Differential Distribution of Virus and Histological Damage in the Lower Respiratory Tract of Ferrets Infected with Influenza Viruses of Differing Virulence

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SUMMARY

The distribution of four strains of influenza virus [A/PR/8/34 (H0N1) and clone 64d (attenuated for ferrets) and clones 64c and 7a (virulent for ferrets) of the recombinant virus A/PR/8/34-A/England/939/69 (H3N2)] in the lower respiratory tract (trachea, bronchi and the hilar, intermediate and outer alveolar zones of the lung) of ferrets was monitored daily for 4 days after intranasal inoculation. On day 1, some animals had high virus titres in all the tissues but in other animals virus was undetectable, irrespective of the virus strain. Two days after inoculation increase of virus contents of all tissues tended to be restricted. On days 3 and 4, the virulent clones (64c and 7a), in contrast to the attenuated strains (A/PR/8/34 and clone 64d), consistently infected the lower respiratory tissues. However, for all infected animals the virus contents of the hilar zones of the lungs were higher than those in the intermediate zones, while the alveolar zones were relatively free from virus. Quantitative estimations of the mild histological damage occurring in the lower respiratory tract 3 to 6 days after inoculation also indicated that bronchial and bronchiolar tissue were more susceptible to influenza virus than alveolar tissue and that clones 64c and 7a produced more damage than the other two strains. In agreement with the relative viral contents of clones 64c and 7a in the bronchi and in the hilar and intermediate zones of the lung, clone 64c produced more damage than clone 7a in the bronchi and less in the bronchioles of the lung parenchyma.

INTRODUCTION

Human influenza is primarily an infection of the upper respiratory tract but tracheobronchitis can occur (Stuart-Harris, 1965). Although influenza pneumonia is rare (Mulder & Hers, 1972; Stuart-Harris, 1965) tests of lung function show that there is a general alteration in many aspects of pulmonary function during influenza infection (Camner et al., 1973; Douglas, 1975; Horner & Gray, 1973; Johanson et al., 1969; Kennedy et al., 1965; Little et al., 1979; Picken et al., 1972) even when the lungs appear normal by clinical and X-ray examination (Douglas, 1975). These studies suggest that subclinical influenza involvement of the lower respiratory tract is prevalent and that the severity of disease may well be determined by the extent of involvement of these areas, especially in the young and old.

In an attempt to establish the viral and host factors which determine the extent of infection in the lower respiratory tract, ferret influenza has been used as a model for the human disease. As in humans, influenza in the ferret is mainly an upper respiratory tract infection

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(Stuart-Harris, 1965; Sweet & Smith, 1980) but tracheitis, bronchitis, bronchiolitis and a rare pneumonitis have been reported (Liu, 1955; Mulder & Hers, 1972; Shope, 1934; Stuart-Harris, 1965). Observations on members of two series of recombinants (Campbell et al., 1979; Matsuyama et al., 1980; Toms et al., 1976, 1977a) and the A/New Jersey/8/76 (Hsw1N1) influenza virus (Toms et al., 1977b) showed that the extent of lung infection as judged by the recovery of infectious virus from macerates of whole lung varied with the strain of virus. Thus, for the recombinant virus system A/PR/8/34–A/England/939/69 (H3N2), the virulent clones 64c and 6 produced peak infections higher than those of the virulent clone 7a and the virulent parent A/England/939/69 (H3N2). An attenuated clone 64d and the attenuated parent A/PR/8/34 (H0N1) produced little lung infection even though with the latter virus the nasal infection was high, if not higher, than that formed by the virulent clones (Toms et al., 1976, 1977a; Matsuyama et al., 1980). However, because the whole lung macerates used included portions of major bronchi and their hilar subdivisions, the capacities of different strains to attack anatomical and histological areas within the lower respiratory tract were not tested in a differential manner.

