Br. J. exp. Path. (1969) **50**, 378.

PERSISTENT RESPIRATORY VIRUS INFECTION IN TRACHEAL ORGAN CULTURES

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Received for publication March 6, 1969

SUMMARY.—Organ cultures of pig or calf trachea were infected with influenza viruses, parainfluenza viruses and a rhinovirus. Infection of calf tracheal cultures with a bovine rhinovirus and with parainfluenza virus type 3 produced considerable epithelial damage. Influenza strains and Sendai virus in calf tracheal cultures and swine influenza virus in pig tracheal cultures damaged the epithelium much less strikingly. In all the infections studied virus production continued undiminished for at least two weeks. Persistent infection of porcine cultures with swine influenza virus was followed for 24 days. Interferon was demonstrable in the infected cultures for 6 days, but on challenge with Sendai virus at various times up to the 19th day of infection these cultures yielded markedly less of the superinfecting virus than did control cultures. Treatment of swine influenza-infected cultures with porcine interferon or with swine influenza antiserum for 7 days did not eliminate the infection. The bearing that these infections in organ culture may have on the mechanisms of respiratory infections *in vivo* is discussed.

METHODS for growing respiratory viruses in organ cultures of respiratory epithelium have been developed by Hoorn and Tyrrell (1965). The use of such cultures prepared from human embryonic tissue has proved very rewarding for isolation and characterization of previously unrecognized human viruses (Tyrrell and Bynoe, 1966), but quantitative studies of virus growth in human embryonic material are difficult because of the scarcity and small size of the organs available. This difficulty can be overcome to some extent in other species, since larger tracheas from which replicate cultures can be made are obtainable. Studies of the infections produced by respiratory viruses in animal trachea organ cultures have been made for example by Hoorn (1966), Craighead (1966), Willems and Van der Veen (1968), and Shroyer and Easterday (1968). The present paper describes the use of calf and pig tracheas for study of the infections produced by several respiratory viruses in differentiated epithelium. Under the conditions used, the infections appear to be persistent rather than rapidly self-limiting; investigations have been made into some factors which may influence the persistence of swine influenza virus infection in pig trachea organ cultures.

MATERIALS AND METHODS

Organ cultures.—Pig tracheas were obtained from 8-week old piglets from a herd known to be free from infection with common pathogens. The tracheas were removed aseptically. Calf tracheas from animals of ages up to about 6 weeks were obtained from the local abattoir. Since these specimens were not removed under aseptic conditions the trachea, with surrounding tissues was clamped at either end and the exterior surface was washed very thoroughly and treated in Hanks' saline containing 1000 u./ml. penicillin and 1000 μg./ml. streptomycin

for 1½ hr at room temperature before cleaning and dissection of the specimen. Cultures were made by the method of Hoorn and Tyrrell (1965). The fragments of pig and calf trachea were about 3 mm. square and 4 mm. square respectively. Four fragments were implanted in each plastic Petri dish of 60 mm. diameter, and Eagle's medium containing 0·1 per cent sodium bicarbonate was added until its surface was level with the epithelium of the tracheal fragment. The cultures were maintained at 33° in an atmosphere of 5 per cent CO_2 in air, and were inoculated between 2–6 days after preparation. Porcine cultures were normally washed once and bovine cultures 2 or 3 times with Eagle's medium before use. Immediately before inoculation the medium was changed to Eagle's medium with 0·2 per cent bovine plasma albumin. Dishes were inoculated by dropping 0·2 ml. per dish of virus suspension on to the surface of the fragments. The medium was changed every 2–4 days depending on the requirements of the experiment. Experimental observations were made on pools of medium obtained from groups of 3 or 4 dishes. Samples for titration of virus infectivity were stored at − 70°.

Viruses.—Influenza virus strains WS and swine, and parainfluenza type 1 (Sendai) were egg-adapted strains. The seed virus used for inoculation of organ cultures was allantoic fluid stored at − 70°, diluted suitably with Eagle's medium.

Parainfluenza virus type 3, bovine strain 33 (Bakos and Dinter, 1960) and the bovine rhinovirus Sd–1 (Bögel and Böhm, 1962) were passed in calf kidney monolayer cultures.

Semliki Forest virus (SFV) was obtained from the Microbiological Research Establishment, Porton, Wilts, and was used as a suspension prepared from suckling mouse brain.

Tissue cultures.—Primary cultures of calf kidney and pig kidney were prepared by standard methods, using a growth medium consisting of 10 per cent ox serum in Eagle's medium.

Virus titrations.—These were performed in calf kidney monolayers in tubes. The cultures were washed three times in Hanks' saline before use and were maintained in Eagle's medium with 0·2 per cent bovine plasma albumin. Ten-fold dilutions of the virus suspension were made, and 4 tubes were inoculated with each dilution. Cultures were incubated at 33° and read after 5–7 days. Endpoints of titrations of the influenza strains and of Sendai virus were determined by the detection of haemagglutinin in the tissue culture fluids. Fowl erythrocytes were added to the cultures and allowed to settle on the rounded bases of the tubes. Haemagglutination was detected by the pattern method. Titrations of parainfluenza type 3 and rhinovirus Sd–1 were read by observation of cytopathic effect.

