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THE PREGNANT FERRET AS A MODEL FOR STUDYING THE CONGENITAL EFFECTS OF INFLUENZA VIRUS INFECTION *IN UTERO*: INFECTION OF FOETAL TISSUES IN ORGAN CULTURE AND *IN VIVO*

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Summary.—Organ cultures of ferret foetal tissues showed a similar pattern of susceptibility to influenza virus to that already observed for human foetal tissues (Rosztoczy *et al.*, 1975); respiratory, alimentary and urogenital tissues supported the replication of influenza virus but nervous and lymphopoietic tissues (those which, in man, are associated with foetal or postnatal abnormalities) were insusceptible. In contrast to corresponding human tissues, ferret foetal placenta and amnion readily supported viral replication although both human and ferret umbilical cord were susceptible.

In limited experiments, neither the membranes nor the susceptible foetal tissues became infected after intranasal inoculation of pregnant ferrets of various gestational ages. However, after intracardial inoculation of pregnant ferrets with high titre virus (*ca* 10^9 $EBID_{50}$) virus was isolated from both foetal membranes and foetuses. The membranes became infected at early, middle and late gestation, but virus appeared to cross the placental barrier to infect foetal tissues only in late gestation. At this stage virus could be isolated not only from those foetal tissues (respiratory, alimentary and urogenital) susceptible in organ culture, but also in small amounts from tissues which were insusceptible in organ culture (heart, lymphopoietic and nervous tissue). Virus was also isolated from foetal membranes and foetuses of late gestation ferrets following intracardial inoculation with a one hundred-fold lower dose of virus which, unlike the higher dose, did not induce a maternal febrile response.

The pregnant ferret appears to be a suitable model for investigating the effects on development of foetal infection with influenza virus but it may have disadvantages with regard to the nature and strength of the placental barrier.

THERE is increasing interest in a possible correlation between influenza during human pregnancy and congenital malformations of the central nervous system, increased incidence of abortion, stillbirths and prematurity or neoplasms of lymphopoietic tissues in childhood (Mackenzie and Houghton, 1974). The evidence for such a correlation comes from retrospective and prospective epidemiological studies, few of which have shown serological confirmation of influenza. It is not surprising that contradictory results have been obtained, due to the dependence upon maternal memory bias, the necessity for large population samples and the difficulties in recognizing subclinical infections. Nevertheless, there are isolated reports of foetal membrane and foetal infection with influenza virus. Thus virus was isolated from foetal tissues in fatal influenza where transplacental passage of virus probably occurred during late gestation (Greenberg *et al.*, 1958; Jewett, 1974; Yawn *et al.*, 1971) and from 10 newborn children who died of influenzal pneumonia during the first hours or within 2 days of birth (Kornyu-

shenko and Maximovich, 1961). Also, following influenza during pregnancy anti-influenza IgM antibody was detected in 4 of 64 cord sera and lymphocyte sensitization in 3 of 16 normal neonates (Ruben, Winkelstein and Sabbagha, 1975). These indications that influenza may have congenital effects has aroused sufficient interest to warrant the search for an animal model to investigate the situation experimentally.

Influenza virus was teratogenic in the developing embryo of the chick (Hamburger and Habel, 1947; Johnson, Klasnja and Johnson, 1971) and rhesus monkey (London, Kent and Sever, 1973; London *et al.*, 1975) when inoculated directly into the embryo. In the pregnant mouse inoculated intranasally, placental and foetal infection occurred with abnormal foetal development manifested by increased foetal resorptions, less viable foetuses that survived less well after birth and occasionally malformed foetuses (Molnarovo and Blaskovic, 1975; Takeyama, 1966). However, in the embryo-inoculation experiments, the influence of host barriers, such as the placenta, to infection was ignored. In addition, mouse influenza with adapted strains of virus is unlike that in man, involving predominantly the lung rather than the upper respiratory tract.

Previous observations demonstrating that influenza in the ferret was similar to the disease in man (Stuart-Harris, 1965; Toms *et al.*, 1976) and that both ferret and human uterus were susceptible to influenza virus in organ culture (Basarab and Smith, 1969, 1970; Rosztoczy *et al.*, 1973, 1975) prompted an investigation of the ferret to study the possible congenital effects of influenza virus infection *in utero*. This paper describes the results of attempts to infect ferret foetal membranes and tissues in organ culture and *in vivo*. An influenza virus (A/PR/8-A/England/939/69 Clone 7a (H_3N_2)) virulent for both man (Beare and Hall, 1971) and ferrets (Toms *et al.*, 1976) was used. A preliminary report has appeared (Sweet *et al.*, 1976).

