PROPAGATION OF FOWL PLAGUE AND OF NEWCASTLE DISEASE VIRUSES IN CULTURES OF EMBRYONIC HUMAN LUNG.

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WITH the object of finding a suitable virus to use for interference experiments in cultures of human tissues, attempts were made to propagate the agents of fowl plague and of Newcastle disease in roller tube cultures of embryonic human lung. Both these viruses are naturally pathogenic for birds. Newcastle disease virus has been reported to cause disease in man (Burnet, 1943 ; Placidi, Santucci and Haag, 1952). To our knowledge no case of human infection with fowl plague virus has been reported.

In the present paper we describe the successful propagation of each of these viruses in roller tube cultures of embryonic human lung and the development of a variant of fowl plague virus differing from the parent strain in its capacity to cause cytopathic effect in these cultures.

MATERIALS AND METHODS.

The viruses used were the Herts strain of Newcastle disease and the Dutch strain of fowl plague.

Cultures of human lung tissue were prepared from embryos up to 16 weeks of age. Roller tube cultures were prepared with 5 explants embedded in chicken plasma clotted by chick embryo extract. In some experiments the explants were placed on plasma-coated coverslips inserted in roller tubes. The nutrient medium (Enders, 1953) consisted of 90 per cent bovine amniotic fluid, 5 per cent inactivated horse serum and 5 per cent bovine embryo extract with the addition of penicillin (100 u./ml.), streptomycin (0·1 mg./ml.), soya bean trypsin inhibitor (0·1 mg./ml.) and phenol red (0·01 mg./ml.). The cultures were incubated at 37° in a revolving drum and the nutrient fluid was changed every 3 or 4 days. To avoid the accidental introduction of fowl cells into the cultures, the plasma and chick embryo extract used for the clot were filtered through a 0·7μ gradocol membrane.

After a few days' incubation a fairly extensive outgrowth, consisting almost exclusively of fibroblasts, could be observed around each explant. Cultures of varying ages were used for the experiments. Virus was added in appropriate dilutions to the medium. Passages were made into fresh cultures by the inoculation of the fluid medium from infected cultures in dilutions of 10^{-1} to 10^{-3}. Coverslips were stained by May-Grünwald-Giemsa according to the method described by Jacobson and Webb (1952).

Virus multiplication was estimated by egg infectivity and haemagglutination tests of the culture fluids. Egg infectivity titrations were carried out by allantoic inoculation of 0·05 ml. of tenfold dilutions of the virus preparations into groups of 4 ten-day embryonated eggs. The end points were calculated by the method of Reed and Muench (1938) and the results given as the number of 50 per cent infectivity doses (EID_{50}) per ml. Haemagglutination tests were performed by the pattern technique using 0·2 ml. of twofold dilutions of the culture material and an equal volume of a 0·5 per cent suspension of chicken red cells.

RESULTS.

Newcastle disease virus was propagated in cultures of embryonic human lung through a series of 8 passages (see Table I). The original inoculum was diluted by

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these passages to 10^{-14} not including the dilution due to fluid changes. A virus titre of $10^{5\cdot3}$ EID_{50}/ml. was obtained in the final passage. No cytopathic effect was observed throughout these passages and haemagglutinating titres were not reached.

TABLE I.—*Passages of Newcastle Disease Virus in Cultures of Embryonic Human Lung.*

Passage.	Inoculum.	Log EID_{50} in inoculum.	Log EID_{50}/ml. in successive fluids. F1.	F2.	F3.	F4.	F5.	Dilution of original allantoic fluid.
1	Allantoic fluid	7·1	—	5·5	3·9	4·3*	4·5	10^{-3}
2	P1–F4	2·3	4·9	5·1*	3·9	4·3	—	10^{-5}
3	P2–F2	3·1	—	—	—	—	>5·0*	10^{-7}
4	P3–F5	>4·0	—	—	3·7*	—	—	10^{-8}
5	P4–F3	2·7	—	3·7*	4·5	—	—	10^{-9}
6	P5–F2	2·7	3·2*	—	—	—	—	10^{-10}
7	P6–F1	2·2	2·8	4·9*	—	—	—	10^{-11}
8	P7–F2	1·9	5·3	4·7	2·3	—	—	10^{-14}

P1, P2....: First, second....passages.
F1, F2....: First, second....fluids removed from cultures.
* Material used as inoculum for next passage.

