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THE PREGNANT GUINEA-PIG AS A MODEL FOR STUDYING INFLUENZA VIRUS INFECTION *IN UTERO:* INFECTION OF FOETAL TISSUES IN ORGAN CULTURE AND *IN VIVO*

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Summary.—Organ cultures of guinea-pig foetal tissues showed a similar pattern of susceptibility to influenza virus to that already observed for human (Rosztoczy *et al.*, 1975) and ferret (Sweet, Toms and Smith, 1977) foetal tissues. Respiratory, alimentary and urogenital tract tissues were susceptible whereas neural and lymphopoietic tissues were insusceptible. However, of the foetal membranes (amnion, chorion, umbilical cord and placenta) only the chorion was susceptible, in contrast to the corresponding ferret tissues, all of which were susceptible. The insusceptibility of the placenta paralleled that of human placenta which is similarly haemomonochorial in structure.

Following intracardial inoculation of high titre virus (*ca* $10^{9.4}$ $EBID_{50}$) into pregnant guinea-pigs virus was isolated from all foetal membranes (amnion, chorion, umbilical cord and placenta), but in low titre. Although sporadic isolations were made from foetal tissues (intestine, kidney, heart, liver and spleen) there was no evidence for viral replication in these tissues.

These results are discussed in relation to possible infection of the human foetus *in utero* with influenza virus.

THE PREGNANT FERRET has advantages and disadvantages as a model for human influenza in studying the role of influenza virus in producing congenital effects. It may be a good model for following possible abnormalities derived from foetal infection; ferret foetal tissues showed the same pattern of susceptibility as human tissues to influenza virus in organ culture (Sweet *et al.*, 1977; Rosztoczy *et al.*, 1975) and foetal infection was established in pregnant ferrets in late gestation by maternal intracardial injection (Sweet *et al.*, 1977). On the other hand it does not seem to reflect the human situation with regard to the nature and strength of the placental barrier to virus infection. The structures of human and ferret placentae are different: the former is haemomonochorial and the latter endotheliochorial. Furthermore, all ferret foetal membranes (placenta, haematoma, umbilical cord, amnion and chorion) were susceptible to virus infection in organ culture (Sweet *et al.*, 1977) whereas, of human membranes, only umbilical cord could be infected reproducibly (Rosztoczy *et al.*, 1975). In ferrets, therefore, the foetal infection observed *in vivo* possibly follows from the susceptibility of the ferret foetal membranes which became heavily infected after intracardial inoculation of virus. However, replication in the membranes does not necessarily result in foetal infection, since in early and middle gestation virus failed to cross the placental barrier despite virus replication in foetal membranes (Sweet *et al.*, 1977).

The guinea-pig has a haemomonochorial placenta similar in structure to that of the human (Enders, 1965). Hence, although this animal does not suffer a typical respiratory infection after intranasal inoculation (Stuart-Harris, 1937;

Hyde, 1942), work with pregnant guinea-pigs might shed some light on the following questions which arise in relation to the human situation. First, is the haemomonochorial placenta of the guinea-pig insusceptible to infection with influenza virus like its human equivalent? Second, does a haemomonochorial placenta provide a greater barrier to foetal infection than an endotheliochorial placenta? Third, is virus replication in the placenta a prerequisite for foetal infection? This paper describes experiments to determine the susceptibility to influenza virus of guinea-pig placenta, foetal membranes and foetal tissues in organ culture and attempts to establish foetal infection *in vivo* by intracardial inoculation of virus.

MATERIALS AND METHODS

Influenza virus.—The recombinant virus A/PR/8–A/England/939/69 Clone 7a (H_3N_2), the preparation of virus stocks and infectivity assays have been described (Sweet *et al.*, 1977).

Guinea-pigs and guinea-pig impregnation.—English guinea-pigs (Dunkin–Hartley strain), obtained from O.L.A.C. (Southern) Ltd, Redhill Farm, Redhill Road, Kings Norton, Birmingham, were bred using a harem system. Male guinea-pigs were housed with 3–4 females and mating was assumed to have occurred at post-partum oestrous. The gestational age was estimated by weighing the foetuses as described by Draper (1920) and Ibsen (1928).

Organ cultures, inoculation and measurement of infection.—These were described by Sweet *et al.* (1977).

Tissue collection and maceration.—These were described by Sweet *et al.* (1977).

