ISOLATION OF A STRAIN OF INFLUENZA-A-VIRUS FROM THE TRACHEA IN A CASE OF INFLUENZA PNEUMONIA IN THE WINTER OF 1947 1) 
by 
J. MULDER and J. VAN DER VEEN
with the co-operation of
MISS S. W. ENSERINK and J. J. BRANS
(Received September 13, 1948).

INTRODUCTION.
After an illness of seventy-two hours' duration a man (K), aged 27, died on February 4, 1947, in the Clinic of Internal Medicine of the University Hospital at Leyden, the cause of death being tracheo-bronchitis, with broncho-pneumonia in both lungs due to Staphylococcus aureus. Particulars about the case history and the pathology of this case have been given elsewhere (MULDER and VERDONK (11)). As this rapid death of a young man, consequent upon infection of the respiratory tract and the lungs with staphylococci made us think of influenza, part of the trachea together with portions of all the lobes of both lungs were post mortem examined for the presence of influenza virus.

ISOLATION OF A STRAIN OF INFLUENZA VIRUS FROM THE MUCOSA OF THE TRACHEA IN FERRETS.
The material was stored for 16 hours at —130° C. The mucosa of the trachea was ground in half saline, half broth with 100 units of penicillin per ml and was next inoculated intranasally in a ferret. The remainder was stored at —130° C. Amniotic inoculations in the

1) With financial support of the Institute for Preventive Medicine, the State Department of Science, the Jan Dekker fund, the Curaçao fund for Preventive Medicine and the Department of Medical Research Philips van Houten, Weesp.
chick embryo could owing to circumstances not be performed until seven months afterwards. Rigid isolation of the animals was possible.

After three days the ferret got fever and severe rhinitis, and a strain of influenza virus was isolated in passage ferrets and seven months later from the turbinate of this first ferret (stored at \(-130^\circ\text{C.}\)) in the amnion of 13 days old chick embryos.

From the third ferret-passage we obtained the strain of the influenza virus by direct inoculation in the allantoic sac of the chick embryo. The strain was regularly passed in the allantoic sac, and after a number of passages it infected eggs in a dilution of \(10^{-8}\) to \(10^{-6}\). A second ferret, infected in February 1947 with the original emulsion of trachea mucosa, also became ill, and a fortnight after the infection the serum contained immune bodies against the isolated strain (hemagglutination-inhibition test).

In September 1947 we did not succeed in isolating the virus immediately in the amnion from the original emulsion of the trachea mucosa (two attempts with respectively 3 and 4 amniotic passages in 13 days old chick embryos).

**Ferret-inoculations with ground lung tissue.**

Not until we had the mouse-passage strain at our disposal (see below), were we in a position to obtain a clear idea of what had happened in the inoculations of lung tissue in ferrets.

The first ferret, inoculated with it on February 5, 1947, did not become ill, but after five days it showed a high fever peak (\(104^\circ\text{F.}\)) without rhinitis. It was then not possible to pass the turbinates of this animal on other ferrets. The post-infectious serum of this ferret had a fairly high titer (96) against the mouse-passage strain (which practically did not show non-specific inhibition by ferret sera), and it also showed a reasonable titer (64) in the mouse protection test with the homologous mouse-passage strain (calculated from the mortality within ten days, four mice being used per dilution).

After six months the lung emulsion (stored at \(-130^\circ\text{C.}\)) was once more passed to ferrets, but we did not succeed in isolating a virus strain. Passage ferrets (5 passages) did not show any immune bodies, and inoculations from the turbinates into the amnion of 13 days old chick embryos remained negative. Nor was it possible after seven months to isolate a virus strain from the lung emulsion into the amnion (3 passages). Hence there is reason to assume that