THE INFANT RAT AS A MODEL FOR ASSESSMENT OF
THE ATTENUATION OF HUMAN INFLUENZA VIRUSES

M. I. A. MAHMUD, R. JENNINGS AND C. W. POTTER

Academic Division of Pathology, Department of Virology,
University of Sheffield Medical School, Beech Hill Road,
Sheffield S10 2RX

Inactivated influenza virus vaccines have been reported to be less effective
than live attenuated vaccines in inducing immunity against influenza (Beare
et al., 1968; Freestone et al., 1972). Furthermore, killed, whole influenza
virus vaccines are known to induce adverse reactions in adults (Smith et al.,
1975; Barry et al., 1976; Jennings et al., 1978), and more particularly in
children (Barry et al., 1976; Marine and Stuart-Harris, 1976). These findings
have led several groups of workers to develop live attenuated influenza virus
vaccines acceptable for general use in man (Mills and Chanock, 1971; Huygelen
et al., 1973; Morris et al., 1975). However, much of this work has been ham-
pered by the lack of reproducible and reliable laboratory tests for assessment of
the attenuation of candidate vaccine strains. At present the only method of
measuring attenuation of influenza virus involves the inoculation of volun-
teers, which is potentially dangerous and slow in a situation where speed is
often essential. Several in-vitro and in-vivo tests of attenuation have been
studied (Mills and Chanock, 1971; Mostow and Tyrrell, 1973; Toms et al.,
1976), but have yet to be proved reliable.

Studies from this laboratory (Michaels et al., 1978) have indicated that
the infant rat may be useful for assessment of the virulence, for man, of can-
didate strains of live influenza virus vaccine. These studies are extended in the
present paper to an investigation of other strains of influenza virus; the growth
of these viruses in the turbinates and lungs of infant rats was determined,
together with the ability of virus infection to promote subsequent bacteraemia
and meningitis after intranasal inoculation with Haemophilus influenzae.

MATERIALS AND METHODS

Virus strains

Influenza virus strains A/Swine/1976/30 (Hsw1N1), A/PR/8/34 (HON1), A/England/939/
69 (H3N2), A/Port Chalmers/1/73 (H3N2), A/Victoria/3/75 (H3N2), and A/New Jersey/8/76
(Hsw1N1) were kindly supplied by Dr J. J. Skehel, National Institute for Medical Research,
Mill Hill, London. Strain RIT4058 (Hsw1N1), a recombinant of A/NJ/76 and A/PR/8/34
was kindly supplied by Dr C. Huygelen, Recherche et Industrie Thérapeutiques, Rixensart,
Belgium. Strain NIB3 (Hsw1N1) a recombinant of A/NJ/76 and influenza X31, and 4A2
(H3N2) a double recombinant of A/Victoria/75 and A/PR/8/34, were obtained from Dr
A. S. Beare, Common Cold Research Unit, Salisbury, Wiltshire. Strain X31 is itself a recombinant of strains A/Hong Kong/1/68 and A/PR/8/34 (Couch et al., 1971).

Virus pools were prepared by the allantoic inoculation of 10-day embryonated eggs. After incubation at 33°C for 72 h, the allantoic fluids were collected and stored at -80°C. The egg-infectivity titre (EIDSO) of each virus pool was determined by titration in 10-day embryonated eggs, and calculated by the method of Reed and Muench (1938).

**Animals**

Wistar strain rats, 48 h old, were obtained from a closed, randomly-bred colony at the University of Sheffield in litters of 8–12.

**Inoculation of infant rats**

*Virus.* Serial 10-fold dilutions were made in phosphate-buffered saline (PBS), pH 7.4, containing bovine serum albumin 1.0%, penicillin 100 000 units/litre, and streptomycin 100 mg/litre; from these, 0.01 ml was inoculated intranasally into the rats from a Hamilton syringe fitted to a scalp-vein infusion set, the stylet of which was cut short to avoid trauma at the inoculation site (Michaels et al., 1978). The syringe was held in a rubber-lined clamp on a stand and the end of the infusion tubing was held close to the anterior nares of the rat during the inoculation.

