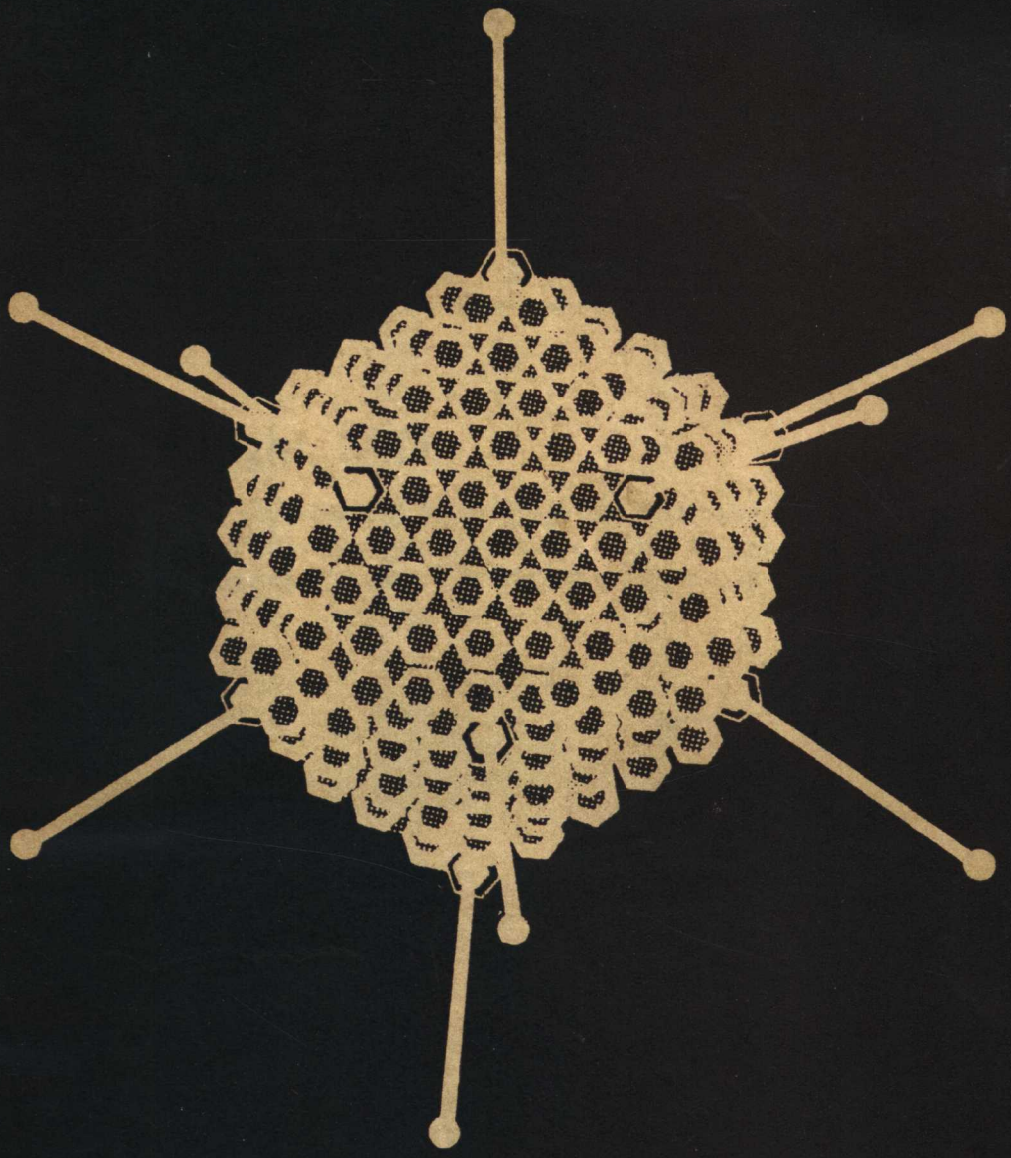

VIRAL AND MYCOPLASMAL INFECTIONS OF THE RESPIRATORY TRACT

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PREFACE

RESPIRATORY VIRAL DISEASES are undergoing an increasingly rapid transition from a collection of often indistinguishable syndromes to a large number of etiologically defined infectious illnesses. New information on the epidemiology, immunology, and properties of the viruses has created a body of knowledge that ultimately must be reflected in the practice of medicine. However, implementation into medical practice is difficult for several reasons. The diagnostic entities are new and numerous; other entities remain to be discovered. These diseases have a reputation for benignity that, while not wholly deserved, diminishes concern for their diagnosis and treatment. Diagnosis usually requires several days, and diagnostic facilities are not readily available. Finally, specific chemotherapy and vaccines presently have only limited usefulness.

Some of these limitations undoubtedly will be removed in the near future by studies in progress. With new methods of air sampling for viruses and generation of experimental aerosols, it seems probable that some of the mystery of transmission of viral infections will be dispelled. Viral chemotherapy is improving. The whole field of vaccine prophylaxis is being productively re-explored. Interferon and interferon-stimulating substances are under study, and the role of immunoglobulin A in respiratory secretions as the first barrier of immunologic defense is being investigated. Finally, increasing avail-

ability of facilities for viral diagnosis will permit physicians to know the disease they are treating.

The authors, for a number of years, have devoted much time and effort to the study of respiratory viral diseases. The work has involved all of the aspects of the problem mentioned earlier and has consisted primarily of observations on infections produced by experimental inoculation of normal human volunteers. Dealing with disease produced under highly controlled conditions gave insights not readily available from study of naturally occurring disease. With this background and an appreciation of the rapid development of knowledge of other aspects of the respiratory viruses, we decided that a text on this subject would fill an important need.

