Br. J. exp. Path. (1985) 66, 217–231

Further studies of the reasons for the lack of alveolar infection during influenza in ferrets

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Received for publication 28 June 1984

Summary. Intratracheal inoculation of influenza virus in the ferret was followed by a more severe airway infection than that produced by nasal infection and was mainly bronchiolar rather than bronchial. Also, virus isolation from the alveolar zone of the lung together with immunofluoresence and immunoperoxidase techniques showed that some virus reached the alveoli. Nevertheless, there was no subsequent alveolitis suggesting the existence of a clearance phenomenon. Alveolar macrophages were shown to have phagocytosed virus *in vivo* and phagocytosis studies *in vitro* showed that two mechanisms could operate to eradicate the virus. First, a rapid destruction of virus and second an abortive cycle of replication which produced virus antigen but not infectious virus. Experiments with large doses of virus indicated that after intranasal inoculation little virus reached the alveoli so it would probably be quickly cleared by the macrophages.

Keywords: alveolar infection, influenza, ferrets

As in adult humans (Mulder & Hers 1972) lower respiratory-tract (LRT) infection with influenza virus in the ferret is predominantly an airway infection with little or no alveolar involvement (Sweet *et al.* 1981; Husseini *et al.* 1983). Following intranasal inoculation with a virulent influenza virus (clone 7a of the recombinant virus A/PR/8/34-A/England/939/69 (H3N2)) virus titres in homogenates of the hilar zones of the lung lobes were generally higher than those in the intermediate zones while those in the alveolar zones were usually much lower. Since the hilar, intermediate and alveolar zones were defined on the basis of decreasing amount of airway epithelium relative to alveolar tissue (Sweet *et al.* 1981) this indicated that *in vivo*, influenza virus replicated in bronchial and bronchiolar epithelium and not in alveolar tissue. This was confirmed by quantitative observation of histological damage (Sweet *et al.* 1981) and by detection of virus antigen using fluorescein-labelled antibody (Husseini *et al.* 1983); both techniques indicated that bronchial epithelium was the most affected tissue.

In investigations of the reasons for the lack of alveolar involvement it was suggested that after intranasal inoculation a small amount of virus does reach the alveoli either from the original inoculum or from the subsequent upper respiratory-tract infection (Husseini *et al.* 1983; Bird *et al.* 1983). In addition, organ culture studies with alveolar tissue from which all major airways had been removed showed that alveolar cells could support virus replication but little virus was released compared with cultures containing airway tissue (Husseini *et al.* 1983). Also, in experiments *in vitro* performed at normal and pyrexial temperatures, ferret lung macrophages adsorbed 72–93% of the virus pre-

sented to them in 1 h and destroyed 52–83% of that adsorbed (Bird *et al.* 1983). It was concluded, therefore, that the lack of alveolar involvement was due to: (1) phagocytosis and destruction of most of the small amount of virus that might reach the alveolar region; and (2) lack of spread of virus from any alveolar cell that did become infected (Husseini *et al.* 1983; Bird *et al.* 1983). To test this hypothesis we have attempted to introduce more virus into the alveolar region by inoculating it directly into the trachea and then to examine whether: (a) there is still no development of alveolar infection; and (b) evidence can be obtained for virus–macrophage interaction *in vivo*.

Materials and methods

Virus. Clone 7a (H3N2) of the recombinant influenza virus A/PR/8/34-A/England/939/69 and the preparation of seed and working stocks were described by Sweet *et al.* (1974*a*,*b*). High-titre stocks were prepared as described by Gould *et al.* (1972).

Infectivity assays. The egg-bit assay of Fazekas de St Groth & White (1958) and the egg assay were as described previously (Sweet *et al.* 1974*a*); titres were expressed in 50% egg-bit infectious doses ($EBID_{50}$) or 50% egg-infectious doses (EID_{50}) respectively.

Inoculation of ferrets. Ferrets were inoculated intranasally under ether anaesthesia as described by Toms *et al.* (1976). For intratracheal inoculation of virus the trachea was exposed under pentobarbitone anaesthesia (Sagatal, May and Baker Ltd, i.p. *ca* 0.5 ml/kg body wt) and 0.5 ml of virus in allantoic fluid or phosphate-buffered saline (Dulbecco A; PBSA) inoculated into the trachea using a 1-ml syringe with a 26 G $\frac{1}{2}''$ needle. The needle was inserted at right angles to the trachea to ensure penetration of the needle into the tracheal lumen; the syringe was then tilted into the horizontal position and virus injected towards the lung. The syringe needle was removed and the wound closed with clips (Michel Clips, Hauptner Instruments, Brookwick, Ward & Co. Ltd, London) and sprayed with plastic dressing (Hibispray, Avelex Ltd, Wigan, Lancs).

Measurement of virus infectivity in macerates of respiratory tract tissues. The lower respiratory tracts were dissected into trachea, main bronchi and the hilar, intermediate and alveolar zones of each of the four main lung lobes as described previously (Sweet *et al.* 1981) and stored at −70°C. After thawing, all samples were macerated in a Sorvall omnimixer as described previously (Sweet *et al.* 1981).

Examination of lung sections by immunofluorescence. The preparation of sections of respiratory tract tissues, antiserum against clone 7a and immunofluorescent staining of sections have been described previously (Husseini *et al.* 1983). Two modifications were made to this procedure. Firstly, whole sera were used instead of purified immunoglobulin and secondly the sera were adsorbed with *Candida albicans* to remove non-specific antibody from rabbit sera which reacted with non-influenza virus antigens present in the respiratory tissues of some ferrets. As a control some sections were treated with anti-influenza clone 7a antiserum which had been adsorbed previously with clone 7a virus.

Examination of lung sections by the immunoperoxidase technique. An indirect immunoperoxidase technique was used (Howie *et al.* 1984). Preliminary experiments showed that periodic acid could not be used to block endogenous peroxidase activity since it virtually abolished antigenic reactivity. Endogenous peroxidase was therefore blocked with hydrogen peroxide in methanol (Howie *et al.* 1984) which did not affect detection of influenza virus antigen. In the first stage of the immunoperoxidase technique sections were covered with rabbit antiserum to clone

7a diluted between 1/20 and 1/40. The second antibody was horseradish peroxidase-conjugated sheep antiserum to rabbit immunoglobulins (Serotec Ltd, Bicester, Oxon), diluted 1/50.

Interaction of virus with macrophages obtained by bronchoalveolar lavage. The preparation of alveolar macrophages and the 1-h *in vitro* phagocytosis test at 39°C were described previously (Bird *et al.* 1983). For the 4-h phagocytosis test, virus was incubated with phagocytes for 1 h at 39°C as in the 1-h test for attachment and phagocytosis to occur. A 0.9 ml amount of HBG [Hank's balanced salt solution with 0.2% bovine serum albumin (BSA) and 0.2% glucose] was added, the contents mixed and after centrifugation as above, the supernatant was removed for infectivity assay of unattached virus. The cell deposit was then resuspended in 0.1 ml of HBG and incubated for a further 3 h at 39°C; 0.9 ml of neuraminidase was then added and subsequent treatments, to determine attached but non-phagocytosed virus and to release phagocytosed infectious virus, were carried out as described previously (Bird *et al.* 1983).

