The Localization of Influenza Virus in the Respiratory Tract of Ferrets: Susceptible Nasal Mucosa Cells Produce and Release More Virus than Susceptible Lung Cells

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SUMMARY

Infectious virus production by ferret nasal mucosa and lung organ cultures has been monitored in both tissue pieces and medium over 24 h following inoculation with an Asian (H₂N₂) strain of influenza virus. Freshly prepared cultures of nasal mucosa produced approx. 10-fold more virus per cell than fresh lung cultures. Also the nasal mucosa cells liberated into the medium a greater proportion (mean 31 %) of the total virus produced than did fresh lung (mean 6 %). Maintenance of lung explants for 24 h prior to inoculation resulted in a 20- to 100-fold increase in the amount of virus released. However, total virus production by fresh and maintained lung was similar. Trypsin did not increase the infectivity of virus released from any of the cultures, indicating that the haemagglutinin in the virus particles was cleaved. Similar results were obtained with a Hong Kong (H₃N₂) virus strain. Hence, one factor operating in the lower susceptibility of the lung compared with the nasal mucosa in vivo may be a lower capacity of lung cells both to produce and release influenza virus.

INTRODUCTION

Influenza in man is predominantly an upper respiratory tract infection with little lung involvement (Stuart-Harris, 1965). A similar syndrome occurs in influenza of ferrets (Stuart-Harris, 1965; Basarab & Smith, 1969) and this animal model for the human disease has been used in attempts to identify the reasons for the different susceptibility of the respiratory tissues. After intranasal infection of ferrets with influenza virus strain A/Moscow/1019/65 (H₂N₂) (abbreviation A₂M) virus yields in the nasal turbinates were up to 1000-fold higher than in the lungs (Basarab & Smith, 1969). The tissue tropism could result from a greater inherent susceptibility (infectious virus producing capacity) of nasal mucosa than lung, limitation of spread to the lung by host defence mechanisms, or a combination of both. Differences in inherent susceptibility were implicated when infected cultures of nasal mucosa produced virus in the medium more rapidly and to higher titre than corresponding cultures of lung alveolar tissue and investigations of the greater susceptibility of nasal mucosa proceeded using such cultures (Basarab & Smith, 1970; Toms et al. 1974; Kingsman et al. 1977a, b). Maintenance of lung explants but not nasal mucosa for 24 h prior to inoculation resulted in significantly increased yields of virus in the culture

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medium (Kingsman et al. 1977a, b). It was considered that a comparison between
maintained and fresh lung as well as one between the latter and nasal turbinates might reveal
the reason for the lower production of virus in the medium by fresh lung (Kingsman et al.
1977a, b). The three tissues had similar total numbers of susceptible cells, adsorbed similar
quantities of virus and showed only small differences in numbers of infected cells at about
10 h after infection with small inocula whereas subsequent virus yields in the medium at
24 h were much less for fresh lung than for the other tissues. Thus, differences in virus yield
were not primarily determined by differences in initiation of infection at the first cycle but
probably by the quantity or quality of virus released in relation to infection of further cells
(Kingsman et al. 1977b).

This paper records the measurement of infectious virus A,M within the tissue pieces as
well as in the medium, from 8 to 24 h p.i. of organ cultures with low virus inocula. The
results have been related to the numbers of infected cells as detected by fluorescent antibody.
Two major points emerged. Firstly, the differences in virus yields from fresh and maintained
lung detected in previous work is a matter of virus release, not total production. Secondly,
individual susceptible cells of nasal mucosa produce more virus and release a greater
proportion of it than the susceptible cells of fresh lung. Similar results were obtained with
a recombinant virus A/PR/8/34-A/England/939/69 (H3N2) Clone 7a which was known to
produce greater yields of virus from ferret nasal mucosa than from lung in vivo (Toms et al.
1976, 1977; Sweet et al. 1978) and in organ culture (Kingsman et al. 1977a).

METHODS

Strains of influenza virus. Strain A2M was described by Basarab & Smith (1969); working
stocks were prepared as described by Kingsman et al. (1977a). Clone 7a of the recombinant
virus A/PR/8/34-A/England/939/69 (H3N2) and the preparation of seed and working
stocks were described by Toms et al. (1976).

Infectivity assays. These were as described by Toms et al. (1974) and results are quoted as
50 % egg-bit infectious doses (EBID50). The virus content of tissue pieces from infected
organ cultures was determined after maceration for 30 s in 2 ml of buffered Eagle's minimal
essential medium (MEM: Wellcome Laboratories Ltd.) using a Sorvall Omnimixer with a
micro-attachment: the macerate was clarified at 750 g for 10 min at 4 °C before titration.

