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EFFECT OF HYPERTONIC MEDIUM ON THE PROTEIN SYNTHESIS IN L-A9 AND *AEDES ALBOPICTUS* CELLS INFECTED WITH MAYARO VIRUS

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Virus infection in general may result in several effects on celular functions such as alteration in membrane potential and fluidity, inhibition of the transport systems for ions and small molecules and modifications of macromolecular metabolism (R.F. Garry et al., 1982, ***Bioscience Reports, 2***: 617-623). An alteration of the intracellular concentration of Na^+ and K^+ was found in cells infected with a number of viruses (R.F. Garry et al., 1979, ***Virology, 96***: 108-112; I.C.P.P. Frugulhetti & Rebello, 1989, ***J. Gen. Virol., 70***: 3493-3499).

Curiously the mRNA specified by a number of DNA and RNA viruses are efficiently translated under altered ionic conditions which block cellular protein synthesis (L. Carrasco & A.E. Smith, 1976, ***Nature***, London, ***264***: 807-809). In the present study we analyzed the synthesis of Mayaro virus specific proteins in cells maintained in hypertonic medium.

Mayaro virus (Alphavirus genus, Togaviridae family) is an arthropod-borne virus antigenically closely related to Semlike Forest virus (J. Casals & L. Whitman, 1957, ***Am. J. Trop. Med. Hyg, 6***: 1004-1011).

Mayaro virus was obtained from American Type Culture Collection, Rockvile, MD, USA. The virus stock was prepared from BHK_{21} cells and stored at $-70°C$. Infectivity titrations of Mayaro virus were performed by plaque assay in L-A9 cells. ***Aedes albopictus*** cells, clone C6/36 (A. Igarashi, 1978, ***J. Gen. Virol., 40***: 531-544) were obtained from Dr R.E. Shope, Arborvirus Research Unit, Yale University, New Haven, Conn, USA. The cells were maintained at 28°C in Dulbecco's modified Eagles medium supplemented with 0.2 mM each of non-essential amino-acids, 2.25% $NaHCO_3$, penicillin (500 U/ml) Streptomycin (100/μg/ml) and 2% fetal bovine serum.

L-A9 and ***A. albopictus*** cells infected and mock infected with Mayaro virus were placed in media containing several concentrations of NaCl (116 to 250 mM) and protein synthesis was estimated by ^{35}S-methionine incorporation. In both cell lines used in these experiments we found a decrease in protein synthesis in cells treated with hypertonic medium. In ***A. albopictus*** cells the synthesis of virus proteins is more resistent even in cells treated with a high concentration of NaCl.

The protein C is easily visualized (arrow) in *A albopictus* cells (Fig.) and the hypertonic medium did not alter the synthesis of this viral protein. In L-A9 cells the protein C is estimulated when the concentration of NaCl increase from 116 mM to 200 mM.

We also observed a decrease in the synthesis of others viral proteins. However these proteins continue to be more resistent to the hypertonicity when compared to the cellular proteins under the same conditions.

Several authors has been demonstrated cellular modifications in Na^+/K^+ influx during the virus infection. In the experiments described in this paper we shown a clear effect of NaCl concentration on the synthesis of cellular and viral proteins.

These data support our hypothesis that synthesis of Mayaro virus proteins is altered by the intracellular concentrations of Na^+ and K^+.