Ciliary activity.—Organ cultures were observed by low power microscopy using reflected light. The ciliary activity of each fragment was scored as either normal, slightly diminished, moderately diminished or severely diminished. In some experiments the "percentage ciliary damage" was calculated. The activity was then scored as either 0, 1, 2 or 3. The total score in each group of cultures was calculated and expressed as a percentage of the total possible score for the group.

Interferon.—Interferon was prepared from primary pig kidney cells, by inoculation of monolayers with a 1 in 10 dilution of allantoic fluid containing a strain of Sendai virus. The fluid was harvested after 2 days when over 50 per cent of the cells had been destroyed. The preparation was brought to pH 2·0 by addition of N HCl. After 18 hr at 4° the pH was restored to neutrality by addition of NaOH. The fluid then showed no infectivity for calf kidney monolayers.

All organ culture fluids to be tested for interfering activity were similarly treated at pH 2·0 and neutralized. All were tested for their ability to infect calf kidney cultures and were found to be non-infective.

Interferon assays were carried out in primary pig kidney monolayers. Serial 2-fold dilutions of the fluids under test were added to pig kidney cultures in tubes. Cultures were incubated at 37° for 18 hr. The fluids were then removed and replaced with Eagle's medium containing approximately $10^{3.5}$ TCD_{50} of SFV per tube. In some experiments the cultures were washed after removal of the test fluids before challenge with SFV, but since this evidently did not influence the final result the procedure was omitted in later experiments. Control tubes, previously untreated, were also inoculated with the same dilution of SFV. Cultures were incubated at 33°. The test was read after about 30 hr when the virus control tubes showed almost complete cytopathic effect. The endpoint of interfering activity was taken as the dilution of organ culture fluid which reduced the cytopathic effect to not more than 50 per cent destruction of the cell monolayers.

Histological preparations.—Organ culture fragments were washed twice in Hanks' saline, fixed in Bouin's fluid, embedded, sectioned and stained with haematoxylin and eosin.

RESULTS

Growth curves in organ cultures

Fig. 1 shows production of infectious virus in calf tracheal cultures. The virus inoculum in each case was about 10^3 TCD_{50} per culture dish. The growth curves show that virus production continued without any marked decline over the period studied. In the case of the WS strain of influenza A the infective titres obtained were low, but the results indicate that small amounts of virus were produced over a period of 15 days. In culture dishes similarly inoculated and

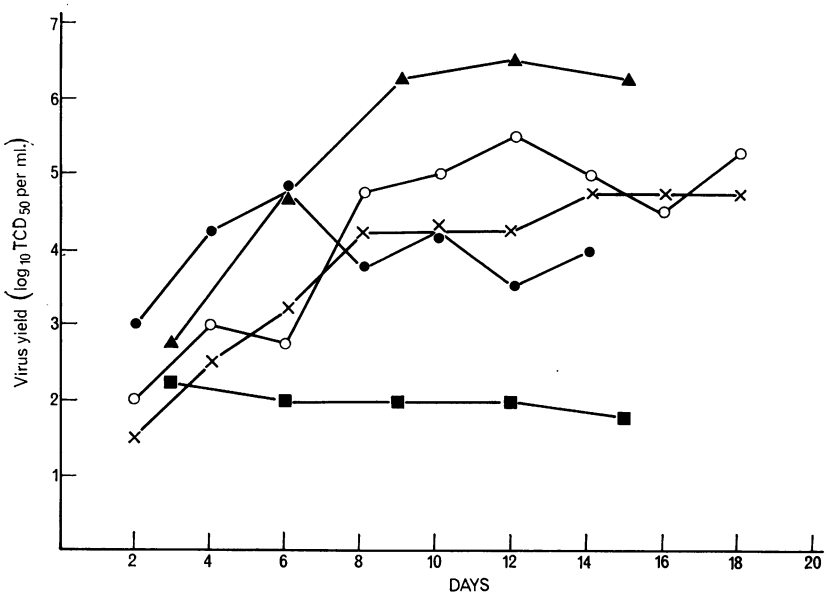


FIG. 1.—Production of infectious virus in calf tracheal organ cultures.

▲—▲ Parainfluenza type 3
○—○ Sendai
●—● Rhinovirus
×—× Influenza A swine
■—■ Influenza A WS

containing culture fluid, but no tissue fragments, virus was not detected for more than two days after inoculation.

Growth curves similar to those shown in Fig. 1 were obtained in pig organ cultures. After infection of pig trachea cultures with $10^{2.8}$ TCD_{50} per dish of Sendai virus the yield of virus was followed for 15 days, at which time the infective titre of the organ culture fluids was $10^{4.0}$ TCD_{50}/ml. When similar cultures were infected with about 10^3 TCD_{50} per dish of swine influenza virus, virus production reached a peak in the first few days of the infection, and was then maintained at a relatively steady level for up to 24 days, this being the longest period studied (see Table I and Fig. 12).