TABLE II.—*Passages of Fowl Plague Virus in Cultures of Embryonic Human Lung.*

Passage.	Inoculum.	Log EID_{50} in inoculum.	Log EID_{50}/ml. in successive fluids. F1.	F2.	F3.	F4.	F5.	Dilution of original allantoic fluid.
1	Allantoic fluid	6·7	—	—	7·3	6·5*	6·8	10^{-3}
2	P1–F4	4·5	>3·0	4·9*	6·7	4·1	—	10^{-5}
3	P2–F2	3·9	—	4·4*	—	—	—	10^{-6}
4	P3–F2	3·4	—	5·7*	>6·0	—	—	10^{-7}
5	P4–F2	4·7	>7·0*	—	—	—	—	10^{-8}
6	P5–F1	>4·0	4·9*	—	—	—	—	10^{-11}
7	P6–F1	3·9	5·5	—*	—	—	—	10^{-12}
8	P7–F2	—	7·5	—	—	—	—	10^{-14}

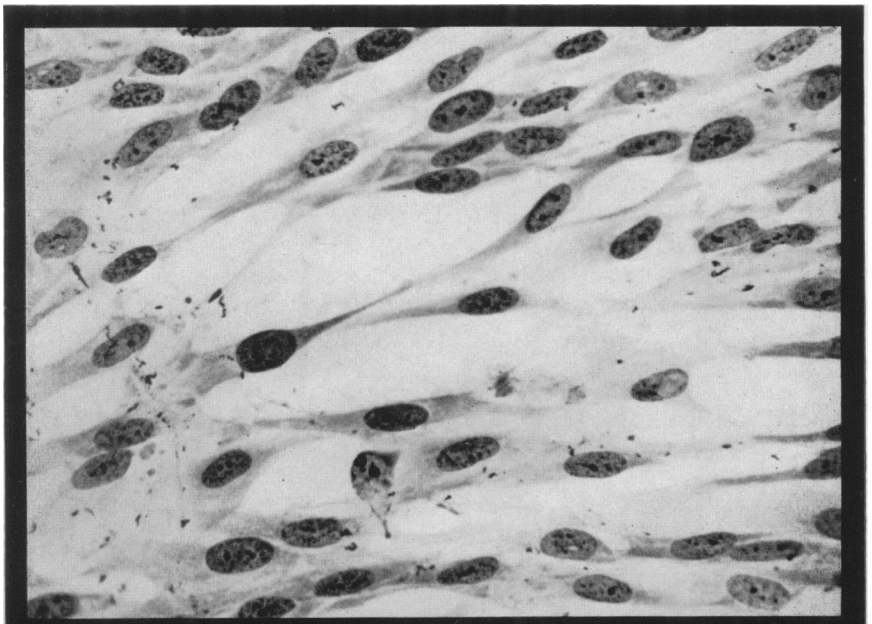
P1, P2....: First, second....passages.
F1, F2....: First, second....fluids removed from cultures.
* Material used as inoculum for next passage.

Fowl plague virus also was propagated through 8 passages in cultures of embryonic human lung (see Table II), the original inoculum having been similarly diluted to 10^{-14}. A virus titre of $10^{7\cdot5}$ EID_{50}/ml. was reached at the final passage. One of 2 experiments at the third passage and 2 of 5 experiments at the seventh passage failed to reveal virus multiplication. No explanation could be found for these failures, which were not related to the size of the inoculum, the origin of the tissues or to the age or microscopical appearance of the cultures at the time of inoculation. Haemagglutination titres were reached very irregularly throughout

EXPLANATION OF PLATE.

FIG. 1.—Uninoculated culture of embryonic human lung. May-Grünwald-Giemsa. × 700.

FIG. 2.—Culture of embryonic human lung 8 days after inoculation with human-adapted (sixth passage) fowl plague virus. May-Grünwald-Giemsa. × 700.



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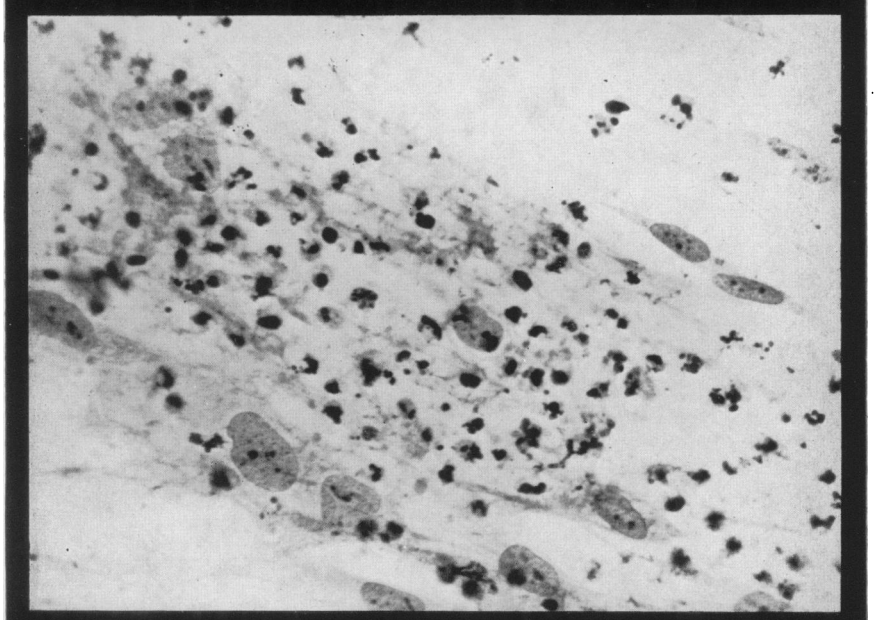

