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Direct Isolation of Human Influenza Virus in Tissue Culture Medium and on Egg Membrane.

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Throat washings in Tyrode's solution were obtained on the second day of illness from a patient acutely ill with influenza. The material was centrifugalized at 2500 revolutions per minute for 30 minutes. The supernatant fluid was then filtered through a graded collodion membrane of 500 mμ average pore size.[1] Flasks containing 4.5 cc. of chick embryo Tyrode medium[2] were inoculated with 2.5 cc. amounts of the filtrate (shown by ferret inoculation to contain active virus). Transfers of 0.5 cc. amounts of the culture material to 4.5 cc. of fresh medium were made at 48-hour intervals. Mice were inoculated intranasally with culture fluid of the 5th transfer. Mouse passages were made at 4-day intervals. No significant lesions were seen in the lungs of mice of the first 3 passage groups. In mice of the 4th passage, however, sugestive lesions were seen; these were more definite in the 5th passage, and in the 6th passage death of the mice with extensive pulmonary involvement occurred. The virus was identified as human influenza virus by means of neutralization tests with known immune serum.

These results indicate that virus was recovered directly from the throat of a human influenza patient by the introduction of the filtered throat washings of the patient into tissue culture medium. The virus not only survived but probably multiplied, since the final dilution of the original material was approximately 1:300,000 at the time the culture material was first given to mice. The behavior of the virus after its inoculation into mice was very similar to that of virus established directly in mice from human throat washings.[3]

Burnet[4] has reported in detail the behavior of human influenza virus introduced after passage through ferrets, onto the chorio-allantoic membrane of the developing chick. The direct cultivation of the virus of common cold on the chorio-allantoic membrane has also been reported.[5] Attempts were therefore made to utilize this procedure in the isolation of virus directly from the human

[1] Bauer, J. H., and Hughes, T. P., *J. Gen. Physiol.*, 1934, **18**, 143.

[2] Li, C. P., and Rivers, T. M., *J. Exp. Med.*, 1930, **52**, 465.

[3] Francis, T., Jr., and Magill, T. P., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 132.

[4] Burnet, F. M., *Brit. J. Exp. Path.*, 1936, **17**, 282.

patient. Throat washings in broth were obtained from two patients acutely ill with influenza. By means of ferret inoculation and subsequent adaptation to mice, virus was shown to be present in the material. Portions of the throat washings of these patients were pooled, centrifugalized at 2500 r.p.m. for 30 minutes and filtered through a graded collodion membrane of 500 mμ average pore size. The chorio-allantoic membrane of a 12-day chick embryo was inoculated with 0.05 cc. of the filtrate. Passages were made at 4-day intervals by inoculating additional embryonic membranes with finely ground emulsions of the previous passage membranes. Thickening of the membranes was observed and white plaque formations were seen. A suspension of the 3rd passage membrane was inoculated into a ferret and into mice. The ferret developed typical experimental influenza and its convalescent serum neutralized human influenza virus. No lesions were seen in the lungs of mice of the first 3 passages. In those of the 4th, 5th, and 6th passages small pulmonary lesions were seen, but they have not been sufficiently extensive to permit of conclusive neutralization tests.

After the 3rd transfer on egg membranes the virus was placed in storage for several days. Since that time the changes in the passage membrane have been less pronounced. These brief observations indicate, however, that human influenza can be isolated directly and maintained for a time, at least, upon the embryonic membranes of developing chick.

The practicability of both the procedures described above is being studied on a larger scale. Certain disadvantages obtain. These consist in the inability to demonstrate the presence of influenza virus in tissue culture medium except by establishing the virus in animals and in the fact that the lesions produced by the virus in the early passages on embryonic membranes are too irregular to enable one to identify the virus. It seems possible that future studies will furnish means of eliminating such difficulties.

[5] Kneeland, Y., Jr., Mills, K. C., and Dochez, A. R., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 213.