MATERIALS AND METHODS

Influenza virus.—The recombinant virus A/PR/8-A/England/939/69 Clone 7a (H_3N_2) and the preparation of virus stocks were described by Sweet, Stephen and Smith (1974*b*) and Gould *et al.* (1972).

Infectivity assays.—The egg-bit assay of Fazekas de St. Groth and White (1958) and the egg assay were used as described by Sweet *et al.* (1974*a*).

Ferret impregnation.—Ferrets obtained from A. S. Roe, Little Fakenham, Norfolk, and tested for absence of serum antibodies to H_3N_2 strains of influenza virus were housed individually and mated throughout the year by manipulation of the photoperiod (Hammond, J. Jr., personal communication). Female ferrets were maintained on a 6-h light cycle which delayed or eliminated the onset of oestrus. When required for mating they were placed on a 14-h light cycle to stimulate the onset of oestrus which took approximately 11 weeks to become apparent as a swelling of the vulva. Mating was carried out after full vulval swelling had been attained. Females were mated with 2 males in a 30-h period to improve the rate of successful matings and Day 1 of pregnancy was taken as the day after the introduction of the first male. Pregnancy was indicated by a reduction in the size of the vulva and successful palpation at Days 17–21 post coitus. Early, middle and late gestation were taken as being from 12–20, 21–29 and 30–42 days post coitus respectively as implantation does not occur until 12 days post coitus (Enders and Schlafke, 1972). Male ferrets received a constant 6-h photoperiod which maintained them sexually active almost indefinitely.

Inoculation of ferrets.—Inoculations were made intracardially under ether anaesthesia with virus suspensions in 1 ml of phosphate-buffered saline (Dulbecco A (PBSA)). Any virus that might have remained on the fur was inactivated by swabbing with 1% iodine in ethanol. Intranasal inoculations were made as described by Toms *et al.* (1976).

Rectal temperatures.—These were taken and the febrile response defined as described by Toms *et al.* (1976).

Nasal and lung washes.—These were performed as described by Toms *et al.* (1976).

Organ cultures, inoculation and measurement of infection.—Organ cultures (Hoorn and Tyrrell, 1965) were inoculated and susceptibility defined as described by Rosztoczy *et al.* (1975).

Tissue collection and maceration for virus assay.—Tissues were collected and macerated as described by Toms *et al.* (1976) but the medium used was Hanks' balanced salt solution (Wellcome Reagents Ltd., Beckenham, Kent) containing 1% w/v bovine serum albumin and

0·16 mg/ml Crystamycin and 0·025 mg/ml Acromycin. Pieces larger than 1–2 g were macerated in 13 ml of medium. The macerates were centrifuged (2000 *g*) for 15 min at 4°. For isolation of virus from foetal tissues, the foetuses were washed 3 times in Hanks' solution and individual foetal tissues removed with separate, clean, sterile instruments. The tissues were macerated as above and centrifuged. The macerates of all tissues were passaged twice in 10–12-day-old embryonated hens' eggs to eliminate any effect of inhibitors in the macerate (Toms *et al.*, 1976).

RESULTS

Susceptibility of ferret foetal membranes and foetal tissues in organ culture to influenza virus infection

Ferret foetal respiratory, alimentary and urogenital tissues supported the replication of influenza virus, as did the kidney, but nervous and lymphopoietic tissues were insusceptible (Table I). In addition, all the foetal membranes examined (placenta, haematoma, umbilical cord, amnion and chorion) supported virus replication (Table I). Most susceptible tissues from foetuses of late gestational age (nasal mucosa, trachea, lung, eosophagus, bladder, kidney, placenta, haematoma, umbilical cord and membranes (amnion and chorion)) produced high titres of virus ($\geqslant 10^{4\cdot5}$EBID$_{50}$/ml) and virus persisted for at least 7 days (Fig. 1). Large and small intestine from foetuses tested at late gestation and whole foetuses of early gestational age produced somewhat lower titres of virus ($\simeq 10^{3\cdot5}$EBID$_{50}$/ml) but again virus persisted for up to 7 days (Fig. 1).