RESULTS

Susceptibility to influenza virus infection of guinea-pig foetal tissues in organ culture

The susceptibility to influenza virus of guinea-pig foetal tissues (Table I) was similar to that for human (Rosztoczy *et al.*, 1975) and ferret (Sweet *et al.*, 1977) foetal tissues. Thus guinea-pig foetal respiratory, alimentary and urogenital tissues readily supported replication of influenza virus, but the nervous and lymphopoietic tissues were insusceptible (Table I). Of the membranes examined, only the chorion allowed some replication (Table I, Fig.) in organ culture while umbilical cord, placenta and amnion were insusceptible (Table I).

TABLE I.—*Comparison of the Susceptibilities of Organ Cultures of Guinea-pig, Ferret and Human Foetal Tissues to Infection with Influenza Virus (Clone 7a)*

Tissue	Susceptibility* Guinea-pig	Ferret†	Human†
Nasal mucosa	+	+	+
Trachea	+	+	+
Lung	+	+	+
Oesophagus	+	+	+
Small intestine	+	+	+
Large intestine	+	+	+
Bladder	+	+	+
Kidney	+	+	−‡
Meninges	−	−	−
Brain	−	−	−
Heart	−	−	−
Thymus	−	−	−
Spleen	−	−	−
Liver	−	−	−
Placenta	−	+	−‡
Umbilical cord	−	+	+
Amnion	−	+	−
Chorion	+	+	ND

Foetal tissues were examined at late gestation (undated) while foetal membranes were examined at both middle (28–29 days) and late (undated) gestation.

* Tissues were designated susceptible if at least 2 samples of organ culture media taken on Days 2, 3 and 4 after inoculation ($10^{4.8}$–$10^{5.5}$ $EBID_{50}$) contained detectable infectivity (>0·5 $\log_{10}$ $EBID_{50}$/ml). All tissues were examined at least twice with similar results.

† Data from Rosztoczy *et al.* (1975) and Sweet *et al.* (1977).

‡ Human foetal kidney and placenta allowed replication in one of 4 and one of 8 experiments respectively.

ND. Not done.

Virus isolations from foetal and maternal tissues following intracardial inoculation of pregnant guinea-pigs

Guinea-pigs of various gestational ages, inoculated intracardially with $10^{9.4}$ $EBID_{50}$ of virus, were killed at intervals after inoculation and foetuses and pooled foetal membranes (amnion, chorion, placenta and umbilical cord taken together) examined for virus infectivity. Virus was isolated from foetal

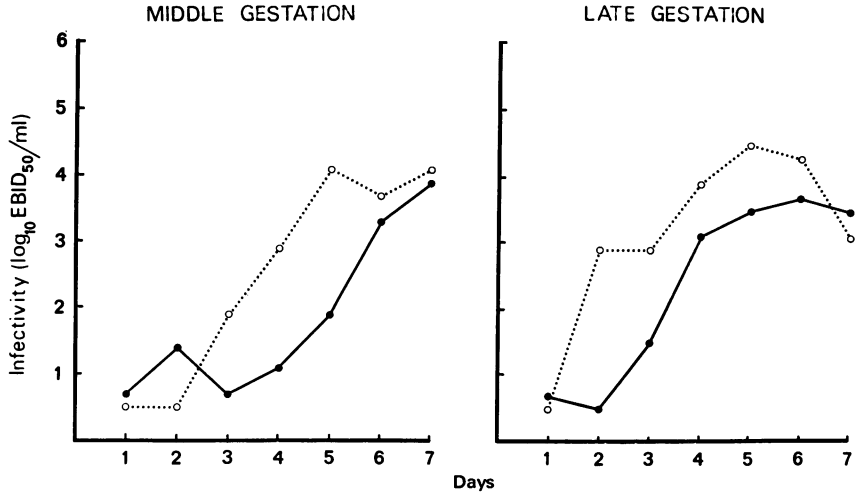

FIG.—Virus released into the medium of organ cultures of guinea-pig chorion, inoculated with $10^{4.8}$ $EBID_{50}$ of A/PR/8-A/England/939/69 Clone 7a (H_3N_2). The graphs show the results of two typical experiments. Chorions were examined at middle (28–29 days) and late (undated) gestation.