In this study, we report the amounts of infectious virus found in different subdivisions of the ferret respiratory tract and a quantitative assessment of the associated damage revealed by histology. Two virulent (clones 7a and 64c) and two attenuated (clone 64d and A/PR/8/34) strains of influenza virus have been used for these comparisons. It should be emphasized that the terms ‘virulent’ and ‘attenuated’ are used here with respect to the viruses’ capacity to produce disease in ferrets. However, for all strains, except clone 64c, the terms also apply to their virulence for man (Matsuyama et al., 1980).

METHODS

Influenza viruses. Clones 7a, 64c and 64d of the recombinant influenza virus A/PR/8/34–A/England/939/69 (H3N2) and the parent virus A/PR/8/34 (H0N1) (A/PR/8) were described previously (Matsuyama et al., 1980; Sweet et al., 1974a) together with the preparation of seed and working stocks.

Infectivity assays. Infectivity assays in eggs (50% egg infectious dose, EID₅₀) were performed as previously described (Sweet et al., 1974b).

Intranasal inoculation of ferrets. This was performed as described by Toms et al. (1976).

Measurement of virus infectivity in macerates of nasal turbinates, trachea, bronchi and subregions of the lung. Taking care to prevent contamination, the nasal turbinates were removed from freshly killed ferrets and stored at −70 °C. The lung, trachea and heart were removed en bloc and placed in a Petri dish.

The lung and trachea were orientated in the Petri dish with the heart uppermost and then the heart and all associated connective and adipose tissue were removed. The trachea was cut off about 1 cm above its bifurcation into the major bronchi and then the bronchi were removed at their entry to the lung lobes (Fig. 1). The two major lobes of both the right and left lung were numbered 1 to 4 anti-clockwise starting from the right upper lobe and each lobe was divided into three subregions (Fig. 1). The hilar zone contained the lobar bronchi, blood vessels, lymph glands and connective tissue with a minimum of parenchyma; the intermediate zone contained mainly bronchi and non-respiratory bronchioles between which lie packed lobules of parenchyma; and the cortical or alveolar zone consisted predominantly of parenchyma (Herrnheiser & Hinson, 1954). Each sample was stored at −70 °C.

After thawing, all samples were macerated in a Sorvall omnimixer in 3 ml Hanks’ balanced salt solution (Wellcome Reagents) containing 1% (w/v) bovine serum albumin, 0.044% (v/v) sodium bicarbonate, 100 units/ml penicillin, 100 μg/ml streptomycin and 120 μg/ml chloramphenicol. The cups of the omnimixer were washed with 1 ml Hanks’ solution, the fluids pooled and centrifuged at 1400 g for 10 min at 4 °C. The supernatants were titrated in
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Fig. 1. Diagram of the regions of the middle and lower respiratory tract that were examined separately for virus content; lung lobe 2 (lower lobe of the right lung) is shown.

10- to 12-day-old hens' eggs. The results were recorded as the total amount of virus in the tissue piece, i.e. the virus titre/ml of homogenate supernatant multiplied by the volume of the latter.

Quantitative histopathology. The lungs, trachea and heart were dissected en bloc and inflated under a pressure of 25 cm H₂O with 10% formal saline fixative containing 3% sucrose. The upper end of the trachea was clamped and the organs placed in sucrose–formal saline for 48 h. After fixation, each lobe from the right and left lung was sectioned sagitally into slices 3 mm in thickness, dehydrated in alcohol, cleaned and embedded flat in Paraplast (Lancer, St. Louis, Mo., U.S.A.). Blocks from the right and left main extrapulmonary bronchi were similarly sampled and processed. An average of five blocks was obtained from each major lung lobe and a single block included the whole of each main bronchus. Sections to include the whole cut surface of each block were cut at 5 μm and stained with haematoxylin and eosin. In preliminary studies blocks were step-sectioned at intervals of 25 μm to determine whether the distribution of histological lesions was adequately sampled by the above method.

