The Effects of Temperature and pH Variations on Plaque Production by Different Virulence Types of Myxoma Virus

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SUMMARY

Eight ‘strains’ of myxoma virus, spanning the complete spectrum of virulence, were tested for ability to produce plaques on rabbit kidney cells at varying temperatures and pH values. A positive correlation was found between virulence in rabbits and ability to produce plaques at supra-optimal temperature and at low pH.

Myxomatosis has been enzootic in the wild rabbit population in Great Britain since 1953-5 and, although the virus type which caused the initial outbreaks was fully virulent, attenuation of virus was found to occur within a short period (Hudson & Mansi, 1955; Fenner & Chapple, 1965). The present position is that virus types or ‘strains’ of widely different virulence can be isolated from wild rabbits (Ross, 1972). A similar pattern of attenuation of virus occurred in Australia and Fenner & Marshall (1957) devised a method of classifying virus types according to virulence, ranging from those causing >99% mortality (Grade I) to <50% mortality (Grade V), among fully susceptible rabbits under laboratory conditions.

It has been shown (Marshall, 1959) that the outcome of infection of rabbits with different virulence types of myxoma virus can be influenced by ambient temperature. The symptoms following infection with moderately virulent ‘strains’ (Grade III) were less severe and mortality rates lower when ambient temperatures were higher than normal and more severe when temperatures were low. Variations in ambient temperature had no significant effect on the course of infection with fully virulent ‘strains’ of virus.

We investigated whether similar effects of temperature variations on the growth of virus could be detected in vitro. The effects of variations in pH were also investigated.

The eight virus types or ‘strains’ used are described in Table 1. All strains were passaged at least once in New Zealand White rabbits, and the virulence grade assessed by the method of Fenner & Marshall (1957) before and after the experiments described. Virus was extracted from lesion material using a pestle and mortar and 2 ml of phosphate buffered saline (PBS), pH 7.2, per g of tissue. After removal of cell debris by centrifuging (1000 g, 5 min), a 10^-2 dilution of the extract in PBS was injected intradermally (0.1 ml) in the flank of a laboratory rabbit. Primary lesions at the site of inoculation were taken at 8 days p.i. or eyelid lesions at 12 to 14 days p.i. Virus was extracted as previously described and titrated by plaque assay (Schwerdt & Schwerdt, 1962) or by inoculation in rabbits.

Rabbit kidney cells (RK13) were grown in Roux bottles containing 100 ml of Earle’s minimum essential medium (MEM) with antibiotics (100 international units penicillin/ml and 100 μg streptomycin/ml, 0.088% NaHCO₃ and 10% new-born calf serum, and in an atmosphere of 5% CO₂. For maintenance medium (MM), the amount of calf serum was reduced to 5%. The pH of media used for passage and maintenance of RK13 cells was 7.2, but for experimental purposes the pH was varied by using different amounts and concentrations of NaHCO₃ and CO₂ and with HEPES buffer (20 mM).
Table 1. *Virus types tested and the effects of 24 h incubation at 39.5 °C on plaque production compared with production at constant 37 °C for complete 5-day incubation*

<table>
<thead>
<tr>
<th>Virus type</th>
<th>Designation</th>
<th>Mean survival time (MST)±s.e.</th>
<th>Virulence grade* and range of MST</th>
<th>Timing of incubation at 39.5 °C (remainder at 37 °C)</th>
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</thead>
<tbody>
<tr>
<td>Cornwall</td>
<td>England/Cornwall/4-54/1 (Fenner &amp; Marshall, 1957)</td>
<td>12.1±0.3 days I (≤13)</td>
<td></td>
<td>Day 0–1</td>
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<td></td>
<td>Glenfield</td>
<td>Australia/Dubbo/2–51/1 (Fenner &amp; Marshall, 1957)</td>
<td>10.5±0.4 days</td>
<td>35.1±3.2 70.1±11.1</td>
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<tr>
<td>Brecon</td>
<td>4298 (Weybridge 1961) (Chapple &amp; Bowen, 1963)</td>
<td>20.4±1.2 days IIIa (16–22)</td>
<td>18.9±4.1 5.3±3.5 19.3±4.8</td>
<td>4.0±0.4</td>
</tr>
<tr>
<td>Stirling</td>
<td>Scotland/TPM–1/66</td>
<td>19.2±1.4 days</td>
<td>9.8±3.6 3.2±1.5</td>
<td>—</td>
</tr>
<tr>
<td>FS98</td>
<td>Australia/</td>
<td>23.0±2.9 days IIIb (22–28)</td>
<td>9.3±6.1 0 1.5±1.4</td>
<td>3.3±5.2</td>
</tr>
<tr>
<td>Rogart</td>
<td>Scotland/V65/71</td>
<td>23.0±2.8 days</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td>Uriarra</td>
<td>Australia/Uriarra/2–43/1 (Mkytowycz, 1953)</td>
<td>32.3±21 days IV (28–50)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Nottingham</td>
<td>England/Nottingham/4–55/1 (attenuated) (Fenner &amp; Marshall, 1957)</td>
<td>—</td>
<td>V (&gt;50) 0.8±0.5</td>
<td>0</td>
</tr>
</tbody>
</table>

