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Experimental Air-Borne Disease. Quantitative Inoculation by Inhalation of Influenza Virus.

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Successful infection of animals with a virus recovered from influenza patients[1] laid the foundation for experimental study of the mode of transmission of this important respiratory disease. Intranasal instillation of influenza virus recovered from artificially infected air[2] proved air conveyance of the virus to be possible, and destruction of the air-suspended virus by ultraviolet irradiation raised hopes of environmental control of epidemic influenza by sanitary ventilation. Demonstration of air-borne influenzal infection of ferrets[3] and introduction of quantitative inhalation technics of inoculation[4] provided experimental means for study of air-borne influenza and air-borne superinfections which frequently complicate the pathologic and epidemiologic patterns.

The experiments to be presented have been conducted during the fall and winter of 1939-40 when other duties delayed the continuation of this study until a preliminary report seems desirable. Of the 25 experiments performed, employing some 1500 mice, protocols have been chosen to illustrate our interpretation of the observed phenomena. These results have been found reproducible in repeated tests, but the exploratory nature of the protocols did not adapt them to statistical summary.

Experimental. I. Air-borne Transmission of Influenza A Virus. Exposure of white mice to air which had been infected by droplet nuclei derived from a suspension of the virus of influenza A produces typical signs of the disease in these animals and causes death with sufficiently high concentration of the virus. All mice exposed 45

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2 Wells, W. F., and Brown, H. W., *Science*, 1936, **86**, 68; *Am. J. Hygiene*, 1936, **24**, 407.

3 Trillat, A., and Beauvillain, A., *Compt. rend. Acad.*, 1936, **205**, 1186; *Rev. d'hyg.*, 1938, **60**, 104; Andrewes, C. H., and Glover, R. E., *Brit. J. Exp. Path., Hygiene*, 1941, **34**, Sec. B, 21.

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TABLE I.
Air-borne Infection of White Mice with Virus of Influenza A and Its Relation to Concentration of Virus in the Air.
From Experiment 12, February 22, 1940.

Dilution of virus (WS-85)	Time of exposure in min.	g of suspension lost	Mouse No.									
			1	2	3	4	5	6	7	8	9	10
Undiluted	45	6.0	D6	D6	D6	D8	D8	D8	D8	D9	D9	D9
10^{-1}	45	5.5	D9	D10	D10	4	3	3	3	2	2	2
10^{-2}	45	5.0	D10	3	2	2	2	1	1	±	±	0
10^{-3}	45	6.0	2	0	0	0	0	0	0	0	0	0

Key to Tables: Mice dying before the 10th day were recorded according to day of death—D4, D5, etc. Surviving mice were sacrificed on the 10th or 12th day and the degree of lung lesions recorded. 1 signifies ¼ consolidated; 2 signifies ½ consolidated, etc.

minutes to air infected by atomizing 1 g of a 10% suspension of mouse lung (infected with PR-8[5] or WS[1] strain) in 50 cubic feet of air died within 6 to 9 days. (Table I.) If one-tenth of this amount was used for the spray, 30% of the mice succumbed and the remaining animals harbored severe lung lesions when autopsied on the tenth day. A dilution of 1:100 of the original suspension resulted in lesions of various degrees in most of the mice, and a 1:1000 dilution produced practically no lesions at all.

This gradation parallels experience with the intranasal inoculation of various dilutions of virus into anesthetized mice. The gross appearance and the microscopical sections of the lung lesions did not seem to differ from those following intranasal infection. Lesions could be prevented by the intranasal administration of a potent mouse anti-influenza A serum previous to the exposure to the virus[6] and the agent could be recovered from the lungs by subsequent passage to normal mice by the intranasal route. No doubt, therefore, influenza A virus has been successfully transmitted to white mice by the air-borne route.

Lesions produced by the air-borne route were, however, decidedly less severe than expected from calculation of the amount of air-borne virus inhaled, as compared with intranasal instillation of similar quantities under anesthesia. The influence of the anesthesia or the fluid medium (broth) on the severity of the lung lesion hardly accounted for the difference.

A few ferrets exposed to ferret-adapted virus showed a rise in temperature 48 hours later. Serum taken 2 weeks afterwards contained a high neutralizing titer for the virus of influenza A, while the serum taken before the start of the experiment was negative.

[5] Francis, T., Jr., *Science*, 1934, **80**, 457.

[6] Henle, W., Stokes, J., Jr., and Shaw, D. R., *J. Immunol.*, 1941, **40**, 201.