Because unpublished observations on animals infected with the A/New Jersey/8/76 (Hsw1N1) virus indicated that only microscopic lesions could be expected, a modification to the point counting method for assessing area and volume proportions in lungs described by Dunnill (1962) was adopted. Sections were projected at a constant magnification using a Leitz projecting microscope on to a screen ruled out in a grid of 2 cm squares so as to produce points 2 cm apart. The number of points overlying areas of acute inflammation [defined as polymorphonuclear (PMN) leucocyte infiltration] were noted and expressed as a percentage of the total number of points counted. The whole of each section was covered by this method. A total of approx. 7000 points were counted in all the lobes of the lung for each animal and approx. 500 points were counted in each bronchus from each animal. In sections
of lung, grid points overlying bronchi (defined as airways containing mural cartilage) were not included in the counts.

RESULTS

Groups of ferrets were inoculated intranasally with $10^5$ 50% ferret infectious doses (FID$_{50}$) (Toms et al., 1976; Matsuyama et al., 1980) of each virus (A/PR/8, clones 64d, 64c and 7a) and three animals were examined for virus in their respiratory tissues at daily intervals for 4 days. Additional groups of ferrets were inoculated as above; three animals were killed 2, 3, 4, 5, 6 and 8 days post-infection and the lower respiratory tract was examined for histopathological changes.

Virus contents of respiratory tissues

One day after intranasal inoculation the nasal turbinates of all animals were heavily infected with each of the four strains (Fig. 2). Some animals (one infected with A/PR/8, one with clone 64c and two with clone 7a) showed virus, often at high level, in all three areas of most lung lobes (Fig. 2). Other ferrets (one for each virus) showed no evidence of virus in any lung lobe and isolations were made only occasionally from the lung lobes of the remaining animals. Thus, at this early stage of infection, the main differences in virus contents of the
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Fig. 3. Total titres of virus given as EID₅₀ at day 2 post-infection for (a) A/PR/8/34, (b) clone 64d, (c) clone 64c and (d) clone 7a in nasal turbinates (NT), trachea (TR), bronchi (BR), and in hilar (L1), intermediate (L2) and alveolar (L3) zones of lung lobes L1, L2, L3 and L4. Three animals (1, 2, 3) were used for each virus. Each histogram represents the titre of virus in an individual tissue or subregion.

lung lobes seemed to be between individual ferrets rather than between the strains of virus (Fig. 2). However, in previous unpublished comparisons of virus contents of the whole lung (i.e. all three areas of all four lobes) five out of 15 ferrets infected with clone 7a contained \( >10^5 \) EID₅₀ of virus in their lungs compared with none of nine ferrets infected with clone 64d, suggesting that clone 7a may have an increased capacity for reaching the lung early in infection.

Two days after inoculation, the virus titres in nasal turbinate macerates were similar to those on day 1 for A/PR/8 and decreased, markedly for clone 64d and slightly for clones 64c and 7a (Fig. 3). Little virus was found in the trachea and bronchi of animals inoculated with A/PR/8 and clone 64d, but titres were moderately high in a few animals inoculated with clones 64c and 7a (Fig. 3). The high levels of virus found in some lung lobes of some animals on day 1 (Fig. 2; nine lobes had virus titres \( >10^5 \) EID₅₀ in at least one lobe of the three zones) were not evident on day 2 (Fig. 3; only two lobes had virus titres \( >10^5 \) EID₅₀). A/PR/8 was present in the subregions of many lung lobes (Fig. 3a) but at barely detectable levels. Clone 64d was rarely detected (Fig. 3b). Clone 64c was sporadically isolated from the hilar and intermediate zones of some lobes (Fig. 3c). Only animals infected with clone 7a consistently showed significant levels of virus in most lung lobes (Fig. 3d).

Three days after inoculation, nasal turbinate titres remained relatively high for A/PR/8 and at the reduced level of day 2 for clone 64d, but those for clones 64c and 7a had declined...
Fig. 4. Total titres of virus given as EID₅₀ at day 3 post-infection for (a) A/PR/8/34, (b) clone 64d, (c) clone 64c and (d) clone 7a in nasal turbinates (NT), trachea (TR), bronchi (BR), and in hilar (†), intermediate (●) and alveolar (□) zones of lung lobes L1, L2, L3 and L4. Three animals (1, 2, 3) were used for each virus. Each histogram represents the titre of virus in an individual tissue or subregion.