Ferrets. These were as described by Kingsman et al. (1977a).

Organ cultures. These were prepared by a modification of the method of Basarab & Smith
(1970) as described by Toms et al. (1976) and Kingsman et al. (1977a). Lung explants were
approx. 1 × 2 × 2 mm in size and nasal turbinates, on which the nasal mucosa is situated,
were cut into fragments 3 mm long and 1 mm wide. Explants were inoculated either immedi-
ately after removal from the animal (fresh cultures) or after 24 h at 37 °C in Petri dishes
(maintained cultures; Kingsman et al. 1977a). Inoculation and incubation at 37 °C was
either in Petri dishes (Toms et al. 1976) or in glass bijou bottles (Sweet et al. 1978). These
techniques produced similar results in 15 experiments with lung tissue (five in Petri
dishes and ten in bottles) and six experiments with nasal mucosa (four in Petri dishes and
two in bottles). In comparisons of fresh and maintained lung tissue using the bijou bottle
method of incubation (Sweet et al. 1978), a modification in inoculation technique
was made. Fifty to sixty explants were inoculated in bijou bottles with 1 ml of MEM
containing 4·6 log10 EBID50 of virus. After 30 to 40 min at 37 °C the pieces were washed
five to ten times (10 ml per wash) with MEM, the medium being decanted through sterile
nylon gauze. The explants were then distributed in groups of six in bijou bottles. In control
samples the tissues were killed by freezing (liquid nitrogen) and thawing (37 °C) three
times and then treated as for experimental samples. At 10 to 12 h p.i. only about 1 log10
EBID50 of the original inocula (4·6 log10 EBID50) survived, but this titre was subtracted
from the titres in the experimental samples. When samples were collected from control cultures at 24 h, less than 0.5 log10 EBID50 survived even if the washing procedure after inoculation was omitted.

In some experiments, nasal mucosa produced less virus than lung in both the medium and tissue pieces at 24 h although the titres in the nasal mucosa cultures were always greater than those of lung cultures at 48 h. Joint experiments by two operators revealed that this delay in virus production in nasal mucosa cultures was not attributable to variation among ferrets, but to some unidentified differences in manipulation of the tissues during the preparation of explants.

Estimation of numbers of infected cells by fluorescent antibody techniques. In comparisons of fresh and maintained lung cultures the proportion of surface cells infected was ascertained using either the impression smear method, or the cell suspension technique with EDTA as described by Kingsman et al. (1977b). When fresh cultures of nasal mucosa and lung were compared, the latter technique was modified as follows. Surface cells were loosened by gentle shaking of the cultures at room temperature in 2 ml of calcium–magnesium-free PBS (Dulbecco A) at pH 7.4, containing 0.25 % (w/v) trypsin (Difco Ltd. 1:250). After 10 min, bovine calf serum (0.5 ml) was added to inactivate the trypsin and the explants were sucked up and down a pipette to remove the surface cells. The large tissue pieces were removed and the cells sedimented (2000 g, 5 min, room temperature) and re-suspended in about 100 μl of the supernatant fluid. The cells were sucked up and down using a syringe (23 g needle). The means of haemocytometer counts of the total numbers of surface cells in three experiments were 7.3×104 and 6.9×104 for nasal mucosa and lung respectively. Single drops of the suspension were spread on slides for staining with fluorescent antibody and differential counting as described by Kingsman et al. (1977a). Three slides from three sets of six explants were observed for each tissue; approx. 500 cells/slide were counted. Influenza virus antigens are detected first in the nuclei and virus particle production follows the appearance of virus antigens in the cytoplasm (Breitenfeld & Schäfer, 1957). Since maximum virus production had been released by 20 to 24 h p.i. (Fig. 1 and 2) it was considered that cells showing predominantly nuclear fluorescence at this time (present in both nasal and lung cultures to about the same extent as the cells showing cytoplasmic staining) were those which had begun the second cycle of infection from virus released from cells that had been initially infected by the inoculum. In order to compare virus yields at 24 h p.i. with the number of cells producing virus antigens following infection by the inoculum virus, only cells showing cytoplasmic fluorescence were recorded.

