Studies on the Replication of Mayaro Virus Grown in Interferon Treated Cells

MCS Rebello, MEF Fonseca*, JO Marinho, MA Rebello

Instituto de Biofisica Carlos Chagas Filho *Instituto de Microbiologia, Universidade Federal do Rio de Janeiro, Centro de Ciências da Saúde, 21941-900 Rio de Janeiro, RJ, Brasil

Mayaro virus grown in interferon treated infected cells has been characterized with regard to its ability to replicate in vertebrate (TC7) and invertebrate (Aedes albopictus) *cells. Virus purified from interferon treated TC7 cells adsorbs and penetrates to the same extent as the control virus. During infection, these virus particles caused inhibition of host protein synthesis and synthesized the same spectrum of viral proteins as normal virus. This population however, was apparently more sensitive to interferon treatment. Electron microscopy of TC7 cells showed the presence of numerous aberrant virus particles budding from the plasma membrane.*

Key words: interferon - Mayaro virus replication

Mayaro virus (Alphavirus genus, Togaviridae family) is an arthropod-borne virus, antigenically related to Semliki Forest virus (Casals & Whitman 1957). The replication of Mayaro virus in vertebrate cells produces acute cytocidal effects leading to cell death in approximately 48 hr. In mosquito cells, however, a persistent infection is developed. This virus has been isolated in Brazil, from human and other mammalian species, especially at frontiers of colonization in the Amazon region. Clinical manifestations of human infection were described as a feverish illness followed by headache, epigastric pain, backache, chills, nausea and photophobia (Casals & Whitman 1957, Causey & Maroja 1957).

The alphaviruses are enveloped RNA viruses with a wide host range. The virus particle consists of a lipoprotein envelope and a nucleocapsid. A great deal of research has been carried out to clarify the ecology, epidemiology and pathology of these viruses since several alphaviruses can be considered of great importance in veterinary and human medicine (Kaariainen & Soderlung 1978).

Recently, we analyzed the interferon (rec-IFN alpha 2b) action on Mayaro virus replication in TC7 cells. A drastic reduction of infective virus production was found. Viral protein synthesis was inhibited as well as virus morphogenesis and virus particle release from plasma membrane (Rebello et al. 1993).

As described for other virus-cell systems (Maheshwari & Friedman 1980, Munoz & Carrasco 1984, Esteban 1984) the population of virus that grows in interferon-treated cells shows a different behavior when compared to the normal population. This report addresses the question of whether IFN has any effect on the Mayaro virus population that was grown in IFN treated cells. For this purpose we analyzed some aspects of the life cycle of these virions in vertebrate (monkey kidney cells) and in mosquito cells.

MATERIALS AND METHODS

Cell cultures and virus - The TC7 cell line is a clone of CV-1 cell line derived from the kidney of a male adult African Green monkey. The cells were grown as monolayers in 60 cm^2 glass bottles at 37°C in Dulbecco's modified Eagle medium supplemented with 2% fetal bovine serum and 8% bovine serum. *Aedes albopictus* cells, clone C6/36, were grown in the same culture medium supplemented with 2% fetal bovine serum and 0.2 mM non-essential amino acids. Mayaro virus was obtained from the American Type Culture Collection (Rockville, MD, USA) and adapted to grow in cell culture as described before (Mezencio et al. 1989).

Preparation of Mayaro virus samples - The normal Mayaro virus (MV) was grown at 0.01 PFU/cell in TC7 cells. After 48 hr the medium was collected and clarified by centrifugation for 10 min at 10,000 x g. Mayaro virus grown in interferon treated TC7 cells (MV_{IFN}) was obtained by the same procedure described above except that TC7 cells were pretreated with interferon (10^3 IU/ml) 18 hr before infection.

Purification of virus samples - Monolayers of TC7 cells were divided in two groups. One of them

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was infected with MV (10 PFU/cell) and the other with MV_{IFN} obtained from interferon treated TC7 cells (10 PFU/cell). The inoculum was removed and fresh medium was added. Six hours later ^{35}S-methionine (20 uCi/ml) was added and 30 hr after infection the culture medium was collected and cell debris sedimented at 10,000xg for 10 min. The supernatant was concentrated at 100,000 x g for 90 min at 4°C. The pellet was resuspended in PBS-albumin (PBS containing 0.4% serum albumin) and further purified in a 5-50% potassium-tartrate gradient in PBS albumin by centrifugation at 100,000xg for 90 min at 4°C. The virus band was collected, diluted in PBS-albumin and sedimented by centrifugation at 100,000xg for 90 min. The pelleted virus was resuspended in PBS.

