Respiratory Infection of Mice with Vaccinia Virus

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Suckling mice readily succumb to infection with small doses of many strains of vaccinia virus, but lethal vaccinial infection in mature mice can be established only with certain virus strains in appropriate doses and by certain routes of inoculation, and only in mice of certain genotypes (1,2). Lethal infection against which mice can be immunized, can be established by the respiratory route after intranasal inoculation (3). Infections established by this route, which have been used in studying antiviral drugs, would be useful in assaying the protective potency of inactivated vaccines. The infection is less unnatural than intracranial inoculation with neurovaccinia strains and possibly simulates the natural mode of infection in smallpox. (4)

The strains of vaccinia virus used (Table I) were obtained from the following sources: LISTER—standard vaccine strain Elstree; LEVADITI—dried rabbit testicular passage Elstree (1939); COPENHAGEN—glycerolated calf lymph Utrecht (1961); TASHKENT—dried vaccine strain Moscow (1962). The mouse neurotropic strains WR and IHD were obtained as dried infected mouse brain through the courtesy of Drs Kingsley Sanders and D. J. Bauer in 1955 and 1966 respectively. The biological characteristics of LEVADITI, WR and IHD have been described by Fenner (5).

Viruses were propagated in either chick cell cultures or chorioallantoic membranes and partially purified by treatment with Arcton 113 (Imperial Chemical Industries) followed by differential centrifugation. Suspensions were assayed by plaque counts in chick cell cultures. Titres were related to a standard dried vaccine preparation of known pock-forming potency and expressed in terms of pock equivalents (pk. eq.). The strains were examined for lethal infectivity in 18 to 21 g. mice inoculated by various routes. In some experiments, four strains of mice were used: National Institutes of Health (NIH), Theiler’s Original (TO), Imperial Chemical Industries (ICI) and PORTON. All were obtained from the Scientific Animal Service, Elstree, who described the derivation and maintenance of the stocks. The first three mouse strains are known to differ; differences between the last two are not clearly established. Mice were examined daily and survivors kept for 3 weeks.

The susceptibility of mice to six strains of vaccinia virus, inoculated in similar doses by various routes, was tested (Table I). No strain produced lethal infection by the subcutaneous route, although mice inoculated this way were subsequently shown to be immune, probably as a consequence of transient dermal infection (6). Intraperitoneal inoculation of four strains was similarly negative. The strains LEVADITI and COPENHAGEN killed a proportion of the mice when inoculated by the intracerebral route. Deaths occurred within 4 days following inoculation. Examination of brain material showed virus to be either absent or much reduced in titre, and no mice died when this material was passaged intracranially. Deaths with these strains could probably be ascribed to toxicity rather than to virus multiplication. Only the mouse neurovirulent strains WR and IHD killed mice by the respiratory route and were examined further. The pattern of respiratory infection with strain WR was identical with that described by...
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Nelson (3). Groups of mice killed at intervals after intranasal inoculation showed that lung consolidation was accompanied by the production of large amounts of virus. Viraemia was present from the 2nd day after inoculation until death between the 5th and 8th days. Virus was present in the brain in the terminal stage of infection, when many mice were paralysed (Fig. 1 A).

Table 1. Effect of route of inoculation on lethal infection of mice with different strains of vaccinia virus

<table>
<thead>
<tr>
<th>Virus strain</th>
<th>Dose (log. pock equivalents)</th>
<th>Number dying/number inoculated by route*</th>
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<tbody>
<tr>
<td></td>
<td>i.c.</td>
<td>i.n.</td>
</tr>
<tr>
<td>LISTER</td>
<td>5-0-6-0</td>
<td>0/10</td>
</tr>
<tr>
<td>LEVADITI</td>
<td>6-0</td>
<td>11/21†</td>
</tr>
<tr>
<td>COPENHAGEN</td>
<td>6-0</td>
<td>7/10†</td>
</tr>
<tr>
<td>TASHKEN T</td>
<td>6-3-6-6</td>
<td>0/5</td>
</tr>
<tr>
<td>WR</td>
<td>6-0</td>
<td>10/10‡</td>
</tr>
<tr>
<td>IHD</td>
<td>6-0</td>
<td>10/10‡</td>
</tr>
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* i.c. = intracranial; i.n. = intranasal; s.c. = subcutaneous; i.v. = intravenous; i.p. = intraperitoneal.
† Toxic death (see text).
‡ Lethal dose i.c. = < 10 pk. eq.
- Not tested.