TABLE I.—*Susceptibility of Organ Cultures of Ferret Foetal Tissues to Infection with Influenza Virus (Clone 7a)*

Tissue	Susceptibility*
Nasal mucosa	+
Trachea	+
Lung	+
Oesophagus	+
Small intestine	+
Large intestine	+
Bladder	+
Kidney	+
Meninges	−
Brain	−
Heart	−
Thymus	−
Spleen	−
Liver	−
Placenta	+
Haematoma	+
Umbilical cord	+
Amnion	+
Chorion	+
Whole foetus	+

Foetal tissues were examined at 37 days' gestation. Whole foetuses at 20 days' gestation were cut into 6 organ culture bits. Foetal membranes were examined at three intervals during gestation (Days 20–37).

* Tissues were designated susceptible if at least 2 of the media samples taken on Days 2, 3 and 4 after inoculation ($10^{4\cdot8}$–$10^{5\cdot5}$EBID$_{50}$) contained detectable infectivity ($>0\cdot5$ log$_{10}$EBID$_{50}$/ml). All tissues were examined at least 3 times with similar results.

Attempts to isolate virus from foetal and maternal tissues following intranasal infection of pregnant ferrets

Groups of ferrets in early, middle and late gestation were inoculated intranasally with 10^{6}EBID$_{50}$ of virus. Nasal washes were taken on Day 1 after inoculation for infectivity titrations to confirm successful infection. Ferrets were killed at various times (1–9 days) post infection and foetal membranes and foetuses examined for virus. Foetal membranes (placenta, haematoma, umbilical cord, amnion and chorion) were pooled for testing but foetuses were examined individually. Although the ferrets exhibited a typical response to intranasal inoculation (Toms *et al.*, 1976) with high titres of virus in nasal washes taken on Day 1, virus in lung washes for 5–7 days and febrile responses (Table II), virus could not be recovered from foetal membranes, foetuses or any maternal extra-respiratory tissue except an isolation from 1 spleen (Ferret 58) only detectable after 2 passages in eggs.

Virus isolations from foetal and maternal tissues following intracardial inoculation of pregnant ferrets

Ferrets in early, middle and late gestation were inoculated intracardially with $10^{9\cdot4}$EBID$_{50}$ influenza virus. A

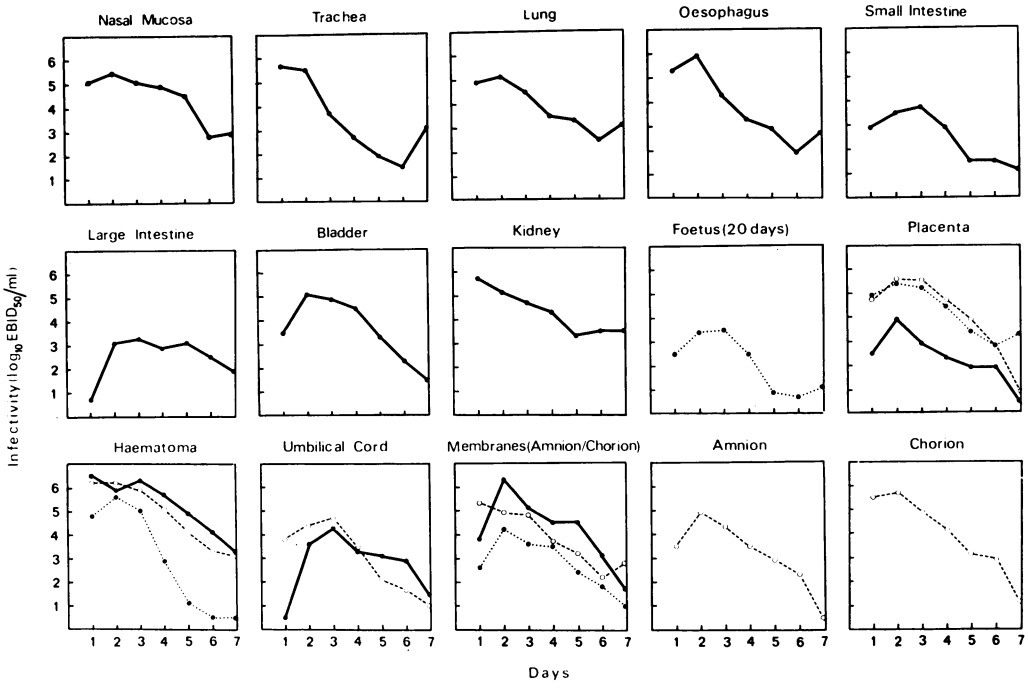

FIG. 1.—Virus released into the medium of organ cultures of ferret foetal tissues, inoculated with $10^{4.8}$–$10^{5.5}$EBID$_{50}$ of A/PR/8-A/England/939/69 Clone 7a (H_3N_2). The graphs show the results of typical experiments. Individual foetal tissues were examined at late gestation (37 days) and whole foetuses at early gestation (20 days). Foetal membranes were examined at intervals throughout gestation (Days 20–37). ●.....● early gestation; ○- - - -○ middle gestation; ●——●——● late gestation.