II. Compound Infection with Influenza A Virus and Pneumococcus Type I. Although recent epidemics have shown that clinical manifestations of uncomplicated infections with the virus of influenza A are not severe in man, bacterial superinfections may entirely alter the pathologic and epidemiologic pattern of the disease. Compound infection with virus and bacterium is decisive in swine influenza,[7] but the relationship in human pathology is not yet clearly understood. Mixed infection of mice with human influenza virus and with hemolytic streptococcus of Lancefield's group C produced higher mortality than either agent alone,[8] and nasal infection of the ferret has been induced by the combined action of influenza A virus and a hemolytic streptococcus (Group C) differing from uncomplicated influenza.[9] Pneumococcus Type I seemed to be particularly fitted for a study of compound infection, for this organism unassisted has little virulence for white mice when administered by the intranasal[10] or the air-borne route.[11] Mortality from compound air-borne infection of mice with pneumococcus (Type I) and sublethal doses of hemolytic streptococcus (Group C) has been shown[4] to exceed the sum of mortalities from infection with either.

Mice infected by inhalation of air-borne influenza A virus were exposed 1 to 5 days later to air-borne pneumococci of Type I from a culture which proved fatal in 10^{-8} to 10^{-9} dilution when intraperitoneally injected. With large doses of virus (resulting in the death of all mice exposed), the pneumococcus, when given 24 hours after the virus, shortened survival times in a number of experimental animals. On the other hand, pneumococci inhaled as late as five days after exposure to sublethal suspensions of the virus increased the severity of lesions and death frequently ensued. Tables II and III illustrate these relationships.

All mice found dead as well as those sacrificed on the twelfth day were examined for the presence of pneumococci in the lungs and the heart's blood, positive findings being designated in the tables by italics. Gross lung lesions usually did not appear to differ from those due to the virus alone. Occasionally a mottled appearance contrasted with the more even brownish-red color of the influenzal lung lesion. Histological studies do not yet justify speculation regarding the specific pathologic changes introduced by superinfection with

7 Shope, R. E., *J. Exp. Med.*, 1931, **54**, 349.

8 Schwab, J. L., Blubaugh, F. C., and Woolpert, O. C., *J. Bact.*, 1941, **41**, 59.

9 Glover, R. E., *Brit. J. Exp. Path.*, 1941, **22**, 91.

10 Webster, L. T., and Clow, A. D., *J. Exp. Med.*, 1933, **58**, 465.

11 Stillman, E. G., *J. Exp. Med.*, 1933, **23**, 58.

TABLE II.
Effect of Pneumococcus Type I on Mice Infected with a Fatal Dose of Influenza A Virus.
From Experiment 16, March 14, 1940.

Suspensions atomized	Mouse No. 1	2	3	4	5	6	7	8	9	10
Virus alone (WS-88, 10% suspension)	D7	D7	D7	D8	D8	D8	D8	D10	D10	D10
Virus + pneumococcus 24 hours later	*D4*	*D5*	*D5*	D8	D8	D8	D8	D9	D9	D9
Pneumococcus alone	*D7	0	0	0	0	0	0	0	0	0

*No lung lesion.
Italics designate the isolation of the pneumococcus from the heart's blood.

TABLE III.
Effect of Pneumococcus Type I on Mice Infected with a Dilute Suspension of Influenza A Virus.
From Experiment 21, April 10, 1940.

Suspensions atomized	Mouse No. 1	2	3	4	5	6	7	8	9	10	11	12
Virus alone (WS-90, 10-2)	D11	D12	4	4	2	2	2	2	0	0	—	—
Virus + pneumococcus 5 days later	*D8*	*D8*	*D8*	*D8*	D9	*D10*	*D11*	*D11*	*D11*	D12	0	0
Pneumococcus alone	0	0	0	0	0	0	0	0	0	0	—	—

Italics designate the isolation of the pneumococcus from the heart's blood.

TABLE IV.
Influence of Ultraviolet Light Radiation on Air Infected with the Virus of Influenza A.
From Experiment 13, February 22, 1940.

Suspension atomized	Ultraviolet radiation	Mouse No. 1	2	3	4	5	6	7	8	9	10
WS-85 (10% suspension)	—	D6	D6	D6	D8	D8	D8	D8	D9	D9	D9
" " "	+	± 0	± 0	± 0	± 0	± 0	0 0	0 0	0 0	0 0	0 0

the pneumococcus. These phenomena did not occur with inhaled sterile nuclei.

III. Sterilization of Infected Air by Ultraviolet Light. Radiant disinfection of air infected with fatal concentrations of influenza A virus (3 seconds exposure to an estimated 120 microwatts per square centimeter of 2537 Å wave length) is shown in Table IV. Only minor lesions were evident in the lungs of mice sacrificed on the tenth day after breathing irradiated air. Five out of 20 mice showed only one or two lesions of pinhead size. Comparison with Table I shows destruction of more than 99% of the virus.