(Fig. 4). Only one of three animals infected with A/PR/8 or clone 64d showed appreciable levels of virus in the trachea and bronchi correlating with significant titres in the lung (Fig. 4a, b). In contrast, much virus was found in the trachea and bronchi of all animals infected with clones 64c and 7a (Fig. 4c, d). Of the eight animals having virus in the trachea and bronchi (Fig. 4a to d), seven had bronchial titres greater than or equal to those in the trachea.

Only single animals infected with A/PR/8 or clone 64d had appreciable titres of virus in their lung lobes (Fig. 4a, b), whereas clones 64c and 7a were found in the lungs of all animals (Fig. 4c, d). However, for all infected animals the titres in the hilar zones were greater than those in the intermediate zones, which in turn were greater than those in the alveolar zones; thus, for 24 lung lobes containing greater than 10³ EID₅₀ of virus in at least one zone (Fig. 4), the titres in the hilar zones were greater than those in the intermediate and alveolar zones in 17 and 22 respectively and, in 20 lobes, the titres in the intermediate zones were greater than those in the alveolar zones. Isolations of clone 64c from the lung lobes were more sporadic than for clone 7a and only four out of 12 hilar zones contained greater than 10⁵ EID₅₀ of clone 64c compared with 10 out of 12 zones for clone 7a; corresponding figures for the intermediate zones were 4 and 10 respectively for clones 64c and 7a. In contrast, all three animals infected with clone 64c had bronchial titres greater than 10⁶ EID₅₀ compared with one animal infected with clone 7a (Fig. 4).
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Fig. 5. Total titres of virus given as EID$_{50}$ at day 4 post-infection for (a) A/PR/8/34, (b) clone 64d, (c) clone 64c and (d) clone 7a in nasal turbinates (NT), trachea (TR), bronchi (BR), and in hilar (■), intermediate (□) and alveolar (□□) zones of lung lobes L1, L2, L3 and L4. Three animals (1, 2, 3) were used for each virus. Each histogram represents the titre of virus in an individual tissue or subregion.

Four days after inoculation, the pattern was similar to that on day 3 but the levels of virus were generally lower (Fig. 5). The important findings were: (i) of 10 animals having virus in the trachea and bronchi nine had bronchial titres greater than those in the trachea; (ii) the amounts of clone 64c and 7a in the lower respiratory tract were larger than those of the other two strains; (iii) of the 26 lung lobes having greater than $10^3$ EID$_{50}$ of virus in at least one zone, the titres for the hilar zones were greater than those for the intermediate and alveolar zones in 20 and 23 lobes respectively and, in 19 lobes, the titres in the intermediate zones were greater than those in the alveolar zones; (iv) five out of 12 hilar zones of animals infected with clone 64c contained more than $10^5$ EID$_{50}$ of virus compared with eight out of 12 hilar zones infected with clone 7a; and (v) in two animals infected with clone 64c, the titres in the bronchi were greater than $10^6$ EID$_{50}$ compared with one animal infected with clone 7a (Fig. 5).

Quantitative assessment of histological damage in the lower respiratory tract

The histopathological effects of intranasal inoculation were similar to classical descriptions of ferret influenza using ferret-adapted influenza strains (Stuart-Harris, 1965): namely, necrosis and degeneration of bronchial and bronchiolar columnar epithelium with focal ulceration and a PMN leucocyte infiltrate associated with oedema; alveolar inflammation, when it occurred, was centred on bronchiolar lesions. In contrast to lesions induced with
Table 1. Quantitative assessment of bronchial inflammation in ferrets 3 to 6 days following infection with influenza viruses

