Acta virol. **36**: 201-203, 1992

SOME PHYSICOCHEMICAL PROPERTIES OF MURINE HERPES VIRUS

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Received February 12, 1991

Summary. - The effect of incubation temperature variation and various pH values on the stability of murine herpesvirus isolate-76 (MHV-76) was investigated. The virus survival in rabbit embryo fibroblasts cells (REF) has been determined. At room temperature (23 °C) 50 % of the virus became inactivated during 12 days and at 37 °C its half life was 9 days. MHV-76 was completely inactivated at 50 °C in 1 hr or at 41 °C in 7 hrs. MHV-76 retains its maximal infectivity at the pH range between 6-9 regardless of the duration of treatment. The pH range 3, 4, 5 and 10, 11, 12 caused either complete or more or less expressed inactivation of the virus. A complete inactivation MHV-76 was also achieved after treatment with ethyl ether, chloroform and 2M urea.

Key words: murine herpesvirus 76; physical and chemical properties; temperature; ether; chloroform; urea and pH stability

From two species free-living rodents *Clethrionomys glareolus* and *Apodemus flavicollis* five viral strains were isolated (Blaškovič *et al.*, 1980). Electron microscopic studies of infected REF provided morphological evidence that these viruses belong to the family Herpesviridae (Čiampor *et al.*, 1981). It was also shown that all five isolates are mutually antigenically related but serologically distinct from murine cytomegalovirus (Svobodová *et al.*, 1982*b*). Isolates also induced cytopathic effect characteristics of herpesvirus infection in various cell lines (Svobodová *et al.* 1982*a*). Viral antigen was detected in many organs of infected animals and its distribution was suggestive of haematogenic spread (Rajčáni *et al.*, 1985). These studies were completed by analysis of viral polypeptides (Stančeková *et al.*, 1987) and by characterization of the genomic DNA (Blaškovič *et al.*, 1988; Efstathiou *et al.*, 1990).

The aim of the present study was to assess the effect of the ethyl ether, chloroform, urea, pH and temperature on the MHV-76 stability.

MHV-76 isolated from *Apodemus flavicollis* (Blaškovič *et al.*, 1980) was used. This strain was routinelly propagated in stable line of REF cells. These cells were used throughout the experiments as well as for virus stock production. Virus stock was prepared by infection at a multiplicity

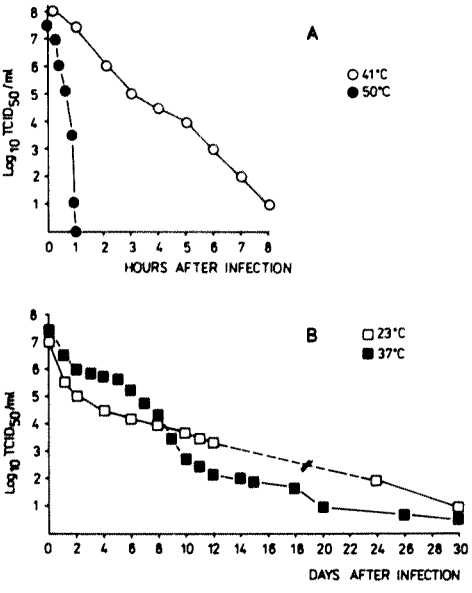

Fig. 1
Effect of temperature on the MHV-76 inactivation
○—○ 41 °C; ●—● 50 °C; ■ 37 °C; □ 23 °C. Abscissa: $\log_{10}$ $TCID_{50}$/ml; ordinate: days post-infection

of the infection (MOI) of 1 $TCID_{50}$ per cell. Virus was obtained 5-7 days post-infection with a titre of 10^5-10^7 $TCID_{50}$ per ml of medium. Purified viral preparations were obtained as described previously (Svobodová *et al.*, 1982*b*). Virus infectivity was measured by titration using REF cells grown in Eagle's basal medium supplemented with 5-10 % inactivated bovine serum, glutamine (3g per 100 ml) and antibiotics (100 units of penicilin and 100 μg of streptomycine per ml) at 37 °C.

Thermal inactivation of the MHV-76 isolate at 23 °C, 37 °C, 41 °C and 50 °C is shown in Fig. 1. Rapid inactivation MHV-76 was achieved at temperatures 50 °C or 41 °C (1 or 7 hr, respectively, Fig. 1-A). At temperature of 23 °C, 50 % of the virus was inactivated during 12 days and at 37 °C its half life was 9 days (Fig. 1-B).

Fig. 2 shows the effect of the various pH values on the inactivation of MHV-76. The following buffers were used: 0.1 mol/l borate buffer pH 8-9; 0.1

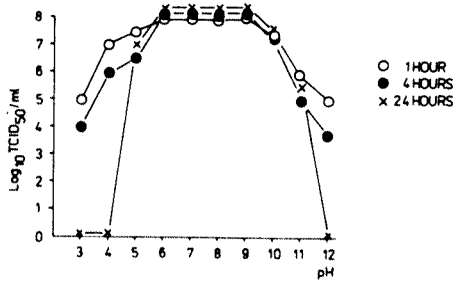

Fig. 2
Effect of pH on MHV-76 inactivation
○—○ 1 hr; ●—● 4 hrs; x—x 24 hrs; Abscissa: pH values; ordinate: $\log_{10}$ $TCID_{50}$/ml

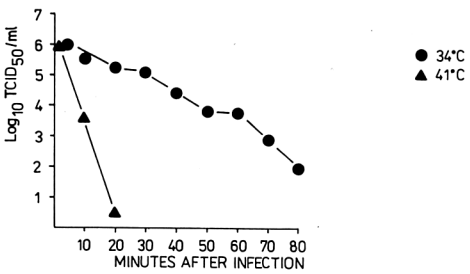

Fig. 3
Effect of 2M urea on the inactivation of MHV-76
○—○ 34 °C; ▲—▲ 41 °C
Abscissa: min after infection; ordinate: log_{10} TCID$_{50}$/ml

mol/l natrium glycine buffer pH 10–12. Purified virus preparations with infectious titre of 10^6 TCID$_{50}$ per ml were mixed with cold buffer solutions of various pH values and held in a refrigerator at 5 °C. Virus infectivity of treated samples was measured by titration in REF cells after exposition time 1, 4, 24 hr. MHV-76 retained its infectivity at pH range from 6 to 9. The pH 3, 4, 5 and 10, 11, 12, respectively, caused either complete inactivation or a marked fall of the MHV-76 titre. MHV-76 was inactivated by 2M urea in eighty minutes at 34 °C and in twenty minutes at 41 °C (Fig. 3). It was also inactivated by contact with ethyl ether and chloroform at 37 °C for 30 minutes as expected by common susceptibility of herpesvirus lipid solvents.

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