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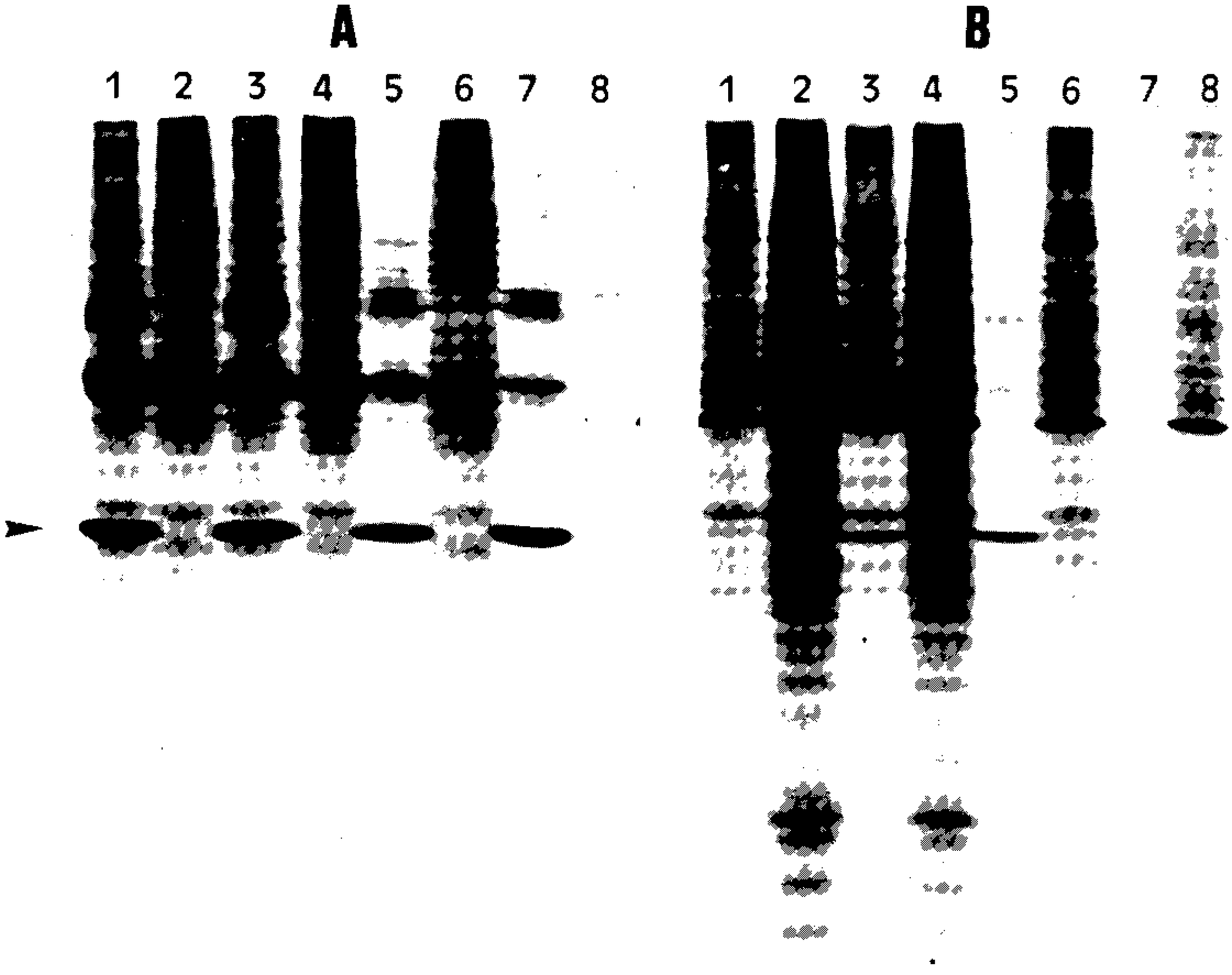

Polyacrylamide gel electrophoresis of Mayaro virus proteins — *Aedes albopictus* cells (A) and L-A9 cells (B) were infected (lanes 1, 3, 5, 7) or mock infected (lanes 2,4, 6, 8) with Mayaro virus (10 PFU cell) and maintained in growth medium during 24h. After this period the cells were treated with isotonic (116 mM NaCl, lanes 1 and 2) and hypertonic medium (150 mM, lane 3-4; 200 mM, lane 5-6 and 250 mM, lane 7-8) for 1h and further labelled with ^{35}S-methinine (10/uCi/ml) for 1h. Cellular extracts were subjected to polyacrylamide gel electrophoresis.