<table>
<thead>
<tr>
<th>Day after inoculation</th>
<th>Ferret no.</th>
<th>A/PR/8</th>
<th>Clone 64d</th>
<th>Clone 64c</th>
<th>Clone 7a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>L</td>
<td>R</td>
<td>L</td>
<td>R</td>
</tr>
<tr>
<td>3</td>
<td>ND†</td>
<td>ND</td>
<td>0</td>
<td>0</td>
<td>25(2-1)</td>
</tr>
<tr>
<td>2</td>
<td>ND</td>
<td>ND</td>
<td>0</td>
<td>0</td>
<td>10.5(1-9)</td>
</tr>
<tr>
<td>3</td>
<td>ND</td>
<td>ND</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>ND</td>
<td>0</td>
<td>0</td>
<td>6(1-1)</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>9.2(1-2)</td>
</tr>
<tr>
<td>2</td>
<td>2.4(0-7)</td>
<td>9.1(1-3)</td>
<td>0</td>
<td>0</td>
<td>22.2(1-8)</td>
</tr>
<tr>
<td>3</td>
<td>9.9(1-2)</td>
<td>8.1(1-3)</td>
<td>0</td>
<td>0</td>
<td>24.3(2-7)</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>15.2(1-9)</td>
</tr>
<tr>
<td>2</td>
<td>3.6(0-9)</td>
<td>3.4(0-8)</td>
<td>0</td>
<td>0</td>
<td>6.3(1-3)</td>
</tr>
</tbody>
</table>

* Assessed as described in Methods; standard errors in parentheses.
† ND, Not done.

ferret-adapted virus strains, the lesions encountered in this study were mild in all animals. Histopathological evidence of damage post-dated rises in the virus contents of the lower respiratory tract by approx. 24 h. Thus, changes were first noted on day 3 after virus inoculation and rose to a peak on day 5 and thereafter declined. Epithelial regeneration started on day 6 and increased by day 8. Bronchial and parenchymal inflammation were qualitatively most evident in animals infected with clones 7a and 64c and almost non-existent in animals infected with clone 64d. Strain A/PR/8 appeared intermediate in this respect. Acute inflammation and necrosis of mural bronchial glands was a prominent feature in many animals.

Quantitative comparison of the acute inflammatory damage induced by viruses was hampered by two factors: (i) the minor degree of damage; and (ii) the extremely focal nature of the changes. In many animals only one lobe or one main bronchus showed any evidence of damage. Counts for individual lobes of each lung were therefore amalgamated in order to minimize standard errors, and expressed as a percentage for the whole lung (right and left). Counts on bronchi were not amalgamated. No attempt was made to express counts in terms of volume proportions of affected lung (Dunnill, 1962) as the standard errors of the percentages were relatively high in most cases (Tables 1 and 2) and measurements of lung volumes would have introduced further errors.

Despite these difficulties, the quantitative results (Tables 1 and 2) confirmed the qualitative observations noted above. Using non-parametric testing, because normal distributions cannot be assumed, the differences between viruses observed on days 3 and 4 were not significant in either the bronchi or parenchyma. However, on days 5 and 6 the differences were significant. In the bronchi, inflammation was significantly greater with clone 64c than with clones 7a (P < 0.05), 64d (P < 0.01) and A/PR/8 (P < 0.01). There was no significant difference between the inflammation induced by clone 7a and A/PR/8; both produced greater bronchial inflammation than clone 64d (P < 0.01 and P < 0.05 respectively). In the parenchyma the relative damage induced by clones 64c and 7a was reversed. Thus, parenchymal inflammation (predominantly bronchiolar) was significantly greater with clone 7a than clone 64c (P < 0.05), clone 64d (P < 0.01) and A/PR/8 (P < 0.01). Also, parenchymal inflammation with clone 64c was significantly greater than with clone 64d (P < 0.05) and...
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Table 2. **Quantitative assessment of parenchymal inflammation in lungs of ferrets 3 to 6 days following infection with influenza viruses**