*Haemophilus influenzae.* Rats were given intranasal inoculation with *H. influenzae* type b (HIlb), strain Pekala, in a volume of 0.01 ml, by the technique described above, given 48 h after virus infection unless stated otherwise. The source of HIlb and its preparation for inoculation into infant rats has been described previously (Michaels et al., 1978). At the time of inoculation, a viable count was done on each suspension of HIlb used for inoculation, and the counts were reproducibly $10^{6.0}-10^{7.0}$ viable organisms/0.01 ml.

**Replication of virus in rat tissues**

Groups of five rats were killed at various times after virus inoculation, and the lungs and turbinates were removed. The lungs were pooled, ground with carborundum powder, and a 40% (w/v) suspension prepared in medium 199; the turbinates were treated in a similar manner. The tissue homogenates were centrifuged at 3000 rpm for 15 min. and the supernatant fluids stored at -80°C. Tittrations of pooled lung and tissue homogenates for infectious virus were done by the allantois-on-shell (AOS) method (Fazekas de St Groth, Witchell and Lafferty, 1958), and the 50% egg-bit infectious dose (EBIDSO) calculated (Reed and Muench, 1938). Tittrations of turbinate and lung suspensions from individual baby rats have shown the variation of the titre of these suspensions to be small enough to allow pooling (Michaels et al., 1978).

**Assessment of H. influenzae infection**

Three days after inoculation with HIlb, the rats were decapitated and a blood sample was collected from the free-flowing blood of each infant rat into a capillary tube. Viable bacterial counts were done on each blood sample diluted with PBS, inoculated on chocolate-agar plates, and incubated at 37°C for 18 h. If few colonies were present after incubation, the organisms were identified by the capsular-swelling test with standard HIlb antiserum (Wellcome Laboratories, Beckenham, Kent). To detect meningitis the rats' heads were skinned and fixed in neutral phosphate-buffered 10% formalin for 7–10 days, followed by decalcification with RDO solution for 2 h (RDO, Bethlehem Instruments Ltd, Paradise, Hemel Hempstead, Herts). A coronal section of each entire head at the level of the external ear and a midline sagittal section were prepared; the two blocks from each head were further decalcified for 1–5 h, processed and sectioned. The sections were stained with haematoxylin and eosin and examined histologically for evidence of meningitis (Moxon et al., 1974).
**Results**

*Infection of infant rats with influenza virus A/England/69*

**Dose of virus.** To determine the optimal concentration of virus needed to promote bacteraemia and meningitis after later H1b challenge, groups of rats aged 48 h were inoculated intranasally with varying concentrations of influenza virus A/England/69. Two days later, each animal was inoculated with H1b at a dose of $4 \times 10^6$ organisms per rat. After another three days the rats were killed and the incidence of bacteraemia and meningitis was determined. The results (table I) show that the greater the dose of virus, the greater was the incidence of bacteraemia and meningitis after H1b challenge. All rats receiving $10^{5.0}$ EID50 of A/England/69 virus showed evidence of bacteraemia, and 60% had meningitis after H1b infection, but decrease of virus inoculum resulted in a decreasing incidence of bacteraemia and meningitis after bacterial challenge; at a virus inoculum of $10^{2.0}$ EID50, only two of 16 (12%) rats showed evidence of bacteraemia and meningitis, whilst only one of 12 (8%) non-virus-infected control rats, given bacteria alone at five days of age, had bacteraemia, and none showed evidence of meningitis.

**Dose of H. influenzae.** The effect of varying doses of H1b on the incidence of bacteraemia in five-day-old infant rats, 48 h after infection with $10^{4.0}$ EID50 of influenza virus A/England/69, is shown in table II. The greatest incidence of bacteraemia followed inoculation of the greatest number of bacteria. Thus, after an inoculum of $4 \times 10^7$ or $4 \times 10^6$ H1b, 10 of 11 (91%) pre-infected infant rats developed bacteraemia; only four of 12 (33%) rats developed a systemic infection after the inoculation of $4 \times 10^4$ organisms.