Effect of trypsin on virus infectivity. Medium, harvested from organ cultures at 24 h p.i. and stored without addition of bovine serum albumin (BSA), was divided into two samples. Trypsin (100 μg/ml; type III, Sigma) was added to one sample and BSA (100 μg/ml) to the other; they were then incubated at 37 °C for the times indicated in Results. Samples were removed from the assay of trypsin using α-N-benzoyl-L-arginine ethyl ester HCl (Sigma) as substrate (Schwert & Takenaka, 1955). Soybean trypsin inhibitor (Sigma) was added (100 μg/ml) to the remainder and to the controls and the samples kept on ice for 1 h before titration.

RESULTS

Inocula of 4.6 log10 EBID50 were used to infect the organ cultures. It was with this and smaller inocula that the big differences in virus yield at 24 h were detected between fresh and maintained lung, and between fresh lung and nasal mucosa (Fig. 1 of Kingsman et al. 1977b). Although synchrony of virus replication was obtained in maintained organ cultures by a high inoculum (6.6 log10 EBID50, Fig. 4 of Toms et al. 1974), this inoculum was not used because of evidence of interference (Fig. 2 of Toms et al. 1974; Fig. 1 of Kingsman et al.
Fig. 1. Replication of influenza virus strain A2M in fresh (■—■) and maintained (□···□) organ cultures of ferret lung; (a) virus in the medium; (b) tissue-associated virus; and (c) total virus. The results are from five experiments; each point is the geometric mean of assays on four replicate cultures and the lines are drawn through the means of the points at each time. Inoculum 4.6 log$_{10}$EBID$_{50}$/ml: before plotting the points the residual virus (about 1.0 to 1.2 log$_{10}$EBID$_{50}$ at 8 to 10 h and less than 1.0 log$_{10}$EBID$_{50}$ later) found in control cultures with dead tissue was subtracted from each experimental reading.

1977b) and because the difference in yield mentioned above was eliminated (Kingsman et al. 1977b; Fig. 1).

Replication of strain A2M in fresh and maintained organ cultures of lung

Fig. 1 records the titres of infectious virus found in the medium, in the tissue and the total in organ cultures of fresh and maintained lung over 8 to 24 h p.i.. The amount of infectious virus in the medium of maintained lung at 8 to 12 h p.i. was more than that in the medium of fresh lung and this difference increased with time (Fig. 1a). Whereas the virus released by maintained lung increased about 100-fold between 10 and 24 h p.i., the corresponding increase for fresh lung was only about fourfold. Hence, by 24 h 20- to 100-fold (geometric mean 63-fold) more virus was released from maintained lung compared with fresh lung in five experiments. These differences were similar to those obtained previously (Kingsman et al. 1977a).

The new and striking result is shown in Fig. 1(b) which records the amount of infectious virus associated with the tissue pieces over 8 to 24 h. There were no marked differences between the fresh and maintained cultures. At the earlier time points there was a tendency for the tissue-associated virus to be greater for fresh than maintained lung but the difference was small and not apparent at later time points. When total infectious virus production was calculated (Fig. 1c) there was no marked difference between the tissues; by 24 h, maintained lung appeared to have produced two- to threefold more virus than fresh lung, but the difference was not significant ($P < 0.1 > 0.05$). Thus maintenance had not markedly increased the capacity of lung tissue to produce virus but to release it; in 24 h during the
Tissue specificity of influenza virus

Table 1. The proportion of cells in fresh and maintained organ cultures of lung which contained virus antigens after inoculation with strain A2M

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Time after inoculation (h)</th>
<th>% cells with virus antigens* in:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Fresh lung</td>
<td>Maintained lung</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>1.0 (0.2)</td>
<td>1.4 (0.3)</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>1.3 (0.4)</td>
<td>4.6 (0.9)</td>
</tr>
<tr>
<td>2</td>
<td>11</td>
<td>1.0 (0.3)</td>
<td>1.4 (0.3)</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>1.7 (0.5)</td>
<td>1.8 (0.4)</td>
</tr>
<tr>
<td>3</td>
<td>18</td>
<td>0.5 (0.1)</td>
<td>0.9 (0.1)</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>1.8 (1.0)</td>
<td>3.4 (1.2)</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Detected by fluorescent antibody; see the Methods.
† Corresponding figures obtained in previous work (Kingsman et al. 1977b) were: fresh lung 0.2, 0.4 and 0.6 %; maintained lung 0.9, 1.5 and 1.1 %.
‡ Standard error in parentheses.