The monograph is intended for medical students and physicians. It seeks to make available, in a usable form, information from the laboratory, the field, and our work with volunteers to increase our ability to detect, confine, alleviate, and prevent these most common of all infectious diseases. While we have indicated the clinical direction of our report, this orientation will not prevent generous reference to discoveries in basic virology that have provided and will continue to be the basis for much new clinical knowledge. An appreciation of the virologic background of respiratory viral disease is becoming essential for the well-trained clinician, and this book is intended to supply that need.

Coinciding with developments in the knowledge of respiratory viruses is an increased understanding of the role of *Mycoplasma pneumoniae* in human disease. Because of the clinical similarity of respiratory involvement with this agent and that due to viruses, a chapter on mycoplasma infection is included in this book.

Houston, Texas VERNON KNIGHT

ACKNOWLEDGMENTS

THE FIRST OF OUR studies of respiratory viral diseases in volunteers were done at the National Institute of Allergy and Infectious Diseases, Clinical Center, National Institutes of Health, Bethesda, Maryland, with the approval of the late Dr. Justin Andrews, Director, and his successor, Dr. Dorland Davis. Normal volunteers for that program were recruited from the federal prison system with the approval of Mr. James V. Bennett, formerly Director, Bureau of Prisons, Department of Justice. Much of our work with viral aerosols was done in collaboration with the staff of the United States Army Biological Laboratories, Fort Detrick, Frederick, Maryland, whose scientific director was Dr. Riley D. Housewright. Ethical and scientific aspects of all studies were reviewed and approved by the Clinical Research Committee, Medical Board, and Director of the National Institutes of Health.