TABLE II.—*Virus Isolations from Maternal and Foetal Tissues after Intracardial Inoculation of Pregnant Guinea-pigs with* $10^{9.4}$ *EBID*$_{50}$ *Clone 7a Influenza Virus*

Guinea-pig (gestational age, days)	Day killed post injection	Virus isolations from (log_{10} $EBID_{50}$)					
		Foetal membranes	Uterus	Foetuses	Nasal mucosa	Lung	Other maternal tissues
1 (19–24)	6	1/8*	—†	1/8	+ (6·2)†	+	—
2 (24–29)	6	2/5	—	1/5	+	—	—
3 (27–29)	8	0/4	—	0/4	—	—	—
4 (29–31)	6	1/2	—	1/2	—	—	—
5 (35–42)	3	4/4§ (1·7)‡	+ (5·1)	0/3§	+ (2·9)	—	+ Bladder, heart, kidney, spleen
6 (38–42)	8	1/5	—	1/3	+	—	—
7 (40–44)	6	7/7 (4·48)	+ (6·9)	7/7 (0·5)	+	—	+ Bladder, heart, blood cells
8 (40–44)	6	4/4 (1·07)	+ (6·5)	0/4	+ (3·4)	—	+ Bladder, spleen, liver, blood cells
9 (42–47)	8	3/5 (2·2)	+	4/5	+ (3·2)	—	—
10 (48–53)	2	1/1	+	0/1	ND	+	ND
11 (51–55)	4	1/3	+	0/3	—	—	+ Bladder, spleen
12 (52–56)	3	1/3	+	0/3	—	—	—
13 (>65)	6	0/4	—	0/4	—	—	+ Bladder, spleen

Guinea-pig 5 aborted 3 days after inoculation and Guinea-pig 10 died 2 days after inoculation.

* Denominator, number examined; numerator, number from which virus was isolated.

† —, Virus could not be isolated after 2 passes in eggs; +, Virus isolated: total virus content ($EBID_{50}$) in parentheses as for ‡.

‡ Average total virus content ($EBID_{50}$) in membranes or foetuses from one animal: when no average is given the total virus content was $<0·5$ $EBID_{50}$.

§ Some of the foetuses were resorbing or resorbed leaving the membranes.

ND. Not done.

TABLE III.—*Virus Isolations from Individual Foetal Membranes after Intracardial Inoculation of Pregnant Guinea-pigs with* $10^{9 \cdot 4}$ *EBID*$_{50}$ *Clone 7a Influenza Virus*

Guinea-pig (gestational age, days)	Day killed post injection	Virus isolations from ($\log_{10}$ EBID$_{50}$)				
		Amnion	Chorion	Umbilical cord	Placenta	Pooled membranes
2 (24–29)	6	0/4*	1/4	1/4	0/4	0/1
3 (27–29)	8	0/2	0/2	0/2	0/2	0/2
4 (29–31)	6	0/2	0/2	1/2	0/2	ND
6 (38–42)	8	0/2	0/2	0/2	1/2	0/3
8 (40–44)	6	4/4 (0·7)†	4/4 (1·7)	2/4	4/4 (1·9)	ND
9 (42–47)	8	3/3	2/3 (2·2)	2/3	2/3	1/2
10 (48–53)	2	0/1	1/1	0/1	1/1	ND

* Denominator, number examined; numerator, number from which virus was isolated.
† Average total virus content (EBID$_{50}$) in membranes from one animal: when no average is given the total virus content was $<0 \cdot 5$ EBID$_{50}$.
ND. Not done.

membranes throughout gestation (Table II) but most frequently from guinea-pigs of 35–47 days gestational age. At this time, virus was frequently isolated from the uterus (Table II). Foetal isolations were less frequent but again mainly occurred in foetuses of guinea-pigs at 35–47 days' gestation and titres were low. As found with ferrets (Sweet *et al.*, 1977) the high dose of virus inoculated intracardially led to respiratory infection. Some isolations from maternal tissues (including blood cells, but not plasma) were obtained 3–6 days after inoculation (Table II).

Virus isolations from individual foetal membranes following intracardial inoculation of pregnant guinea-pigs

The virus isolation from pooled membranes from inoculated animals together with the observed insusceptibility of these membranes, except chorion, in organ culture, stimulated an investigation to determine which foetal membranes contained virus. Virus was isolated from all foetal membranes (amnion, chorion, umbilical cord and placenta) (Table III).

Virus isolations from individual foetal tissues following intracardial inoculation of pregnant guinea-pigs

Virus could only rarely be isolated from individual foetal tissues and then only by two passages in eggs (Table IV). Virus was occasionally isolated from whole foetuses or foetal remnants but the titres ($<0 \cdot 5 \log_{10}$ EBID$_{50}$/ml) did not suggest that replication had occurred and the possibility of contamination from corresponding foetal membranes and/or uterus cannot be ruled out.

DISCUSSION

Organ cultures of guinea-pig foetal tissues showed a similar pattern of susceptibility to influenza virus to that previously described for human (Rosztoczy *et al.*, 1975) and ferret (Sweet *et al.*, 1977) foetal tissues. Respiratory, alimentary and urogenital tissues were susceptible while nervous and lymphopoietic tissues, the tissues in which malformations or malignancy have been observed in man, were insusceptible. However, of the guinea-pig foetal membranes only the chorion was susceptible. This is in contrast to the ferret where all membranes (amnion, chorion, haematoma, placenta and umbilical cord) were susceptible but similar to the human situation where the placenta was insusceptible and only the umbilical cord supported replication.