Interferon - Recombinant human interferon alpha-2b (10^8 IU/mg protein) was a generous gift from Dr Paulo H Leal (Schering-Plough). Concentrations of interferon are expressed in International Reference Units.

Virus infection and measurement of protein synthesis - Confluent monolayers of TC7 or *A. albopictus* cells were infected with Mayaro virus. After 1 hr adsorption at 37°C, the inoculum was removed and replaced by fresh medium. At indicated times, the medium was removed and the cells were pulse labelled for 30 min with 20 uCi/ml of ^{35}S-methionine. The cell proteins were analyzed by SDS-polyacrylamide gel electrophoresis as described by Laemmli (1970). Gel slabs were autoradiographed on Kodak SK-1 films and densitometric scans were made in a laser densitometer LKB 2202 Ultrascan.

Electron microscopy - TC7 cells were processed for electron microscopy as previously described (Mezencio et al. 1989).

Virus binding and penetration - These experiments were performed as described by Belkowski and Sen (1987). Purified ^{35}S-methionine labelled Mayaro virus was used to analyse the process of virus binding and penetration into TC7 cells. Briefly, the monolayers were infected with purified virions. After adsorption at 37°C for 1 hr, the cultures were washed three times with a buffer containing 150 mM NaCl and 10 mM Tris pH 7.4 and lysed in the same buffer containing 0.5% sodium dodecyl sulphate. The radioactivity of each lysate was determined in TCA precipitable material in a liquid scintillation counter.

For the penetration experiments the monolayers were infected as described above. After adsorption, inoculum was removed, the cells were washed with 150 mM NaCl, 10 mM Tris pH 7.4, fresh medium was added and then incubation continued for 90 min at 37°C. Monolayers were then washed with the same buffer and the remaining viruses on the cell surface were removed by incubation with 0.25% trypsin, 0.25% EDTA (5 min at room temperature). Trypsinization was stopped by adding fresh medium. Cells were harvested by centrifugation, washed with culture medium and the pellet was solubilized. The radioactivity was determined by liquid scintillation counter.

RESULTS

Virus infectivity - We first designed experiments to test the infectivity of the virus population grown in IFN-treated cells. TC7 cells were infected with MV or with MV_{IFN}. Using optical microscopy, a typical cytophatic effect leading to cell death was observed in the cultures infected with both preparations of Mayaro virus. After cell lysis, the culture fluid was collected and virus titer was measured by plaque assay. The virus titer resulting after infection of TC7 cells with MV or with MV_{IFN} (m.o.i. = 0.01) respectively reached similar values ($2x10^7$ PFU/ml). These results indicate that apparently these virions are infectious and able to generate new infectious virus particles.

Virus binding and penetration - The process of binding and penetration for both virus populations was tested in TC7 and in *A. albopictus* cells as described in Materials and Methods. We found that MV_{IFN} was as effective as the MV in regard to the process of binding (98% of control) and entry (97%