Fig. 2. Replication of influenza virus strain A2M in fresh organ cultures of ferret lung (■ --- ■) and nasal mucosa (□ • • □); (a) virus in the medium; (b) tissue-associated virus; and (c) total virus. The results are from seven experiments; each point is the geometric mean of assays on four to six replicate cultures and the lines are drawn through the means of the points at each time. Inoculum 4.6 log_{10}EBID_{50}/ml; before plotting the points the residual virus (about 1.0 to 1.2 log_{10}EBID_{50} at 8 to 10 h and less than 1.0 log_{10}EBID_{50} later) found in control cultures with dead tissue was subtracted from each experimental reading. Statistical analyses of the results in (a), (b) and (c) showed in each case the broken and full lines were significantly different.

experiments summarized in Fig. 1, maintained lung released an average of 40 % of its total virus whereas fresh lung released only 2 %.

Table 1 shows that during the organ culture infection the proportions of cells which were producing virus antigens were small and not unduly different from maintained and fresh
Table 2. The numbers of cells in fresh organ cultures of nasal mucosa and lung which contained virus antigen 24 h after inoculation with strain $A_2M$ or clone 7a; relation to virus yield

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Virus</th>
<th>Tissue</th>
<th>Total surface cells recovered ($\times 10^{-4}$)</th>
<th>Cells with virus antigens</th>
<th>Total virus yield ($\log_{10}$ EBID$_{50}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$A_2M$</td>
<td>Lung</td>
<td>7.9 (1.0)*</td>
<td>0.9 (0.1)</td>
<td>2.7 (0.2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nasal mucosa</td>
<td>5.6 (0.2)</td>
<td>2.3 (0.6)</td>
<td>4.1 (0.1)</td>
</tr>
<tr>
<td>2</td>
<td>$A_2M$</td>
<td>Lung</td>
<td>4.5 (0.1)</td>
<td>2.0 (0.2)</td>
<td>4.2 (0.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nasal mucosa</td>
<td>4.5 (0.5)</td>
<td>1.7 (0.5)</td>
<td>4.7 (0.1)</td>
</tr>
<tr>
<td>3</td>
<td>7a</td>
<td>Lung</td>
<td>6.3 (0.4)</td>
<td>3.4 (0.5)</td>
<td>4.9 (0.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nasal mucosa</td>
<td>3.8 (0.6)</td>
<td>4.3 (0.5)</td>
<td>6.2 (0.1)</td>
</tr>
</tbody>
</table>

* Standard error in parentheses.

The numbers of cells in fresh organ cultures of nasal mucosa and lung which contained virus antigen 24 h after inoculation with strain $A_2M$ or clone 7a; relation to virus yield.

As might be expected, more cells appeared to be infected at later times. With one exception, the figures indicated that more cells were infected in the maintained tissue than in the fresh tissue. Statistical analysis showed the differences were not significant ($P < 0.1 > 0.05$) but the tendency for more cells to be infected in maintained tissue was consistent with the signs of a slightly greater production of virus in maintained tissue (see above).

Infectious virus production per infected cell of fresh and maintained tissue was similar.

**Replication of strain $A_2M$ in fresh organ cultures of nasal mucosa and lung**

Fig. 2 shows the time course of virus production and release in fresh organ cultures of nasal mucosa and lung infected with strain $A_2M$. Virus release from nasal mucosa was significantly greater than that from lung at earlier time points (difference about 10-fold) as well as at 24 h (difference about 30-fold; Fig. 2a). The fresh pieces of nasal mucosa contained statistically significantly ($P < 0.01$) more virus than fresh pieces of lung at all time points (Fig. 2b); and total production of virus (Fig. 2c) after 15 h was about 10-fold higher for fresh nasal mucosa than for lung. Although in these experiments (Fig. 2) fresh lung released more (about 10%) of the total virus produced than previously (about 2%; Fig. 1), nasal mucosa released an even greater proportion (about 10% at earlier times but 30% at 24 h) of its production. Clearly nasal mucosa tissue produced and released virus better than lung tissue.

The differences in virus production were then related to the numbers of cells infected as evidenced by possession of virus antigens. At 10 h p.i. the mean proportion of lung and nasal mucosa surface cells, the total numbers of which were not unduly different (Kingsman et al. 1977b), which produced virus antigens was 0.3 and 0.4, respectively (Kingsman et al. 1977b). Similarly at 24 h p.i. the numbers of lung and nasal mucosa cells which had virus antigens in the cytoplasm were similar (Table 2). The results in Fig. 2(c), in Table 2 and of Kingsman et al. (1977b) indicate that the virus yield per infected cell was approx. 10-fold greater for nasal mucosa than lung cells.

