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SOME PHYSICAL AND CHEMICAL PROPERTIES OF BHANJA VIRUS

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Summary. — Bhanja virus is acid-labile, relatively thermostable, resistant to trypsin and heparin; a complete inactivation was achieved with chloramine B or formaldehyde, while phenol was ineffective, and UV radiation only partially effective.

Key words: *Bhanja virus*; *physical and chemical properties*; *disinfectants*

Introduction

Sensitivity of Bhanja virus (*Bunyaviridae*) to diethyl ether, chloroform, sodium deoxycholate, its haemagglutinating activity, the virion size (90—120 nm) and shape (spherical to slightly oval particles with spikes 5—10 nm in length) have been already described (Shah and Work, 1969; Lopes *et al.*, 1970; Verani *et al.*, 1970; Murphy *et al.*, 1973).

Strain Bg 326, an antigenically typical European representative of Bhanja virus (Hubálek and Halouzka, 1985), was used at its 4th or 5th passage in suckling mice brain (SMB) throughout this investigation. The virus stock was prepared as a 10% or 20% suspension of infected SMB in Dulbeco's phosphate buffered saline pH 7.4 with 0.75% of bovine serum albumin and antibiotics (PBS/BSA), clarified, and stored at −60 °C. The virus properties were tested using 1% SMB suspension in PBS/BSA (unless otherwise indicated). Infectivity titrations of exposed and corresponding control suspensions were carried out in Vero cell tube cultures, and median cytopathic doses (CD_{50}) were estimated.

Nucleic acid type (Hamparian *et al.*, 1963). 5-bromodeoxyuridine (40—60 μg/ml maintenance medium) did not decrease the virus titre in Vero cultures, while the control herpesvirus HSV-1 was inhibited by 1.7—2.0 log CD_{50}. This indirect test shows that Bhanja virus contains RNA.

Thermostability. Three cycles of freezing-thawing led to a decrease of 0.3 log CD_{50} in the 1% SMB suspension. The virus (initial titre $10^{6.7}$ CD_{50}/ml) survived in PBS/BSA for at least 374 days at −20 °C (the titre decreased of only 1.2 log after a 5-year storage at −60 °C), for more than 180 (but less than 374) days at 5 °C, 63—69 days at 20 °C, 42—48 days at 28 °C, 16—20 days at 37 °C, more than 160 min at 56 °C, for 5 min (in traces 20 min) at 60 °C, for 5 min (in traces 10 min) at 65 °C, in traces 5 min at 70 °C, and a rapid inactivation was achieved by temperatures of 75 °C or higher (Table

Table 1. Thermal inactivation rate of Bhanja virus (log CD_{50}/ml)

Temperature: ± tolerance °C	−60 °C 1.5	−20 °C 1.5	+5 °C 1.5	+20 °C 1.5	+28 °C 0.5	+37 °C 0.5
0 hr	6.7	6.7	6.7	6.7	6.7	6.7
24 hr	6.5	6.4	6.2	6.1	6.0	5.9
16 days	6.5	6.3	5.5	5.0	3.5	2.5
28 days	NT	6.0	5.0	4.5	2.5	< 1.0
42 days	NT	NT	NT	3.5	1.0	< 1.0
49 days	NT	5.5	4.8	2.7	< 1.0	NT
63 days	NT	5.0	4.5	1.5	< 1.0	
70 days	NT	NT	NT	< 1.0	NT	
180 days	NT	NT	3.0	NT		
374 days	NT	2.0	< 1.0			
540 days	6.0	NT	NT			
5 years	5.5	NT	NT			

	+56 °C 0.1	+60 °C 0.1	+65 °C 0.1	+70 °C 0.1	+75 °C 0.1	+80 °C 0.1
0 min	6.7	6.7	6.7	6.7	6.7	6.7
5 min	NT	1.0	0.7	(0.5)*	< 0.5	< 0.5
10 min	NT	(0.7)*	(0.5)*	< 0.5	< 0.5	< 0.5
20 min	5.8	(0.5)*	< 0.5	< 0.5	< 0.5	< 0.5
30 min	5.5	< 0.5	< 0.5	NT	NT	NT
160 min	4.0	NT	NT	NT	NT	NT

* Virus traces, detected only by the i.c. assay in suckling mice
NT — not tested

1). However, Grešíková and Vachálková (1971) and Karas (1977) observed an almost complete inactivation of Bhanja virus at 50 °C already after 60 min, and at 45 °C after 3 hr, respectively.

Freeze-drying. The infectivity of a centrifuged 10% SMB suspension in PBS/BSA decreased only tenfold or less after lyophilization (Edwards apparatus). Also freeze-dried preparations of various Bhanja virus strains, received from abroad, all contained a fully viable virus.

pH stability. Average titre values (log CD_{50}/ml) after exposure (60 min/ /37 °C) of 1% SMB to particular pH values were: pH 10.0: 7.8; pH 7.0 (control): 8.5; pH 4.1: 3.3; pH 3.0 < 3.0. In one of the experiments, virus traces were detected even at pH 3.0. Grešíková and Vachálková (1971) found a titre decrease of 2 log at pH 5 (as compared with pH 7) in Bhanja and tick-borne encephalitis (TBE) virus, while 4 log in Ťahyňa virus and 5 log in Uukuniemi virus; this indicates that Bhanja and TBE viruses fall among the less acid-susceptible arboviruses.

Trypsin resistance. Two experiments showed a low sensitivity of Bhanja virus to this proteolytic enzyme: the infectivity decrease was only 0.3—1.0

log after 60 min treatment with 0.05% trypsin at 37 °C (in PBS without BSA).

Heparin sensitivity. About 10 CD_{50} of the virus were mixed with various concentrations (final 0.0, 0.5, 2.5, 5, 10 and 20 I.U./ml) of heparin and incubated at 37 °C for 60 min. No virus decrease was found. In another experiment, various concentrations of heparin were included into maintenance medium of Vero cell cultures and the virus was titrated on them in parallel: the concentrations from 0.6 to 2.5 I.U./ml enhanced susceptibility of Vero cells to the virus in that the titres were a little higher, and the CPE appeared sooner.

UV radiation. The infectivity decrease of only 2.0 log against unexposed control was found after UV irradiation with a routine 15-W germicide lamp at a distance of 60 cm for 15 min at 20 °C.

Disinfectants. The 5-min exposure at 37 °C of the virus to various (final) concentrations of chloramine B resulted in the following residual titres (log CD_{50}/ml): 0.00% (control): 8.5; 0.01%: 8.2; 0.1%: 5.0; 1.0%: < 3.0 (no virus was detected). An exposure for 60 min at 37 °C to 0.1% chloramine B or 0.1% formaldehyde caused a full inactivation of 1% SMB suspension of Bhanja virus (control titre $10^{6.2}$ CD_{50}/ml), whereas 0.1% phenol decreased the infectivity of the same suspension negligibly (by 0.2 log).

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