Ciliary activity

Observations on ciliary activity are shown in Fig. 2. The bovine rhinovirus Sd-1 (which did not grow in pig tracheal cultures) regularly eliminated the ciliary

TABLE I.—*Infectious Virus and Interferon in Fluids from Pig Tracheal Cultures Infected with Swine Influenza Virus*

Day of experiment	2	4	6	8	10	12	14	16	18
Mean infectivity titre (log_{10} units per ml.)	6·1	6·1	4·9	5·0	4·9	4·75	4·75	4·75	5·0
Mean interferon titre	1/4	1/6	1/3	<1/2	<1/2	<1/2	<1/2	<1/2	<1/2

activity of calf tracheal epithelium. Sendai virus had a much more severe effect on the cilia of pig than of calf tissue. The other viruses used produced varying degrees of destruction ranging from the moderately severe damage of parainfluenza 3 to the undiscernable effect of influenza WS.

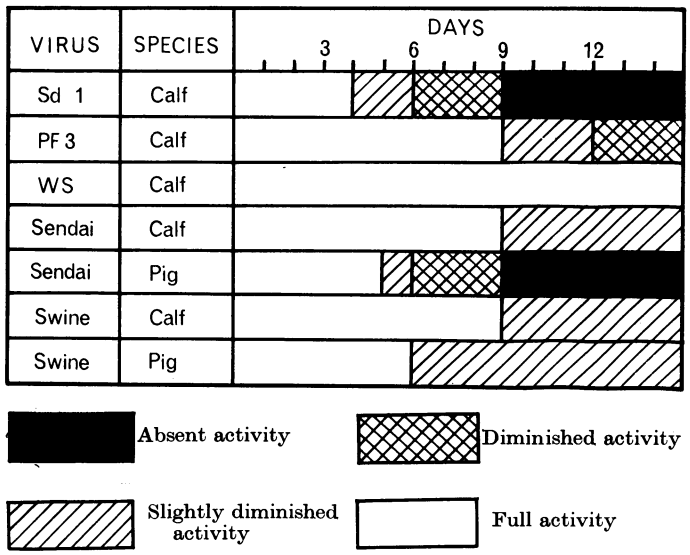


FIG. 2.—Ciliary activity in virus-inoculated tracheal organ cultures. Uninoculated controls maintained full activity.

Histological appearances

In uninoculated control cultures the histological structure was well maintained. Normal calf tracheal cultures showed an even, regular pattern of epithelium with well-preserved cilia and mucus-secretory cells (Fig. 3). A few dead or pyknotic cells were present in the epithelium, and mitoses were very occasionally seen in the basal layer. Submucous glands appeared normal. There was good preservation of all structures in cultures maintained for 30 days.

In pig tracheal cultures the basal layer of epithelial cells was well defined and the cilia were well preserved (Fig. 7). Goblet cells were abundant in cultures maintained for up to a week, but were less obvious in older cultures. Large submucous glands were present, but these sometimes became distended with degenerate cell debris in older cultures. In uninoculated porcine cultures maintained for 26 days there were occasional areas of thinning of the epithelium with some loss of cilia.

Damage to the superficial layers of epithelial cells correlated well with diminu-

tion or cessation of ciliary activity. Histologically, the most severe damage was seen in bovine cultures infected with rhinovirus Sd-1 or parainfluenza 3, and in porcine cultures infected with Sendai virus. The rhinovirus Sd-1 produced severe damage, mainly to the superficial layers of the epithelium (Fig. 4). Bovine tracheal cultures infected with parainfluenza virus type 3 showed moderately severe damage to cells in all layers of the epithelium (Fig. 5). Cell debris and vacuoles occurred within the epithelium, and dead cells were shed from the surface. This virus produced large syncytia in calf kidney monolayer cultures, but these structures were not certainly identified in the tracheal epithelium, though possible syncytia are shown to the left of Fig. 5. Sendai virus in pig tracheal cultures also produced severe damage, mainly to the superficial layers of the epithelium, the basal layer being well preserved (Fig. 8).

In cultures infected with the WS or swine strains of influenza virus the damage was less severe. Bovine cultures infected with WS virus produced only low titres of virus and there was no discernable effect on ciliary activity. Histologically the abnormality was minimal (Fig. 6). Bovine cultures infected with swine influenza or Sendai virus showed slightly more abnormality than that produced by WS virus, but despite the fact that these cultures produced moderately high titres of infectious virus the histological abnormalities were not striking.

The effect of swine influenza virus on pig tracheal cultures is shown in Fig. 9 and 10. The degree of damage varied considerably in different cultures. Fig. 9 shows an area of moderate damage in a culture fixed 9 days after infection. There is thinning of the epithelium and loss of its normal pattern, evidently due to destruction of cells throughout the layers of the epithelium. Abnormal and pyknotic nuclei are present and there is some loss of cilia. In Fig. 10 the abnormality is less marked, being confined to a single small focus. This preparation, made from a culture fixed 14 days after inoculation, shows that much normal-looking epithelium was present even in cultures in which the infectious process was well established.