**Cleavage-state of haemagglutinin of strain $A_2M$ released in infected organ cultures**

The apparently small release of virus from fresh lung organ cultures may have been due to the release of virus whose haemagglutinin was in an uncleaved form and therefore of low infectivity (Klenk et al. 1975; Lazarowitz & Choppin, 1975). Hence $A_2M$ virus released to the medium from fresh lung organ cultures and from fresh nasal mucosa and maintained lung cultures, was titrated before and after treatment with trypsin. No increase
Table 3. Effect of trypsin on the infectivity of strain A_{z}M released from organ cultures of fresh nasal mucosa and fresh and maintained lung tissue

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Source of virus</th>
<th>Concentration of trypsin (μg/ml)</th>
<th>Incubation time (min)</th>
<th>Infectivity (log_{10} EBID_{50}/ml) after incubation*</th>
<th>Without trypsin</th>
<th>With trypsin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Fresh lung</td>
<td>100</td>
<td>30</td>
<td>2.2 (0.1)</td>
<td>2.3 (0.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nasal mucosa</td>
<td>100</td>
<td>30</td>
<td>4.0 (0.1)</td>
<td>4.2 (0.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 Maintained lung</td>
<td>10</td>
<td>30</td>
<td>2.8 (0.1)</td>
<td>2.6 (0.1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Virus was incubated at 37 °C with either trypsin or bovine serum albumin at the same concentrations (w/v).
† Each figure is the mean of those obtained from three to four individual organ cultures.
‡ Standard error.

Table 4. Replication of influenza virus clone 7a in fresh organ cultures of ferret nasal mucosa and lung

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Inoculum (log_{10} EBID_{50})</th>
<th>Medium</th>
<th>Tissue</th>
<th>Total</th>
<th>Medium</th>
<th>Tissue</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.6</td>
<td>4.0 (0.2)</td>
<td>4.9 (0.1)</td>
<td>4.9 (0.1)</td>
<td>5.9 (0.1)</td>
<td>6.0 (0.2)</td>
<td>6.2 (0.1)</td>
</tr>
<tr>
<td>2</td>
<td>4.6</td>
<td>2.0 (0.4)</td>
<td>4.2 (0.1)</td>
<td>4.2 (0.1)</td>
<td>5.3 (0.2)</td>
<td>5.7 (0.2)</td>
<td>5.9 (0.2)</td>
</tr>
<tr>
<td>3</td>
<td>3.6</td>
<td>≤ 1.4 (0.5)</td>
<td>3.3 (0.4)</td>
<td>3.3 (0.4)</td>
<td>4.4 (0.1)</td>
<td>4.9 (0.1)</td>
<td>5.1 (0.1)</td>
</tr>
<tr>
<td>4</td>
<td>3.6</td>
<td>≤ 1.0 (0.1)</td>
<td>≤ 2.8 (0.4)</td>
<td>≤ 2.8 (0.4)</td>
<td>4.5 (0.1)</td>
<td>5.0 (0.2)</td>
<td>5.1 (0.1)</td>
</tr>
</tbody>
</table>

* Standard error of mean of four to six replicates.

in infectivity occurred for virus from any of the three cultures (Table 3). The organ cultures may have been associated with serum which contains an inhibitor of trypsin (Reynolds & Newball, 1976). However, assay of trypsin after incubation with the culture media showed that the enzyme had not been inactivated.

Replication of clone 7a in fresh organ cultures of nasal mucosa and lung

The pattern with clone 7a was similar to that for strain A_{z}M. At 24 h fresh nasal mucosa produced more total infectious virus than fresh lung tissue (mean difference 32-fold) and released more (mean about 35 % of total for nasal mucosa; mean about ≤ 5 % of the total for lung; Table 4). However, at 24 h the number of cells showing virus antigen was similar for the two tissues (Table 2) indicating that, again, individual susceptible nasal mucosa cells produced more virus and released it more efficiently than lung cells. In previous work with a different inoculation technique (Sweet et al. 1978), nasal turbinates produced more virus than lung at 37 °C but the lesser release from the latter was not apparent 24 h after inoculation; it was, however, observed (culture medium contained 6% of the total virus) at 48 h (C. Sweet, unpublished data).