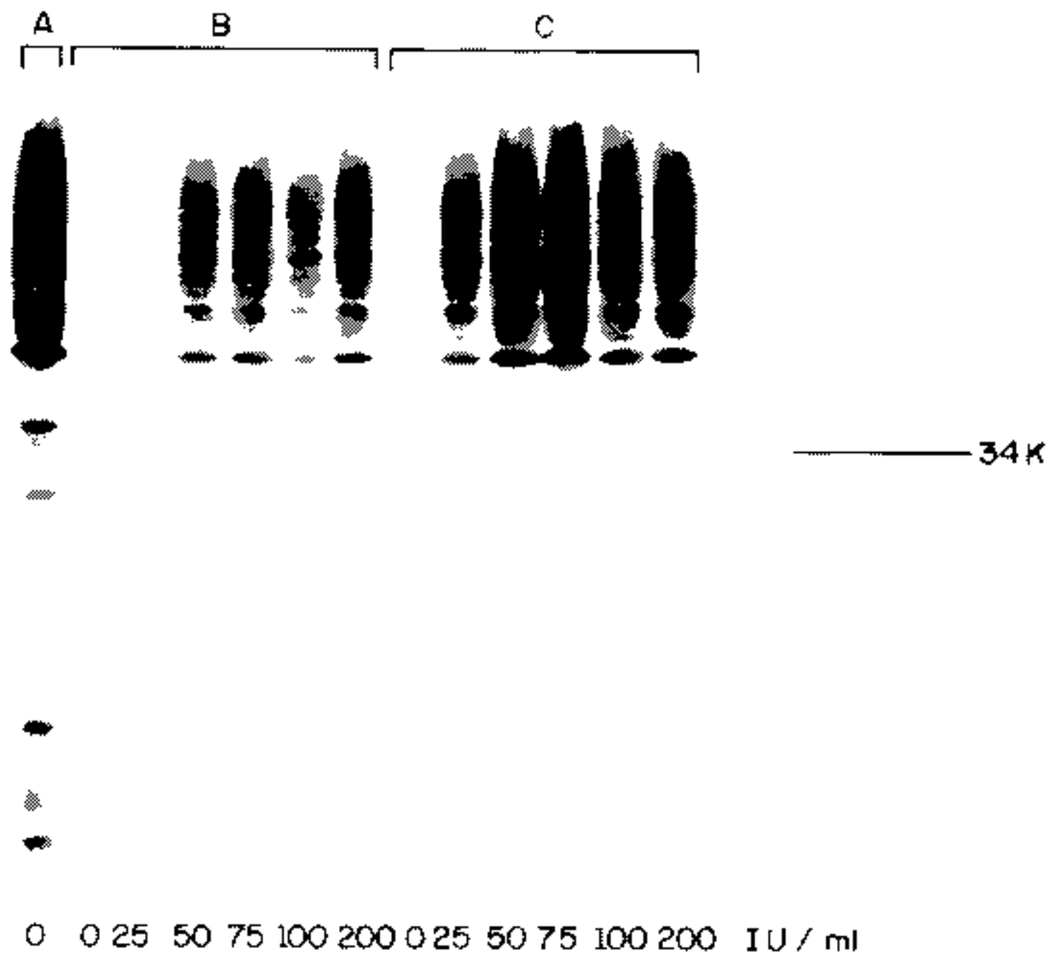

Fig. 1: effect of IFN on Mayaro virus proteins. TC7 cells were treated with different doses of IFN and infected with the same number of particles (m.o.i. = 0.01 PFU/cell) of MV or MV_{IFN}. Twenty-four hours post-infection the cells were pulse-labelled with ^{35}S-methionine (20 µCi/ml) for 1 hr and cellular extracts subjected to 12% polyacrylamide gel electrophoresis. (A) Mock infected cells. (B) Cells treated with different doses of interferon and infected with MV. (C) Cells treated with different doses of interferon and infected with MV_{IFN}.