To examine viral antigen production at 1, 4 and 21 h after infection, virus was allowed to interact with phagocytes in tubes (10^6 macrophages/tube) as described by Bird *et al.* (1983). After 1 h incubation at 39°C all tubes were centrifuged (1200 *g*, 15 min, 4°C) and the cells in two tubes resuspended in 0.5 ml FA PBS (Husseini *et al.* 1983); 10-μl samples (i.e. 2×10^4 macrophages) were then added to wells of a 12-well multi-test slide (Flow Laboratories, Irvine, Scotland). The cells in the other tubes (four) were resuspended in 0.1 ml of HBG and incubated for a further 3 h at 39°C. Two of the tubes were then centrifuged, resuspended in FA PBS and sampled as above; 0.9 ml of HBG containing crystamycin (100 U/ml) was added to each of the two remaining tubes before their incubation for a further 17 h at 39°C, at which time they were centrifuged, resuspended in FA PBS and sampled. All slides were then stained for immunofluorescence (Husseini *et al.* 1983).

To examine for virus replication in alveolar macrophages, the cells were collected as described previously (Bird *et al.* 1983), and suspended in Eagles Minimal Essential Medium (MEM) supplemented with 0.18% sodium bicarbonate, 100 U/ml penicillin and 100 μg/ml streptomycin. Approximately 10^5 cells were then added to each well of 100-mm square Repli dishes (Sterilin) and then incubated for 3 h at 37°C in 5% CO_2/95% air for cells to attach to the plastic. The medium was then removed and the cells washed with 1 ml of MEM followed by addition of 1 ml MEM containing 10^6 $EBID_{50}$ to each well. After 1 h incubation at 37°C, the inoculum was removed and the cells washed once with 1 ml of MEM; 1 ml of Medium 199 (Wellcome Research Laboratories, Beckenham, England), supplemented as above, was added and the plate incubated further at 37°C in 5% CO_2/95% air. The culture medium was harvested at 24, 48, 72 and 96 h after infection.

Results

Distribution of virus infectivity in the LRT immediately following intratracheal inoculation of virus

Within 20 min of intratracheal inoculation of two ferrets with $10^{7.6}$ EID_{50} or $10^{5.6}$ EID_{50} of clone 7a surprisingly low amounts of virus were found in the trachea and main bronchi (Table 1). The hilar region of the lung contained appreciable amounts of virus (about 10% of the inoculum) and significant but lesser quantities were found in the intermediate and alveolar regions (Table 1). With a smaller inoculum ($10^{3.6}$ EID_{50}) the same pattern was seen with virus being highest in the hilar region and detectable in the intermediate and alveolar regions but not so in the trachea and bronchi (Table 1). Thus, even with small doses of virus introduced intratracheally some of the inoculum reached the alveolar region, although not necessarily the alveolar spaces.

Table 1. Virus distribution in the lower respiratory tract of ferrets 20 min after inoculation

Virus (log_{10} EID_{50})	Animal no.	Total virus titre in log_{10} EID_{50} (% recovery)					
		Nasal turbinates	Trachea	Main bronchi	Lung zones‡		
					Hilar	Intermediate	Alveolar
7.6	1	ND*	5.2(0.4)	4.5(0.1)	6.4(6.5)	5.2(0.4)	2.8(0.001)
	2	3.5(0.01)	5.5(0.7)	4.7(0.1)	6.6(11)	5.7(1.4)	5.5(0.7)
5.6	3	UD†	3.5(0.7)	1.0(0.002)	4.7(11.2)	3.9(2.1)	3.2(0.4)
	4	UD	3.0(0.2)	2.7(0.1)	4.4(6.5)	2.9(0.2)	2.1(0.03)
3.6	5	UD	UD	UD	3.1(32.4)	1.0(0.2)	1.0(0.2)
	6	UD	UD	UD	3.0(22.4)	1.2(0.4)	1.4(0.6)

* ND = Not done.

† UD = Undetectable.

‡ The lung zones were titrated separately for each of the four main lobes and the yields recorded above are the geometric means for the four lobes.

The isolation of virus from the nasal turbinates of one animal (Table 1) indicates that an aerosol is set up during the inoculation procedure.

Distribution of virus antigen in the LRT immediately following intratracheal inoculation of virus

Within 20 min of intratracheal inoculation of ferrets with $10^{7.6}$ EID_{50}, $10^{5.6}$ EID_{50} or $10^{3.6}$ EID_{50} of clone 7a animals were killed and sections of the various regions of the LRT were examined by the immunofluorescence and immunoperoxidase techniques for influenza virus antigen. Virus antigen could not be detected in the bronchi or bronchioles of the lung of any ferret inoculated with any of the three doses of virus. However, in the ferrets inoculated with $10^{7.6}$ EID_{50}, virus was regularly detected in, or associated with, alveolar macrophages (Fig. 1a) found distributed throughout the lung (Fig. 1*b*). Hence, virus reached the alveolar spaces and was phagocytosed by lung macrophages when $10^{7.6}$ EID_{50} virus was inoculated. Virus antigen was not detected in alveolar macrophages of ferrets inoculated with lower doses of virus but small amounts were probably present.

The development of LRT infection after intratracheal inoculation as indicated by virus infectivity in various lung zones at 24 and 72 h after inoculation

Fig. 2 shows the virus titres in various parts of the respiratory tract of two ferrets 24 h after intratracheal inoculation with $10^{7.6}$ EID_{50}, $10^{5.6}$ EID_{50} or $10^{3.6}$ EID_{50} clone 7a. For the highest two inocula virus titres in the various regions of the LRT (Fig. 2*a*: 1, 2; *b*: 1, 2) were at similarly high levels and the most striking aspect of the pattern was that titres in the hilar zones of the lung lobes were usually higher than those in the intermediate zones and that those in the alveolar zones were lower than those in the other two zones. With the smallest inoculum (Fig. 2*c*: 1, 2), although the levels of virus found at 24 h in the various regions were lower than those for the larger inocula, the pattern of virus distribution was similar. In the total of 22 infected lobes from the six animals (Fig. 2) hilar titres were greater than or equal to intermediate and alveolar titres in 16 and 20