DISCUSSION

If organ cultures reflect the virus-producing capacity of the tissues in vivo then two factors which may contribute to the predominance of an upper over a lower respiratory tract infection are a greater capacity of individual nasal mucosa cells than lung alveolar cells to
produce and release virus. In organ culture, nasal turbinates produced significantly more virus than did fresh lung at all time points investigated (13 to 24 h), the difference being about 10-fold for most of the period (Fig. 2). However, similar numbers of cells were infected in the two tissues both early, when cells were in the first cycle of infection, and late, when some may have been in the second cycle (Tables 1 and 2). By 24 h p.i., nasal mucosa cells had released about 30% of the total virus produced whereas lung tissue had released only 10% or less (Fig. 1 and 2). At 24 h, some of the cells of both tissues may have still been involved in the first cycle of infection. Although with high inocula (6.6 log10 EBID50) synchrony of virus production was achieved with maximum virus production at 8 to 10 h (Toms et al. 1974), with the low inocula used here maximum virus production was delayed until 20 to 24 h after inoculation (Fig. 1 and 2). Asynchrony of infection (White et al. 1965) had probably occurred, the growth curves resembling those produced after cell cultures had been inoculated at low multiplicity (Choppin, 1969; Mills & Chanock, 1971).

The greater production by nasal mucosa than lung cells of influenza virus is not due to differences in the initiation of infection (Kingsman et al. 1977b), and the kinetics of virus replication in the two tissues are similar (Toms et al. 1974). Hence the difference appears to lie in factors that influence either the amount and quality of virus components synthesized or their subsequent assembly. Influenza virus infects many cell-types but the yield of virus varies (Choppin, 1969) and multiplication can be abortive (Henle et al. 1955; Franklin & Breitenfeld, 1959; Choppin & Pons, 1970). Although type I and II lung alveolar cells are susceptible to influenza virus infection (Kingsman et al. 1977a) they have characteristics which might limit virus production. In cell monolayers of kidney and other cell types, haemagglutinin and possibly other envelope proteins of influenza virus are synthesized on rough endoplasmic reticulum (Compans, 1973; Klenk et al. 1974) and translocated along the smooth endoplasmic reticulum (Compans, 1973; Stanley et al. 1973; Klenk et al. 1974) to the plasma membrane (Stanley et al. 1973). Alveolar type II cells appear to have little smooth endoplasmic reticulum for translocation (Williams & Mason, 1977) which may be further hindered by surfactant. This surface-active material is probably formed in the endoplasmic reticulum (Mason, 1976) and is composed in part of dipalmitoylphosphatidyglycerol which are rare in other mammalian cells (Rooney, 1976). Alveolar type I cells are relatively devoid of ribosomes and mitochondria (Mason & Williams, 1977) and this may limit virus production. These factors may have resulted in the production of a disproportionate quantity of incomplete virus by lung cells and this possibility has not yet been investigated.

Maintenance of lung tissue in organ culture increased the proportion of virus released to approx. 40% of the total produced, similar to that of nasal mucosa (Fig. 1 and 2). However, total virus production by maintained lung was not significantly greater than that of fresh lung (Fig. 1). This effect of maintenance was not due to obvious artefacts like cell necrosis and fibroblast outgrowth (Kingsman et al. 1977a). Furthermore, fresh lung cultures from unborn and 2-day-old ferrets released virus as efficiently as maintained adult lung and did not release more virus on maintenance (D. Cavanagh, M. H. Collie, C. Sweet & H. Smith, unpublished data). Thus, a comparative study of fresh and maintained lung in relation to virus release might lead to an explanation for the inefficient release of virus from fresh lung.

Mucus and surfactant might have interfered with virus release but appeared to have little influence (Kingsman et al. 1977a, b). Virus might have been released from fresh lung tissue in a form which was uninfecctious in the assay system. However, cleavage of the haemagglutinin (Klenk et al. 1975; Lazarowitz & Choppin, 1975) appears to have occurred (Table 3). Defects of the virus surface other than non-cleavage of the haemagglutinin (Laver, 1963; Klenk & Choppin, 1970; Meier-Ewert & Dimmock, 1970; Scholtissek, 1975) might have impaired infectivity or an association of the virus surface with cellular material from
organ cultures. Both appear unlikely however, since the titres of the tissue virus were similar for fresh and maintained lung.

The lower production and release of virus by lung than nasal mucosa would be expected to result in a less extensive infection of the lower rather than the upper respiratory tract. However, since lung cells are capable of modest virus production and release, other factors, particularly host defence mechanisms such as mucociliary action, phagocytes and fever (Sweet et al. 1978), probably play a role. Clearly the present organ culture experiments must be supplemented by further experiments in vivo.

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REFERENCES


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