Human Influenza Resulting from Aerosol Inhalation. (31255)

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In the years following the discovery of the viral etiology of human influenza, a number of studies were performed utilizing aerosol inoculation of volunteers(1-5). The doses were large, 10^5 egg or mouse infectious doses or greater, and information on the physical properties of the aerosols was limited. Although infection and illness were produced in these experiments, little information was gained concerning the site of deposition of the particles or the minimum infectious dose of the agent. Since epidemic influenza of man is probably transmitted, at least in part, by droplet nuclei of very small dimensions from infected persons, and it is likely that the virus content of such droplets is low, a study was initiated to determine the minimum infectious aerosol dose and the resulting patterns of infection and illness.

Techniques for the production, administration, and quantitation of small-particle bacterial aerosols have been developed(6). These techniques have been modified recently for use with viral agents(7). The following study was conducted using this system for administration of small-particle viral aerosols with amounts of virus similar to those discharged in coughs and the respiratory events of man(8).

Materials and methods. Virus strains. The virus used for human inoculation was influenza A2/Bethesda/10/63. An inoculum pool which had been passaged 5 times in primary African green monkey kidney tissue culture* was used initially. Nine subjects were subsequently given virus which had been passaged an additional 4 times in human embryonic kidney tissue culture. The virus inoculum pools were tested for safety as previously described(9).

In preliminary calibration experiments an influenza A2/Japan/305/57-related strain which had been passaged 5 times in eggs† was also used.

Aerosol generation. Aerosol produced from a liquid suspension of virus was produced using previously described equipment and procedures(10). The desired dilutions of virus were made in Eagle's basal medium (Hanks' salt solution base) with 0.2% bovine serum albumin. Ten to 15 ml of the liquid suspension of virus were placed in a Collison atomizer to which 0.2 ml of an antifoam agent‡ had been added. Aerosol particles predominantly of 1 to 3 μ in diameter were dispersed in a copper tube 7 feet by ½ foot in diameter by an airflow of 200 liters per minute. Humidity was maintained at 45-55%. Nineteen-liter samples of the aerosol for virus assay were drawn by vacuum into modified Shipe impingers containing 10 ml of veal infusion broth with 0.5% bovine serum albumin. At least 4 impinger samples were collected for each spray suspension atomized.

Virus titrations. Liquid virus suspensions and impinger fluid specimens were assayed for virus content as soon as they were obtained. Undiluted specimens and serial 10-fold dilutions in Eagle's basal medium were routinely assayed for virus content in primary rhesus monkey kidney tissue culture. Due to toxicity for the tissue culture, undiluted broth from impingers was decanted after 3 to 4 hours' adsorption at 36°C and replaced with Eagle's basal medium. After 5 to 6 days' incubation at 36°C, the presence of virus was determined by the hemadsorption method using guinea pig red cells. Virus concentration was determined by the method of Fisher(11) and expressed as 50% tissue culture infectious doses ($TCID_{50}$). Assays of egg propagated virus were performed in 10-day embryonated eggs by inoculation of 0.1

* Obtained from Dr. J. Anthony Morris, DBS, Nat. Inst. Health, Bethesda, Md.

† Obtained from Dr. Fred M. Davenport, University of Michigan, School of Public Health, Ann Arbor.

‡ Type A, Dow-Corning Co., Midland, Mich.

ml of specimens into the chorioallantoic cavity. Presence of virus in harvested chorioallantoic fluid was determined after 3 days' incubation at 36°C by the hemagglutination method using guinea pig red cells. Doses were calculated as above and expressed as 50% egg infectious doses.

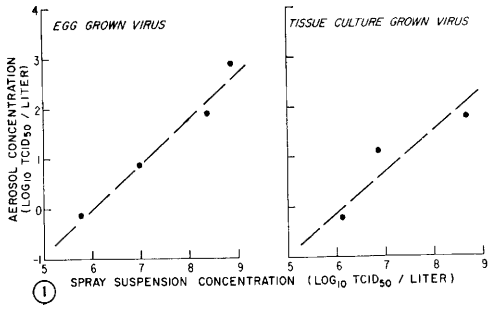
The mean virus suspension concentration for each aerosol experiment was calculated from the concentration before and after atomization. The mean impinger fluid virus concentration was calculated from at least 4 specimens for each aerosol generated. The concentration of virus in the aerosol was calculated on the assumption that each impinger specimen recovered all of the virus in the aerosol sampled. Shipe impingers recover 95% of inert particles of this size(12).

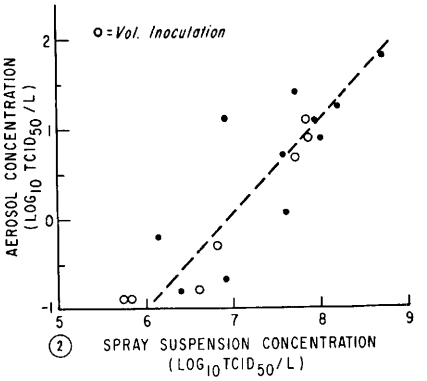
Preliminary experiments. Serial dilutions of a given liquid virus suspension were made and aerosols were generated from them. The virus titers of the liquid virus dilutions were found to be linearly related to the titers of the resulting aerosols. This was found for both tissue culture and egg propagated virus (Fig. 1). The slope was approximately 1. This relationship allowed volunteer dosages to be estimated prior to inoculation.