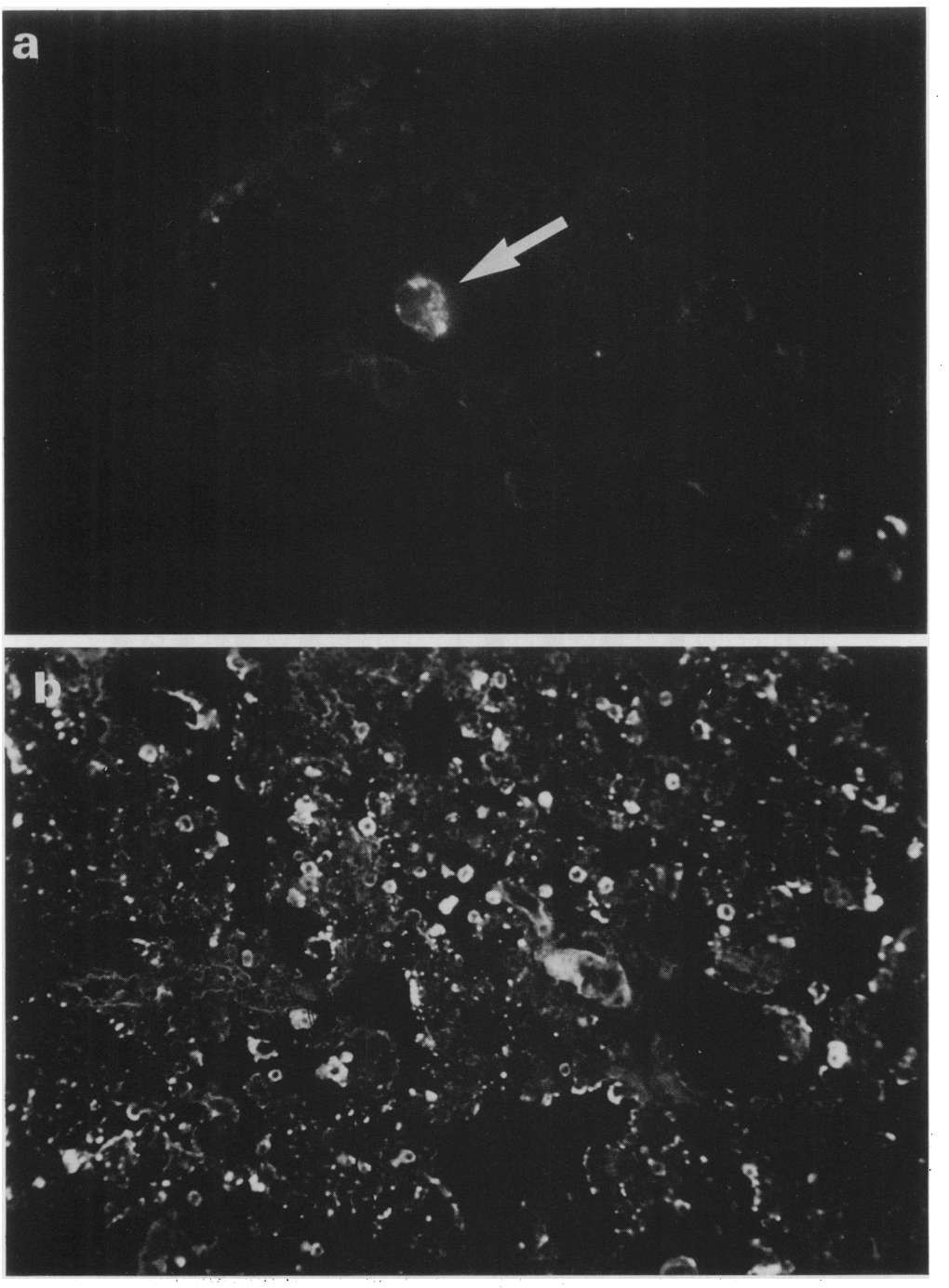

Fig. 1. Virus antigen in the lower respiratory tract of ferrets 20 min after intratracheal inoculation with $10^{7.6}$ EID_{50} clone 7a. (*a*) Alveolar macrophage (arrowed), immunofluorescence technique, ×430; (*b*) lower magnification showing scattered fluorescence throughout the lung, ×110.