Interferon production

It was considered that production of interferon by infected cultures might be relevant to persistence of infection; an attempt was therefore made to demonstrate

EXPLANATION OF PLATES

FIG. 3.—Uninoculated calf tracheal culture, maintained for 12 days. The epithelium is of even and regular structure, with abundant cilia. H. and E. ×500.

FIG. 4.—Calf tracheal epithelium infected with rhinovirus Sd-1. There is severe damage to the superficial layers of the epithelium. H. and E. ×620.

FIG. 5.—Calf tracheal epithelium, 10th day of infection with parainfluenza virus type 3. There is moderately severe damage to all layers of the epithelium with vacuolation, possible formation of syncytia, and loss of cilia. H. and E. ×500.

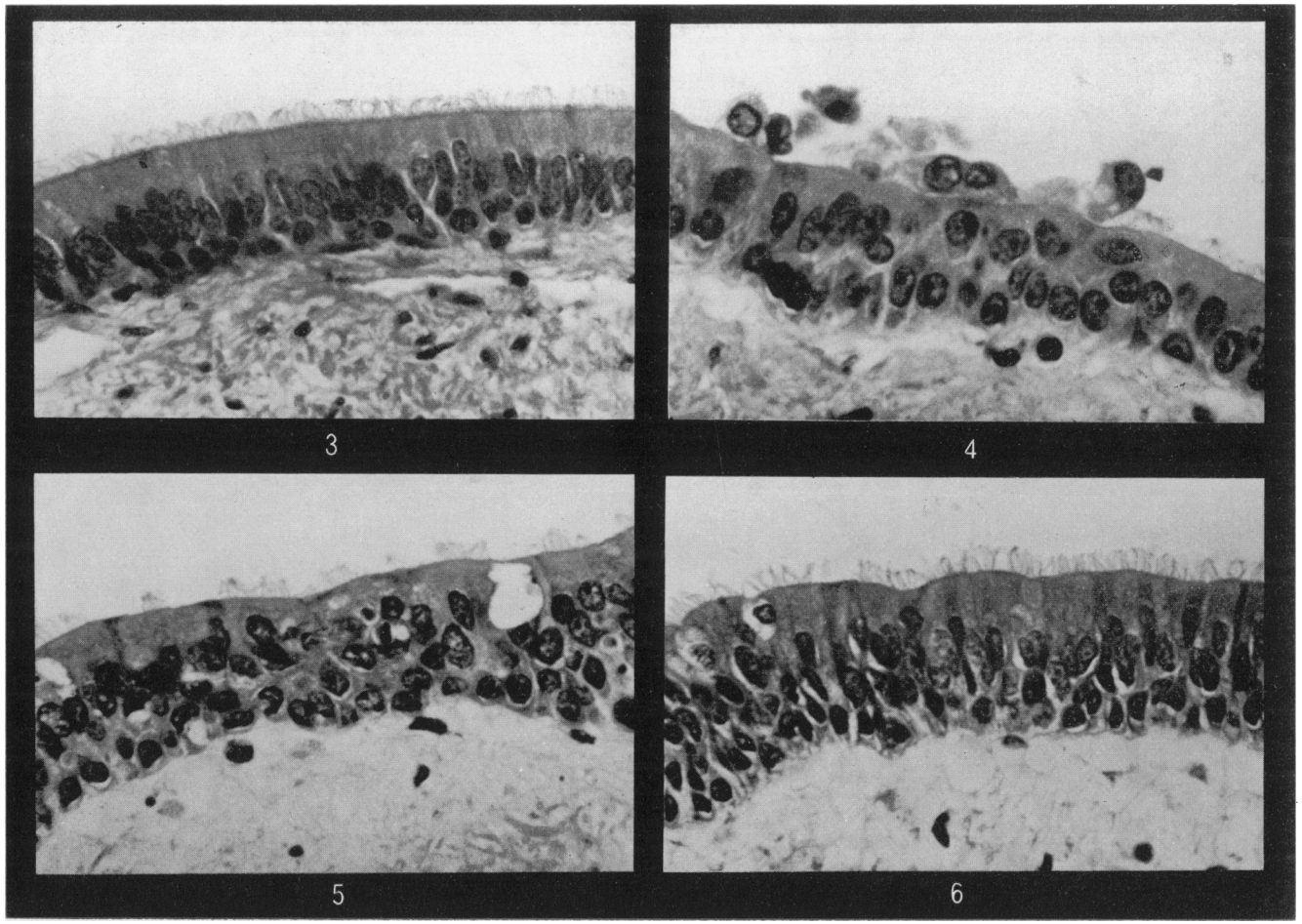
FIG. 6.—Calf tracheal epithelium, 13th day of infection with WS strain of influenza virus. Only minor abnormalities are present. H. and E. ×500.