* Virulence grades and ranges of MST were taken from Fenner & Marshall, (1957).
† Plaque production as percentage of plaque numbers at constant 37 °C: mean±s.e.
‡ Mean survival times calculated by method of Sampford (1954) since several rabbits survived infection.
§ Mean survival time for Nottingham was not calculated since all rabbits infected with this 'strain' survived.
Fig. 1. Effects of (a) temperature and (b) pH on plaque production in RK13 cells by different virulence grades of myxoma virus. (a) Virus-infected RK13 cell monolayers were incubated at the temperatures shown for 5 days with the medium in each case being held at pH 7·2. Results are shown as percentages of the maximum numbers of plaques (mean values with standard errors represented by vertical lines where they are larger than the symbols). Incubation at 39·5 °C was restricted to the final 24 h, following 4 days at 37 °C. (b) Virus-infected cell monolayers were incubated at 37 °C with varying pH values for 5 days. Results are shown as percentages of the maximum number of plaques (mean values with standard errors represented by vertical lines where they are larger than the symbols). △—△, Grade I; ○—○, Grade III; ■—■, Grade IV; □—□ Grade V.

For plaque assay, virus extracts were diluted in MM and 0·5 ml of the dilutions inoculated on to RK13 cell monolayers on 50 mm Petri dishes. Virus was allowed to adsorb for 2 h at room temperature, after which time the cell sheets were washed and covered with 5 ml of MM. The cells were incubated for 5 days at 37 °C or at other temperatures as indicated below. The cell sheets were then stained with 0·5 % crystal violet and plaques were counted using a photographic enlarger.

Each strain of virus was tested for ability to produce plaques at temperatures between 30 and 39·5 °C. Unfortunately, incubation at 39·5 °C for the 5 days required for plaque production was found to damage the RK13 cells and so incubation as this temperature was limited to 24 h periods within the 5 day incubation, the remainder being at 37 °C. The results of incubation at 39·5 °C are shown in Table 1, with plaque production at 39·5 °C being expressed as a percentage of plaque production at a constant 37 °C.

It can be seen that all virulence types tested were more sensitive to inhibition at 39·5 °C in the earlier stages of incubation. However, fully virulent viruses (Grade I) were only moderately inhibited, while the single Grade V virus was completely inhibited and Grade III viruses were intermediate.

In Fig. 1(a), plaque production of viruses from each virulence grade at temperatures between 30 and 39·5 °C is shown as a percentage of the maximum production at the optimal temperature for each grade. The figures for 39·5 °C represent production resulting from incubation at that temperature for the final 24 h (i.e. day 4 to 5). All strains tested appeared to have optimal temperatures for the production of plaques in the range 32 to 34 °C, but there is a clear correlation between virulence and ability to produce plaques at sub-optimal and supra-optimal temperatures, \( F = 17·87, \) d.f. 2, 27, \( P < 0.001 \) for 39·5 °C; \( F = 42·48, \) d.f. 2, 7, \( P < 0.001 \) for 30 °C.

The effects of variations in the pH of MM during incubation at a constant 37 °C were also
investigated using the same strains of virus except Uriarra (Grade IV). The results are shown in Fig. 1(b), with plaque production at different pH values being shown as percentages of maximum production. Grade I strains produced plaques equally well throughout the range pH 6.9 to 7.5. Grade III strains and the single Grade V strain had optimum production at pH 7.5 or higher and all were inhibited at pH 6.9; Grade III strains by 50% and Grade V almost completely. The differences are highly significant (F = 64.4, d.f. 2 and 26).

The observed relationship between virulence and ability to produce plaques at supra-optimal temperatures, fits well with the observations of Marshall (1959) on the effects of high ambient temperatures on infections in rabbits, and with the findings of Baxby (1974) using smallpox vaccine strains. In the first 2 to 3 days after infection of a rabbit, virus replication is confined to the injection site and the local skin temperature is generally about 38 °C; later when infection becomes general, rectal temperatures reach 39 to 41 °C (J. Ross and M. F. Sanders, unpublished observations). At high ambient temperatures skin and rectal temperatures are at least 1 °C higher. Thus the temperatures used in the tissue culture experiments are similar to those encountered during infection of rabbits.

Lwoff (1959) gave other examples in which an increase of temperature and a decrease of pH can depress virus multiplication. One of the reactions of an animal to virus infection is the inflammatory response and two features of this response are an increase in body temperature (fever) and, in some cases at least, a local decrease in cellular pH. The results presented here show that different strains of myxoma virus react differently to these two features of the inflammatory response and that the reactions are related to the virulence of the strains.

REFERENCEs


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