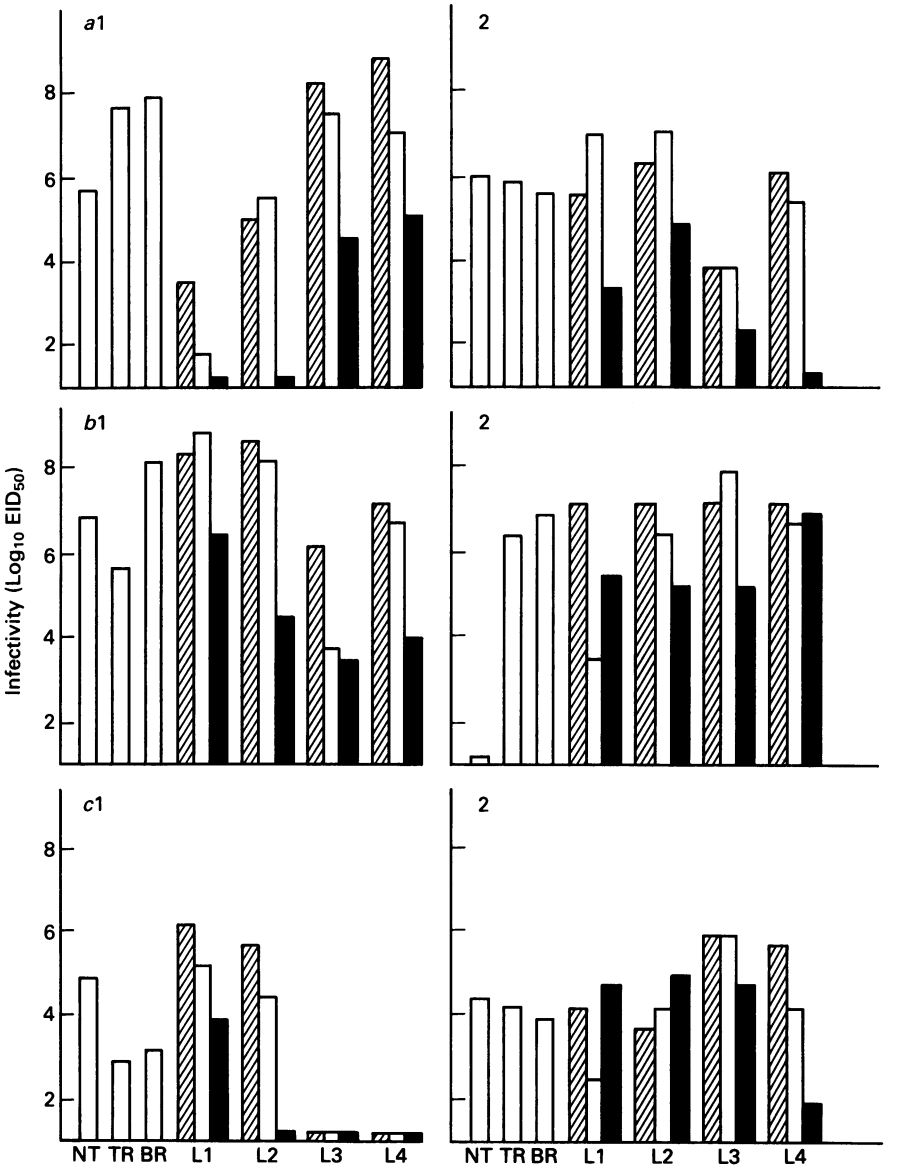

Fig. 2. Total titres of virus given as 50% egg-infectious dose (EID_{50}) at day 1 after infection in nasal turbinates (NT), trachea (TR), bronchi (BR), and in hilar (■), intermediate (□) and alveolar (■) zones of lung lobes L1, L2, L3 and L4 for two animals (1 and 2) inoculated with (a) $10^{7.6}$ EID_{50}; (*b*) $10^{5.6}$ EID_{50}; or (*c*) $10^{3.6}$ EID_{50} clone 7a. Each histogram represents the titre of virus in an individual tissue or subregion.

lobes respectively and in 18 of 22 infected lobes titres in intermediate zones were greater than those in alveolar zones. For all inocula the titres in the trachea and bronchi were similar to one another and often less than those found in the hilar zones. The presence of nasal turbinate infection in all animals again indicates that aerosols are

generated during the inoculation procedure or perhaps from the spread of virus up the respiratory tract.

By 72 h after intratracheal inoculation of $10^{7.6}$ EID_{50} clone 7a little virus was found in the LRT. While total virus titres found in the nasal turbinates of two animals were $10^{6.7}$ and $10^{5.0}$ EID_{50} respectively, those found in the tracheas, bronchi and combined hilar, intermediate and alveolar zones of the four lung lobes were 1.8, U.D. (undetectable); 2.7, 1.9; 2.3, U.D.; 2.2, U.D; and U.D., U.D. respectively. Clearly the LRT infection did not persist for long.

The development of infection in the LRT after intratracheal inoculation; distribution of viral antigen and tissue damage in lung zones at 24 and 72 h.

Both the immunofluorescence and immunoperoxidase techniques showed the infection induced 24 h after intratracheal inoculation with both $10^{7.6}$ EID_{50} and $10^{5.6}$ EID_{50} was very focal. Virus antigen was found in some lobes but not others and affected lobes varied in degree of involvement; some lobes had virus antigen in $> 50\%$ of their airways (Fig. 3*a*, *b*) while others had only one or two airways containing antigen in the hilar regions. The focal pattern may have reflected the mode of inoculation (Brain & Valberg 1979). Heavily affected lobes contained some fluorescing alveolar cells and possibly macrophages but these were seen mainly around affected airways (Fig. 3*c*). The latter were predominantly bronchioles (Fig. 3*a–c*) although bronchial cells were infected in some animals (Fig. 3*d*). As early as 24 h after inoculation inflammation was evident in the bronchioles (Fig. 3*e*) and the lumens of many contained plugs of inflammatory exudate (Fig. 3*a*).

After 72 h, antigen-containing cells were difficult to detect by immunofluorescence. However, despite the fact that many airways were denuded of ciliated epithelial cells (Fig. 4*a*), the immunoperoxidase techniques detected antigen in those bronchiolar epithelial cells which still remained attached to the basement membrane (Fig. 4*a*, *b*). Few antigen-bearing cells could be detected in alveolar tissue with either technique and, in contrast to the situation in the airways, no damage or inflammatory response was evident in the alveoli (Fig. 4*a*, *b*).

Attempts to infect the alveolar region with large intranasal inocula

The success of the intratracheal inoculation prompted attempts to demonstrate virus in the alveolar regions of the lung after large intranasal inocula. Twenty minutes after intranasal inoculation of $10^{9.3}$ EID_{50} of clone 7a into each of two ferrets the virus yields in the nasal turbinates were $10^{8.0}$ EID_{50} and $10^{8.2}$ EID_{50}, which was about 6% of the original inoculum; 1.2, 0.7, 1.9, 1.6 and 2.3 $\log_{10}$ EID_{50} were detectable in the trachea, bronchus and hilar, intermediate and alveolar lung zones respectively for one animal, virus being undetectable in the LRT of the other animal. In sections of such lungs virus antigen could only be detected as weak immunofluorescence in the occasional alveolar macrophage. Hence very little virus reaches the alveoli from intranasal inoculation even with large inocula. When a smaller inoculum ($10^{7.6}$ EID_{50}) was used no virus could be detected in any of the lung zones.

Observations of prolonged virus–macrophage interaction

Since the experiments with intratracheal inoculation had supported the idea that virus reaching alveoli may be phagocytosed and removed by macrophages our previous work where macrophage–virus interaction was studied for only 1 h was extended over a longer period to determine whether phagocytic killing increased or virus replicated in the macrophages. As previously observed (Bird *et al.* 1983), after 1 h interaction about 85% of the virus was adsorbed to the macrophages and, of that adsorbed, 43–77% could not be recovered by treatment with neur-