FIG. 7. Uninoculated pig tracheal culture, maintained for 20 days. The basal layer of epithelial cells is well defined. H. and E. ×500.

FIG. 8.—Pig tracheal epithelium, 9th day of infection with Sendai virus. Destruction has occurred mainly in the superficial layers of the epithelium, leaving an intact basal layer. H. and E. ×500.

FIG. 9.—Pig tracheal epithelium, 9th day of infection with swine influenza virus. The damage appears to affect all layers of the epithelium, with some loss of cilia. H. and E. ×500.

FIG. 10.—Pig tracheal epithelium, 14th day of infection with swine influenza virus. There is a small focus of apparent damage in a generally well-preserved culture. H. and E. ×500.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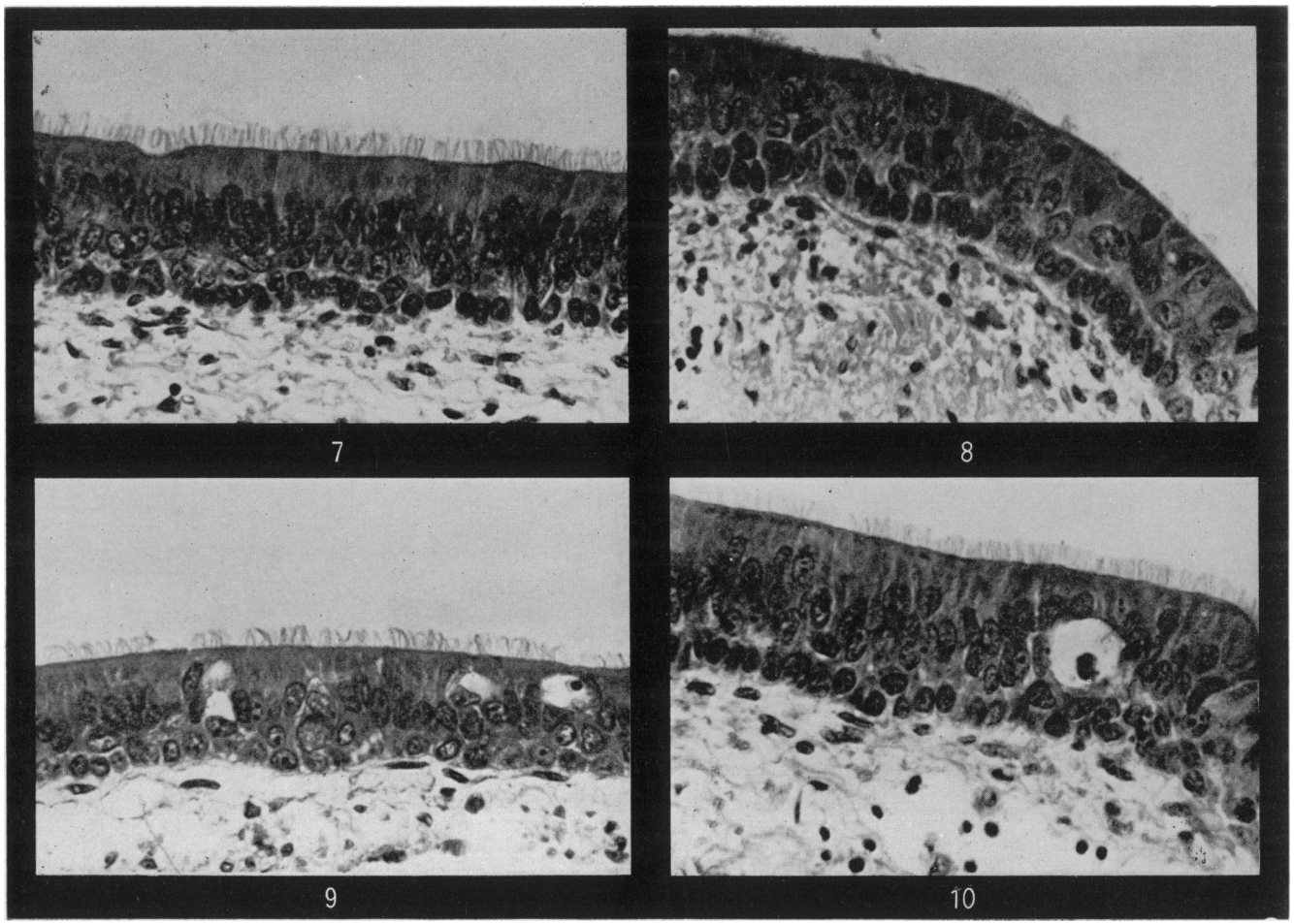

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interference and interferon production in pig tracheal cultures persistently infected with swine influenza virus. A series of replicate cultures of pig trachea was inoculated with $10^{2.8}$ TCD_{50} per dish of swine influenza virus. At intervals of two days the medium was harvested and renewed and pools of medium from groups of 3 dishes were made for titration of infectivity and interferon. Table I shows the mean of infective titres of swine influenza virus found in groups of infected cultures over a period of 18 days. Samples of the same fluids were titrated for interfering activity. After acid treatment and neutralization, dilutions of the fluids were tested for their ability to interfere with the growth of Semliki Forest virus in primary pig kidney monolayers. As shown in Table I, fluids harvested in the first 6 days of the infection, but not later, showed interfering activity. After acid treatment and neutralization these fluids showed no infectivity for calf kidney monolayers. Fluids from uninoculated control cultures showed no interfering activity. Similar experiments were also done on fluids from pig tracheal cultures infected with Sendai virus. Again, low titres of interferon were detected in the first week of the infection but not later.