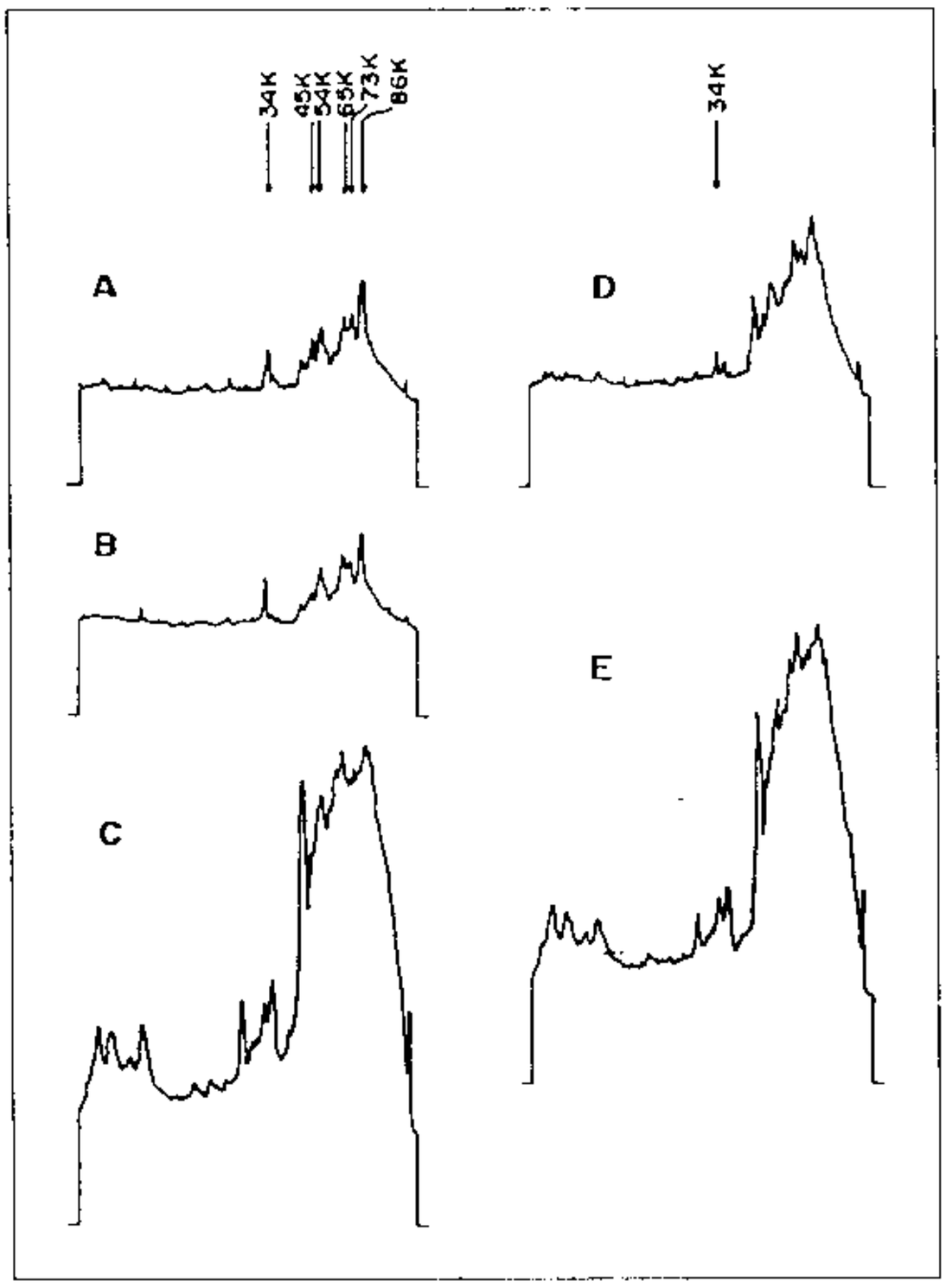

Fig. 2: densitometric profile of proteins. (A) TC7 cells not treated with IFN and infected with MV. (B) TC7 cells not treated with interferon and infected with MV_{IFN}. (C) Control cells (not treated with interferon and not infected). (D) TC7 cells treated with interferon (25 IU/ml) and infected with MV. (E) TC7 cells treated with interferon (25 IU/ml) and infected with MV_{IFN}.