<table>
<thead>
<tr>
<th>Day after inoculation</th>
<th>Ferret no.</th>
<th>A/PR/8</th>
<th>Clone 64d</th>
<th>Clone 64c</th>
<th>Clone 7a</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>1</td>
<td>ND</td>
<td>0.27(0.06)</td>
<td>0.06(0.03)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>ND</td>
<td>0.3(0.08)</td>
<td>0.97(0.12)</td>
<td>0.04(0.04)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>ND</td>
<td>ND</td>
<td>0</td>
<td>0.03(0.03)</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0.09(0.09)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0</td>
<td>0.06(0.08)</td>
<td>0.08(0.08)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.02(0.01)</td>
<td>0.46(0.08)</td>
<td>0.16(0.16)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>0.02(0.02)</td>
<td>0.7(0.09)</td>
<td>2.43(0.25)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.03(0.01)</td>
<td>0.2(0.06)</td>
<td>1.4(0.14)</td>
<td>0.3(0.09)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.04(0.02)</td>
<td>0.02(0.02)</td>
<td>0.46(0.08)</td>
<td>1.59(0.22)</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>0.01(0.01)</td>
<td>0.64(0.07)</td>
<td>2.66(0.06)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0</td>
<td>0.02(0.02)</td>
<td>0.08(0.02)</td>
<td>3.81(0.06)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0</td>
<td>0.06(0.03)</td>
<td>0.05(0.02)</td>
<td>1.96(0.25)</td>
</tr>
</tbody>
</table>

*Assessed as described in Methods. The table includes bronchioles where the main inflammatory changes occurred and the focal alveolar changes around the bronchioles, but the bronchi were excluded. Standard errors are in parentheses.

† ND, Not done.

A/PR/8 (P < 0.01) but there was no significant difference between clone 64d and A/PR/8.

Results from day 2 and day 8 are not shown in the tables and were not analysed as there was no acute inflammatory reaction in tissues on these days.

**DISCUSSION**

In contrast to previous studies where infection was assessed at only two sites (i.e. in nasal washings and in homogenates of whole lung) and numerous ferrets were examined (Toms et al., 1976, 1977a; Matsuyama et al., 1980), the present work concentrated on gaining more precise information about virus distribution and histological damage in the respiratory tract and necessarily (there were 15 virus estimations on tissues from each animal) only relatively few animals were surveyed. Nevertheless, the results were sufficiently consistent for valid conclusions to be drawn, particularly the fact that, in the lower respiratory tract, more virus and histological damage was found in the bronchi and in those regions of the lung (hilar and intermediate zones) where ciliated epithelium was present than in the alveolar region.

The presence of virus in homogenates of any part of the respiratory tract does not necessarily mean that virus replication has occurred there because virus can be moved by mucociliary action or drainage. However, the fact that histological evidence of acute inflammation and necrosis was seen in the sites where most virus was found suggests that replication was in fact occurring there.

The titres of all four strains in homogenates of nasal turbinates confirmed the patterns of nasal infection that were indicated by virus contents of nasal washings. The reasons for these patterns are known, namely, the restrictive influence 2 days after inoculation of inflammatory phagocytes, non-specific inhibitors and interferon on all virus strains coupled with the differential effect of fever on their replication (Husseini et al., 1981; Sweet et al., 1977, 1978; Toms et al., 1976, 1977a).

With regard to the lower respiratory tract, high levels of virus were found 1 day after inoculation, irrespective of strain, in all parts of the lower tracts of some animals, although it was virtually absent from these sites in others. This suggested that the efficiency of mucociliary clearance differed in individual ferrets and, in some cases, could not correct...
‘spillover’ of virus from the high levels in the upper respiratory tract. However, present and previous experiments indicated that, with clone 7a at least, replication may have occurred in the lower respiratory tract during the first day of infection.

