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The role of cellular susceptibility in the declining severity of respiratory influenza of ferrets with age

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Summary. A comparison was made, both *in vivo* and in organ culture, between newborn (1-day-old) and suckling (15-day-old) ferrets of lower respiratory tract tissue infected with a virulent strain (clone 7a) of influenza virus. Newborn ferrets were killed by influenza virus following intranasal inoculation but suckling ferrets were almost as resistant as adult ferrets. In newborn ferrets there was a rapid, severe and progressive infection of lung tissue with infection of alveolar cells as well as those of bronchial and bronchiolar epithelium (assessed by monitoring virus infectivity and by fluorescent antibody staining). In suckling ferrets, as previously shown for adult animals, the lung infection was less severe, less persistent and confined to the epithelium of bronchi with only a small bronchiolar involvement and even less alveolar cell infection. These differences observed *in vivo* were repeated in organ cultures obtained from various areas of the lung, i.e. alveolar and airway epithelial cells of newborn ferrets exhibited a greater susceptibility than those of older ferrets. Thus, it appears that one factor determining the greater susceptibility of the lower respiratory tract of newborn ferrets is a greater inherent susceptibility of alveolar and airway epithelial cells to infection with influenza virus. Other factors may also be involved and have yet to be investigated.

Keywords: Cellular susceptibility, respiratory influenza, resistance

Influenza is a common respiratory infection of young children and, although in general its effects may be mild (Douglas 1975), convulsions and croup can occur (Stuart-Harris & Schild 1976) as can bronchiolitis, bronchiectasis and pneumonia, the latter leading to death in some cases (Laraya-Cuasay *et al.* 1977; Paisley *et al.* 1978; Kim *et al.* 1979; Glezen 1980; Murphy *et al.* 1981).

As a possible model for infant human infection newborn ferrets were infected intranasally with clone 7a of the recombinant influenza virus A/PR/8/34-A/England/939/69 (H3N2) (Collie *et al.* 1980). This infection proved to be invariably fatal. All influenza virus-infected newborn ferrets showed severe involvement of the upper respiratory tract and some died, apparently from obstruction of the airways (Collie *et al.* 1980). However, a significant proportion appear to have died of uncomplicated influenzal pneumonia (Collie *et al.* 1980). This contrasted with the mild, transient illness that occurred with infected adult animals where viral growth in, and damage of, the lower respiratory tract was minimal and restricted to airway (particularly bronchial) epithelium not alveoli (Toms *et al.* 1976;

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Sweet *et al.* 1981; Husseini *et al.* 1983). The greater susceptibility of the lower respiratory tract of newborn ferrets might result from: a greater susceptibility of alveolar and/or airway epithelial cells compared with corresponding adult cells; a greater proportion of airway (i.e. ciliated) epithelium relative to alveolar tissue in the lungs of newborn animals; fewer or less mature alveolar macrophages; or a combination of these three factors. This paper describes an investigation of the first of these possible reasons for the greater susceptibility.

Previous studies (Cavanagh *et al.* 1979*a*) indicated that, by 2 weeks of age, ferrets might be approaching the adult situation with regard to the susceptibility of their lung tissue to influenza virus infection. Hence comparisons have been made between the pattern of infection in newborn (1-day-old), suckling (15-day-old) and adult (> 3-month-old) animals. Firstly, the lethality of clone 7a for suckling ferrets was determined for comparisons with those previously recorded for newborn and adult ferrets. Secondly, the amount and distribution of influenza virus in the respiratory tract regions of newborn and suckling ferrets after influenza virus infection was determined, both by isolation of infectious virus and by fluorescent antibody staining of tissue sections for comparison with previously published data for the adult ferret (Sweet *et al.* 1981; Husseini *et al.* 1983). Thirdly, the ability of tissues to support influenza virus replication in organ culture was determined for different regions of the lungs of newborn, suckling and adult ferrets and the nature and extent of the infected cells in these cultures were investigated by fluorescent antibody staining.