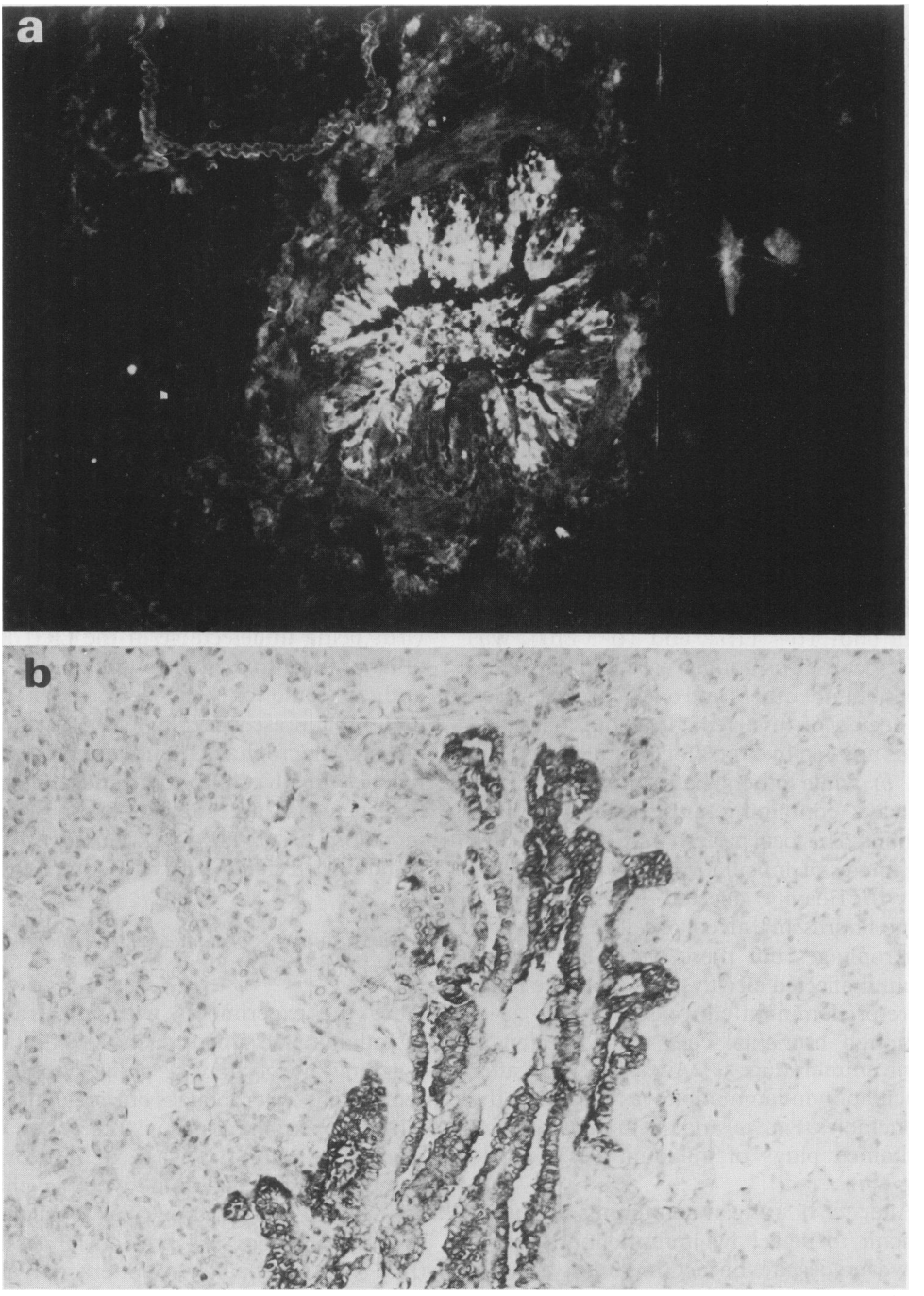

Fig. 3. Virus antigen in the lower respiratory tract of ferrets 24 h after intratracheal inoculation with clone 7a. (*a*) Bronchiole, × 110, with fluorescing epithelial cells, $10^{5.6}$ EID_{50}; (*b*) bronchiole, × 110, immunoperoxidase technique showing no alveolar involvement, $10^{5.6}$ EID_{50}.

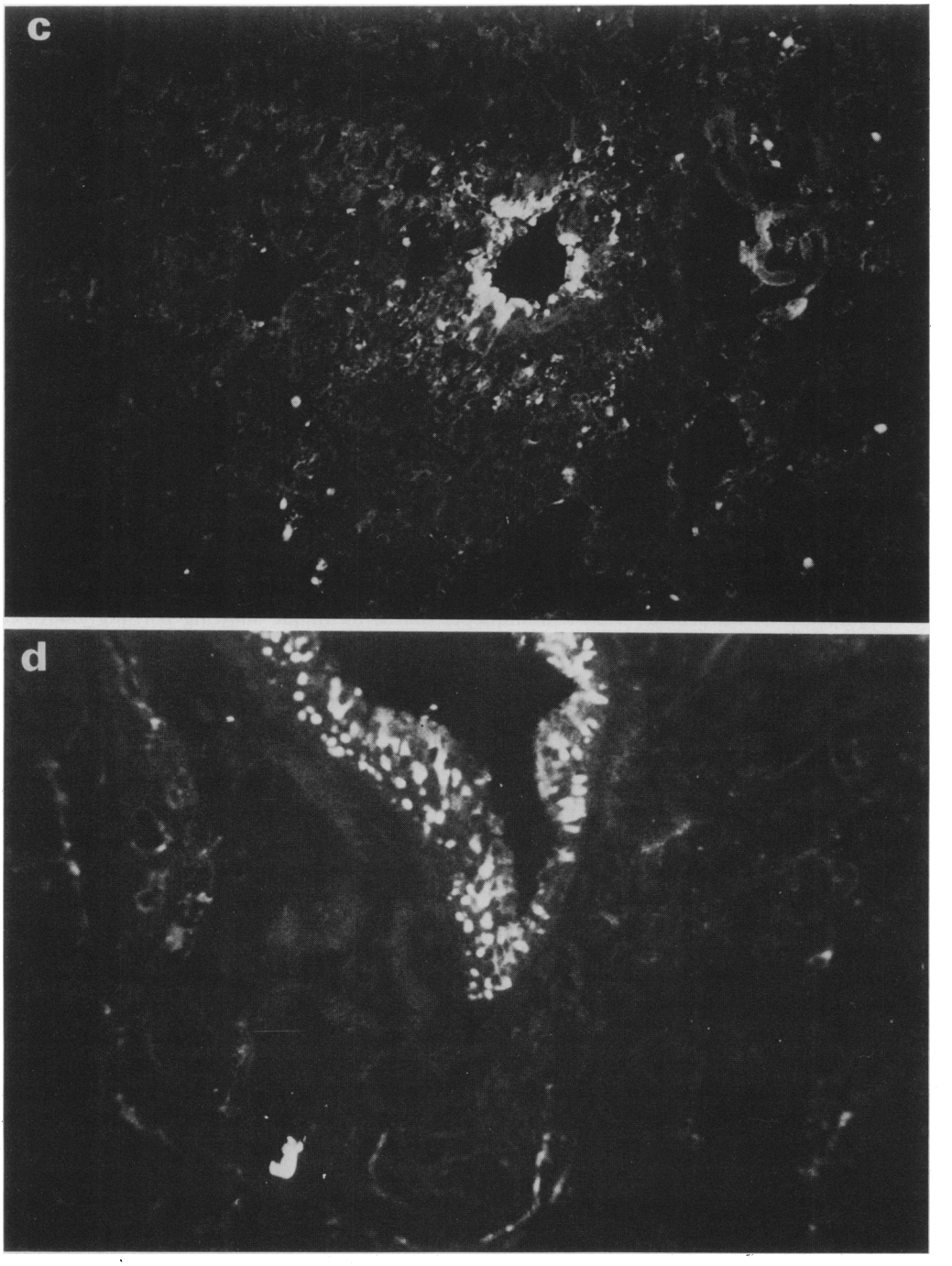

Fig. 3 continued. (*c*) Bronchiole, × 110, with associated fluorescing alveolar cells and/or macrophages; (*d*) bronchus, × 270, immunofluorescence technique, $10^{5.6}$ EID_{50}.