TABLE II.—*Virus Isolations from Maternal and Foetal Tissues and Temperature Responses after Intranasal Inoculation of Pregnant Ferrets at Early, Middle and Late Gestation with $10^{6.0}$ EBID$_{50}$ Clone 7a Influenza Virus*

Ferret no.	Day killed post infection	Virus ($\log_{10}$EBID$_{50}$/ml) in nasal wash on Day 1	Virus ($\log_{10}$EID$_{50}$/ml) in lung wash when killed	Febrile* response	Virus isolations from: Foetal membranes	Foetuses	Maternal† tissues
			Early gestation (Day 17)				
51	7	4·3	⩽1·45	—	0/6‡	0/6	—
52	9	5·1	—	+	0/9	0/9	—
			Middle gestation (Day 25)				
72	1	4·1	⩽0·7	—	0/10§	0/8§	—
70	3	5·1	3·7	+	0/12	0/11	—
61	5	5·1	3·2	+	0/11	0/11	—
60	7	5·5	—	—	0/11	0/11	—
59	9	4·9	—	+	0/6	0/5	—
			Late gestation (Day 34)				
32	2	5·3	4·7	+	0/7	0/7	—
7	3	4·9	4·7	+	0/10	0/10	—
42	5	5·1	3·2	+	0/10	0/10	—
58	6	4·9	1·7	+	0/6	0/6	+**

* A rise in rectal temperature > two standard deviations above the preinfection mean.
† Tissues examined were uterus, spleen, kidney, heart, liver, bladder, red blood cells and citrated plasma.
‡ Denominator, number examined; numerator, number from which virus was isolated.
§ Some foetuses were resorbing or resorbed leaving the membranes.
** Virus was isolated from spleen by 2 passes in eggs.
— No response or no virus isolated.

ferret was killed on Days 1, 2, 3, 5 and 7 post injection and foetal membranes (placenta, haematoma, cord, amnion and chorion pooled) and foetuses examined for virus as previously described. Virus was isolated from foetal membranes and foetuses of those ferrets killed on Days 1–5 post injection in early gestation and on Days 1–7 in middle and late gestation (Table III). The titres of virus found in the membranes (Fig. 2) were generally high on Days 2–5 after inoculation and lower after 5 days for early and middle gestational stages. In late gestation a similar pattern was observed with high virus titres on Days 2 and 3 after inoculation, but the individual titres in some of the membrane pools at 7 days post injection were still high, whereas others could not be titred. In the foetuses from early and middle gestation only small amounts of virus were found 2–5 days after inoculation. Some variation was observed, however, with some foetuses having virus contents of $> 10^2 EBID_{50}$/ml, but the possibility that this was due to contamination from the membranes cannot be ruled out. However, in late gestation foetuses there was evidence of viral replication, the virus content of those examined at 3 days being generally higher than the contents of foetuses from earlier gestational stages and, more significantly, the content of those examined at 7 days being similar to or greater than that of the corresponding membranes. Indeed some foetuses yielded high virus titres in the absence of titratable virus in corresponding membranes.

Following a high intracardial inoculum, virus was frequently isolated from maternal tissues such as nasal mucosa, lung, spleen, kidney, uterus, heart and occasionally bladder and liver (Table III). Virus found in foetal membranes and foetuses was probably not a result of

TABLE III.—*Virus Isolation from Foetal and Maternal Tissues and Temperature Responses after Intracardial Inoculation of Pregnant Ferrets at Early, Middle and Late Gestation with* $10^{9.4}$ *EBID*$_{50}$ *Clone 7a Influenza Virus*