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the series, but developed earlier and occurred more frequently at higher passage levels. No cytopathic effect was observed in the first two passages. At the third passage this effect was observed in 1 of 2 successful experiments after 7 days' incubation. In further passages the cytopathic effect occurred with more regularity and its time of onset was reduced to 2 to 3 days. In two experiments in the seventh passage in which there was no cytopathic effect, virus multiplication did not occur.

The cytopathic effect caused by fowl plague virus is shown in Fig. 1 and 2. The first evidence was the appearance of basophilic granules in the nucleus. As degeneration progressed the nucleus became increasingly basophilic until the nucleolus and granules were no longer visible. At this stage the nucleus, often lobed or dumb-bell shaped, came to occupy one side of the cell and had by then become pyknotic. These changes were followed by complete cellular disintegration. Not all the cells were destroyed by the virus. The surviving cells continued to multiply, and after prolonged incubation the proportion of degenerating cells decreased until finally only débris and healthy cells could be seen.

Multiplication of fowl plague virus in cultures of human lung could be demonstrated in all passages by egg infectivity tests. The cytopathic effect in human lung cultures, however, was only observed from the third passage onwards and it occurred in cultures inoculated with lower doses than that used in the first passage. This result was interpreted as being due to the selection of a human-adapted variant of the virus. Material from the eighth human lung passage of fowl plague virus after three limit-dilution passages in embryonated eggs showed no change in its acquired cytopathic activity for human lung cultures. The same material was titrated in eggs and in human lung cultures, giving titres of $10^{-7.5}$ and $10^{-5.8}$ respectively. These end-points were calculated on the basis of haemagglutination tests in the egg titration and of cytopathic effect in the human lung cultures.

The human-adapted strain of fowl plague virus was compared with the parent strain for cytopathic effect in cultures of chick embryo fibroblasts and no differences could be detected.

Fowl plague and Newcastle disease viruses after 8 passages in human lung were each tested by haemagglutination-inhibition against immune sera and no differences from the parent strains were detected.

COMMENTS.

Evidence for the multiplication of fowl plague and of Newcastle disease viruses in the system under study was afforded by the frequent increase of virus titres in the cultures and by the demonstration of viral multiplication at the end of each series of passages, where the original inocula had been diluted to 10^{-14}. In the course of these passages no change of properties of Newcastle disease virus could be detected, but a variant of fowl plague virus, differing from the parent strain in its cytopathic effect in human lung cultures, was developed. This variant did not revert to the parent form after three passages in embryonated eggs at limit dilutions. A feature of most cultures showing cytopathic effect of fowl plague virus was the survival of some of the cells which continued to multiply and remain healthy after relatively long periods of incubation. This phenomenon may have been due to variations in the susceptibility of different cells or to interference, either by non-infectious virus retained in the solid phase of the culture

(Pereira and Gompels, 1954) or by the original non-cytopathogenic virus which might have accompanied the variant through the series of passages. Interference with poliomyelitis virus by a non-cytopathogenic variant was observed by Sabin (1954) and similar findings have been reported by Le Bouvier (1954). One experiment designed to demonstrate interference between the original and the human-adapted strains of fowl plague gave a negative result.

Recent work in our laboratory (unpublished) revealed that neither the parent nor the human-adapted strain of fowl plague virus was able to multiply or cause any alteration in cultures of HeLa cells. Newcastle disease virus, however, as shown by Henle (1954) and by Tyrrell (1955), can be easily propagated in HeLa cells, causing a striking cytopathic effect.

The capacity of fowl plague virus to multiply with cytopathic effect in cultures of human lung does not warrant the conclusion that it is a potential human pathogen. This possibility, however, should be kept in mind, and the fact that no human infections by this virus have been reported may be due to the restricted number of laboratories in which it is handled. In our own laboratory no antibodies against fowl plague virus were found in the blood of workers exposed to risk of accidental infection.

The ability to multiply in human tissues is one more character which fowl plague and Newcastle disease viruses share with others of the group of *Myxovirus* (Andrewes, 1954) in which they are included.

SUMMARY.

Fowl plague and Newcastle disease viruses were successfully propagated in cultures of embryonic human lung.

Newcastle disease virus did not give rise to cytopathic effects throughout the series of 8 passages in these cultures.

Fowl plague virus became cytopathogenic on the third passage and this property was enhanced in successive cultures.

The human-cytopathogenic variant of fowl plague virus did not revert to the parent form after three limit-dilution passages in embryonated eggs.

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