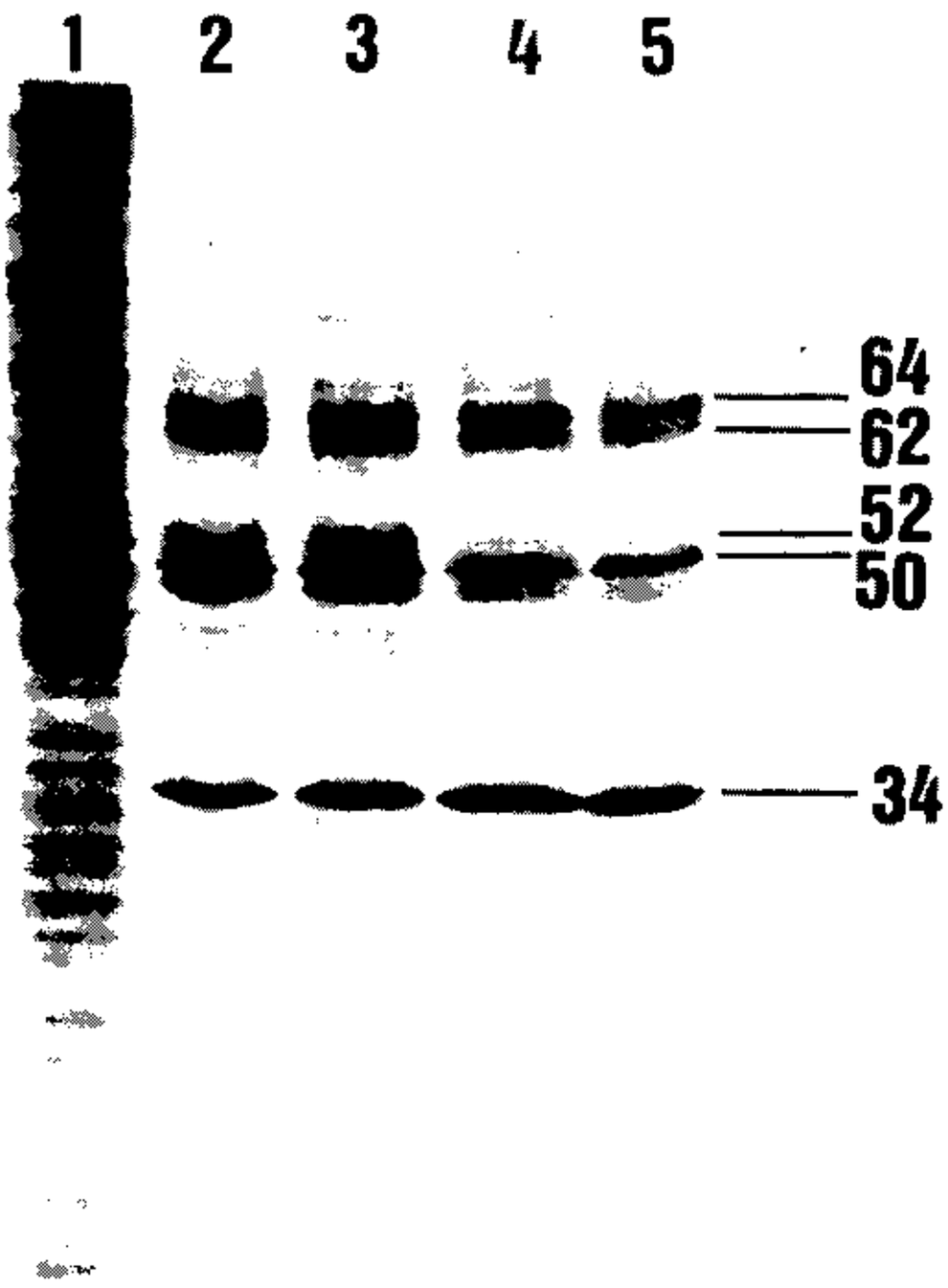

Fig. 3: protein synthesis in *Aedes albopictus* cells. Cells were mock infected (lane 1) or infected with MV (lanes 2, 4) or infected with MV_{IFN} (lanes 3, 5) and maintained in growth medium for 24 hr (lanes 2, 3) or 48 hr (lanes 4, 5). After this period the cells were labelled with ^{35}S-methionine (20 µCi/ml) for 1 hr and cellular extracts subjected to 12% polyacrylamide gel electrophoresis.

of control) into a second round to initiate a new cycle of infection.

Protein synthesis in vertebrate cells - The degree of viral protein synthesis in the cells infected with MV or with MV_{IFN} was determined after pulse labelling with ^{35}S-methionine followed by SDS-PAGE and autoradiography. We also treated TC7 cells with different doses of IFN and then infected with both populations of virions.

Figure 1 (lanes 2 and 8) reveals that MV_{IFN} inhibited host protein synthesis and synthesized the same espectrum of viral proteins as MV.

Comparing the densitometric profiles obtained from the autoradiogram showed in Fig. 1 of Mayaro virus proteins synthesized by both Mayaro virus populations we did not find any significant difference either quantitatively or qualitatively (Figs 2A, 2B). On the other hand, both viruses inhibited cellular protein synthesis to the same extent.

Effect of interferon treatment on Mayaro virus grown in IFN-treated - As can be observed in Fig. 1, MV_{IFN} was apparently more sensitive to IFN treatment since lower doses of IFN were required to obtain the same effect observed with MV. These results can be best appreciated in the densitometric analyses of the autoradiogram (Fig. 2). When we treated TC7 cells with IFN (25 IU/ml) and infected with Mayaro virus, we observed that cellular protein was less reduced (reltive increase up to 126%) (Figs 2A, 2D) and the viral proteins were not totally inhibited. On the other hand, this relative increase of cellular protein synthesis in cultures infected with MV_{IFN} was 204% (Figs 2E, 2B) and virus proteins were not detected.