Materials and methods

Strain of influenza virus. The recombinant influenza virus A/PR/8/34-A/England/939/69 clone 7a (H3N2) was described previously together with its assay and the preparation of seed and working stocks (Gould *et al.* 1972; Sweet *et al.* 1974*a,b*).

Ferrets. Adult female ferrets, obtained from A.S. Roe, Little Fakenham, Norfolk and tested for absence of serum antibodies to H3N2 strains of influenza virus, were housed individually and mated throughout the year as described by Sweet *et al.* (1977).

Inoculation of newborn and suckling ferrets and assessment of virus in the respiratory tract. Newborn (1-day-old) ferrets were obtained as described previously (Sweet *et al.* 1977; Collie *et al.* 1980) and inoculated intranasally, without anaesthesia, with 0.05 ml of phosphate-buffered saline (Dulbecco A) (PBSA) containing $10^{0.9}$ $EBID_{50}$ (50% egg-bit infectious doses) of clone 7a as described by Collie *et al.* (1980). Respiratory tissues were removed from dead or killed newborn ferrets on days 1–9 after inoculation and the virus infectivity in homogenates of nasal turbinates, trachea and hilar and outer (a combination of the intermediate and alveolar) zones of the lung determined as described previously (Sweet *et al.* 1981). Infectivity assays were performed in eggs and results quoted as 50% egg infectious doses (EID_{50}) (Sweet *et al.* 1974*a*). Suckling (15-day-old) ferrets were inoculated intranasally with 0.1 ml of PBSA containing $10^{0.9}$ $EBID_{50}$ or $10^{6.0}$ $EBID_{50}$ of virus as described above. In some experiments the animals were observed for 9 days and the number of deaths recorded. In others, animals were killed at intervals and infectious virus estimated in the respiratory tract tissues as described for the newborn animals.

Immunofluorescent examination of sections of lung tissue from infected animals. This was performed as described by Husseini *et al.* (1983) using rabbit anti-7a IgG and fluorescein-labelled sheep antiserum to rabbit IgG (Wellcome Reagents, Beckenham, England).

In an attempt to quantify airway and alveolar cell fluorescence sections were examined from both the hilar and outer zones of the lung lobes of newborn and suckling ferrets at intervals after inoculation.

Organ cultures. Tissue was obtained from the hilar, intermediate and alveolar zones of adult ferret lungs (Sweet *et al.* 1981). Due to their smaller size, lungs from newborn and suckling ferrets were divided into only two zones, outer (intermediate plus alveolar) and hilar. Small pieces of tissue were prepared as described previously (Kingsman *et al.* 1977; Cavanagh *et al.* 1979*b*; Husseini *et al.* 1983) and inoculated with $10^{4.7}$ $EBID_{50}$ of virus as soon as possible after removal from the animal ('fresh' cultures; see Kingsman *et al.* 1977). The Petri dishes containing the inoculated tissue were enclosed in air-tight containers, gassed with 5% CO_2 in air and completely immersed in water baths at either 34 or 39°C to simulate the temperatures of the lungs of newborn and adult ferrets respectively (Belshe *et al.* 1978). Virus yields in the culture medium and in homogenates of tissues were assayed 24 h after inoculation.

Sections of lung organ culture tissue pieces were stained with fluorescent antibody, as described above, and the distribution of specific fluorescence assessed.

Results

Lethality of influenza virus clone 7a for suckling (15-day-old) ferrets

No deaths were recorded in 17 suckling ferrets inoculated intranasally with $10^{0.9}$ $EBID_{50}$ of clone 7a, an inoculation dose which Collie *et al.* (1980) found to be invariably fatal for newborn ferrets. A few deaths (three of 25 animals) were seen, however, by 9 days after inoculation in suckling ferrets inoculated with $10^{6.0}$ $EBID_{50}$ of influenza virus clone 7a; this dose is not normally fatal for adult ferrets (Toms *et al.* 1976).