The ability of pig tracheal cultures persistently infected with swine influenza virus to resist superinfection with Sendai virus was also investigated. On the 3rd, 7th, 11th, 15th and 19th day of the experiment described above, groups of swine-influenza infected cultures and comparable groups of previously uninfected cultures were challenged by inoculation of $10^{4.1}$ TCD_{50} of Sendai virus per dish. Three days after the challenge the medium was harvested and the yield of Sendai virus was titrated in calf kidney monolayers. The samples which were presumed to contain both swine influenza virus and Sendai virus were treated before titration with a 1 in 10 dilution of a hyperimmune rabbit serum against swine influenza virus for 1 hr at room temperature. This serum was inactivated at 56° for 30 min. before use. Suitable controls showed that this treatment with swine influenza antiserum did not reduce the titre of Sendai virus in samples containing Sendai virus alone, and that the same treatment was capable of neutralizing 10^{7} TCD_{50} of swine influenza virus. The yields of Sendai virus obtained 3 days after each challenge are given in Table II which shows that throughout the experiment

TABLE II.—*Yields of Sendai Virus Obtained* 3 *Days after Each Challenge from Cultures Infected with Swine Influenza Virus and from Control Cultures*

No of days after initiation of swine influenza infection on which Sendai challenge was made		3	7	11	15	19
Titre of Sendai virus (log_{10} TCD_{50} per ml.)	Swine influenza infected cultures	<0·75	<0·75	1·0	<0·75	0·75
	Control cultures	2·75	3·0	3·0	2·25	2·75

the swine influenza infected cultures yielded markedly less Sendai virus than did control cultures. The ciliary activity of these cultures was also observed and the percentage of ciliary damage calculated. The results are shown in Fig. 11. In cultures which were infected with swine influenza virus and not challenged with Sendai virus ciliary destruction was slight, and was not progressive after the first week of the experiment. Uninfected control cultures challenged with Sendai virus on the 3rd, 7th and 11th days of the experiment (challenges 1, 2 and 3 in Fig. 11) showed complete destruction of ciliary activity within seven days after

each challenge. Cultures which had been infected with swine influenza virus at the start of the experiment were also challenged with Sendai virus on the same days, and in these latter cultures the destruction of ciliary activity by the Sendai virus was sometimes delayed in comparison with the controls. This delay was as much as 4 days after challenge 1 and 1–2 days after challenge 2; no delay occurred after the 3rd or subsequent challenges.

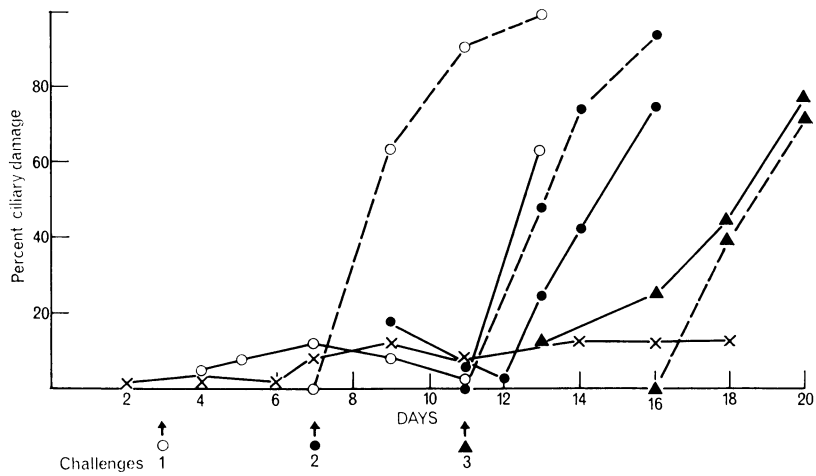

FIG. 11.—Ciliary damage in cultures infected with swine influenza virus and in control cultures, each challenged with Sendai virus.

○— —○ Control cultures after challenge 1
●— —● Control cultures after challenge 2
▲— —▲ Control cultures after challenge 3
○—○ Influenza infected cultures after challenge 1
●—● Influenza infected cultures after challenge 2
▲—▲ Influenza infected cultures after challenge 3

Treatment of swine-influenza infected cultures with interferon and antiserum

Since interferon was demonstrably present in the early stages of the infection, but not later, the possibility was considered that persistence of the infection might be related to this late failure of interferon production. Infected cultures were therefore treated with additional interferon, and also with antibody, in an attempt to modify the infection.