In 1966 the program was continued at Baylor College of Medicine in Houston, Texas. Subjects for experiments were inmate volunteers from the Texas Department of Corrections. Their participation was authorized by its Director, Dr. George Beto. Studies were made in the General Clinical Research Center (NIH grant RR-00350) at Methodist Hospital with the approval of Mr. Ted Bowen, its President, and at various units of the prison system. Studies at the prison units were largely supported by contracts from the Infectious Disease Branch, National

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Dr. Herbert E. Landahl, Department of Biochemistry and Biophysics, School of Medicine, University of California at San Francisco, made helpful criticisms of Chapter 1 *Airborne Transmission and Pulmonary Deposition of Respiratory Viruses;* and Dr. Arnold Monto, School of Public Health, University of Michigan, Ann Arbor, Michigan, critically read Chapter 12 *Coronaviruses.* Miss Leola Owings, Department of Microbiology, Baylor College of Medicine, supervised the preparation of manuscripts. The authors wish to acknowledge the assistance provided by these individuals and many others who contributed directly and indirectly to the work. The authors wish to thank the volunteers for an almost uniform record of friendly cooperation and concern for the success of experiments that were the basis for much of the work described in the book.

V.K.

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CONTENTS

CHAPTER 1

Airborne Transmission and Pulmonary Deposition of Respiratory Viruses

Vernon Knight

ONE OF THE OBSTACLES to prevention of viral and mycoplasmal respiratory diseases is an inadequate understanding of their means of dissemination. With most of these organisms infection must be transmitted by infected respiratory or oral secretions, directly or indirectly, to the respiratory tract of a susceptible person. With a few, such as adenoviruses and some enteroviruses, in which gastrointestinal infection occurs, fecal-oral or fecal-respiratory transmission may take place. The extent to which this form of transmission occurs, however, is unknown, although it seems less important than transmission by infected respiratory or oral secretions.

Given an infected case, how does virus inhabiting the nasopharynx or the lower respiratory tract get to the respiratory tract of the next victim? At one extreme, infected secretions may be transmitted by personal contact, i.e., kissing, contaminated hands, handkerchiefs, and the like, and by direct impaction of large droplets produced by coughing and sneezing and other forceful exhalations. While the latter is obviously a form of airborne transmission, the fact that it must occur at the same time as the exhalation, because the heavy droplets sediment rapidly, and because it can occur only at short range, it is convenient to consider it as a form of transmission by personal contact.

In contrast, infection may be transmitted at some distance by the large populations of

infected small particles, or aerosols,* generated by coughs and sneezes. Such particles, ranging in size from 1 to more than 20 microns in diameter, by remaining airborne for long periods, may disseminate widely in the local environment such as a schoolroom, home, or hospital ward, and potentially can infect large numbers of people. This, of course, assumes a sufficient dose and continued viability of the organism, points to be considered later.

Particles that settle out may become airborne again as, for example, when floors are swept or beds are made; but the common respiratory viruses probably lose most of their viability by this time, and it is doubtful that re-suspended viruses play much role in transmission.

Investigations in aerobiology, the branch of biology that deals with the occurrence, transportation and effects of airborne microorganisms or other biologic substances, have disclosed significant new data on the distribution and properties of viral aerosols as they relate to human respiratory viral disease. It is convenient to review the data according to (1) the effect of viral aerosols on the susceptible host, (2) the generation of viral aerosols by infected persons, and (3) the occurrence of viral aerosols in the air of the environment.

Deposition of Aerosol in the Respiratory Tract

The site of deposition of aerosol particles after inhalation is a function of their size; the principal forces at work on inhaled particles are inertia resulting from the respiratory effort, sedimentation due to gravity, and diffusion, an action that is important only when particles are quite fine.

* Aerosols are dispersions in air of solid or liquid particles, of fine enough particle size, and consequent low settling velocities to have relative airborne stability. If the air were perfectly quiet, it would take a spherical unit density particle of a 100 micron diameter 10 seconds to fall the height of a room (3 meters). For 40, 20, and 10 micron particles, the times would be 1, 4, and 17 minutes respectively.