The WR virus was taken through twelve successive mouse lung passages. At each passage, the concentration of virus varied between $10^8$ and $10^9$ pk.eq./g. wet weight of lung tissue. Passage did not reduce the numbers of pk.eq./lung LD $50$ which varied from $10^{2.5}$ to $10^{4.5}$ pk.eq. in the last four passages. Comparison of unpassaged material with 11th passage material showed that the pattern of multiplication in the lung was unaltered (Fig. 1 A). The intracranial LD $50$ of the passaged strain remained constant at $10^{6.5}$ pk.eq.; the lung LD $50$ was the same in four different strains of mice, and was consistently about 4 log units higher than the intracranial dose. The strain WR inoculated intranasally, regularly gave mortalities consistent with the calculated dose and in this respect was better than IHD.

The IHD strain of vaccinia virus was taken through four serial lung passages in each of four different strains of mice and five further passages in each of three strains of mice. The yield of IHD virus in successive lung passages was similar to WR. Titres varied between $10^9$ and $10^8$ pk.eq./g. wet weight of lung tissue and lung LD $50$ titres between $10^{3.5}$ and $10^{4.8}$ pk.eq. Eighth passage material and unpassaged virus had the same infectivity for mouse lungs; and again the ratio of pk.eq. to lung LD $50$ was not reduced after lung passage. Adequate dosage, therefore, seems more important than lung adaptation, if indeed the latter occurs. Different susceptibilities were not apparent in four strains of mice used with WR virus and were doubtful with IHD virus. The present observations differ in these respects from those of Link et al. (10).

Observations were made on 10 mice killed at intervals after the intranasal inoculation of the non-neurovirulent strains LEVADITI and COPENHAGEN. Abortive multiplication of virus occurred without macroscopic changes in the lungs (Fig. 1 B). No virus could be detected in the blood or brain of animals infected with either strain. Similar results were obtained with the Lister strain in CBA and SCHNEIDER mice (Kaplan,
personal communication). Those mice not killed remained symptomless during the experiment. Lung material taken at the peak of virus multiplication was without effect when passaged intranasally and virus titres rapidly declined to undetectable levels.

Attempts to induce mouse neurovirulence and possibly lung infectivity of the Lister Institute strain of vaccinia virus were unsuccessful. Intracerebral passage in suckling mice of increasing age and size (7, 8, 9) yielded virus of $10^8$ to $10^9$ pk.eq./g. wet weight of brain tissue at each passage. However, samples of the 6th, 10th and 15th brain passages in suckling mice which had a high virus content had only minimal neurovirulence and no lung infectivity in adult mice. Neurovirulence was not maintained on subsequent adult passage in different mouse strains and virus was undetectable in the brain after three further intracranial passages.

![Graph](image)

Fig. 1. Growth of vaccinia virus in mice inoculated intranasally. Inoculum $10^8$-5 pk.eq. in 0.05 ml. intranasally. Groups of three mice killed at intervals. Ten % (w/v) suspensions made of lungs, heart blood or brains and titrated for virus. A. Mouse neurovirulent strain wr. Closed symbols unpassaged virus. Open symbols after 11 lung passages; □ = virus in lungs; △ = virus in brain; □ = virus in blood. B. Strain COPENHAGEN, closed symbols; LEVADITI, open symbols. ○ = virus in lungs. No virus was detectable in the blood or brains of these mice.

The experiments suggest that only those strains showing neurovirulence were capable of producing lethal infection by the respiratory route (Table I). The present findings with the strain wr and those of Link et al. (10) with IHD show that virus can be found in the brains of mice inoculated intranasally with these strains. The terminal event may be infection of the central nervous system to which the respiratory tract provides an indirect route of entry as well as a site for virus reproduction. The spread of virus to the central nervous system may be haematogenous since it follows the early viraemia (Fig. 1 A). However, intravenous, subcutaneous and intraperitoneal doses of wr virus, sufficient to produce blood concentrations similar to that of the viraemia, were not lethal (Table 1). It is possible, therefore that infection of the central nervous system could occur directly via the nasal mucosa and cribriform plate. The insuscept-
ability of adult mice to lethal infection by the subcutaneous route permits the direct comparison of the immunogenicity of both live and inactivated vaccines. In experiments to be reported, different strains inactivated by different methods and administered in varying doses were examined. The assay of a variety of circulating antibodies or the modification of skin lesions in rabbits reflects some aspects of the immunological response to such vaccines. Challenge of immunized mice by the respiratory route could provide a direct index of the protection afforded.

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REFERENCES


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