The Pathogenesis of Pseudorabies in Mice: 
Virus Replication at the Inoculation Site and 
Axonal Uptake

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SUMMARY

Three-week-old mice were inoculated in the right ear pinna with pseudorabies virus. Ears were surgically removed at various times after inoculation and changes from the normal pathogenesis were observed. Virus replication in the ear tissue and cervical dorsal root ganglia was also monitored. Following inoculation with a small dose of virus, local multiplication of the virus was necessary before the infection spread to the nerves. With larger infecting doses there was probably direct uptake of virus from the inoculum into the nerve endings. After these larger doses virus was first detected in the dorsal root ganglia 17 h after infection, suggesting a retrograde axonal flow rate of at least 1.7 mm/h.

We have established (Field & Hill, 1974) that in the early stages of infection with pseudorabies virus, the virus is transmitted from the inoculated hind foot pad via the sciatic and femoral nerves and the lumbar dorsal root ganglia to the spinal cord. The rate of virus translocation from the inoculation site to the dorsal root ganglia was estimated to be approx. 1.7 mm/h. However it was not certain whether the inoculated virus itself travelled to the dorsal root ganglion or whether the virus replicated at the inoculation site before entering the nerve endings. Hence it was not possible to make precise estimates of the axonal flow rates of the virus, assuming this to be the likely mode of transport. In the present study, further experiments were made to determine the importance of local virus multiplication in the early stages of infection and spread to the peripheral nerves. A new inoculation site, the ear pinna, was chosen since it allowed complete and easy surgical removal of the inoculated tissue.

The pseudorabies virus used was strain N1A-2, kindly given to us by Dr J. B. McFerran, Belfast (Field & Hill, 1974). Before inoculation, three-week-old, random bred mice were anaesthetized by intra-peritoneal injection of sodium pentobarbitone. A drop of virus suspension (approx. 0.002 ml) was placed on the right ear, which was then pricked firmly through the drop 20 times using a 26-gauge needle. This was done within an area of 2 mm² at the outer margin of the ear. Excess fluid was then gently removed using a sterile swab. At various times following inoculation the mice were again anaesthetized and the whole area of inoculated tissue was removed by amputation at a point 2 to 3 mm proximal to the site of inoculation. This operation resulted in little or no bleeding. Ear fragments were stored separately at -60 °C in Eagle's minimal essential medium (containing 2 % foetal calf serum, antibiotics, and buffered with sodium bicarbonate). The tissue was then ground in a Griffith's tube, sonicated and titrated in tissue cultures of VERO cells. When dorsal root ganglia were removed, they were either treated in a similar manner, or more often, transferred whole without storage to a tissue culture vessel and maintained in culture on a
monolayer of VERO cells to indicate the appearance of released virus (Field & Hill, 1974). The latter method was more sensitive for detecting low levels of infectious virus. Using the inoculation method described above, one LD$_{50}$ was equivalent to $2 \times 10^5$ p.f.u./ml. Further dosages will be expressed in terms of LD$_{50}$.

Mice were inoculated in the right ear with 1, 10, 100, and 500 LD$_{50}$ (Table I) and at times shown, the right ear was amputated; a control group infected with each dose was left with ears intact. At doses of 1 and 10 LD$_{50}$, amputation of the ear completely protected the mice up to 36 and 24 h after inoculation respectively. Mice which developed clinical signs following amputation at later times showed similar pathogenesis to the control groups, suggesting that the infection did not result from spread by a non-neural route (Field & Hill, 1974). With larger doses, a small proportion of animals died despite the removal of the ear within 30 min of inoculation. The proportion of mice dying despite ear amputation at intervals during the first 12 h after infection was quite constant (Table I).

Control experiments were made at the large dose to test whether the proportion of mice which escaped protection resulted from a breakdown of the experimental technique. In a group of 16 mice the virus suspension was placed on the ear and the drop allowed to dry, but no pricking was done. In 6 of these mice the ear was left intact and in the remaining 10 the ear was amputated 30 min after application of the virus. No mice in either group developed pseudorabies. This suggested that the inoculum was not spreading on the surface of the skin and gaining entry either into the cut edge or through the intact skin.

After grinding, the suspensions of ear tissue were titrated for infectious virus (Table I). With a dose of 10 LD$_{50}$, virus was just detectable for about 3 h after infection. There followed a period of eclipse, with reappearance of virus in samples taken 24 h after inoculation. With 500 LD$_{50}$ correspondingly higher levels of infectious virus were detected in the ear tissue and there was little or no change in titre until a rise occurred about 12 h after infection. When 10 p.f.u. or less were detected in the ear fragments mice could be protected by amputation of the ear up to 24 h after infection. In mice receiving 10 LD$_{50}$ or less there was a lag of at least 12 h before the level of virus reached this titre. It was thus established that virus multiplication occurred at the inoculation site. In mice given very large doses (> 1000 LD$_{50}$) there is probably sufficient virus in the tissue immediately after inoculation to pass directly into the nerve endings without replication.
Table 2. The isolation of pseudorabies virus from cervical dorsal root ganglia following ear inoculation

<table>
<thead>
<tr>
<th>Time after inoculation (h)</th>
<th>10 LD&lt;sub&gt;50&lt;/sub&gt; right ear</th>
<th>Approx. 5000 LD&lt;sub&gt;50&lt;/sub&gt; right and left ear</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2nd and 3rd cervical</td>
<td>4th cervical</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>-</td>
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<tr>
<td>17</td>
<td>-</td>
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<tr>
<td>18</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>24</td>
<td>0/6</td>
<td>0/6</td>
</tr>
<tr>
<td>36</td>
<td>0/6</td>
<td>1/6</td>
</tr>
<tr>
<td>41</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>48</td>
<td>3/6</td>
<td>2/6</td>
</tr>
</tbody>
</table>

* Ganglia ground, sonicated and titrated in VERO cells directly. (In all other cases, ganglia were maintained in tissue culture in the presence of VERO cells.)
† Ratio of the number of mice from which virus was isolated from the ganglia to the number tested at that time post infection.

At the dose of 10 LD<sub>50</sub> virus could usually be detected in the cervical dorsal root ganglia 48 h after infection and occasionally as soon as 36 h (Table 2). At the higher dose of 5000 LD<sub>50</sub> virus was isolated at the earlier time of 17 h after inoculation. Assuming direct uptake of virus into nerve endings this suggests a retrogradely axonal flow rate of at least 1.7 mm/h (measured over 30 mm nerve) which confirms our previous result for the sciatic nerve (Field & Hill, 1974). This would be a high enough rate to allow virus particles to escape from the area of inoculation before the earliest time of amputation (30 min). This rate is comparable to that obtained by Hendry et al. (1974) for the retrogradely axonal transport of nerve growth factor in the mouse (2.5 mm/h) and by Kristensson, Lycke & Sjöstrand (1971) for the retrogradely transport of proteins in the rabbit hypoglossal nerve (5 mm/h). However our rate is considerably slower than the rate of 10 mm/h indicated by the results of McCracken, McFerran & Dow (1973) for pseudorabies infection in the calf (measured over 750 mm nerve).

In most instances of natural infection, pseudorabies virus is probably introduced into the tissues at low dose levels. Our results therefore suggest that under normal circumstances replication of virus occurs at the point of inoculation before entry of virus into the nerve endings. However, the use of virus at high doses may prove valuable in further studies on axonal uptake and transport of herpes viruses.

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Department of Bacteriology
The Medical School
University Walk
Bristol BS8 1TD
England

H. J. FIELD
T. J. HILL
Short communications

REFERENCES


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