Protein synthesis in invertebrate (mosquito) cells - *A. albopictus* cells were infected with MV or with MV_{IFN}. The cultures were observed by optical microscopy for two weeks and no alteration was detected in the morphology of the cells for both viral populations indicating, as expected for this type of virus, the development of a persistent infection in these cells. At 24 and 48 hr post infection, the cells were pulse labelled with ^{35}S-methionine (25 uCi/ml) for 30 min and then processed for electrophoresis. As can be observed in Fig. 3, the analyses of the proteins shows the same profile for both populations.

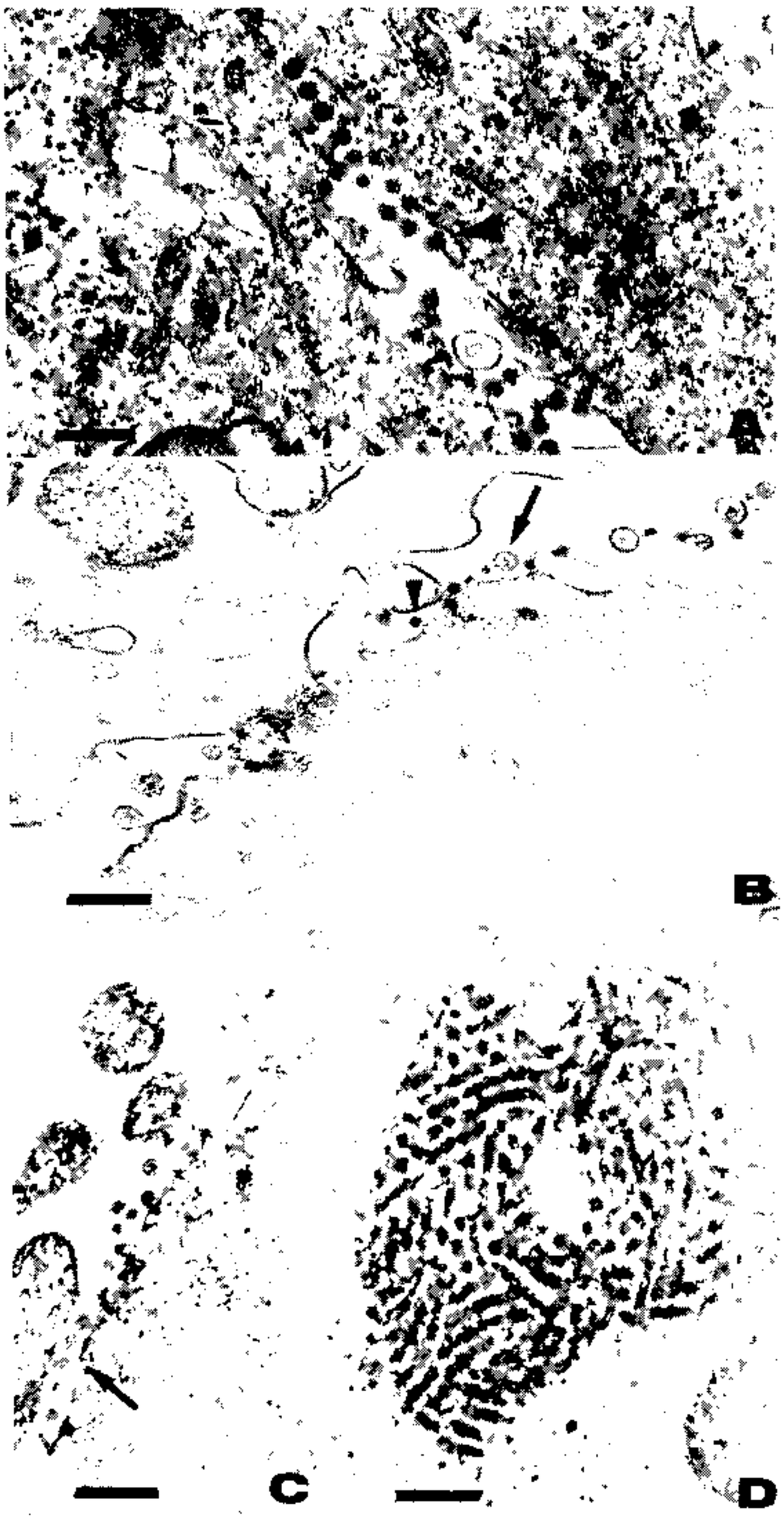

Fig. 4: electron microscopy of TC7 cells infected with MV_{IFN^-}. (A) Cells infected with MV 75,000 X Bar 135 nm. (B), (C), (D) Cells infected with MV_{IFN^-}. (B), (C) Budding of complete virus particles with 43 nm diameter (arrow head) and presence of aberrant forms of viruses (60 - 120 nm diameter) with electron luscent center (arrow): (B) 33,300 X Bar 300 nm. (C) 45,500 X Bar 220 nm. (D) Aggregation of tubular proteins, possibly of virus origin, found in the cytoplasm. Average size of transversally cut proteins. 71,250 X Bar 140 nm.