Effect of Particle Size and Hygroscopicity. Particles derived from the respiratory tract are hygroscopic: when they are discharged into the ambient air, they lose moisture and shrink in size; when they are inhaled they take up moisture from the saturated air within the respiratory tract and regain their original dimensions. Thus, it is estimated that 1.5-micron hygroscopic particles, a size present in large numbers in coughs and sneezes, will increase to 2 microns in diameter when passing through the relatively dry air of the anterior nose, where the greatest amount of filtration by nasal hairs occurs, but will increase to 4 microns in the highly saturated air of the nasopharynx and the lung. The estimated pattern of deposition, on this basis, of a 1.5 micron hygroscopic aerosol within the respiratory tract is described in Table 1–1. The effect of hygroscopicity and the consequent increase in size of particles is to increase retention in tertiary bronchioles and alveolar ducts, an effect that might be significant in connection with viral aerosols that

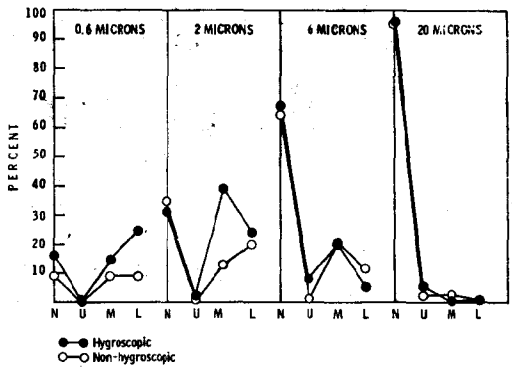

Fig. 1–1. The effect of hygroscopicity and consequent increase in particle size on the site of deposition and percentage of retention of particles. (N = nose breathing [6 per cent Tidal Air]; U = upper respiratory tract—pharynx to and including bronchi [10 per cent TA]; M = middle respiratory tract—bronchioles [20 per cent TA]; L = lower respiratory tract—including alveolar ducts [63 per cent TA].) (Data from Landahl, H. D.: The effect of gravity, hygroscopicity and particle size on the amount and site of deposition of inhaled particles, with particular reference to hazard due to airborne viruses. In Mercer, T. T., Morrow, P. E., and Stober, W. (eds.): *Assessment of Airborne Particles.* Springfield, Illinois, Charles C Thomas, 1972, pp. 421–428.)

Table 1-1. Deposition of 1.5-Micron Hygroscopic Particles within the Respiratory Tract

	% of Total Air	% Deposition Hygroscopic Particles	% Deposition Nonhygroscopic Particles
Nose	±6	36*	25
Pharynx to secondary bronchi	10	1†	0
Tertiary bronchi to respiratory bronchioles	21	25†	10
Alveolar ducts	63	21†	13
Total retained		83	48

* 24% of 2-μ particles retained on inhalation; 12% of total inhaled particles, 4μ in diameter due to accretion of water, retained on exhalation.

† Retention as 4-μ particles.

are highly infectious for this area of the respiratory tract.

In Figure 1–1 is shown the calculated pattern of deposition of aerosols, inhaled through the nose, of a range of particle sizes that is generated by coughs and sneezes. The effect of accretion of water in the respiratory tract, calculated as described earlier, is also presented. The effect of hygroscopicity is negligible with aerosols whose particles are 6 microns or greater in diameter. Below that size, deposition of hygroscopic particles is considerably increased in the tertiary bronchi (M, 2 microns) or alveolar ducts (L, 0.6 microns). Of significance is the fact that particles 6 microns and greater in size are trapped increasingly in the nose. Through the range of 0.6 to 6.0 microns, appreciable deposition occurs in the nose and also at one or more sites in the lower respiratory tract. The site of greatest deposition of aerosol might not necessarily be the site of initiation of infection with an agent that has greater capacity to infect one level of the respiratory tract than another.

Patterns of Deposition in Zero Gravity. Another point of interest in consideration of airborne infection is the effect of lack of gravity in space flight on patterns of deposition of aerosol.[8] Estimates indicate that in the absence of gravity, deposition of particles 2 to 6 microns in diameter is reduced by about two thirds in the lower respiratory tract, which would tend to reduce the hazard of infection with agents that might otherwise cause infection at these sites. The tendency for particles of large size to remain suspended in the absence of gravity, however, would increase the exposure of the nose and upper respiratory tract to airborne infection. No information is yet available to indicate that these theoretic possibilities actually alter the pattern of response to airborne infectious agents in space.