Infection with swine influenza virus was established in pig tracheal cultures, the medium being changed on the 2nd day. On the 5th day the normal maintenance medium was replaced in groups of 4 culture dishes by maintenance medium with one of the following modifications: (a) addition of 5 per cent rabbit antiserum against swine influenza virus, (b) addition of 5 per cent normal rabbit serum and 50 per cent of a pig interferon preparation, (c) addition of 5 per cent rabbit antiserum against swine influenza virus and 50 per cent of pig interferon preparation, (d) addition of 5 per cent normal rabbit serum. Uninoculated controls were also treated with medium containing 5 per cent normal rabbit serum. All media contained a final concentration of 0·2 per cent bovine plasma albumin. The antiserum used showed an initial titre of 1/960 by the haemagglutination-inhibition technique using fowl red cells. The initial titre of the

interferon preparation was 1/32. The four media described were renewed after 4 days. After a total of 7 days' treatment all cultures were washed 3 times with Eagle's medium and fed with Eagle's medium with 0·2 per cent bovine plasma albumin. Subsequently, the medium was changed and samples collected for titration of infectivity every 2–4 days. The production of infectious virus by these cultures is shown in Fig. 12. It is evident that treatment for a week with medium containing either pig interferon at a final titre of 1/16, or swine influenza antiserum at a final titre of 1/48, or a combination of the two did not cure the infection permanently. Addition of interferon alone produced no significant reduction in virus titre. In samples taken during treatment with antiserum

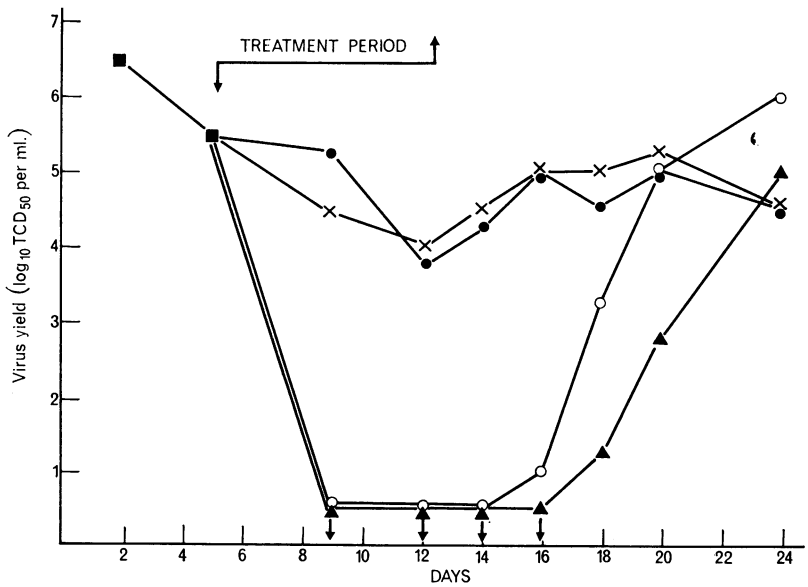

FIG. 12.—Virus yield in porcine cultures infected with swine influenza virus, treated with antiserum and interferon.

×—× Untreated control cultures
●—● Interferon treatment
○—○ Antiserum treatment
▲—▲ Interferon + antiserum treatment

virus infectivity was, of course, undetectable, and remained so for 2–4 days thereafter. However, the cultures subsequently regained infectivity titres equal to or greater than the pre-treatment levels. Cultures treated with both antiserum and interferon took longer to recover than those treated with antiserum alone.

Treatment of swine influenza infected cultures with aminoadamantane

Pig tracheal cultures in which swine influenza virus infection was already established were treated for 5 days with medium containing aminoadamantane 30 μgm./ml. No significant reduction in titre of swine influenza virus occurred. Confirmation of the sensitivity, under more favourable conditions, of this strain of virus to the action of aminoadamantane was obtained in an experiment in

which a reduction of virus titre of 1·7 $\log_{10}$ was obtained in calf kidney monolayers pre-treated with 30 μg./ml. of the drug, as compared with untreated monolayers.

DISCUSSION

The infection in an organ culture is more complex than that in a monolayer tissue culture. Fully differentiated cells are present which presumably vary widely in their susceptibility to the virus. Cultures of respiratory mucous membrane also secrete and transport mucus. The influence of this on experimental infections is uncertain, but the secretion has been shown to contain inhibitors of haemagglutination by influenza viruses (Tyrrell and Reed, unpublished). On the other hand, the absence in organ cultures of a circulation bearing both humoral factors (including antibody), and inflammatory and immune cellular responses implies that the complexity of the infection falls far short of that which occurs *in vivo*. It is therefore perhaps not surprising that infections in organ cultures show features which are apparently unlike either tissue culture or *in vivo* infections, but which may nevertheless bear on the mechanisms operating *in vivo*.