		Virus isolations from					
				Maternal tissues			
Ferret no.	Day killed post injection	Foetal membranes	Foetuses	Nasal mucosa	Lung	Other	Febrile* response
				Early gestation (Day 19)			
82	0†	4/7‡	1/7	+	—	Uterus, spleen, kidney, heart	—
79	2	8/8	5/8	—	—	Uterus, spleen, heart	+
312	3	1/6§	0/2§	+	+	Spleen, kidney	+
78	5	10/11	10/10	+	+	Uterus	+
83	7	0/8	0/8	—	—		+
				Middle gestation (Day 26)			
84	1	3/6	0/5	+	—	Kidney, heart	+
319	2	5/6	2/5	+	—	Uterus, kidney, heart	+
317	3	4/4	3/4	+	+	Uterus, spleen, kidney, heart, bladder, liver	+
316	5	4/4	1/4	+	+	Uterus, kidney	+
314	7	3/5	1/3	—	—		+
				Late gestation (Day 32)			
320	1	3/4	2/3	+	—	Uterus, spleen, kidney, heart	+
81	2	5/7	1/5	+	+	Uterus, kidney, heart	+
306	3	8/8	5/8	—	—	Uterus, liver	+
87	7	8/9	9/9	+	—	Uterus	+

* A rise in rectal temperature > two standard deviations above the preinjection mean.
† Animal died from cardiac thrombosis 2 h after intracardial inoculation.
‡ Denominator, number examined; numerator, number from which virus was isolated.
§ Some foetuses were resorbing or resorbed leaving the membranes.
— No response or no virus isolated.

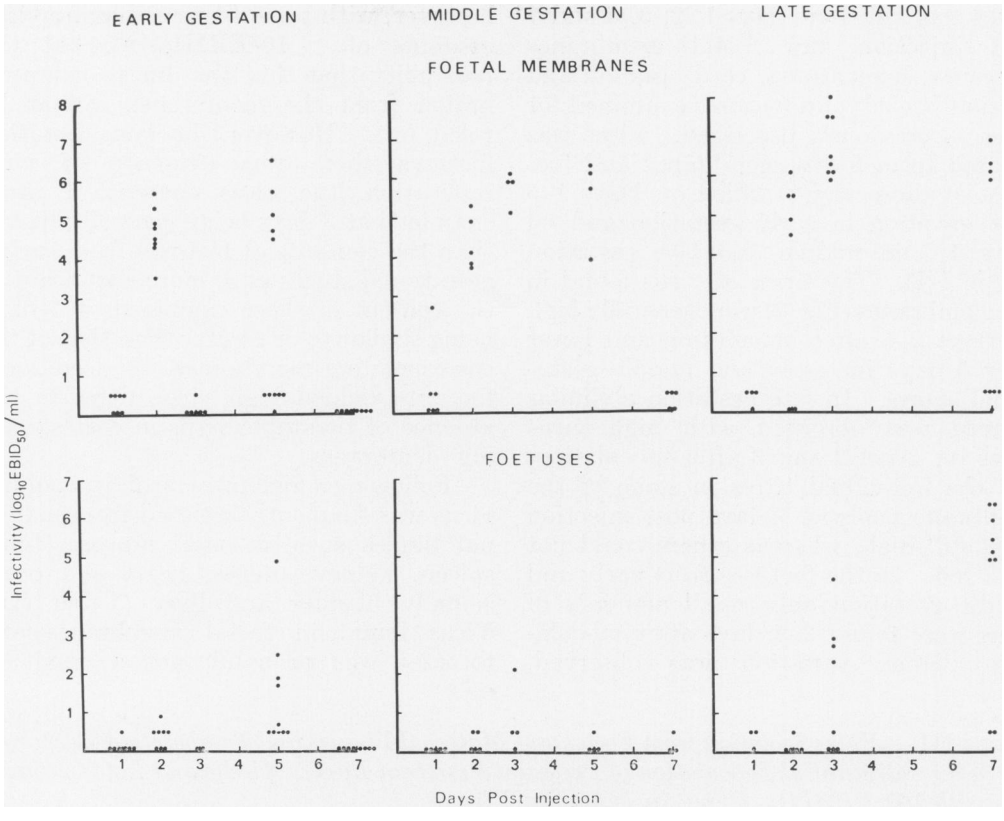

FIG. 2.—Virus content of foetal membranes (placenta, haematoma, cord, amnion and chorion pooled for homogenization) and foetuses after intracardial inoculation of pregnant ferrets at early, middle and late gestation with $10^{9.4}$EBID$_{50}$ influenza virus Clone 7a. Single ferrets were killed on most days up to 7 days and all the membranes and foetuses were examined: each point represents the titre of virus (EBID$_{50}$/ml) of a membrane pool from one foetus or an individual foetus from the ferret killed on that particular day.

contamination from the infected uterus because occasionally much higher titres were present in foetal membranes (*e.g.* Ferrets 316, 81, 87, Table III, Fig. 2) than in the uterus where virus was isolated only after 2 passages in eggs.