Inoculation of volunteers. Twenty-three male volunteers between 21 and 40 years of age from Federal correctional institutions participated in this study. Selection was based on serum antibody titers to the challenge virus. Supervision and clinical evaluation of these volunteers have been previously described(9).

Approximately 10 liters of the aerosol were inhaled by a face mask attached to a port in the copper tube. Inhalation was through the nose and exhalation was through a mouthpiece from which air was exhausted to prevent rebreathing of exhaled virus.

Virus isolation from clinical specimens. Throat swabbings were placed in 3 ml vials of veal infusion broth with 0.5% bovine serum albumin and antibiotics. Specimens were also obtained by gargling 10 ml of the same broth. Three-tenths ml of each specimen was inoculated into 4 rhesus monkey kidney tubes containing 1.0 ml of Eagle's basal medium. Presence of virus was determined by hemad-





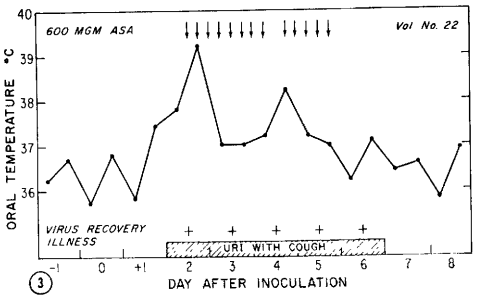


FIG. 1. Relation of liquid virus suspension concentrations and aerosol concentrations of A2 influenza.

FIG. 2. Summary of concentrations of tissue culture propagated A2 influenza virus in liquid virus suspensions and related aerosols.

FIG. 3. Clinical course of volunteer inoculated with A2 influenza by aerosol.

sorption with a 0.4% saline suspension of guinea pig red blood cells in 2 tubes after 5 days' and 2 tubes after 10 days' incubation at 36°C.

Serology. Neutralizing antibody in serum obtained before and 28 days following inoculation was determined employing rhesus monkey kidney tissue culture by the hemadsorption-inhibition method(13). Sera were inactivated at 56°C for 30 minutes prior to test-

TABLE I. Aerosol Administration of Influenza A2/Bethesda/10/63 to Volunteers.

Inhaled virus ($TCID_{50}$)	Vol #	Illness	Virus recovery (days after inoc)	Neutralization antibody: Before inoculation	Neutralization antibody: 28 days after
126	1	*	*	1280	2560
	2			2560	2560
	3			640	1280
78	4			160	160
	5			320	320
	6			320	320
59	7			40	1280
	8			80	80
	9			80	80
1	10	+	3–7	<5	80
2	11			<5	<5
	12			<5	<5
	13			<5	<5
	14		4–7	<5	320
5	15			40	40
	16			80	80
	17			40	40
	18	+	4, 7	10	1280
	19			20	1280
	20	+	2–6	5	5120
	21			<5	<5
	22	+	2–6	<5	640
	23			<5	<5

* Blank spaces indicate no response.

ing. Thirty-two to 100 $TCID_{50}$ of virus were used in the tests.

Results. Aerosol. A summary of the liquid virus suspension concentrations and aerosols from tissue culture propagated virus used in these studies is shown in Fig. 2. The particular aerosols administered to volunteers are indicated. These values also tended to follow a linear relationship. The slope of the line in the Figure was determined by the method of Wald(14). More scatter was noted than when consecutive values determined on a single day were compared. Also, comparable liquid virus suspension concentrations usually yielded lower aerosol concentrations than shown in Fig. 1. Recovery of virus from impinger specimens in volunteer studies was not enhanced by the use of embryonated eggs for assay of impinger specimens. Additional inoculation of 0.1 ml of this material into the amnionic and 0.2 ml into the chorioallantoic cavities of 4 eggs per specimen with subpassage of harvested material yielded no virus from the specimens in which it was readily recovered by the tissue culture methods employed.

Administered virus. The mean volume of inhaled aerosol was 10.1 liters (range 9.5 to 10.9). This volume was inhaled in an average of 10 inhalations (5 to 14) in 50.8 seconds (23 to 80). Approximately 0.02 ml of the liquid virus suspension was atomized in each 10 liters of aerosol. The approximate dose of virus per volunteer based on observed impinger results and an inhaled volume of 10 liters was from one to 126 $TCID_{50}$ (Table I). In 14 men the dose was 5 $TCID_{50}$ or less.

