14251 P

Experimental Air-Borne Influenza Infection. I. Influence of Humidity on Survival of Virus in Air.*

C. G. Loosli, H. M. Lemon, O. H. Robertson, and E. Appel.
From the Department of Medicine, the Douglas Smith Foundation for Medical Research, and the Bartlett Memorial Fund of the University of Chicago.

The ease with which infections with influenza A virus can be produced in susceptible animals by allowing them to breathe virus-containing air suggests that this may be an important mode of dissemination of the disease in man.[1-3] Its importance would depend on whether the virus survived in the air sufficiently long for an infective level to be built up. Previous studies have shown that the air in small chambers into which virus had been sprayed was infective for mice up to one hour.[3] Further studies have been made concerning the physical factors which influence the survival of virus in larger spaces simulating room environments. The data reported here include the effect of relative humidity on the persistence of virus in the air.

The experiments were performed in a room of 800 cubic feet capacity (10 x 10 x 8 feet). The air of the room was gently and continuously agitated by means of a centrally placed, slowly rotating vertical fan. The temperature varied from 27 to 29°C. The low humidity experiments were carried out during the winter months. The higher relative humidities were obtained by vaporizing steam into the room before each experiment. Influenza A virus (PR-8 strain) was employed and prepared from infected mouse lungs. The amount of virus used in each experiment varied from 2.4 to 3.6 cc of a 10^{-1} dilution by weight of ground lungs in 10% horse serum and broth. It was sprayed into the room with an atomizer which produced fine uniform-sized droplets. Following the atomization of the virus, groups of 10 mice were placed in the room for 20-minute periods at increasing intervals of time. As a more efficient means for the detection of air-suspended influenza virus 20 minute air samples (representing 20 cubic feet of air) were taken simultaneously with the Moulton air sampler.[4] The virus-containing broth was then inoculated intranasally into groups of 10 mice.† All mice dying were autopsied and examined for lung lesions. Those surviving were killed on the 12th to 14th day.

It was found that room atmospheres of high humidity (80-90%) into which virus suspension had been sprayed were no longer infective after one hour, although the mice exposed to this air 10 to 30 minutes after spraying all died with extensive consolidation of the lungs. Atmospheres of 45% to 55% relative humidity remained infective for 6 hours and were lethal for mice up to one hour and 10 minutes. At low humidities (17 to 24%) similar amounts of virus continued to produce influenzal pneumonia in the exposed mice for at least 24 hours. During the first 6 hours after introduction of the virus into this dry atmosphere all the mice died. At 12 hours 40% died and the remainder showed extensive pulmonary involvement. Among the mice exposed at 24 hours there were no deaths but pulmonary lesions were present in 30% of them. Experiments illustrating the influence of relative humidity on the per-

* This investigation was aided in part through the Commission on Cross Infections in Hospitals, Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, Preventive Medicine Division, Office of the Surgeon General, U. S. Army.

1 Wells, W. F., and Henle, W., Proc. Soc. Exp. Biol. and Med., 1941, **48**, 298.

2 Loosli, C. G., Robertson, O. H., and Puck, T. T., *J. Bacteriology*, 1942, **43**, 648.

3 Loosli, C. G., Robertson, O. H., and Puck, T. T., *J. Inf. Dis.*, 1943, **87**, 126.

4 Moulton, S., Puck, T. T., and Lemon, H. M., *Science*, 1943, **97**, 51.

† The recovery of influenza virus from the air by this method will be described in a separate publication by M. Hamburger, Jr., and C. G. Loosli.

Persistence of Influenza Virus in Air at Different Relative Humidities.

