COMPARATIVE TITRATIONS OF STREET RABIES VIRUS ACCORDING TO THE CYTOPATHIC EFFECT AND BY IMMUNOFLUORESCENCE IN TISSUE CULTURES AND IN MICE

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We are reporting data on the possibility of street rabies virus titration in tissue culture according to the cytopathic effect (CPE).

The Mochalin strain of street rabies virus was isolated in our laboratory from a person who died of hydrophobia after an incubation of 714 days in spite of having received a complete series of injections of antirabic gamma-globulin and Fermi vaccine. After 2 intracerebral (ic) passages in puppies, the virus was adapted by serial passaging to primary cultures of 4-week Syrian hamster kidney (SHK) cells. Starting with the 21st passage, the virus produced a regular CPE in SHK cells. Therefore, we carried out titration experiments in SHK cell cultures, evaluating the results according to the CPE and by the fluorescent antibody (FA) technique; parallel ic titration in 5—6 g white mice was used for comparison.

Bottle cultures of SHK cells, obtained by trypsinization, were grown in Hanks' solution (pH 7) with 0.5% lactalbumin hydrolysate and 10% normal bovine serum. Virus adsorption onto 4—5 days old monolayers lasted for 2 hr at 37° C. After removal of unadsorbed virus, the cultures were supplied with maintenance medium (Earle's solution, pH 7.6, with 0.5% lactalbumin hydrolysate and 5% normal bovine serum) and incubated further at 37° C, changing the medium every 5 days (*1*).

The results were read 8—10 days after inoculation of SHK cell cultures and 21 days after inoculation of mice. The reciprocal of the highest dilution of virus that caused degeneration in half of the cell cultures was taken for the titre of virus based on the CPE; it was expressed in TCD_{50}/ml values. The reciprocal of the highest dilution of virus that still caused the appearance of fluorescent granules in cell cultures was taken for the titre of virus based on FA assay. The titre values according to the CPE, FA assay and ic inoculation of mice were calculated by the formula of Reed and Muench. The specificity of the CPE was checked by parallel neutralization tests in SHK cell cultures and in mice.

The results of parallel titrations at several passage levels were as follows:

SHK cell passage No.	Days after inoculation	Virus titres (log values per ml)		
		CPE (TCD_{50})	FA	ic mouse LD_{50}
23	10	4.0	5.0	4.0
27	8	5.0	4.0	6.0
28	10	7.0	3.0	7.0
29	10	6.0	4.0	6.0
34	11	7.0	2.0	7.0
36	8	7.0	7.0	7.0

The titres determined according to the CPE and in mice corresponded to each other in all instances. The FA assay proved to be less sensitive. In passages 23, 27, 29 and 36, the FA technique revealed 70—100% infection of cells with numerous inclusions of various size (0.5—40 μ) and their strong fluorescence (4+) only after massive inocula (10^4—10^7 TCD_{50}/ml); with an inoculum of 10^3 $TCID_{50}$/ml, only 10—30% of the cells contained small (0.5—1 μ) and few inclusions showing less intensive fluorescence (1+); after inoculation of 100 TCD_{50}/ml or less, no fluorescence was observed. In passages 28 and 34 with inocula of 10^3—10^4 and 10^3—10^5 TCD_{50}/ml, respectively, the FA assay was negative, in spite of the presence of a CPE and a positive infectivity assay of culture fluid from the respective bottles: the brains from the dead mice always contained Babes-Negri bodies and FA-positive inclusions. The high titres according to FA assay in passages 23 and 36 were probably due to nonspecific fluorescence in the cytoplasm of cells.

So far, the Mochalin strain of street rabies virus underwent 70 serial passages in SHK cell cultures and the appearance of the CPE proved to be stable.

Reference

1. Selimov, M. A., and Ilyasova, R. Sh., *Vop. Virus.* **11** : 602, 1966.