Two days after inoculation, when restrictive influences curbed nasal infection (see above), the high levels of virus found after 1 day in some lung zones of a few animals were not seen (Fig. 2 and 3) and only in the case of clone 7a were there signs of consistent infection and then at a moderate level (Fig. 3). If Fig. 2 and 3 are fairly representative of the positions on days 1 and 2, bearing in mind the small number of ferrets involved, it appears that similar restrictive influences operate on the second day in the lower respiratory tract to hinder replication or inactivate some virus. Of the probable restrictive factors in the lung, such as alveolar macrophages, interferon and fever, only the latter has been studied and then only for alveolar tissue (Matsuyama et al., 1980; Sweet et al., 1978). Infection of organ cultures of alveolar tissue at normal and pyrexial temperatures indicated that fever, which occurs from about 1.5 to 2.5 days post-inoculation for all strains, could restrict their replication in the alveoli at that time. However, the effect of pyrexial temperatures on replication in bronchial and bronchiolar tissue in organ culture has not yet been investigated.

Three and 4 days after inoculation, the distribution of virus in the zones of lung-infected animals suggested that, irrespective of strain, the epithelium of the bronchi and bronchioles was more easily and heavily infected than the cells of the alveolar zone. This is supported by previous studies using fluorescent antibody in which replication in the ciliated epithelial cells of the bronchi and bronchioles was indicated by cytoplasmic and nuclear fluorescence and desquamation of some fluorescing bronchiolar cells (Liu, 1955). In the alveolar region fluorescence was noted only in alveolar macrophages (Liu, 1955) although, in some studies with ferret-adapted strains, fluorescence occurred in alveolar cells (Mulder & Hers, 1972; Stuart-Harris, 1965) indicating that, as in organ cultures (Cavanagh et al., 1979; Kingsman et al., 1977), these cells will support some replication. The infection pattern was further supported by the fact that histological damage in alveoli appeared to be secondary to damage in adjacent ciliated epithelium in bronchioles and bronchi and involved a greater relative percentage area in bronchi than parenchyma. The reasons for the differential infection of the lower respiratory tissues are unknown. Alveolar cells can support virus replication both in organ cultures and in vivo (see above) so the relative lack of alveolar involvement might be due to destruction of virus or virus-infected cells by alveolar macrophages or other host defence mechanisms. The strong adherence of influenza virus to cilia (Dourmashkin & Tyrrell, 1970; Gould et al., 1972) may promote infection of bronchial and bronchiolar tissue in vivo. Also, the ciliated epithelial cells of the bronchi and bronchioles may, like those of the nasal mucosa (Cavanagh et al., 1979; Kingsman et al., 1977), be better able to support virus replication and to release virus than alveolar cells. Thus, infection might develop and spread more easily in ciliated epithelium. Bronchial replication probably supplied some of the virus found in the trachea as a result of mucociliary action. Taking this into account, the lower virus titres in the trachea compared with those of the bronchi (Fig. 4 and 5) and the fact that histological damage in the trachea was minimal and focal (J. C. Macartney et al., unpublished observations) support conclusions from scanning electron microscopy that virus replication in the trachea is restricted to small foci (Chevance et al., 1978). If true, this restriction is interesting since it occurs in ciliated epithelium.

The virus distributions on days 3 and 4 not only clearly indicated that clones 64c and 7a infected bronchial tissue better than clone 64d and A/PR/8 but also suggested that clone 64c infected the bronchi better than clone 7a and the lower regions (hilar and intermediate zones) less well. These differences in distribution of the strains were substantially in agreement with the relative amount of histological damage induced by them in either the major bronchi or lung parenchyma. Thus, clones 64c and 7a induced greater overall damage than either clone
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64d or A/PR/8, and clone 64c damaged the bronchi more than clone 7a and the bronchioles of the lung parenchyma less. The differences were statistically significant despite the low levels of damage and the high standard errors on measurements. Overall, the relative percentage bronchial inflammation appeared more significant than parenchymal damage (Tables 1 and 2). Clearly, the reasons for the differences between strains in their ability to infect the lower respiratory tract must be sought in their relative capacities to infect bronchial and bronchiolar tissue and not alveolar tissue, which seems little affected by even the virulent strains.

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REFERENCES


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