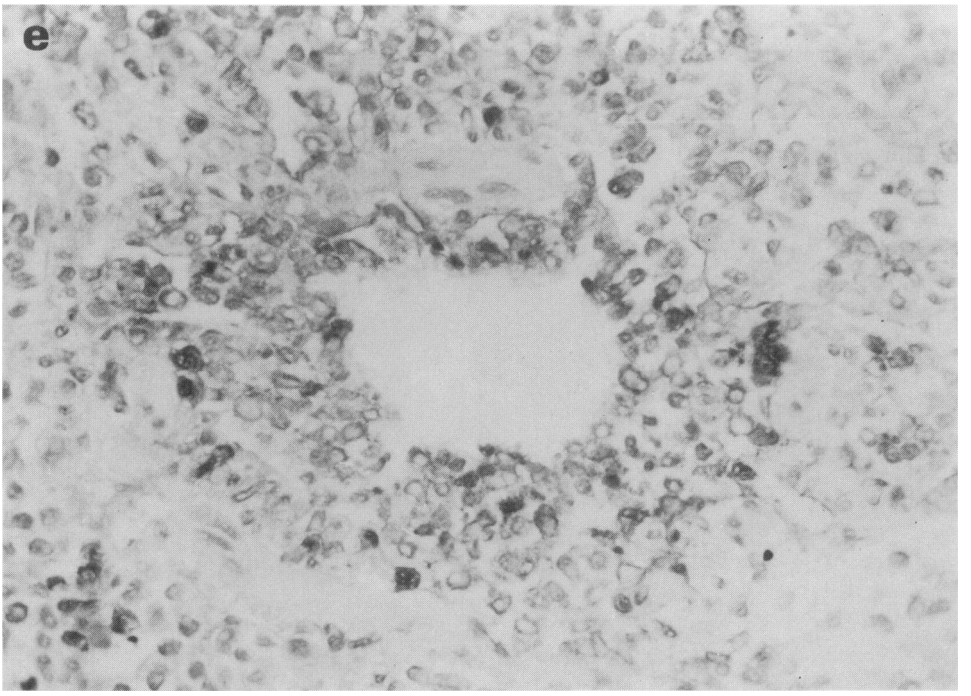

Fig. 3 continued. (*e*) Bronchiole, ×270, immunoperoxidase technique showing polymorphonuclear phagocyte infiltration, $10^{5.6}$ EID_{50}.

aminidase and freeze–thawing (Table 2). After removing the inoculum and incubating the cell-associated virus for a further 3 h the amount of virus that could be recovered had not decreased significantly (Table 2). Thus prolonged interaction with macrophages did not increase the amount of virus destroyed. In three separate experiments, where added virus was not removed after the first 1 h and remained in contact with the macrophages throughout the 4 h phagocytosis period, similar results were obtained. The mean (of the three experiments) cell associated virus was 87% (SEM ± 4) and 73% (± 7) after 1 h and 4 h respectively and, of that adsorbed, 33% (± 9) and 29% (± 1) respectively was recovered by neuraminidase and freeze–thawing.

When the virus-infected macrophages were incubated over a prolonged period (see methods) no infectious virus was detected in the culture medium harvested at 24, 48, 72 and 96 h after infection. However, using the immunofluorescent and immunoperoxidase techniques virus was seen to be taken up by the macrophages (Fig. 5*a*) and to produce much antigen by 24 h post inoculation (Fig. 5*b*) indicating an abortive replication cycle.

Discussion

These experiments would have been performed better by aerosol inoculation but this needs complex and expensive apparatus and much technical experience (Brain & Valberg 1979). Nevertheless, intratracheal instillation achieved the objective of the experiments, namely to deliver virus to the alveoli and then to examine the outcome.

Intratracheal inoculation placed surprisingly little virus in the trachea and main external bronchi (Table 1) and this might be due to relatively inefficient adherence of virus to the epithelium or to very rapid

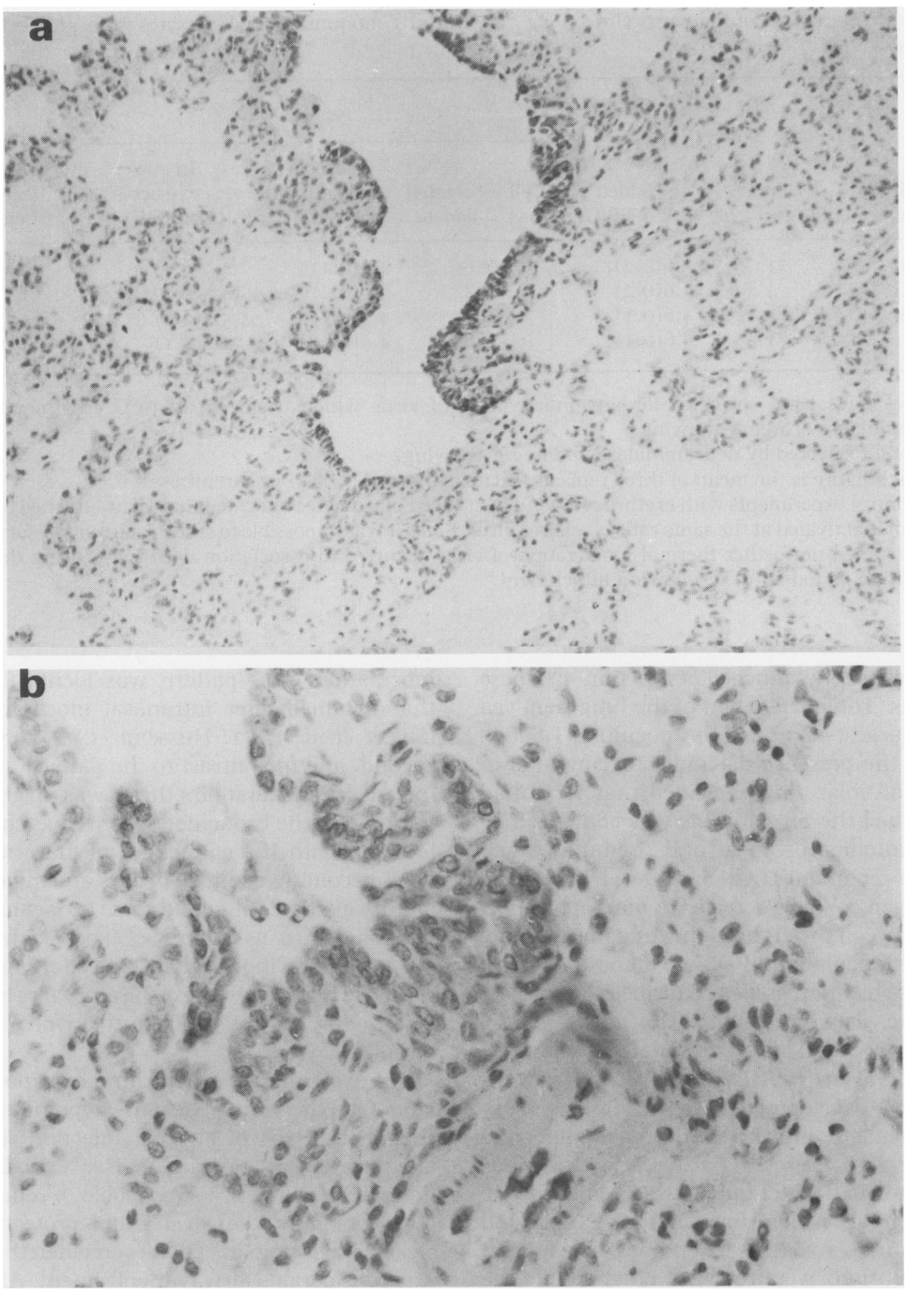

Fig. 4. Virus antigen in the lower respiratory tract of ferrets 72 h after intratracheal inoculation with $10^{7.6}$ EID_{50} clone 7a. (*a*) Bronchiole, × 110, immunoperoxidase technique showing viral antigen in airway only; (*b*) bronchiole, × 270, immunoperoxidase technique. Note loss of ciliated epithelial cells and lack of alveolar involvement.

