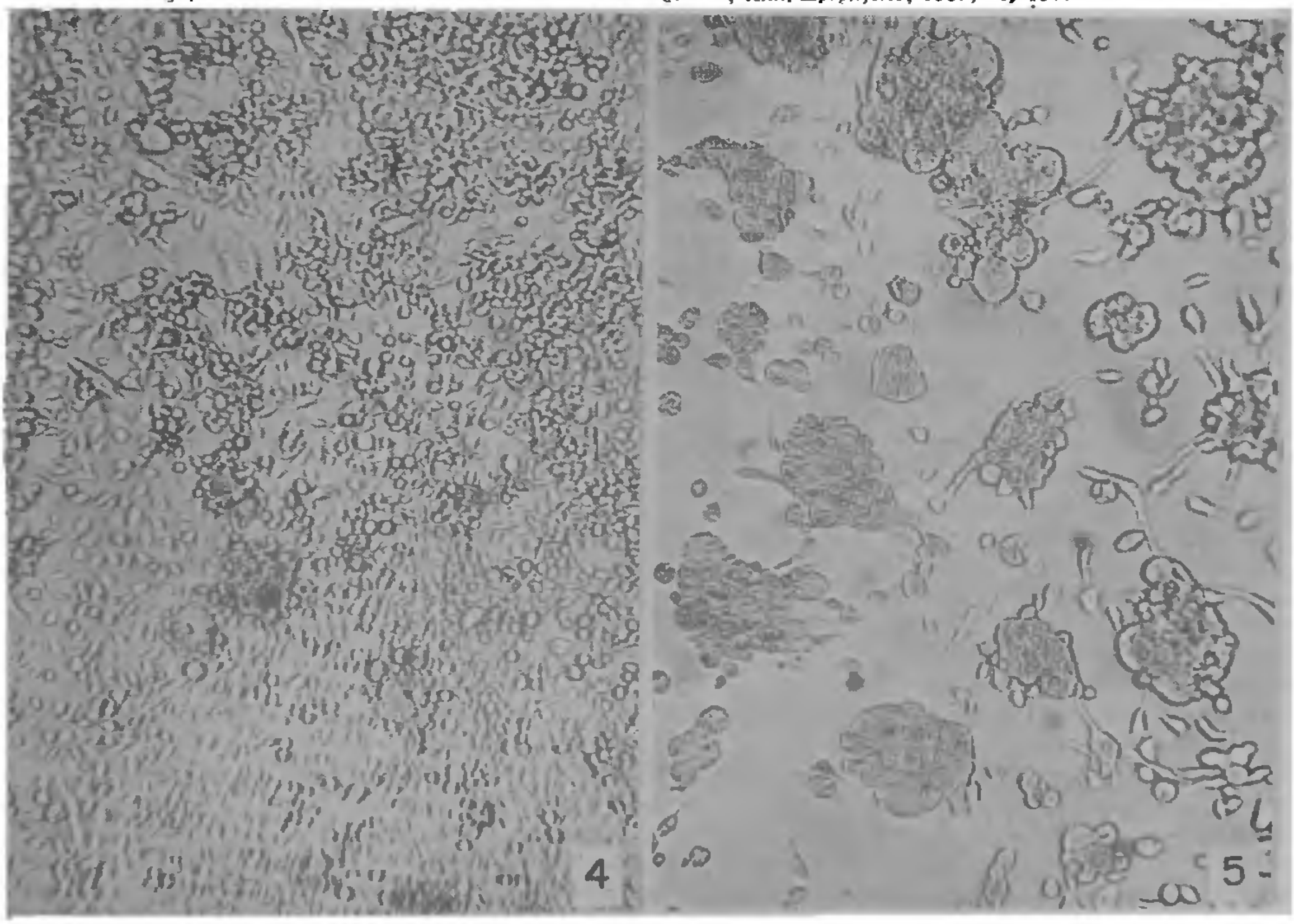

FIGS. 4-5. Fig. 4. Normal cell culture of *A. albopictus*, × 100. Fig. 5. Cell culture of *A. albopictus* showing cytopathic effect, 10 days after the inoculation of the West Nile virus, × 100.

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