Virus content of the respiratory tissues of newborn (1-day-old) and suckling ferrets after inoculation with clone 7a

Newborn ferrets were inoculated intranasally with $10^{0.9}$ $EBID_{50}$ of clone 7a and the respiratory tract tissues removed from dying or killed ferrets on days 1–9 after inoculation. The yields of virus in the nasal turbinates, trachea and the pooled hilar and outer regions of all lung lobes were determined and the mean results from two to five animals on each day are shown in Fig. 1*a*. Titres in all four respiratory tract tissues reached high levels (approximately $10^{6.0}$ $EBID_{50}$) 3 days after inoculation and high titres were still present in animals dying 8 and 9 days after inoculation. Generally, virus titres tended to be higher in the lower respiratory tract tissues than in the upper respiratory tract with titres from hilar lung tissue exceeding those from nasal turbinates by approximately 10-fold on days 4–8 after inoculation.

In suckling ferrets inoculated intranasally with $10^{0.9}$ $EBID_{50}$ of clone 7a, virus titres in the nasal turbinates were similar, up to 7 days after inoculation, to those seen in ferrets inoculated at 1 day old (Fig. 1*b*). However, markedly lower titres, compared with those found in newborn animals, were present in the trachea and hilar and outer lung tissues. Furthermore, lower respiratory tract infection was decreasing by 7–8 days after inoculation and by 9 days virus was virtually undetectable, contrasting with the high levels present in all four respiratory tissues of ferrets inoculated at 1 day old. In suckling ferrets inoculated with $10^{6.0}$ $EBID_{50}$ of clone 7a (Fig. 1*c*), as expected the infection proceeded more rapidly than that for the lower dose. However, although virus titres reached similar levels to those seen in newborn ferrets (Fig. 1*a*) in all four respiratory tissues on days 2–4 after inoculation, rapid elimination of

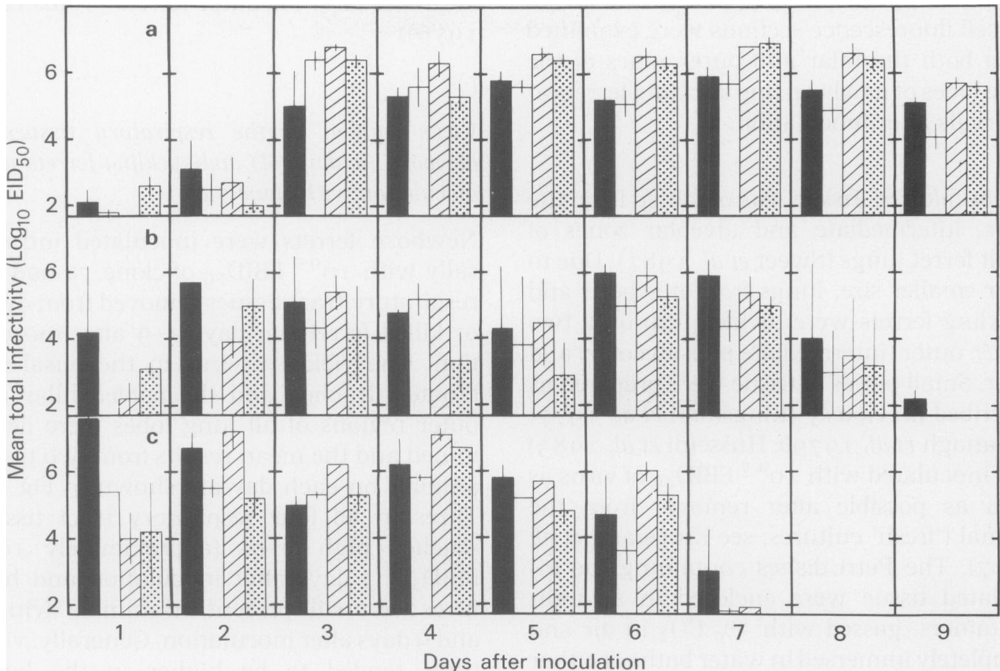

Fig. 1. Mean total virus titres (Log_{10} EID_{50}) on days 1 to 9 after inoculation in the nasal turbinates (■), trachea (□) and hilar (▨) and outer (⊡) zones of lungs from (*a*) newborn ferrets (39 animals in total) inoculated intranasally with $10^{0.9}$ $EBID_{50}$ of influenza virus clone 7a, (*b*) suckling ferrets (18 animals in total) inoculated intranasally with $10^{0.9}$ $EBID_{50}$ of clone 7a and (*c*) suckling ferrets (16 animals in total) inoculated intranasally with $10^{6.0}$ $EBID_{50}$ of clone 7a. Virus titres for (*a*) are means from four to five animals either killed or dying per day after inoculation. Virus titres for (*b*) and (*c*) are means from two animals killed per day after inoculation.

virus occurred thereafter and none could be detected by day 8 after inoculation.