Table 2. Interaction of influenza virus clone 7a with lung mononuclear phagocytes from uninfected ferrets

Experiment no.	Time of phagocytosis	Virus: Added (log_{10} $EBID_{50}$)	Cell associated as % added	Eluted by neuraminidase (log_{10} $EBID_{50}$)	In cell extract (log_{10} $EBID_{50}$)	Recovered* as % cell associated
1	1	5.6(0.2)†	88	4.4(0.1)	4.7(0.1)	23
	4	5.6(0.2)	85	3.9(0.2)	4.5(0.1)	14‡
2	1	5.6(0.1)	83	4.7(0.1)	5.1(0.1)	57
	4	5.6(0.1)	66	4.7(0.1)	4.8(0.1)	40‡

Titres have been corrected for any inactivation of virus which occurred at 39°C, treatment with neuraminidase or freeze–thawing.

* Total recovered by neuraminidase and freeze–thawing.

† Each figure is the mean of three replicate incubated samples (SEM in parentheses).

‡ Control experiments with erythrocytes and heat-killed phagocytes indicated that virus attached to cells was not inactivated at the same rate as cell free virus. Since it was impossible to control adequately for this it was assumed no further thermal inactivation of virus occurred in association with cells during the 3 h incubation period at 39°C following attachment.

clearance by mucociliary action in these areas. The hilar region of the lung removed the largest amount of the inoculum (Table 1) and the presence of significant amounts in the alveolar zone indicated that virus had reached the alveoli. This was confirmed by immunofluorescence and immunoperoxidase techniques which showed that virus antigen could be detected in macrophages in the alveoli throughout the lung immediately after inoculation of a large amount of virus. These techniques also confirmed that at least some virus was engulfed by macrophages *in vivo* and the postulated clearance mechanisms were in operation.

The development of the infection subsequent to intratracheal inoculation was followed by assessing virus titres in various areas of the LRT and also by microscope examination using immunofluorescence and immunoperoxidase techniques. Two main conclusions were reached. First, despite the fact that some of the inoculated virus had reached the alveoli, virus infection in that area did not proceed; significant alveolitis was not observed either at 24 h or 72 h after inoculation. This pattern was identical to that obtained after intranasal inoculation (Sweet *et al.* 1981; Husseini *et al.* 1983). Second, and in contrast to the pattern after intranasal inoculation, there was a gross infection of the bronchioles, as well as some bronchi, with the epithelium of many airways becoming denuded of cells and inflammatory exudates plugging some bronchiolar lumens. Thus, as might be expected with intratracheal inoculation, infection proceeded further down the respiratory tree but, despite this, did not significantly involve the alveoli.

The experiments with intratracheal inoculation had therefore upheld the hypothesis to explain the lack of alveolar infection seen after intranasal inoculation (see introduction). Macrophages were capable of removing virus from the alveoli and protecting alveolar cells from infection even when there was considerable airway involvement. Also, any alveolar cell infection that occurred around the infected airways after intranasal inoculation was focal; there appeared no tendency for spread fitting in with the lack of