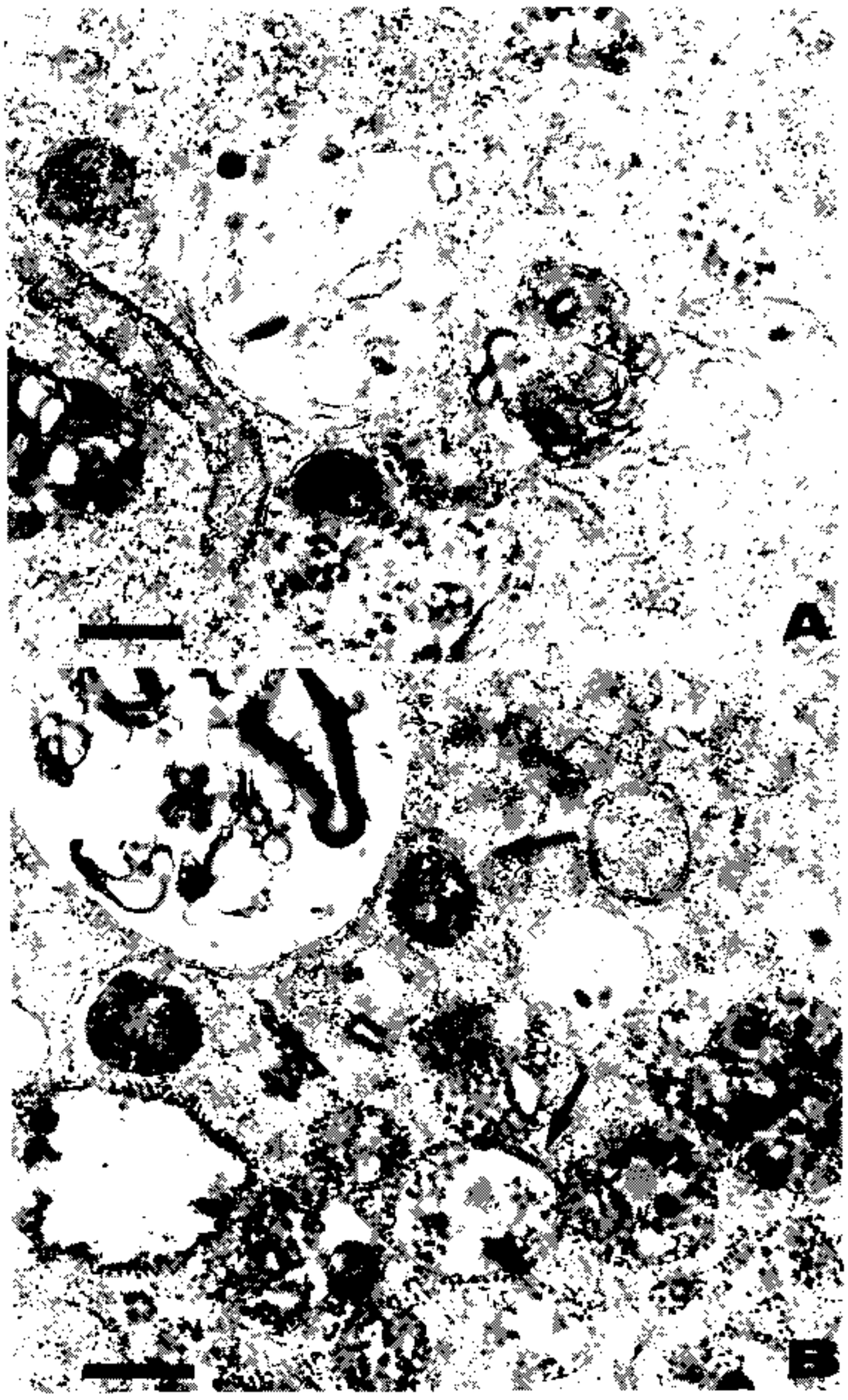

Fig. 5: electron microscopy of *Aedes albopictus* cells. (A) Cells infected with MV. 61,000 X Bar 165 nm. (B) Cells infected with MV_{IFN} 27,000 X Bar 370 nm. Both (A) and (B) show presence of vacuoles containing virus particles. Nucleocapsids are seen around vacuole membranes (arrows) in (B).

The Mayaro infection of mosquito cells leads to a transitory inhibition of cellular protein synthesis as described for other alphaviruses (Koblet 1990). We found an inhibition of 45% relative to the control uninfected cells caused by both virus populations.

Virus assembly - In TC7 cells, we had already observed that Mayaro virus was released by budding from the plasma membrane. No viral components or vacuoles containing virus were seen in the cytoplasm (Rebello et al. 1993). In TC7 cells infected with MV_{IFN} we also observed (Figs 4B, 4C) virus budding. Curiously most of the virus particles showed aberrant forms (60 nm diameter) without electron opaque centers and only a few normal virions (43 nm diameter) were observed. Accumulation of viral precursors (probably nucleocapsid proteins) were also noted in the cell cytoplasm (Fig. 4D).

As shown in Fig. 5A in *A. albopictus* cells infected with MV, the presence of cytoplasmic vesicles containing viral nucleocapsids and mature viral particles was detected. Budding of virus through the plasma membrane was rarely observed. Comparing these results with those obtained with mosquito cells infected with MV_{IFN} (Fig. 5B) we observed no differences in the process of assembly of the two populations. We also want to emphasize that no aberrant virus like particles were seen in invertebrate cells.

DISCUSSION

It was described for some enveloped viruses that IFN treatment causes a decrease in the production of virus particles followed by a marked reduction in infectivity of virions released (Maheshwari & Friedman 1980, Esteban 1984, Gendelman et al. 1990).

Our results on the replication of MV_{IFN} indicate that these virions retain their ability to infect a new cell. They adsorb and penetrate to the same extent as control virions do. In the course of infection these particles caused shut-off of cellular protein synthesis and synthesized similar proteins apparently in equal amounts as MV.