Distribution of influenza virus-infected cells in lungs of newborn and suckling ferrets after inoculation with clone 7a

Table 1 summarizes the results of total and fluorescing bronchi, bronchioles, respiratory bronchioles and alveolar cells in the lungs of newborn ferrets inoculated with $10^{0.9}$ $EBID_{50}$ of clone 7a and killed 3, 5 and 7 days after inoculation.

Results were similar for both the hilar and outer zones, with most of the bronchi, about a quarter of the bronchioles and approximately 10% of the respiratory bronchioles showing fluorescence (Table 1). There was no evidence of a progressive pattern to the fluorescence since the numbers of airways fluorescing on days 3, 5 and 7 were similar. The lumens of some bronchioles, especially at later times, contained plugs of fluorescing cell debris, and fluorescing epithelial cells appeared to be detached from the nasal membrane (Fig. 2*a*). In contrast to previous results with adult ferrets (Husseini *et al.* 1983) many fluorescing cells were seen in the alveolar region on days 3 and 5 after inoculation, decreasing by day 7 (Table 1). These cells were usually seen in close association with each other, indicating a focus of infection, and often in regions where the alveoli contained cell debris (Fig. 2*b*).

Table 1. Enumeration of bronchi, bronchioles, respiratory bronchioles and alveolar cells containing antigen in the two lung zones of newborn ferrets infected with $10^{0.9}$ $EBID_{50}$ of clone 7a

Lung region	Days after inoculation	Bronchi		Bronchioles		Respiratory bronchioles		No. of fluorescing alveolar cells per section
		Total†	% fluorescing*	Total†	% fluorescing*	Total†	% fluorescing*	
Hilar	3	185	80(9)	1433	14(2)	856	11(2)	81.2(21.0)
	5	131	87(6)	1505	28(9)	778	13(5)	45.0(21.4)
	7	239	89(6)	987	30(17)	819	17(11)	23.2(17.3)
Outer	3	109	74(8)	966	21(7)	1002	15(7)	159.6(84.8)
	5	117	95(4)	796	27(3)	621	14(5)	34.4(15.1)
	7	171	78(9)	1111	21(15)	862	9(7)	8.4(7.5)

* Mean (SEM in parentheses) % bronchi, bronchioles and respiratory bronchioles showing infected (antigen-containing) cells.

† The figures represent the total number of bronchi, bronchioles and respiratory bronchioles counted for all lung lobes of four animals at each time point. Between three and 10 sections (30 to 50 fields/section) from the hilar and outer regions were examined for each animal. Bronchi were defined as airways totally or partially surrounded by mural cartilage; bronchioles were not surrounded by cartilage and respiratory bronchioles were bronchioles which opened into alveolar ducts.

In suckling ferrets infected with $10^{0.9}$ $EBID_{50}$, however, fluorescence was restricted almost exclusively to bronchial epithelium with in general $<50\%$ of the bronchi involved (Table 2). Fewer than 10% of the bronchioles were involved, even fewer of the respiratory bronchioles and only the occasional alveolar cell (Table 2). There appeared to be a gradual decrease in the proportion of bronchi showing fluorescence from days 3 to 7 (Table 2). The lack of alveolar involvement was similar to that observed in adult ferrets (Husseini *et al.* 1983). This picture remained essentially unchanged in suckling ferrets inoculated with $10^{6.0}$ $EBID_{50}$ (Table 3).

Production and release of virus from organ cultures of ferret lung tissue

In organ cultures of lung from newborn ferrets virus production was high for both hilar and outer tissue, irrespective of the temperature of incubation (Table 4). Release of virus into the culture medium was generally higher from hilar than from outer lung tissue, but only marginally so.