In the ferret intracardial inoculation of high titre virus ($10^{9 \cdot 4}$ EBID$_{50}$) produced infection of ferret foetal membranes of all gestational ages and foetal infection in late gestation (Sweet *et al.*, 1977).

TABLE IV.—*Virus Isolations from Foetal Tissues after Intracardial Inoculation of Pregnant Guinea-pigs with* $10^{9 \cdot 4}$ *EBID*$_{50}$ *Clone 7a Influenza Virus*

Guinea-pig (gestational age, days)	Day killed post injection	Foetus no.	Virus isolations from foetal tissues: Thymus	Bladder	Kidney	Intestine	Heart	Lung	Spleen	Liver	Brain	Whole foetuses or remnants
2 (24–29)	6	1	ND	ND	—	—	—	—	—	—	—	—
		2										—
		3	ND	ND	—	—	—	—	—	—	—	—
		4										—
		5	ND	ND	—	±	—	—	—	—	—	±
3 (27–29)	8	1	—	ND	—	—	—	—	ND	—	—	—
		2										—
		3	ND	ND	—	—	—	—	ND	—	—	—
		4										—
4 (29–31)	6	1	ND	ND	—	—	—	—	—	—	—	—
		2	ND	ND	—	—	—	—	±	±	—	—
6 (38–42)	8	1	ND	ND	—	—	—	—	—	—	—	—
		2										—
		3	ND	ND	—	—	—	—	—	—	—	±
8 (40–44)	6	1	ND	—	—	—	—	—	—	—	—	—
		2										—
		3	ND	—	—	—	—	—	—	—	—	—
		4										—
9 (42–47)	8	1	ND	—	±	—	—	—	±	—	—	+
		2										+
		3	ND	—	—	—	±	—	—	—	—	+
		4										+
		5	ND	—	—	—	±	—	—	—	—	±

—, Virus could not be isolated after 2 passes in eggs.
±, Virus could be isolated only by 2 passes in eggs.
+, Virus could be isolated on first pass in eggs but titre $<0 \cdot 5 \log_{10}$ EBID$_{50}$/ml.
ND. Not done.

Following a similar inoculation of pregnant guinea-pigs, virus was isolated from all foetal membranes. Virus was also isolated from foetuses but less frequently than from corresponding membranes and always in low titre. Occasionally virus was found in individual foetal tissues but the titres did not suggest that viral replication had occurred even though foetuses are susceptible (Table I; Woolpert *et al.*, 1938; Dettwiler, Hudson and Woolpert, 1940). With regard to a route for foetal membrane infection, virus was isolated from the uterus. Viral replication in the uterus following intracardial inoculation may lead to chorion infection and possibly contamination of placental tissue. Foetal infection *via* a similar route has been suggested for reovirus in rats and hamsters (Kilham and Margolis, 1975, 1976). As the umbilical cord and amnion are insusceptible in organ culture virus isolated from these tissues *in vivo* probably represents contamination from the chorion *in utero* or from the chorion and uterus at post-mortem. Hence, both the guinea-pig placenta and amnion, because of their insusceptibility, may act as strong barriers to foetal infection. The low level of virus found in foetuses could again represent post-mortem contamination or possibly virus may have been ferried across the placenta either free or on maternal blood cells (Mims, 1968). Virus was detected on maternal blood cells in the present work and blood cells have been reported to cross the placental barrier in animals (Holliday and Barnes, 1973) and man (Anderson, 1971). In limited experiments, Kornyushenko and Maximovich (1961) also isolated influenza virus from foetal membranes but not neonates following intranasal inoculation of pregnant guinea-pigs 3–4 days before parturition. They failed to isolate virus from amniotic fluid, suggesting a barrier between the foetal membranes and the foetus.

If the haemomonochorial placenta and membranes of man act similarly to those in the guinea-pig, then their insusceptibility in organ culture (Rosztoczy *et al.*, 1975), together with these studies with pregnant guinea-pigs, indicate that foetal infection will probably be a rare event in man. The susceptibility of human chorion, however, remains to be determined. The reported isolations of virus from human placenta and membranes (Yawn *et al.*, 1971; Jewett, 1974) may be genuine infection. However, they could also represent contamination from virus in the uterus as human endometrium and decidua are susceptible to influenza virus infection (Rosztoczy *et al.*, 1975).

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