**Age of rats.** To determine the effect of age on the incidence of systemic bacterial infection induced by the inoculation of H1b in infant rats pre-infected with influenza A/England/69 virus, rats of different ages were given inoculations of either PBS or $10^{4.0}$ EID50 of A/England/69 virus; two days later the animals were given $4 \times 10^6$ H1b organisms and the incidence of bacteraemia and meningitis was determined after three days. Table III shows that the incidence of

<p>| Table I |
|---|---|---|
| <em><em>Effect of dosage of influenza virus A/England/69 on the response of infant rats to challenge</em> with H. influenzae</em>* |</p>
<table>
<thead>
<tr>
<th>Dose of A/Eng/69 (EID50)</th>
<th>Number (%) of rats with <em>H. influenzae</em> bacteraemia</th>
<th>meningitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^{5.0}$</td>
<td>10/10 (100)</td>
<td>6/10 (60%)</td>
</tr>
<tr>
<td>$10^{4.0}$</td>
<td>10/12 (83)</td>
<td>6/12 (50%)</td>
</tr>
<tr>
<td>$10^{3.0}$</td>
<td>3/12 (25)</td>
<td>2/12 (17)</td>
</tr>
<tr>
<td>$10^{2.0}$</td>
<td>2/12 (17)</td>
<td>2/12 (17)</td>
</tr>
<tr>
<td>Nil</td>
<td>1/12 (8)</td>
<td>0/12 (0)</td>
</tr>
</tbody>
</table>
|* Dose = $4 \times 10^6$ cells.**
HIb bacteraemia and meningitis induced in pre-infected rats decreased with increasing age. Thus, 83% of rats infected with A/England/69 virus at two days of age suffered bacteraemia on subsequent inoculation with HIb; five of 12 (42%) showed evidence of meningitis. Virus infection at four days and seven days of age followed by bacterial challenge two days later, produced bacteraemia respectively in 40% and 8%, and meningitis in 20% and 0% of the infant rats. Rats receiving PBS before bacterial infection showed minimal incidence of bacteraemia irrespective of age.

**H. influenzae infection in infant rats pre-infected with influenza virus H3N2 strains**

Optimal conditions for the comparison of influenza strains in the infant rat were chosen on the basis of the results set out in tables I, II and III. Thus, influenza viruses were inoculated into rats aged 48 h, at a concentration of $10^{4.0}$ EID50. Two days later the animals were inoculated with approximately $2-4 \times 10^6$ viable cells of HIb. The incidence of bacteraemia and meningitis was assessed 72 h later.

**TABLE II**

Effect of challenge dosage of *H. influenzae* on the development of bacteraemia in infant rats pre-infected* with influenza virus A/England/69

<table>
<thead>
<tr>
<th><em>H. influenzae</em>; number of cells inoculated</th>
<th>Number (%) of rats developing bacteraemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>$4 \times 10^4$</td>
<td>4/12 (33)</td>
</tr>
<tr>
<td>$4 \times 10^5$</td>
<td>7/11 (63)</td>
</tr>
<tr>
<td>$4 \times 10^6$</td>
<td>10/11 (91)</td>
</tr>
<tr>
<td>$4 \times 10^7$</td>
<td>10/11 (91)</td>
</tr>
</tbody>
</table>

* Dose = $10^{4.0}$ EID50.

**TABLE III**

Effect of age of infant rats on the development of bacteraemia and meningitis after inoculation with influenza virus* A/England/69 and *H. influenzae†

<table>
<thead>
<tr>
<th>Age of animals on day of virus inoculation</th>
<th>Rats inoculated with phosphate buffered saline + <em>H. influenzae</em>: number (%) developing</th>
<th>Rats inoculated with A/Eng/69 + <em>H. influenzae</em>: number (%) developing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>bacteraemia</td>
<td>meningitis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 days</td>
<td>1/10 (10)</td>
<td>1/10 (10)</td>
</tr>
<tr>
<td>4 days</td>
<td>0/10 (0)</td>
<td>4/10 (40)</td>
</tr>
<tr>
<td>7 days</td>
<td>1/12 (8)</td>
<td>1/12 (8)</td>
</tr>
</tbody>
</table>