Virus production in lung organ cultures from suckling ferrets was 10- to 100-fold lower than that from newborn ferrets and again showed no significant variation with lung region or temperature of incubation (Table 4). Release of virus into the culture medium was higher in cultures of hilar lung tissue than in cultures of outer lung tissue, especially those incubated at 34°C.

Results obtained for organ cultures of adult ferret hilar, intermediate and alveolar lung tissue (Table 4) were consistent with those described by Husseini *et al.* (1983) and similar to those obtained with suckling ferret lung tissue (if the outer lung zone of the latter is taken to be equivalent to a mixture of the intermediate and alveolar zones of the adult lung).

Tissue from lung organ cultures of newborn and suckling ferrets incubated at 34°C was sectioned and stained as described above and the numbers of bronchi, bronchioles and alveolar cells containing fluorescence deter-

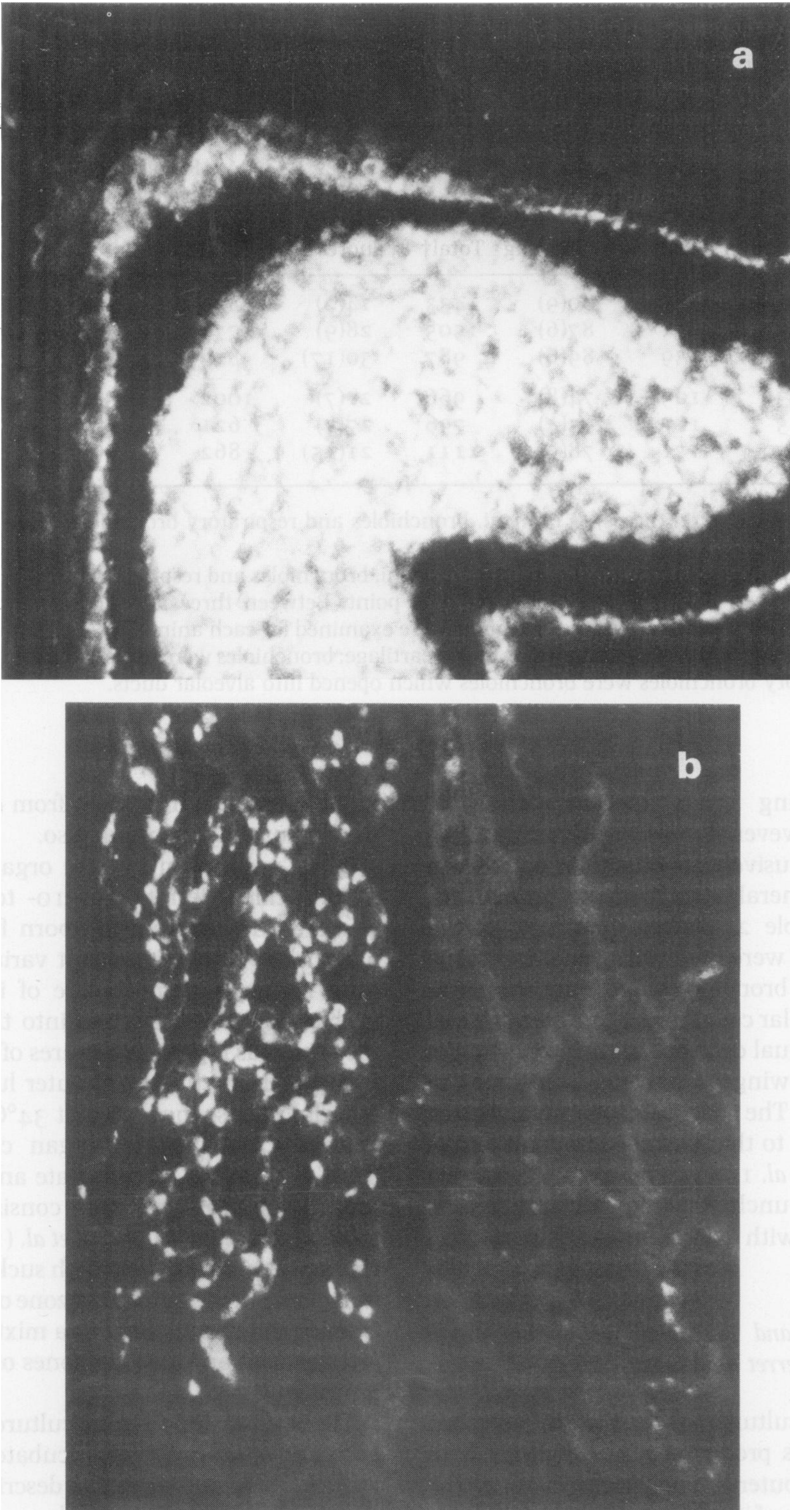

Fig. 2. Fluorescence in cells of different regions of the lower respiratory tract of newborn ferrets infected with clone 7a. (*a*) Bronchiole in hilar region, × 170, showing a plug of fluorescing cell debris; (*b*) alveolar cells in outer region, × 170.

mined. The results (Table 5) showed that similar numbers of bronchi exhibited fluorescence in the hilar zones of both newborn and suckling ferrets but a striking difference was that approximately 10-fold more alveolar cells fluoresced in the lung cultures of newborn ferrets than in similar cultures of suckling animals. In addition a much greater proportion of bronchioles fluoresced in the lung cultures of newborn ferrets.

Table 2. Enumeration of bronchi, bronchioles, respiratory bronchioles and alveolar cells containing antigen in the two lung zones of suckling ferrets infected with $10^{0.9}$ $EBID_{50}$ of clone 7a

Lung region	Days after inoculation	Bronchi		Bronchioles		Respiratory bronchioles		No. of fluorescing alveolar cells per section