Intracardial inoculation of pregnant ferrets with smaller doses of influenza virus

Ferrets in late gestation (Day 30) were injected intracardially with $10^{9.4}$, $10^{7.4}$ or $10^{5.4}$EBID$_{50}$ influenza virus. Virus could be isolated from foetal membranes and foetuses after inoculation with $10^{7.4}$EBID$_{50}$ as well as $10^{9.4}$EBID$_{50}$, but not with $10^{5.4}$EBID$_{50}$ (Table IV). With the lower doses of $10^{7.4}$EBID$_{50}$ and $10^{5.4}$ EBID$_{50}$, virus could not be isolated from maternal respiratory tissues. The highest dose ($10^{9.4}$EBID$_{50}$), but not the lower doses, produced a febrile response (Tables III and IV) which lasted 4–6 h and occurred 2–3 h after virus was injected.

Isolation of virus from the tissues of foetuses following intracardial inoculation of pregnant ferrets

Ferrets of 30 days' gestation were injected intracardially with $10^{9.4}$EBID$_{15}$ influenza virus. At various times after injection ferrets were killed and individual tissues were removed from foetuses and examined for virus as described in Materials and Methods. Virus was isolated from all

TABLE IV.—*Virus Isolations from Foetal Tissues after Intracardial Inoculation of Pregnant Ferrets at Late Gestation with Various Doses of Clone 7a Influenza Virus*

Ferret no.	Dose ($log_{10}EBID_{50}$)	Virus isolation from: Foetal membranes	Foetuses	Maternal nasal mucosa and lung	Febrile* response
414 (4)†	9·4	4/7‡	5/7	—	+
415 (4)		8/8	7/8	—	+
412 (4)	7·4	3/10§	0/9§	—	—
408 (5)		6/8	2/5	—	—
411 (5)		10/10	6/10	—	—
413 (5)		0/8	0/6	—	—
403 (4)	5·4	0/8	0/8	—	—
405 (5)		0/7	0/6	—	—
407 (5)		0/3	0/3	—	—
406 (5)		0/3	0/0	—	—

* A rise in rectal temperature > two standard deviations above the preinjection mean.
† Day after inoculation when ferret was examined.
‡ Denominator, number of foetuses examined; numerator, number from which virus was isolated.
§ Some foetuses were resorbing or resorbed leaving the membranes.
— No response or no virus isolated.

foetal tissues irrespective of their susceptibility in organ culture. However, virus was generally present in higher titres in the susceptible tissues (lung, intestine, bladder, kidney) than in the insusceptible tissues (liver, thymus, spleen, brain, heart). In ferrets killed 8 days post infection or later, isolations from individual foetal tissues of some foetuses occurred in the absence of virus in corresponding membrane pools (*e.g.* Foetuses 7 and 8 of Ferret 367, Foetus 2 of Ferret 531, Foetuses 1 and 5 of Ferret 536) again suggesting that viral replication has occurred in these foetuses.

DISCUSSION

Using organ culture, ferret foetal tissues showed a similar pattern of susceptibility to influenza virus to that already observed for human foetal tissues (Rosztoczy *et al.*, 1975): respiratory, alimentary and urogenital tract tissues were susceptibile but neural and lymphopoietic tissues were not. The susceptibility of ferret foetal kidney, in contrast to human foetal kidney tissue, may have been due to its maturity since it was taken from late gestation. The human tissue was taken from early gestation and Dowdle and Schild (1975) have noted that as the foetal kidney undergoes maturation early insusceptible fibroblast-like cells are replaced later by susceptible epithelial-like cells.

With regard to a possible route for foetal infection from the bloodstream, the ferret foetal membranes (placenta, haematoma, umbilical cord, amnion and chorion) were all susceptible, in contrast to the corresponding human membranes of which only umbilical cord was consistently susceptible and placenta became infected in only 1 of 8 experiments (Rosztoczy *et al.*, 1975). The difference in susceptibility may or may not be correlated with the different structure of the placentae: ferrets possess an endotheliochorial-type and humans a haemomonochorial-type (Parkes, 1952).