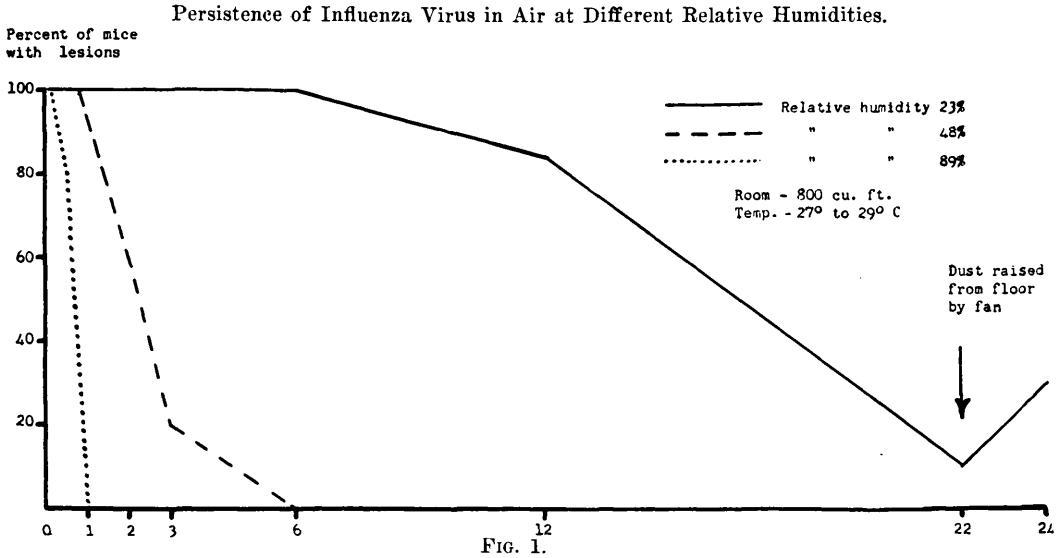

FIG. 1.
Time in hours following introduction of virus.

sistence of viable influenza virus in the air are shown graphically in Fig. 1.

In order to determine whether prolonged sojourn in atmospheres containing relatively low concentrations of influenza virus would result in a higher percentage of infections than occurred in mice exposed for the customary 20-minute period, groups of mice were placed in the experimental room 24 hours after introduction of virus into dry air and allowed to remain there for 2 hours. All these mice exhibited widespread consolidation of the lungs at autopsy while only one-third of the animals exposed for 20 minutes became infected and their lesions were limited in extent. These and other experiments reported in an earlier paper[3] indicate that intrapulmonary dosage which depends principally on the concentration of the infectious agent in the air and the amount of air inhaled, plays a very important role in determining the inception and severity of air-borne influenzal infection.

Our studies suggest that the disappearance of the virus from atmospheres of high humidity is due more to inactivation of the virus in the air rather than to a rapid settling out of the infectious particles as Edward and his colleagues[5] conclude, although both factors probably play a role. Evidence for the prolonged suspension of virus particles in the air was provided by the persistence of a marked Tyndall effect 12 hours after the introduction of the virus spray into an atmosphere of high humidity at a time when the air was no longer infective for mice. Further corroboration of the persistence of fine droplets in air of high relative humidity was derived from experiments in which mice exposed in the experimental room 4 hours following dispersal of streptococci, Group C, into the air, all became ill and died of streptococcal infection. Also large numbers of streptococci could be collected on settling plates and with the Moulton sampler for as long as 12 hours. The survival of the virus in atmospheres of low humidity is probably due to the rapid drying of the infectious particles as is indicated in the experiments of Edward.[6] Another indication of the survival of influenza virus in desiccated form was provided by certain experiments in which vigorous sweeping of the floor of the experimental room, 22 hours after spraying in the virus, enhanced the infectivity of the air. (Fig. 1). Preservation of viruses, for experimental purposes, by quick drying procedures gives further support to this view. These observations have an important bearing on the spread of infectious agents by the aerial route.

[5] Edward, D. G., Elford, W. J., and Laidlaw, P. P., *J. Hygiene*, 1943, **43**, 1.

[6] Edward, D. G., *Lancet*, 1941, **2**, 664.