* Dose = $10^{4.0}$ EID50. † Dose = $4 \times 10^6$ cells.
By this procedure, four H3N2 influenza virus strains and influenza virus A/PR/8/34 (HON1) were inoculated into infant rats. These were subsequently challenged with H1b and the incidences of bacteraemia and meningitis were determined. The results (table IV) show that bacteraemia or meningitis was not observed in non-virus-infected rats given H1b infections, but all animals pre-infected with A/England/69 or A/Port Chalmers/73 and 75% of the animals pre-infected with A/Victoria/75 showed evidence of bacteraemia after bacterial challenge. Meningitis after H1b inoculation was detected in 30%, 45%, and 25% of rats pre-infected with A/England/69, A/Port Chalmers/73, and A/Victoria/75 viruses respectively.

Influenza virus strain 4A2 produced significantly less enhancement of H1b bacteraemia than the previous three virus strains. Thus, only three of 11 (27%) rats pre-infected with strain 4A2 showed bacteraemia after H1b challenge, and only one of these animals showed histological evidence of meningitis. In contrast, infection with A/PR/8/34 influenza virus followed by intranasal H1b induced bacteraemia in 100% and meningitis in 92% of the rats.

**H. influenzae infection in infant rats pre-infected with influenza HSw1N1 virus strains**

Table V shows the ability of various HSw1N1 influenza viruses to promote subsequent infection by H1b. None of these strains promoted bacterial infection to the same extent as the H3N2 viruses. Thus, the incidence of bacteraemia ranged from 11 to 23%, and in all experiments only four rats showed evidence of meningitis.

**Influenza virus replication in infant-rat turbinates and lungs**

The growth of various H3N2 and HSw1N1 influenza virus strains and A/PR/8/34 was examined in infant rats infected with 10^{4.0} EID50 of each virus at two days of age. At various intervals the rats were killed and the amount of virus present in 40% (w/v) lung or turbinate homogenates was determined.

### TABLE IV

<table>
<thead>
<tr>
<th><strong>Virus</strong></th>
<th><strong>H. influenzae; number of cells inoculated</strong></th>
<th><strong>Number (%) of rats developing H. influenzae bacteraemia</strong></th>
<th><strong>Number (%) of rats developing meningitis</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>A/England/69</td>
<td>3.4 × 10^6</td>
<td>11/11 (100)</td>
<td>4/11 (36)</td>
</tr>
<tr>
<td>A/Port Chalmers/73</td>
<td>3.4 × 10^6</td>
<td>11/11 (100)</td>
<td>5/11 (45)</td>
</tr>
<tr>
<td>A/Victoria/75</td>
<td>2.0 × 10^6</td>
<td>9/12 (75)</td>
<td>3/12 (25)</td>
</tr>
<tr>
<td>Recombinant 4A2</td>
<td>2.0 × 10^6</td>
<td>3/11 (27)</td>
<td>1/11 (9)</td>
</tr>
<tr>
<td>A/PR/8/34</td>
<td>3.4 × 10^6</td>
<td>12/12 (100)</td>
<td>11/12 (92)</td>
</tr>
<tr>
<td>None</td>
<td>2.0 × 10^6</td>
<td>0/12 (0)</td>
<td>0/12 (0)</td>
</tr>
</tbody>
</table>

* Dose = 10^{4.0} EID50.
TABLE V
Incidence of bacteraemia and meningitis induced by H. influenzae in infant rats pre-infected* with swine or "swine-like" influenza viruses of known human virulence

<table>
<thead>
<tr>
<th>Virus</th>
<th>H. influenzae; number of cells inoculated</th>
<th>Number (%) of rats developing H. influenzae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>bacteraemia</td>
</tr>
<tr>
<td>A/swine/1976/30</td>
<td>2.0 x 10^6</td>
<td>2/13 (15)</td>
</tr>
<tr>
<td>A/New Jersey/8/76</td>
<td>2.0 x 10^6</td>
<td>1/9 (11)</td>
</tr>
<tr>
<td>Recombinant RIT4058</td>
<td>2.0 x 10^6</td>
<td>3/13 (23)</td>
</tr>
<tr>
<td>Recombinant NIB3</td>
<td>2.0 x 10^6</td>
<td>2/14 (14)</td>
</tr>
<tr>
<td>None</td>
<td>2.0 x 10^6</td>
<td>0/12 (0)</td>
</tr>
</tbody>
</table>

* Dose = 10^4.0 EID50.