		Total†	% fluorescing*	Total†	% fluorescing*	Total†	% fluorescing*	
Hilar	3	149	38(2)	553	1(0)	356	0(0)	0.2(0.2)
	5	144	31(2)	871	3(3)	436	0(0)	0.15(0.15)
	7	174	16(12)	836	2(2)	584	0(0)	0(0)
Outer	3	109	49(9)	954	2(1)	398	0(0)	0.6(0.3)
	5	121	5(5)	1395	0(0)	841	0.5(0.5)	0.9(0.9)
	7	106	4(1)	1088	1(1)	610	0(0)	0(0)

* Mean (SEM in parentheses) % bronchi, bronchioles and respiratory bronchioles showing infected (antigen-containing) cells.

† The figures represent the total number of bronchi, bronchioles and respiratory bronchioles counted for all lung lobes of two animals at each time point. Between six and 10 sections (60 to 70 fields/section) from the hilar and outer regions were examined for each animal. The distinction between bronchi, bronchioles and respiratory bronchioles is outlined in Table 1.

Table 3. Enumeration of bronchi, bronchioles, respiratory bronchioles and alveolar cells containing antigen in the two lung zones of suckling ferrets infected with $10^{6.0}$ $EBID_{50}$ of clone 7a

Lung region	Days after inoculation	Bronchi		Bronchioles		Respiratory bronchioles		No. of fluorescing alveolar cells per section
		Total†	% fluorescing*	Total†	% fluorescing	Total†	% fluorescing*	
Hilar	3	166	25(7)	1171	1(0)	407	1(0)	0(0)
	5	254	53(2)	1082	7(4)	538	0(0)	0.25(0.25)
	7	230	0(0)	1090	0(0)	500	0(0)	0(0)
Outer	3	121	1(1)	830	0(0)	325	0(0)	0(0)
	5	131	14(6)	847	4(3)	295	0(0)	0.8(0.8)
	7	141	0(0)	1158	0(0)	462	0(0)	0(0)

* Mean (SEM in parentheses) % bronchi, bronchioles and respiratory bronchioles showing infected (antigen-containing) cells.

† The figures represent the total number of bronchi, bronchioles and respiratory bronchioles counted for all lung lobes of two animals at each time point. Between eight and 10 sections (60 to 70 fields/section) from the hilar and outer regions were examined for each animal. The distinction between bronchi, bronchioles and respiratory bronchioles is outlined in Table 1.