Clearly a potential for foetal and foetal membrane infection exists in ferrets but these tissues did not become infected *in vivo* following intranasal infection of a limited number of animals. To produce foetal infection from the respiratory focus virus must escape into the blood and reach placental tissue there to replicate progressively through the cells of the barrier or be ferried across either free or on maternal blood cells (Mims, 1968). Alternatively, viraemia-initiated replication in

TABLE V.—*Virus Isolations from Foetal Tissues after Intracardial Inoculation of Pregnant Ferrets at Late Gestation with* $10^{9\cdot4}$ $EBID_{50}$ *Clone 7a Influenza Virus*

Ferret no.	Day killed post injection	Foetus no.	Virus isolations from foetal tissues: Lung	Intestine	Bladder	Kidneys	Heart	Liver	Spleen	Thymus	Brain	Whole foetuses or remnants	Foetal membranes
415	4	1										+	6·5*
		2	−	−	NT	+	−	−	NT	NT	−	−	4·1
		3										⩽2·7	6·5
		4	−	−	NT	−	−	−	NT	−	−	−	6·5
		5										⩽1·9	6·5
		6	⩽0·7	⩽0·7	NT	⩽0·7	+	−	NT	NT	±	+	5·9
		7										+	5·3
		8	±	−	NT	±	−	±	NT	NT	−	−	6·5
529	7	1	±	−	±	−	±	−	±	±	−	−	±
		2										+	4·6
		3	−	+	+	+	+	+	−	+	+	⩽1·7	5·5
		4										4·9	5·9
		5	2·1	⩽1·9	+	+	+	⩽1·1	−	⩽0·9	+	3·6	6·5
		6	−	+	+	−	+	−	−	+	+	+	6·5
		7	+	+	−	+	+	±	+	+	+	3·3	3·9
367	8	3	3·3	4·9	NT	+	+	+	+	NT	⩽1·9	4·4	±
		7	+	±	NT	−	±	+	NT	+	+	+	−
		8	6·5	4·7	NT	⩽1·8	2·7	⩽2·1	NT	NT	3·7	6·5	−
531	8	1	+	−	+	−	−	−	+	+	−	+	5·7
		2	−	+	+	−	−	⩽1·1	+	+	+	+	−
536	9	1	+	−	+	−	±	−	−	−	±	⩽2·7	±
		2	−	−	−	−	−	−	−	−	−	−	−
		3	−	−	−	−	−	−	−	−	±	+	−
		4	−	−	−	−	−	−	−	−	−	−	−
		5	−	±	−	−	−	−	±	±	−	+	−
535	10	6	−	−	−	−	−	±	−	−	−	−	±

* Titre in $\log_{10}EBID_{50}$/ml.
− Virus could not be isolated.
\+ Virus isolated on first pass in eggs but titre $<0\cdot5$ $\log_{10}EBID_{50}$/ml.
± Virus isolated only by two passes in eggs.
NT Not tested.

uterine epithelium could lead to membrane infection with consequent foetal infection (Mims, 1968). Thus, there are at least 3 possible barriers to spread of infection from the respiratory tract: the respiratory, blood and foetal membrane (mainly placental) barriers. Virus can escape from the respiratory tract in the ferret. Liu (1955) found influenza virus antigens in the mediastinal lymph node; Barker and Small (1974) observed infection of a tracheal pouch *via* the bloodstream and Toms *et al.* (1976) found infective virus consistently in the cervical lymph node and sporadically in liver, spleen, kidney and citrated plasma. Nevertheless, viraemia has not been consistently found and a technique which detected blood cell-borne virus antigen in man (Wilson *et al.*, 1976) has so far failed to do so in ferrets (Planterose, personal communication). This suggests that the blood (*e.g.* non-specific inhibitors) and the reticulo-endothelial system acting as a continual blood filter may present considerable barriers to spread of virus, especially if only relatively small amounts are liberated from the respiratory tract (Toms *et al.*, 1974). The lack of a sufficiently high viraemia could therefore be an important factor in preventing a

breach of the third barrier to foetal infection and this was tested by inducing a viraemia artificially by by-passing the respiratory barrier.