Fig. 1 shows the replication of the H3N2 strains and A/PR/8/34 viruses in infant rat turbinates and lungs. It shows that A/England/69, A/Port Chalmers/73 and A/Victoria/75 influenza viruses have similar patterns of replication in rat turbinates. Peak virus concentrations of > 10^5.0 EBID50/0.05 ml were observed one or two days after infection, but decreased sharply thereafter. A similar pattern was observed for A/PR/8/34 in infant-rat turbinates; the amount of virus recovered was maximal, 10^4.25 EBID50/0.05 ml, at 24 h and declined thereafter. Influenza virus strain 4A2 grew more slowly in turbinates but otherwise showed a growth pattern similar to those of the other H3N2 strains, although the peak concentration at 48 h was only 10^3.5 EBID50/0.05 ml.

In the lungs, viruses replicated more slowly and peak concentrations were usually lower than in the turbinates for all strains except A/PR/8/34. The peak concentrations for the H3N2 strains ranged from 10^2.8 to 10^3.5 EBID50/0.05 ml at 48 h. They declined at the same rate as in the turbinates. Influenza virus A/PR/8/34, however, showed an initial peak concentration of 10^4.3 EBID50/0.05 ml at three days and a second lower peak of 10^3.0 EBID50/0.05 ml at five days after infection; this pattern of growth was distinctive.

Fig. 2 shows the growth patterns of influenza HSw1N1 viruses in infant-rat turbinates and lungs. The growth curves of these viruses were different from those of the H3N2 strains. Virus replication in the turbinates was low throughout the experiments and rarely rose above 10^1.5 EBID50/0.05 ml. However, strain RIT4058 showed a concentration of 10^3.0 EBID50/0.05 ml at 24 h, but replication had decreased sharply by 48 h. Concentrations of 10^2.6 EBID50/0.05 ml were found at 24 and 48 h in the turbinates of rats infected with A/Swine/30 influenza virus, but were lower thereafter. For strains A/NJ/76 and NIB3, the peak concentrations were 10^2.0 and 10^1.5 EBID50/0.05 ml, respectively (fig. 2).

Replication of HSw1N1 influenza viruses in the lungs of infant rats was also different from that of the H3N2 viruses (fig. 1). For A/NJ/76, virus concentrations were <10^1.0 EBID 50/0.05 ml for four days after infection, but rose sharply on day five, to 10^3.0 EBID50/0.05 ml and remained high until the end of the
INFLUENZA VIRUS IN THE INFANT RAT

Fig. 1.—Replication of H3N2 influenza viruses in infant-rat lung (○—○) and turbinate (●—●) tissue: (a) strain A/England/939/69; (b) recombinant strain 4A2; (c) strain A/Port Chalmers/1/73; (d) strain A/PR/8/34; (e) strain A/Victoria/3/75.

experiment. Virus replication of NIB3 in the lung also occurred late, with a peak of 10^{3.5} EBID50/0.05 ml at four days.

Influenza viruses RIT4058 and A/Swine/30 also grew well in infant-rat lungs, but, unlike A/NJ/76 and NIB3, they reached relatively high concentrations soon after infection. Thus, at 24 h, concentrations of 10^{4.0} and 10^{2.6} EBID50/0.05 ml were found in suspensions of lungs from rats given RIT4058 or A/Swine/30, respectively. The concentrations remained at 10^{2.3} to 10^{3.7} EBID50/0.05 ml until six days after infection.
Fig. 2.—Replication of HSsw1N1 influenza viruses in infant-rat lung (○-○) and turbinate (●-●) tissue: (a) strain RIT4058; (b) strain A/Swine/30; (c) strain A/New Jersey/8/76; (d) recombinant strain NIB3.