The degree of epithelial damage revealed in the present studies varied widely. Parainfluenza virus type 3 and rhinovirus Sd-1 in bovine trachea produced generalized and severe damage, but even though the most susceptible cells were rapidly destroyed, virus production continued. The influenza virus strains used were less severely destructive, though it is possible that some regeneration of epithelium may have helped to retain a normal structure. The damage produced by swine influenza virus in pig tracheal cultures appeared, on histological grounds, to be focal, and bovine cultures infected with the swine or WS strains were barely distinguishable from normal. The influenza and Sendai strains used were egg-adapted and wild strains might perhaps have given somewhat different results. Nevertheless, the present experiments confirm that influenza viruses can multiply in respiratory epithelium of an unaccustomed host without producing major histological abnormalities. The ability of influenza strains to infect species other than their natural host is of some interest in relation to epidemiological studies, and organ culture experiments may perhaps provide one approach to this problem.

Several authors have found infectious virus in organ cultures over a period of weeks. For example Craighead (1966) showed persistence of parainfluenza virus type 3 in guinea pig lung organ culture for 28 days. Best, Banatvala and Moore (1968), Shroyer and Easterday (1968) and Fogel and Plotkin (1968) showed persistence for similar or longer periods of, respectively, rubella virus in human embryonic nasal tissue, infectious bovine rhinotracheitis virus in bovine foetal trachea, and poliovirus in human foetal intestine. The present experiments involved viruses which under natural conditions cause relatively short-lived infections, yet in organ culture the infections were prolonged. It seems probable that persistent infection commonly occurs if organ cultures can be maintained in a healthy state for a sufficiently long time.

An attempt was made to define the role of interferon in the maintenance of swine influenza virus infection in porcine cultures. Smorodintsev (1968) using virus-infected calf tracheal organ cultures, showed that interferon was produced in the first week of the infection, and that addition of extraneous calf interferon to the medium 24 hr before infection with rhinovirus Sd-1 or Sendai virus reduced the virus yield in comparison with that obtained from untreated control cultures. In the present experiments interferon was found in the culture medium during

the first few days of the infection and not later, although it is nevertheless possible that small amounts of interferon, undetectable by the assay method used, may have been present after the first week. Furthermore, it was found that addition of moderately high titres of interferon on the 5th day of the established infection did not influence the output of infectious virus. The present experiments thus provide no evidence that interferon is present in persistently infected cultures or that it is relevant to the mechanism of this persistence. Nevertheless, challenge with Sendai virus of cultures infected with swine influenza virus revealed a resistance to superinfection not only in the first week, but throughout the three weeks of the infection. Observations of ciliary activity in these cultures (Fig. 11) suggest that the mechanism of the interference found in the later stages of the experiment may have been different from that found after the early challenges, when interferon was demonstrably present. Damage to the epithelium by the long-continued infection with swine influenza virus must be considered as a factor contributing to the depression of yield of swine influenza-infected cultures as compared with control cultures. However, the histological evidence suggests that this damage was very unlikely to have been sufficient to account entirely for the low yields of Sendai virus obtained. Interference mediated by mechanisms other than interferon has been noted in various tissue culture systems. Marcus and Carver (1967) have described " intrinsic interference " induced by several noncytopathic viruses. However, the interference investigated by these authors was demonstrable only by challenge with Newcastle disease virus (NDV) and not by a number of other viruses tested. Baluda (1959) also described interference with replication of superinfecting NDV. In this case the interference was induced by pre-treatment of the cells with ultraviolet-inactivated NDV, and may have been due to enzymic destruction of cell receptors. A similar phenomenon may be relevant to the present experiments, since cell receptors for Sendai virus are likely to be susceptible to the neuraminidase of swine influenza virus.

Treatment of swine influenza infected cultures with antibody for 7 days failed to cure the infection permanently. This result is in agreement with Craighead (1966) who found that 4 days' treatment with antiserum was insufficient to cure infection with parainfluenza type 3 in guinea pig nasal mucosa. It would be interesting to know how long the antibody treatment must be maintained if the infection is to be completely eliminated; persistence of infection in the presence of antibody implies intracellular survival of virus, perhaps with direct cell-to-cell spread. Persistent infection resistant to treatment with antisera occurs in tissue culture systems, and is well documented, particularly with regard to the paramyxoviruses (Fraser, 1967), but the mechanisms of such persistence in respiratory epithelium are less easily defined. The exact location of the virus in organ cultures can so far only be deduced from the histological changes, which may be very slight. Studies using fluorescent antibody are likely to give further evidence on this point.

In attempting to relate the findings to respiratory virus infections *in vivo*, it may be questioned whether these latter infections are always so rapidly self-limiting as is commonly assumed. Development of antibody will normally mask the infection but it is possible that in occasional individuals intermittent virus excretion may persist for much longer periods.

I should like to thank the Director of the A.R.C. Institute for Research in Animal Diseases, Compton, and Dr. R. N. Gourlay for arranging my access to

specimens from a " minimal disease " herd of pigs. I am also grateful to Mrs. Susan Finniss for valuable technical assistance and to Dr. D. A. J. Tyrrell for much helpful advice and discussion.

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