Observations on the Plaque Assay of Visna Virus

(Accepted 17 March 1969)

Visna virus is the cause of a slowly evolving neurological illness of sheep (Sigurdsson, Pálsson & Grimsson, 1957; Sigurdsson & Pálsson, 1958). The virus has been propagated in cell cultures derived from sheep choroid plexus and quantified by 50% end-point determinations of cytopathic effects in sheep choroid plexus cells 14 to 21 days after inoculation (Sigurdsson, Thormar & Pálsson, 1960; Harter & Choppin, 1967b).

Attempts to obtain a plaque assay for visna virus have been hampered by the poor affinity which sheep choroid plexus cells have for neutral red and other vital dyes. To avoid this difficulty, an assay involving the development of plaques in a secondary cellular overlay, which could be stained with neutral red, was developed (Harter & Choppin, 1967a). An infected sheep choroid plexus monolayer of cells was maintained for 12 to 14 days under a semisolid overlay containing carboxymethylcellulose; the overlay was then removed and the sheep choroid plexus cells were covered with a sufficient number of baby hamster kidney cells (BHK 21-F) to establish a second confluent monolayer. Foci of degenerated giant cells formed in the BHK 21-F cells by virus released from underlying infected sheep choroid plexus cells could be recognized after staining with neutral red. Although well-defined plaques were obtained, a large number of baby hamster kidney cells were required to form the indicator monolayer and, therefore, experiments requiring the simultaneous assay of many virus samples were difficult to perform.

I report here plaque development in monolayers of sheep choroid plexus cells infected with visna virus under gelatinous overlays, followed by direct staining of the cell sheet and present data on the adsorption of visna virus to sheep choroid plexus cells using the plaque assay technique.

Confluent monolayers of sheep choroid plexus cells were grown in 60 mm. plastic Petri dishes as previously described (Harter & Choppin, 1967b), washed twice with phosphate buffered saline (PBS, Dulbecco & Vogt, 1954), pH 7.2, and inoculated with 0.5 ml. of serial dilutions of virus in reinforced Eagle's medium (Bablanian, Eggers & Tamm, 1965) with 0.5% bovine plasma albumin. After a four-hour period of adsorption at 37°, the inoculum was removed and the cell sheet was overlaid with 5.0 ml. of double strength reinforced Eagle's medium containing 8% heat-inactivated foetal calf serum, which had been mixed with equal parts of either 0.8% carboxymethylcellulose (Hercules Powder Company, Wilmington, Delaware), 0.6% agar (Difco Noble), or 0.5% agarose in distilled deionized water. The cultures were incubated at 37° in a humidified atmosphere of 5% CO2 for 12 to 14 days. The overlay was then removed and the cell sheet was washed, fixed with absolute ethanol and stained with a 1% solution of crystal violet. To facilitate removal of carboxymethylcellulose, plates maintained under this overlay were incubated with a 1% cellulase solution for 30 min. at 37° before ethanol fixation. Discrete plaques 2 to 3 mm. in diameter with irregular edges which could easily be distinguished from unaffected portions of the cell sheet were obtained with each overlay.
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To determine the relative efficiency of the three overlays, eight separate experiments assaying the same virus preparation were performed using both the plaque count and cytopathic end-point methods. Tissue culture infective units (TCIU) were calculated by multiplying TCD 50 titres by 0.69. The highest p.f.u.:TCIU ratio resulted when carboxymethylcellulose was used as the gelling agent (Table 1). Carboxymethylcellulose was chosen, therefore, as the overlay in subsequent experiments.

To determine the relationship between virus concentration and the number of plaques, serial twofold dilutions of virus were assayed. There was a linear relationship between the number of plaques obtained and the concentration of virus (Fig. 1).

Experiments on the development of visna virus cytopathic effect after different

![Graph showing the relationship between virus concentration and number of plaques.](image)

**Table 1. Effect of overlay on plaque count of visna virus in sheep choroid plexus cells**

<table>
<thead>
<tr>
<th>Overlay</th>
<th>Infective virus* ($\times 10^{-8}$ per ml)</th>
<th>p.f.u.:TCIU ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4% carboxymethylcellulose</td>
<td>2.5 ± 1.0 p.f.u.</td>
<td>2.5</td>
</tr>
<tr>
<td>0.3% agar</td>
<td>1.0 ± 0.5 p.f.u.</td>
<td>1.0</td>
</tr>
<tr>
<td>0.3% agarose</td>
<td>1.1 ± 0.9 p.f.u.</td>
<td>1.1</td>
</tr>
<tr>
<td>Cytopathic end-point</td>
<td>1.0 ± 0.6 TCIU</td>
<td>—</td>
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</tbody>
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* Arithmetic mean of eight determinations ± standard deviation.
periods of virus adsorption showed that maximum adsorption occurred within 2 hr after inoculation, but the lack of a plaque assay for the virus prevented a direct measurement of virus adsorption (Thormar, 1963). To determine the rate of adsorption of visna virus to sheep choroid plexus cells, replicate monolayers were washed with PBS and inoculated with 0.5 ml. (50 to 75 p.f.u.) of virus prepared in reinforced Eagle’s medium with 0.5% bovine plasma albumin. At intervals the inoculum was removed from four cultures and the monolayers were washed three times. Overlay containing carboxymethylcellulose was added, and the plaques produced were subsequently counted. Fifty per cent adsorption of the virus occurred after 30 min. at 37°C and 90% after 2 hr (Fig. 2).

![Graph](image)

**Fig. 2.** Adsorption of visna virus to sheep choroid plexus cell monolayers at 37°C; results expressed as percentage of six-hour value.

Visna virus could be assayed reproducibly by the plaque technique under semisolid overlays containing carboxymethylcellulose, agar or agarose. Of the three overlays, carboxymethylcellulose gave the best results and permitted direct measurement of the virus adsorption rate. The plaque assay described is relatively simple to perform and should be useful in experiments requiring quantification of the virus in a large number of samples.

I wish to thank Mrs Karen K. Funk and Mrs Viola Mahoney for excellent technical assistance.

This work was supported by Public Health Service Research Grant no. NB-06989,
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Public Health Service Career Research Development Award 1K3NB34,900 and the Miles Hodson Vernon Foundation.

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REFERENCES


(Received 6 March 1969)