Table VI

Virulence of influenza virus strains for man, their growth in infant-rat tissues, and their ability to potentiate H. influenzae infection

<table>
<thead>
<tr>
<th>Virus strain</th>
<th>Virulence for man</th>
<th>Peak virus concentration (EBID50 per 0.05 ml) 48 h after infection in turbinate homogenate</th>
<th>Percentage of rats developing H. influenzae homogenate lung homogenate bacteraemia meningitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/England/69</td>
<td>Virulent</td>
<td>$10^{5.7}$</td>
<td>100</td>
</tr>
<tr>
<td>A/Port Chalmers/73</td>
<td>Virulent</td>
<td>$10^{4.8}$</td>
<td>100</td>
</tr>
<tr>
<td>A/Victoria/75</td>
<td>Virulent</td>
<td>$10^{6.3}$</td>
<td>75</td>
</tr>
<tr>
<td>A/New Jersey/76</td>
<td>Attenuated</td>
<td>$&lt;10^{1.9}$</td>
<td>11</td>
</tr>
<tr>
<td>A/PR/8/34</td>
<td>Non-infectious</td>
<td>$10^{3.3}$</td>
<td>100</td>
</tr>
<tr>
<td>A/swine/30</td>
<td>Attenuated</td>
<td>$10^{2.5}$</td>
<td>15</td>
</tr>
<tr>
<td>Recombinant 4A2</td>
<td>Attenuated</td>
<td>$10^{3.5}$</td>
<td>27</td>
</tr>
<tr>
<td>Recombinant NIB3</td>
<td>Attenuated</td>
<td>$10^{1.5}$</td>
<td>14</td>
</tr>
<tr>
<td>Recombinant RIT4058</td>
<td>Attenuated</td>
<td>$10^{4.5}$</td>
<td>23</td>
</tr>
</tbody>
</table>
Correlation of virus virulence for man and for rats

The virulence for man of the influenza viruses used in the present study are known. The H3N2 strains tested, A/England/69, A/Port Chalmers/73 and A/Victoria/75, are isolates from cases of natural influenza; strain A/PR/8/34 is non-infectious for man (Beare and Hall, 1971), and the virulence for man of strains 4A2, NIB3, A/NJ/76, A/Swine/30 and RIT4058 has been established in volunteer studies. The virulence for man of these strains, their ability to grow in rat turbinates and lungs, and to promote bacteraemia and meningitis after subsequent H1b challenge are shown in table VI.

DISCUSSION

A practical laboratory test would be of great value in the development of live, attenuated influenza virus vaccines.

We have studied the behaviour of several influenza viruses, of varying virulence for man, in infant rats. This model was first used in a study of the pathogenesis of H. influenzae meningitis (Moxon et al., 1974) which can be potentiated by prior influenza virus infection (Michaels, Myerowitz and Klaw, 1977). Earlier studies from this laboratory showed good correlation between the growth of four strains of influenza in infant rat turbinates, the ability of virus infection to enhance subsequent H. influenzae infection, and virulence for man (Michaels et al., 1978); these results have been extended in the present study. Thus, influenza viruses A/England/69, A/Port Chalmers/73 and A/Victoria/75 are virulent for man (Mostow and Tyrrell, 1973; Beare, Schild and Craig, 1975; Florent et al., 1977), replicate to high concentrations in rat turbinates, and considerably enhance subsequent H1b infection. In contrast, influenza virus strains A/Swine/1976/30, A/NJ/76, 4A2, RIT4058 and NIB3 are partly or completely attenuated for man (Beare et al., 1975; Beare and Craig, 1976), replicate relatively poorly in infant-rat turbinates and do not promote systemic infection by H. influenzae to the same extent as the above virus strains. There was less difference between the attenuated and virulent viruses in their ability to replicate in rat-lung tissue; however, all strains attenuated for man, including A/PR/8/34, showed either equal or greater growth in the lungs than in the turbinates. For the virulent strains, growth in the infant-rat lung was significantly less than in the turbinates. In addition, several of the attenuated virus strains showed a phase of late replication in the infant-rat lung.