Table 4. Production and release of influenza virus clone 7a by fresh cultures of lung tissue from newborn, suckling and adult ferrets incubated at 34°C and 39°C

Type of ferret	Tissue	Experiment no.	Virus yield at 34°C: Total* (log_{10} $EBID_{50}$)	34°C: In culture medium (% of total)	Virus yield at 39°C: Total* (log_{10} $EBID_{50}$)	39°C: In culture medium (% of total)
Newborn	Hilar	1	5.7 (0.1)	20 (6)	6.4 (0.1)	15 (5)
		2	6.2 (0.1)	21 (6)	6.0 (0.1)	23 (7)
		3	5.9 (0)	19 (5)	6.1 (0.1)	21 (3)
	Outer	1	5.7 (0.1)	13 (3)	6.3 (0.1)	6 (3)
		2	5.8 (0.1)	18 (5)	6.0 (0)	19 (7)
		3	5.8 (0.1)	6 (2)	5.9 (0.1)	8 (2)
Suckling	Hilar	1	3.7 (0.2)	34 (13)	3.8 (0.4)	16 (9)
		2	4.7 (0.1)	14 (4)	4.8 (0.1)	13 (7)
		3	4.6 (0.2)	9 (4)	4.5 (0.2)	9 (3)
	Outer	1	3.6 (0.1)	2 (1)	4.3 (0.1)	12 (8)
		2	4.6 (0.1)	5 (2)	4.9 (0.1)	3 (2)
		3	4.4 (0.3)	4 (1)	5.0 (0.1)	13 (7)
Adult†	Hilar	1	4.3 (0.3)	11 (4)		
		2			4.6 (0.2)	26 (7)
	Intermediate	1	3.8 (0.1)	10 (3)		
		2			4.3 (0.2)	18 (5)
	Alveolar	1	3.7 (0.2)	4 (1)		
		2			4.1 (0.1)	2 (1)

* Organ cultures were prepared and inoculated ($10^{4.7}$ $EBID_{50}$/culture) as described in materials and methods. Figures represent the mean of yields from six replicate cultures (SEM in parentheses) 24h after infection.

† Only one experiment at each temperature is included because the results were similar to those published previously (Husseini *et al.* 1983).

Table 5. Enumeration of bronchi, bronchioles and alveolar cells containing antigen in clone 7a-infected organ culture tissue from the two lung zones of newborn and suckling ferrets

Type of ferret	Lung region	Experiment no.	Bronchi: Total	Bronchi: % fluorescing	Bronchioles: Total	Bronchioles: % fluorescing	Fluorescing alveolar cells: No. per section	Fluorescing alveolar cells: Total no. of sections examined*
Newborn	Hilar	1	6	33	145	49	49.1	9
		2	24	88	53	38	26.8	6
	Outer	1	0	—	125	86	62.2	10
		2	0	—	95	73	64.6	5
Suckling	Hilar	1	8	50	210	6	6.0	5
		2	13	15	23	0	2.5	4
	Outer	1	6	0	99	10	5.2	5
		2	0	—	89	12	16.8	5

* Each section consists of the transects of the six organ culture tissue pieces from one culture taken 24 h after inoculation.

Discussion

The present and previous results clearly demonstrate an age-related susceptibility of ferrets to infection with influenza virus. Adult ferrets infected with $10^{6.0}$ $EBID_{50}$ of clone 7a suffered a transient infection of both upper and lower respiratory tracts (Toms *et al.* 1976; Sweet *et al.* 1981). In contrast newborn ferrets infected with approximately 100 000-fold less virus ($10^{0.9}$ $EBID_{50}$) all died (Collie *et al.* 1980). By 15 days of age suckling ferrets had become resistant to the lethal effects of influenza virus even when the high inoculum was used. Clearly many factors may be involved in these age-related differences but one factor seems to be an increased inherent susceptibility of the cells of the lower respiratory tract of newborn ferrets.