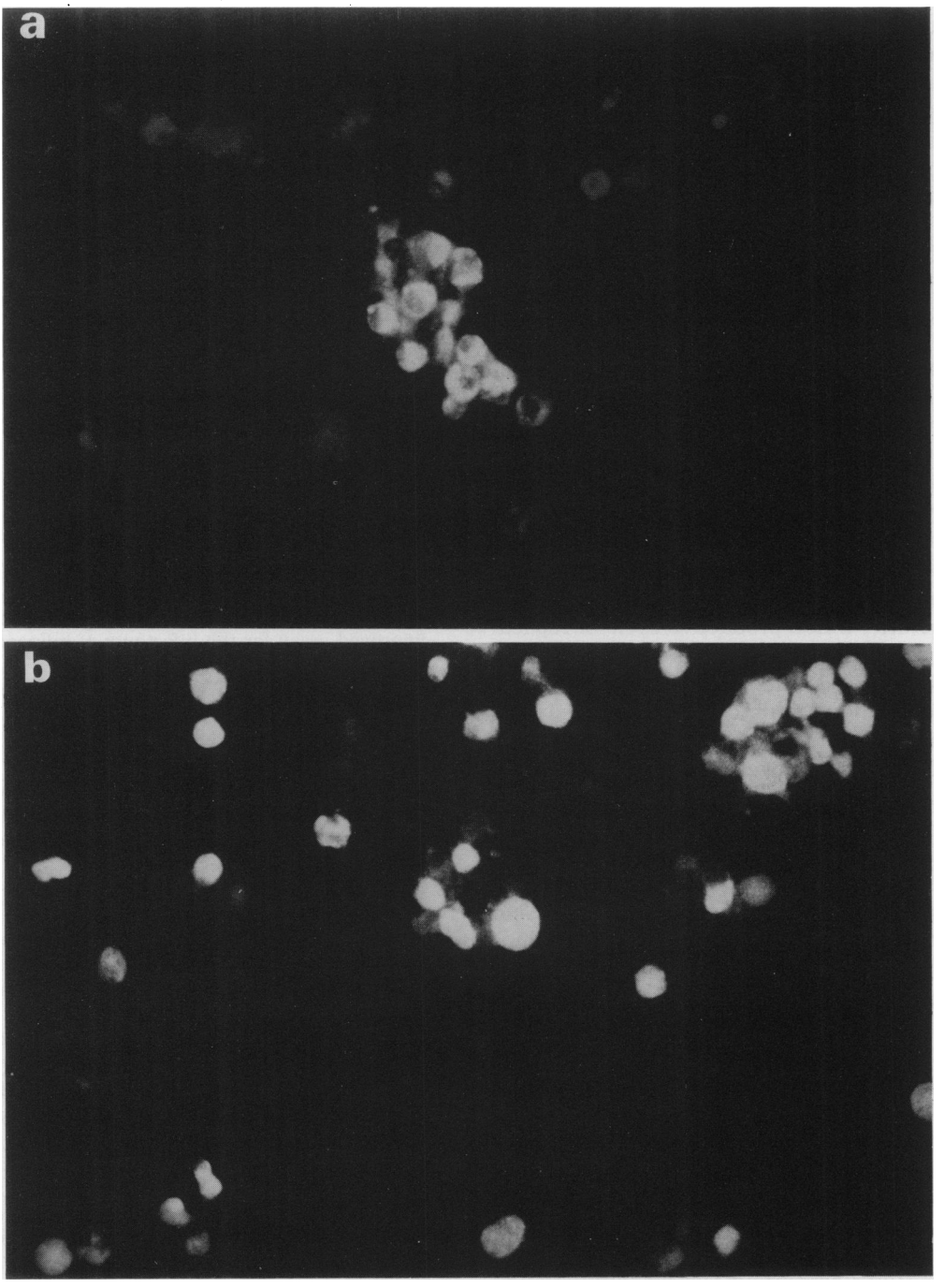

Fig. 5. Fluorescence in broncho-alveolar lavage macrophages inoculated *in vitro* with clone 7a, ×430. (*a*) 30 min after inoculation; (*b*) 21 h after inoculation.

release of virus (Cavanagh *et al.* 1979; Husseini *et al.* 1983). In addition, the experiments with large intranasal inocula showed that little virus reaches the alveoli so the clearance mechanisms which operate efficiently after intratracheal inoculation are by no means overburdened after intranasal inoculation.

Lung macrophages clearly take up virus efficiently both *in vitro* and *in vivo*. However, the mechanism of eradication of virus is less clear. In the extension of our original phagocytosis experiments (Bird *et al.* 1983), although 70–90% of the virus becomes associated with the macrophages, about 30% of it was recoverable after 4 h (Table 2) in contrast to the 2–11% recovered after 1 h with ferret polymorphonuclear (PMN) cells (Sweet *et al.* 1977). Hence the initial destruction of virus in macrophages appears less efficient than for PMN phagocytes. However, another mechanism of removing virus could operate—a non-productive cycle of replication; 24 h after infection of the ferret lung macrophages, and subsequently up to 96 h, no infectious virus could be detected over and above that detectable in the presence of killed macrophages but immunofluorescence and immunoperoxidase techniques demonstrated uptake of virus and production of abundant virus antigen by 24 h. Abortive cycles of replication of influenza virus have been demonstrated in mouse and human macrophages (Rodgers & Mims 1981, 1982*a*, *b*). Two other considerations regarding clearance should be noted. Firstly, migration of macrophages containing virus, either up the mucociliary escalator or to the regional lymph nodes, could remove virus. Secondly, the production and processing of virus antigen during the abortive cycle of replication may be essential for stimulating immune responses; bronchoalveolar macrophages from guinea-pigs inoculated intratracheally with influenza virus were effective as antigen-presenting cells for stimulation of T lymphocyte responses (Lipscomb *et al.* 1983).

Acknowledgements

This work was supported by a grant from the Medical Research Council. We gratefully acknowledge the technical assistance of Mrs R. Hunt, Mrs S. Chalder, Mrs J. Kit and Mrs J. Gem.

References

Bird R.A., Sweet C., Husseini R.H. & Smith H. (1983) The similar interaction of ferret alveolar macrophages with influenza virus strains of differing virulence at normal and pyrexial temperatures. *J. gen. Virol.* **64**, 1807–1810.

Brain J.D. & Valberg P.A. (1979) Deposition of aerosol in the respiratory tract. *Am. Rev. resp. Dis.* **120**, 1325–1373.

Cavanagh D., Mitkis F., Sweet C., Collie M.H. & Smith H. (1979) The localization of influenza virus in the respiratory tract of ferrets: susceptible nasal mucosa cells produce and release more virus than susceptible lung cells. *J. gen. Virol.* **44**, 505–514.

Fazekas de st Groth S. & White D.O. (1958) An improved assay for the infectivity of influenza viruses. *J. Hyg.* **56**, 151–162.

Gould E.A., Ratcliffe N.A., Basarab O. & Smith H. (1972) Studies on the basis of localization of influenza virus in ferret organ cultures. *Br. J. exp. Path.* **53**, 31–36.

Howie A.J., Brown G., Fisher A.G. & Khan M. (1984) Widespread distribution in human tissues of an antigenic determinant of granulocytes. *J. clin. Path.* **37**, 555–559.

Husseini R.H., Sweet C., Bird R.A., Collie M.H. & Smith H. (1983) Distribution of viral antigen within the lower respiratory tract of ferrets infected with a virulent virus: production and release of virus from corresponding organ cultures. *J. gen. Virol.* **64**, 589–598.

Lipscombe M.F., Yeakel-Houlihan D., Lyons C.R., Gleason R.R. & Stein-Streilein J. (1983) Persistence of influenza as an immunogen in pulmonary antigen-presenting cells. *Infect. Immun.* **42**, 965–972.

Mulder J. & Hers J.F. Ph. (1972) *Influenza.* Wolters-Noordhoff, Groningen.

Rodgers B.C. & Mims C.A. (1981) Interaction of influenza virus with mouse macrophages. *Infect. Immun.* **31**, 751–757.

Rodgers B.C. & Mims C.A. (1982*a*) Role of macrophage activation and interferon in the resistance of alveolar macrophages from in-

fected mice to influenza virus. *Infect. Immun.* **36**, 1154–1159.

RODGERS B.C. & MIMS C.A. (1982*b*) Influenza virus replication in human alveolar macrophages. *J. med. Virol.* **9**, 177–184.

SWEET C., STEPHEN J. & SMITH H. (1974*a*) Purification of influenza viruses using disulphide-linked immunosorbents derived from rabbit antibody. *Immunochemistry* **11**, 295–304.

SWEET C., STEPHEN J. & SMITH H. (1974*b*) The behaviour of antigenically related influenza viruses of differing virulence on disulphide-linked immunosorbents. *Immunochemistry* **11**, 823–826.

SWEET C., BIRD R.A., TOMS G.L., WOODWARD C.G. & SMITH H. (1977) Thermal stability and interaction with ferret inflammatory exudates of two clones of influenza virus of differing virulence for both ferrets and man. *Br. J. exp. Path.* **58**, 635–643.

SWEET C., MACARTNEY J.C., BIRD R.A., CAVANAGH D., COLLIE M.H., HUSSEINI R.H. & SMITH H. (1981) Differential distribution of virus and histological damage in the lower respiratory tract of ferrets infected with influenza viruses of differing virulence. *J. gen. Virol.* **54**, 103–114.

TOMS G.L., BIRD R.A., KINGSMAN S.M., SWEET C. & SMITH H. (1976) The behaviour of two closely related clones of influenza virus of differing virulence for man. *Br. J. exp. Path.* **57**, 37–48.