Similar results were found for vaccinia virus grown in IFN-treated cells as far as shut-off and synthesis of viral proteins was concerned (Esteban 1984), although the author described a reduction in the relative amounts of some viral proteins in the cells infected with these virions.

As reported by Munoz and Carrasco (1984), Herpes simplex I virus grown in IFN-treated HeLa cells, was as efficient as control virions in the processes of penetration and uncoating in a new cycle of infection but no late viral proteins could be detected indicating that the replication was blocked at some stage after virus penetration. The authors investigated the replication of Herpes simplex I virus in cells pre-treated with IFN and found that no viral proteins were detected.

We also found an enhanced sensitivity to interferon. This is an important observation since the infective virions produced in IFN-treated cells were not "resistant" to IFN so they could not be selected out when they start a new infective cycle.

The most important feature that we observed in TC7 cells infected with MV_{IFN} was the presence of a great number of aberrant virion forms together with normal particles budding from the plasma membrane. Curiously these virus-like particles were not seen in mosquito cells.

From this work we can conclude that the differences between the population of MV_{IFN} and MV must be very subtle since we only detected in vertebrate cells an enhanced sensitivity to IFN and aberrant virus-like particle formation. As reported for vaccinia virus (Esteban 1984), Herpes simplex virus (Munoz & Carrasco 1984) and vesicular stomatitis virus (Maheshewari & Friedman 1980, Jay et al. 1983, Debrot et al. 1984) alterations in the structural proteins could be responsible for the differences in the behavior of the IFN-grown virus population. Studies in our laboratory are in progress in order to explain what exactly are the molecular differences between normal virions and those obtained from the IFN-treated cells.

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