

SENSITIVITY OF TRIBEČ ARBOVIRUS TO HEATING AND pH

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Received January 24, 1969

Summary. — The effects of heating and pH on Tribeč virus were investigated. The virus was inactivated at 60—65° C for 30 minutes; it showed a narrow zone of stability at pH from 7 to 8. A rapid drop in infectivity occurred after lowering the pH below 7 or increasing it above 8.

Since Tribeč virus was isolated from *Ixodes ricinus* ticks and proved to be pathogenic for suckling mice after intracerebral inoculation (Grešíková *et al.*, 1965) it has been tentatively grouped with arboviruses.

According to its antigenic properties, Tribeč virus was included into the Kemerovo virus group (Casals, unpublished data). Physico-chemical properties along with antigenic properties can offer more reliable criteria for arbovirus classification (Andrewes, 1964). Tribeč virus was found to contain ribonucleic acid (Žemla *et al.*, 1968), which fact also supports the inclusion of Tribeč virus into the arbovirus group.

In the present work we studied the effect of heating and pH on Tribeč virus.

Preparation of virus suspension. Tribeč virus isolated from *Ixodes ricinus* ticks (Grešíková *et al.*, 1965) was propagated in suckling white mice and chick embryo cell cultures. Suckling mice were inoculated intracerebrally with 0.01 ml volumes and killed when starting to show disease symptoms. Their brains were dissected and frozen at —60° C. The frozen brains were homogenized to obtain a 20% suspension in phosphate buffered saline at pH 7.2. After ultrasonic disintegration (3 times for 15 seconds in a Czechoslovak apparatus 1 Mc/sec, 250 W), the virus suspension was clarified at 13000 × g for 1 hour and the resulting supernatant (further on designated mouse brain virus) used in the experiments.

Chick embryo cell monolayers were inoculated with an input multiplicity of 1000 LD₅₀ of Tribeč virus per cell. After 2 hours of adsorption at 37° C, the non-adsorbed virus was removed and fresh medium 199 added to the cultures. After 24 hours at 37° C, the cultures showed a distinct cytopathic effect; the extracellular virus-containing medium was clarified by low speed centrifugation (further on designated cell culture virus) and used in the experiments.

Virus titration. All virus-containing materials were titrated by inoculating 0.01 ml volumes of serial 10-fold dilutions intracerebrally into suckling white mice. The LD₅₀ values were calculated by the formula of Reed and Muench.

The effect of heating was investigated in the range from 40 to 70° C. The viral suspensions in sealed glass ampoules were heated for 30 minutes at each temperature in a water bath operated by a Wobser ultrathermostat. After cooling, the virus was immediately titrated in suckling mice.

The effect of pH was investigated in the range from pH 3 to pH 11. Sørensen's buffers, namely 0.1 M sodium citrate buffers at pH 3 and 4, 1/15 M phosphate buffers at pH 5—8, and 0.1 N glycine buffers at pH 9—11 were used. The buffer solutions were sterilized by autoclaving at 1.5 atmospheres for 30 minutes and their pH values accurately measured using a "Multoscop" pH-meter with a glass electrode at room temperature. One part of mouse brain or cell culture virus was mixed with 9 parts of the appropriate buffer and the mixture incubated for 24 hours at 4° C. Then the pH was adjusted to 7.0 and the materials were titrated in suckling mice.

The results of experiments on the effect of heating on Tribeč virus are illustrated in Fig. 1. Infectivity of virus decreased with increasing the temperature above 40° C. After heating at 65° C for 30 minutes, the virus was inactivated.

The effects of pH on Tribeč virus infectivity are evident from Fig. 2. The infectivity showed a maximum between pH 7 and 8; it dropped rapidly on

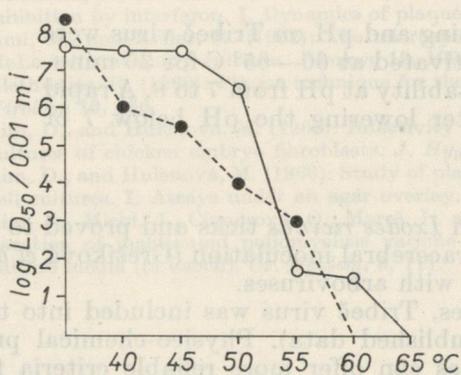


Fig. 1.

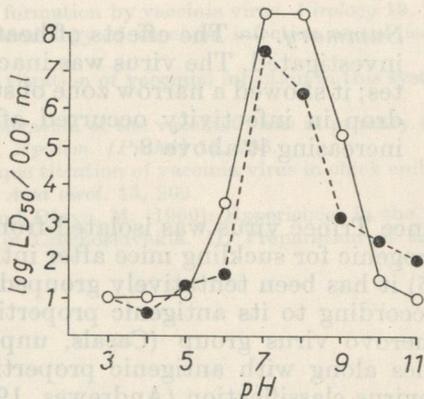


Fig. 2.

The effect of heating on Tribeč virus

- Mouse brain virus
- Cell culture virus

Control values (before treatment) are given on the ordinate

Fig. 2.

The effect of pH on Tribeč virus

- and ● as in Fig. 1

shifting the pH to both the acid and alkaline side. The course of inactivation curves was similar with both mouse brain and cell culture virus.

The effects of heating on some arborviruses belonging to groups A and B according to Cacal's (1957) classification are well known (Lépine, 1931; Kudo *et al.*, 1937; Melnick, 1946; Olitsky *et al.*, 1950; Grešíková-Kohútová, 1959). Our experiments showed that, similarly to A and B group arboviruses, the infectivity of Tribeč virus decreased on heating at 40–50° C and was lost on heating at 60–65° C for 30 minutes.

Studies on the effect pH on arboviruses have shown that the optimum for A and B arboviruses lies between pH 7 and 8.8 (Finkelstein *et al.*, 1938; Duffy, 1946; Duffy and Stanley, 1945; Grešíkova, 1959). Tribeč virus showed a marked optimum around pH 7–8. Lowering the pH of the medium below 7 or increasing it above 8 resulted in a rapid drop in infectivity.

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