Toms *et al.* (1974) demonstrated a short viraemia in ferrets after intracardial inoculation of a dose ($10^{9\cdot4}$EBID$_{50}$) of virus sufficiently high to saturate non-specific inhibitors in the blood and to escape total sequestration by the reticulo-endothelial system. In pregnant ferrets such a dose produced infection of the foetus and the foetal membranes. Foetal membrane infection occurred at all gestational ages but significant and consistent foetal infection ensued only in late gestation despite organ culture evidence of the susceptibility of foetuses of early gestational age. A restricted time for transplacental passage has also been observed in mice (Siem *et al.*, 1960) and possibly in man (Greenberg *et al.*, 1958; Yawn *et al.*, 1971; Kornyushenko and Maximovich, 1961) suggesting that the placental barrier acts at certain stages during gestation. The route to foetal infection in ferrets is not clear because all foetal membranes are susceptible. Infection could occur by spread of virus from (*a*) a placental focus along the umbilical cord or (*b*) a uterine or placental infection *via* foetal membranes, as occurs with reovirus infection in rats (Kilham and Margolis, 1973; Margolis and Kilham, 1973). In the infected foetuses most virus was found in those tissues shown to be susceptible in organ culture. However, virus was also isolated from neural (brain) and lymphopoietic tissues (liver, thymus and spleen) which were insusceptible in organ culture but which in man have been associated with developmental or postnatal abnormalities. The virus may have resulted from replication but more probably it was due to contamination from other tissues or represented blood-borne virus in these tissues; a foetal viraemia could occur by spread of virus from a placental focus along the umbilical cord or from replication elsewhere, *e.g.* in respiratory and alimentary tissues. If abnormalities of lymphopoietic and neural tissue are found they may follow from limited or abortive cycles of replication in the tissues themselves—such abortive infections with influenza virus are known to be toxic (Barker and Hoyle, 1972; Mims, 1960)—or, indirectly, from virus replication in other sites. In chick embryos neural abnormalities were produced but viral replication was limited to extraneural sites (Johnson *et al.*, 1971).

In ferrets both the blood and the placenta can present strong barriers to foetal infection and these barriers, coupled with the relatively small amounts of virus that seem to escape from the respiratory tract, may explain the failure to produce foetal infection after intranasal challenge. The blood and placental barriers can be breached in late gestation if a sufficiently high viraemia (about 10^5EBID$_{50}$ virus/ml) is produced. Perhaps foetal infection resulting from a respiratory focus will occur in a small minority of animals where escape of virus into the blood stream is excessive. Whether it might occur with a more virulent strain of virus, under immunosuppression or environmental stress such as high or low temperature or humidity, or when bacterial pneumonia complicates influenza, is a matter for future research.

Thus, in some respects, but not in all, infection of the pregnant ferret appears to be a good model for providing information that might apply to congenital infection in man. There are three main areas of comparison. There are similarities between the respiratory disease in man and ferrets (Stuart-Harris, 1965; Toms *et al.*, 1976) and escape of virus from the respiratory tract occurs in both cases. Viraemia (Naficy, 1963; Lehmann and Gust, 1971; Stanley and Jackson, 1966; Khakpour *et al.*, 1969) has been detected in man and may occur in ferrets (Toms *et al.*, 1976). Thus, the ferret should provide a good model for studying factors that influence escape of virus from the respiratory tract and possibly for investigating the nature of the blood and reticulo-endothelial barrier. On the other hand the ferret may not be an appropriate model for studying

the strength of the placental and foetal membrane barriers. In organ culture, the susceptibilities of ferret and human placentae and other foetal membranes to infection with virus are different. The ferret membranes are susceptible to infection while human membranes are not; the reported isolations from human placenta or membranes (Yawn *et al.*, 1971; Jewett, 1974) may indicate a different situation existing *in vivo* though virus could have been present on maternal blood cells (Mims, 1968). Furthermore, the structures of ferret and human placentae are different (Parkes, 1952). A more suitable model for studying the placental and membrane barriers might be the pregnant guinea-pig, which possesses a haemomonochorial placenta similar in structure to that of man (Enders, 1965), and results obtained with this model will be described in a subsequent paper. Despite the apparent insusceptibility of human foetal membranes, virus has been isolated from foetal tissues following influenza in pregnant women (Greenberg *et al.*, 1958; Yawn *et al.*, 1971; Kornyushenko and Maximovich, 1961) and immunological evidence of infection obtained in progeny (Ruben *et al.*, 1975). The ferret seems to provide a good model for following the effects of such foetal infection. There is a similarity in pattern of susceptibility of human and ferret foetal tissues in organ culture to a strain of influenza virus known to be virulent for man and, in ferrets, this same pattern of infection occurs *in vivo* after transplacental infection in late gestation. Hence continued observation of ferret progeny following influenza infection *via* the maternal blood may bear relevance to the outcome of foetal infections in influenza of pregnant women.

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