Illness. Typical clinical influenza occurred in 4 men. A representative clinical course is shown in Fig. 3. The mean duration of illness was 6 days and the mean maximum temperature elevation (with frequent administration of aspirin) was 38.8°C orally. Systemic symptoms of headache, malaise, and myalgia were prominent. Vomiting occurred 30 times in subject no. 20 on the third day of illness. Respiratory involvement was indicated by nasal obstruction, rhinorrhea, sore throat and cough. Inspiratory and expiratory wheezes, lasting 6 days, occurred in volunteer no. 10.

Virus recovery. Virus was isolated from the

4 men who were ill and from one additional subject. Days on which virus was recovered are indicated in Table I.

Serological results. Serum neutralizing antibody titers before and 28 days after inoculation are shown in Table I. No subject with pre-inoculation antibody titer of 80 or greater was infected as indicated by 4-fold or greater antibody increase. Infection without demonstrable virus shedding occurred in 2 of 5 men with antibody levels of 1 to 20 or 1 to 40. Antibody levels of 1 to 10 or less were associated with infection in 5 of 10 instances as indicated by both significant antibody rise and virus shedding. These infections in men essentially free of antibody were achieved with an administered virus dose of approximately 1 to 5 $TCID_{50}$.

Discussion. In the present investigation the dosage of virus was low, and in several instances it approached the limits of sensitivity of the sampling and assay systems. The comparison of virus concentrations of liquid suspensions and the aerosols produced from them yielded results similar to those in studies utilizing aerosols containing other viruses(15). The absolute sensitivity of the system is not known. Only about 10% of the infectivity of the liquid virus suspension which was atomized was recovered from each aerosol. Effects of humidity and thermal inactivation were probably minimal as the aerosols traversed the confining tube in about 10 seconds. Considerable loss of infectivity of the virus probably occurred in the atomization process and also possibly in the sampling procedure. If destruction of virus infectivity occurred as a result of the sampling process, estimated minimum infectious doses are falsely low. There was also variability in the tissue culture used for assay which may have affected the results.

The results obtained here may be used to define the approximate minimum infective dose of A2 influenza virus for man when administered in an aerosol. This dose should be considered only as an approximation due to the limitations of the system which were mentioned above. Sixty per cent of inert particles of the size of the aerosols used in this study have been shown to be retained in the respiratory tract, predominantly the lower tract(16). Assuming a similar retention of virus aerosol particles, half of the men with very low or nondetectable pre-inoculation antibody titers were infected with 0.6 to 3 $TCID_{50}$. This range agrees with that found for mice infected with A2 influenza by aerosol(17). In mice the mean LD_{50} was 10 and the 50% infectious dose was 0.5 mouse infectious doses.

A comparison of effects of administration of small doses of the same strain of A2 influenza by the aerosol and nasopharyngeal routes is needed. The illness produced in this study was of a severity equal to that produced previously by administration of 80,000 to 180,000 $TCID_{50}$ of this strain nasopharyngeally(18). It has been reported that illness occurred in 40% of susceptible volunteers given 30 $TCID_{50}$ of this strain by the nasopharyngeal route(19).

The demonstration that typical clinical influenza can be produced by low doses of virus when administered by the aerosol route suggests that very small amounts of virus may be sufficient for natural transmission. This finding is of particular interest because of recent findings of viral air contamination from infected persons(8). Also, it was found that low levels of pre-existing serum neutralizing antibody to the infecting virus were not sufficient to protect from infection and illness despite very low inoculation dosages.

Summary. Volunteers were given A2 influenza virus in a small-particle aerosol. Infection and typical influenza resulted from low doses of virus administered in this manner. Low levels of serum neutralizing antibody were not completely effective in preventing infection and illness. The human infectious dose of this influenza strain when administered by aerosol to subjects free of serum neutralizing antibody was approximately 3 $TCID_{50}$.

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Augmented Natriuretic Response to Infusion of Saline in Dogs Rendered Acutely Hypertensive with Metaraminol. (31256)

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Hypertensive subjects respond to infusion of sodium with an exaggerated natriuresis(1). This response is observed in patients with essential hypertension(2,3) and also in those with hypertension due to Cushing's syndrome (4,5), primary aldosteronism(6,7), pheochromocytoma(5) or unilateral renovascular disease(8). This natriuresis therefore appears to be a nonspecific response related only to the presence of elevated blood pressure. Indeed normal subjects exhibit an augmented natriuretic response to infusion of sodium when a temporary hypertensive state has been pharmacologically induced by administration of metaraminol(9).

The mechanism by which human hypertension alters the renal response to sodium loading is not known and no intact animal model has been available for study of this phenomenon(10). In the present study an attempt has been made to determine if animals rendered hypertensive by metaraminol respond to saline loading with a greater increase in urinary sodium output than occurs when the same animals are normotensive. The data demonstrate that dogs exhibit an enhanced natriuretic response to infusion of sodium during pharmacologically-induced hypertension.

Methods. Six non-pregnant mongrel bitches were maintained on a constant diet without restriction of fluid. Studies were performed in the morning after food and fluids had been withheld for 18 hours. Dogs were anesthetized with pentobarbital, 30 mg per kilogram, which was supplemented as necessary during the study. Aqueous vasopressin, 5 u, was