In newborn animals, virus replicated in both the airways and alveolar cells producing high titres of virus in the lower respiratory tract. In contrast, in suckling and adult ferrets virus replicated relatively poorly in the lower respiratory tract being limited mainly to bronchial epithelium with little or no alveolar involvement. That these differences in age-related susceptibility were partially, at least, a reflection of differences in cell susceptibility was established when similar differences were observed in organ cultures. Cultures of tissue from both hilar and outer regions of the lungs of newborn ferrets produced 10- to 100-fold more virus compared with similar cultures from suckling and adult ferrets (Table 4; Husseini *et al.* 1983). In addition, immunofluorescent studies indicated that, as *in vivo*, this was due to increased virus replication in bronchiolar epithelial cells and alveolar cells (Table 5; Husseini *et al.* 1983) indicating that both ciliated and alveolar cells were more susceptible in newborn animals than in older animals.

Another factor possibly contributing to enhanced spread of infection in the alveoli of newborn ferrets *in vivo* is greater release of virus by cells in the outer region of the lung as observed in organ culture. Such cultures released into the medium about 12% of the total virus produced while similar cultures from suckling ferrets released only about 2–4% (Table 4). Organ cultures of alveolar tissue from adult ferrets also showed a similar poor release of virus into the culture medium (Husseini *et al.* 1983). However, such adult tissue contained mainly alveolar tissue with few bronchioles and no bronchial tissue while the cultures of the outer regions of both newborn and suckling ferrets did contain airway epithelium. Thus, whether these differences in release reflect differences in the amount of ciliated epithelium in the various cultures or differences in alveolar cells between newborn and suckling or adult ferrets is unknown at present.

The age-dependence of influenza virus growth in ferret lung demonstrated in the present study is strikingly similar to that observed with respiratory syncytial virus (RSV), another important pathogen of human infancy (Prince & Porter 1976; Porter *et al.* 1980). The differential susceptibility of animals with age was reflected in organ cultures suggesting that the age-dependence of RSV replication in ferret lung *in vivo* was a reflection of cellular or tissue maturation rather than immunologic factors.

However, while differences in cell susceptibility play an important role in early stages of infection with influenza virus, other factors may be equally important later. In newborn ferrets, although there is a progressive decrease in the number of infected alveolar cells after day 3, possibly because of loss of damaged cells, virus is not eradicated and the animals die whereas older animals recover from the infection. This difference is not reflected in the amount of virus replication since virus titres in the lungs of suckling ferrets inoculated with $10^{6.0}$ $EBID_{50}$ attain levels similar to those in newborn ferrets. They are more likely to reflect differences in host responses since the host response in newborn animals is immature and inadequate to eradicate virus infections and differ-

ent components of the host response mature at different times after birth. Thus, macrophages from neonatal animals are generally defective in their ability to kill virus; most studies have shown that macrophages lose this defectiveness by 3–4 weeks of age (Mogensen 1979). Natural killer-cell activity is also low at birth (Welsh 1981) and newborn animals are generally poor at regulating body temperature (Roberts 1979) and produce less interferon (Smith 1977). Specific immune responses, too, are not fully functional until well after birth (Andersson *et al.* 1981). In addition factors in maternal milk may also play a role in the recovery of suckling ferrets. While transmission of specific anti-influenza antibody and T lymphocytes could not occur because mothers were non-immune, other factors such as macrophages, lymphocytes and neutrophils are present in milk and can be transmitted to the gut during suckling (Pittard 1979), and such lymphocytes may be induced by viruses to produce interferon *in vitro* (Emodi & Just 1974). In addition, a soluble factor in milk has been shown to stimulate the local secretory immune response of breast-fed human infants (Pittard 1979). However, whether such factors contribute to the age-related susceptibility of ferrets must remain conjectural since the leucocyte content of milk declines over the first few days and, furthermore, the actual migration of milk leucocytes through the wall of the gastrointestinal tract of newborn animals or human infants has never been reported (Pittard 1979). Also, since such changes occur early, newborn ferrets, which in some cases may be suckled for up to 10 days before death, might be expected to be protected by such factors. Whether one or more of these host responses (neonatal or maternal) is involved in the age-related differences reported here is unknown.

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