Thermo-Efficient Strains of Variola Major Virus

(Accepted 12 March 1967)

Thermo-efficient and thermo-sensitive mutants of pox viruses are obtained, without the aid of mutagens, only with difficulty. Kirn and his colleagues (1, 2) have described both a thermo-sensitive and a thermo-efficient mutant of vaccinia virus. This note reports the derivation and properties of two thermo-efficient strains of variola major virus.

The first strain (\(\text{Ht} \)) was adapted by serial passage in the chick chorioallantois (CAM) at temperatures which were increased from 37\(^\circ\) to 39.8\(^\circ\) in small steps as adaptation occurred. After 44 passes a clonal stock (\(\text{Ht} \)) was prepared. The other strain (\(\text{Bt} \)) was adapted by a similar process except that occasional passes at 35\(^\circ\) were made to increase the titre of the passage virus. This enabled bigger incremental steps to be made in the growth temperature than was possible when using continued passages at elevated temperature. After 22 passes a clonal stock (\(\text{Bt} \)) was prepared.

Efficiency of pock formation at different temperatures. Suspensions containing \(10^4\) pock forming units (pk.f.u.) per ml. were prepared from the stocks of parent and adapted viruses and titrated with appropriate dilution on the chorioallantoic membrane (CAM) at different temperatures of incubation (Table 1). Although all four viruses showed decreased efficiency of pock formation at elevated temperatures, \(\text{Ht} \) and \(\text{Bt} \) clearly differed from the parent viruses in producing pocks at 39\(^\circ\) and visible lesions even at 40\(^\circ\).

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<tr>
<th>Virus</th>
<th>Temperature of incubation</th>
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<tbody>
<tr>
<td></td>
<td>38.5(^\circ)</td>
</tr>
<tr>
<td>(\text{Ht} )</td>
<td>3.7</td>
</tr>
<tr>
<td>(\text{Ht} )</td>
<td>3.7</td>
</tr>
<tr>
<td>(\text{Bt} )</td>
<td>3.4</td>
</tr>
<tr>
<td>(\text{Bt} )</td>
<td>3.4</td>
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The figures quoted are the titres in log\(_{10}\) pk.f.u./ml. for suspensions containing \(10\) pk.f.u./ml. when titrated at 35\(^\circ\).

Growth of virus in CAM. Chick embryos were inoculated with approximately \(10^8\) pk.f.u. of virus and incubated at 35\(^\circ\), 38\(^\circ\) and 39\(^\circ\). At intervals CAM's were harvested and extracts of these were titrated for virus content by pock counts at 35\(^\circ\). The results are shown in Fig. 1, 2. Both \(\text{Ht} \) and \(\text{Bt} \) showed evidence of growth at 39\(^\circ\). At 35\(^\circ\) the growth of \(\text{B} \) and \(\text{Bt} \) was equal but the growth of \(\text{Ht} \) was significantly less than that of \(\text{H} \). The decreased growth at elevated temperatures is due in part to the lower efficiency with which infective centres are formed. There is also a decreased growth at each centre which was shown by excising individual pocks making extracts of them and titrating their content of infectious virus (Table 2). It is worth noting that a visible pock may yield a remarkably small amount of infectious virus. Evidence will
Table 2. Virus content of pocks on the CAM at different temperatures for two strains of variola (H and B) and their thermo-efficient mutants (Ht and Bt)

<table>
<thead>
<tr>
<th>Virus</th>
<th>Temperature of incubation</th>
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<tbody>
<tr>
<td></td>
<td>35°</td>
</tr>
<tr>
<td>H</td>
<td>6.2</td>
</tr>
<tr>
<td>Ht</td>
<td>4.6</td>
</tr>
<tr>
<td>B</td>
<td>7.0</td>
</tr>
<tr>
<td>Bt</td>
<td>6.7</td>
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</table>

The figures quoted are the log_{10} pk.f.u. of virus extracted per sample of 10 individual pocks taken at 72 hr.

Fig. 1, 2. Growth curves on the CAM at different temperatures for strains H and B and their thermo-efficient mutants Ht and Bt. Fig. 1. ◊--◊, H; ●--●, Ht. Fig. 2. □--□, B; ■--■, Bt.

be presented elsewhere that some functions of variola virus proceed at temperatures where new infective virus is not formed. Stocks of all strains grown at 35° revealed similar particle/infectivity ratios, of the order of 10:1, when counted by the loop-drop method (3).

Virulence for chick embryo was measured as previously described (4). The virus inoculum (log_{10} pk.f.u.) required to kill 12-day-old chick embryos in 4 days at 35° was found to be 5.4 for H and more than 8.0 for Ht. This marked reduction in virulence for Ht was not paralleled by strain Bt. Both B and Bt gave values in the range 5.1 to 5.9. There was thus no evidence of increase in virulence of the thermo-efficient strains. The relative avirulence of Ht is probably attributable to its lowered growth potential at 35°.

In other laboratory properties the thermo-efficient strains were found to behave as typical variola. In particular it is to be noted that they gave no lesions following intradermal injection of rabbits with inocula of 10 pk.f.u.
Strain Ht was tested for its genetic stability. Six serial passes with large inocula were made at 35°C. The virus stock from the 6th passage and three clonal isolates made from it were tested for efficiency of pock formation. All behaved as Ht.

The two thermo-efficient strains described represent the only successes from several attempts to adapt variola strains to growth at higher temperatures. No success was achieved with a strain of variola minor virus.

The findings of Kirn and his colleagues (1, 2) with vaccinia have suggested a direct relation between ceiling temperature and virulence. Our results with variola contrast with this and suggest that the relationship may not always be simple: a finding in keeping with earlier observations on genetic hybrids of pox viruses (5).

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REFERENCES

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