The ability of HSw1N1 influenza viruses to grow well in the lower respiratory tract has been noted in ferrets (Toms, Sweet and Smith, 1977), and it was suggested that these strains may be pneumotropic. Because of this it is not possible to say whether the ability to replicate in lung tissue shown by the HSw1N1 viruses is a feature of viruses with these surface antigens or is a property of viruses attenuated for man. However, the good growth in infant rat lungs relative to turbinates observed for strains 4A2 and A/PR/8/34 in the present study favours the latter hypothesis.

Influenza virus A/PR/8/34 is non-infectious for man (Beare and Hall, 1971); however, the growth pattern of this virus in rat tissue, and its ability
to promote systemic \textit{H. influenzae} infection resembled that of the virulent influenza viruses tested. In addition, A/PR/8/34 produces a severe disease in ferrets (McLaren, Potter and Jennings, 1974), animals also studied as experimental models for determining the virulence for man of strains of influenza virus (Toms \textit{et al.}, 1976; Fenton, Jennings and Potter, 1977). The extensive laboratory manipulation that the A/PR/8/34 virus has received since its isolation (Massaab, Kendal and Davenport, 1972) may have contributed to its untypical behaviour. Influenza A/PR/8/34 virus was chosen as a parent for the production of recombinant vaccine viruses 4A2, NIB3 and RIT4058, because it is non-infectious for man and could provide the recombinant strain with this property. However, neither A/PR/8/34 nor A/Victoria/75 viruses are attenuated for the infant rat, but the recombinant strain derived from them, 4A2, is attenuated. Thus, the property of attenuation is more complex than the selection of strains for vaccine production implies.

The replication of influenza viruses in infant-rat turbinates and their ability to enhance H\textit{I}b bacteraemia and meningitis are reproducible, and may be useful for assessment of the degree of attenuation of candidate live virus vaccine strains. Of the total of 13 strains examined in this study and the previous one (Michaels \textit{et al.}, 1978), only A/PR/8/34 did not behave as expected. However, more virus strains should be tested in infant rats before the value of this animal for estimating the virulence of influenza viruses for man can be confirmed.

\textbf{Summary}

The intranasal infection of infant rats with \textit{Haemophilus influenzae} type b can be considerably enhanced by prior infection of the rats with influenza virus. When influenza virus A/England/939/69 was used to infect the animals a minimum of $10^{4.0}\ \text{EID}_{50}$ was required to enhance \textit{H. influenzae} infection; infection with $4 \times 10^{6} \ \text{H. influenzae}$ bacteria was needed to reveal this enhancement and infant rats two days old at the time of virus inoculation had to be used. By this method, nine strains of influenza virus were assessed for their ability to enhance \textit{H. influenzae} infection, and the results were compared with their known virulence for man. The results showed a close correlation in this respect for all of the viruses, except strain A/PR/8/34.

The replication of these viruses in infant-rat turbinates and lungs was also studied; virus concentrations in turbinate tissues 48 h after infection showed a close correlation with virulence for man. Thus, three influenza virus strains known to be virulent for man reached concentrations in infant-rat turbinates ranging from $10^{4.8}$ to $10^{5.7}\ \text{EBID}_{50}/0.05 \text{ ml}$ at 48 h; the concentrations of six viruses known to be attenuated or non-infectious for man grew less well in infant rat turbinates, and reached concentrations at 48 h of $10^{1.0}$ to $10^{3.5}\ \text{EBID}_{50}/0.05 \text{ ml}$.

The results are discussed in relation to the use of the infant-rat model for assessment of the attenuation of candidate live influenza virus vaccine strains.

The authors wish to thank Dr C. Huygelen and Dr A. S. Beare for supplying the recombinant viruses, and would also like to acknowledge the excellent technical assistance of Mr Andrew Platts.
REFERENCES


