The Differentiation of Herpes Simplex Virus Type 1 and Type 2 by Temperature Markers

(Accepted 7 July 1971)

Herpes simplex viruses have been divided into two types. These types may be differentiated by serological tests (Shubladze et al. 1960; Plummer, Waner & Bowling, 1968) and by the character of the lesions produced on the chorioallantoic membrane (CAM) of fertile hens' eggs (Parker & Banatvala, 1967) and in tissue culture (Lowry, Melnick & Rawls, 1971). It has been suggested recently that the serological classification into type 1 and type 2 may be too rigid and that a whole spectrum of variants may exist (Roizman et al. 1970).

The two types have a characteristic biological differentiation, type 1 being associated mainly with lesions of the face and type 2 with lesions of the genital tract. The observation that genital strains may be linked in some way with carcinoma of the cervix (Naib, 1966) has increased interest in these viruses.

Growth at different temperatures has proved a useful marker for several groups of viruses. Among the pox viruses, temperature differentiates alastrim from variola and both from vaccinia (Bedson & Dumbell, 1961; Nizamuddin & Dumbell, 1961). Wild neurovirulent strains of poliovirus can be distinguished from vaccine strains by their ability to grow at higher temperatures (Lwoff, 1962). It was therefore of interest to test the influence of temperature on the multiplication of the two serotypes of herpes simplex virus.

Thirty-one strains of herpes simplex virus were tested for their ability to produce cytopathic effects at various temperatures in Vero cells. These strains included both recent isolates and laboratory strains with long passage histories in eggs and tissue culture. Sixteen strains were classified as type 1 and 15 strains as type 2 on the basis of neutralization tests, pock size on the CAM, or a combination of both tests.

Tenfold dilutions from $10^{-1}$ to $10^{-4}$ of each virus strain were made in distilled water and 0.1 ml. samples of each dilution were inoculated into tubes containing monolayers of Vero cells. Each strain of virus at each dilution was incubated stationary at 35°, 39.2°, 39.8° and 40.3°. Temperatures were stable within ±0.2°. Cell sheets were examined for cytopathic effect after 2 and 5 days.

All the type 1 strains produced cytopathic effect at all of the temperatures tested, but the replication of the type 2 strains was markedly inhibited at temperatures above 39° (Table 1). At 40.3° the infectivity of all the type 2 strains was reduced to 0.01% or less of that at 35° (Fig. 1).

Both recent isolates and strains with long passage histories in eggs and tissue culture were used, but such factors did not influence the ability of strains to produce cytopathic effect at supra-optimal temperatures. Of the three type 2 strains showing some survival at 39.8°, two were recent isolates.

An interesting effect was observed in some tubes inoculated with high concentrations of type 2 strains and incubated at 40.3°. After 48 hr small foci of refractile rounded cells appeared, but after several days this effect receded. A very few naked and disintegrating particles were shown by electron microscopy in the supernatant fluid, but no virus particles were seen in the cells. The nature of the effect is unknown.

So far no investigation has been made to determine which stage of the virus replication
Table 1. Effect of temperature on the growth of type 1 and type 2 strains of herpes simplex virus

<table>
<thead>
<tr>
<th></th>
<th>Type 1</th>
<th>Type 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. of strains tested</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>Recent isolates</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Laboratory-adapted strains</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>Cytopathic effect produced in Vero cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35.0°C</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>39.2°C</td>
<td>16</td>
<td>9</td>
</tr>
<tr>
<td>39.8°C</td>
<td>16</td>
<td>3</td>
</tr>
<tr>
<td>40.3°C</td>
<td>16</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig. 1. The reproductive capacity of strains of herpes simplex virus at various temperatures. The reproduction of type 1 strains is almost unaffected within the range 35-40°C, whereas that of type 2 strains is progressively inhibited. ■, type 1; □, type 2.

The cycle is inhibited by higher temperatures. It is hoped that this temperature marker test, which has interesting theoretical implications, may be used as a simple alternative test for the differentiation of type 1 and type 2 strains.

Since this paper was submitted for publication similar findings have been reported by Longson (1971).

I should like to thank Professor P. Wildy and Dr G. T. Cook for providing some of the strains of herpes simplex virus, Dr Anne M. Field for help with electron microscopy, and Dr Yvonne E. Cossart for useful help and discussion.

Virus Reference Laboratory
Central Public Health Laboratory
Colindale Avenue
London, N.W. 9

HAZEL RATCLIFFE
Short communications

REFERENCES


(Received 10 May 1971)