Effect of High Pressure Environment. The high pressures incident to working at great depth under the ocean could conceivably create hazards of airborne infection different from those on the earth's surface. In a theoretic consideration of this problem, Gussman and Beeckmans estimated that overall retention of particles 1 to 10 microns in diameter at a depth of 1000 feet (34 atmospheres) would not be greatly changed.[7] Deposition in the lower pulmonary tract would generally be less by about 10 per cent, the difference being most conspicuous at aerosol diameters of 3 to 5 microns.

Human Response to Inoculation with a Viral Aerosol

The effect of inoculation of infectious agents in aerosols depends not only on the physical

properties of the aerosols, but also on their biologic properties. Some important biologic features are the concentration of virus in the aerosol, the persistence of viability, the susceptibility of cells at various levels of the respiratory tract to infection, and infectivity of the virus used.[3] Related to the host is the possible presence of humoral antibody or cellular hypersensitivity to the agent and nonspecific factors of resistance. Not well studied in relation to response to infection is the role of abnormalities of pulmonary function, especially as it may relate to pulmonary clearance mechanisms, allergy, and present or recent infection with other agents. Other factors of host resistance undoubtedly exist that might influence susceptibility to infection.

Quantitative information on the infectivity of viral aerosols has been obtained in which uniform viability was maintained by the use of a flowing aerosol a few seconds old at the time of administration.[6] Host factors were controlled by the use of healthy young adult volunteers of known antibody status with respect to the agent under examination.

Table 1–2 shows the infectious dose of four agents for antibody-free adult volunteers following inoculation with a 1.5-micron viral aerosol and by nasal instillations. Since aerosol deposits to some extent throughout the respiratory tract, the site or sites responsible for the initiation of infection cannot be determined without other information. This limitation is further complicated by the fact that detection of infection is based on samples of the nasopharynx or oral secretions, while the initiation of infection and site of greatest involvement could be the lower respiratory tract. These limitations are partly obviated by a knowledge of the infectious dose following intranasal inoculation.

When the infectious dose deposited in the nose by aerosol is equal to or greater than the infectious dose deposited in the nose by nasal drops, it is not possible to implicate the lower respiratory tract as the site of initiation of infection. When the infectious dose deposited by aerosol in the nose is smaller than the infectious dose by nasal drops, it is probable that the lower respiratory tract is the site of initiation of infection.

Aerosol inoculation with rhinovirus and coxsackievirus (Table 1–2) led to deposition of doses of virus greater than the infectious dose by nasal drops in the nose and at each of two sites in the lower respiratory tract. Thus no conclusion regarding the site(s) of successful inoculation is possible. (For further discussion see "Pathogenesis," p. 159, Chapter 10.)

With a strain of adenovirus type 4 and apparently with the strain of influenza used, the infectious dose by aerosol was considerably smaller than that by nasal drops, indicating a greater susceptibility of the lower respiratory tract to infection with these viruses. The bronchioles and alveolar ducts (columns 3 and 4, Table 1–2) received about the same amount of virus; therefore, no distinction between these sites is possible.

Since the number of virions constituting an infectious dose of adenovirus was of the order of magnitude of 7, it is probable that most of the virus was deposited in the bronchioles and the alveolar ducts, the probable site(s) of initiation of infection.[2] The circumstances may be similar with influenza.

In any event, the number of particles required to produce infection in man with all four agents is small, a characteristic that fits well with their transmission by the airborne route. With the coxsackievirus and the rhinovirus, the lower dose required by the nasal drops than by aerosol suggests that some form of personal contact could lead to implantation of virus in the nose. With adenovirus and influenza, however, it appears that small-particle aerosol should be the most likely route to produce infection. This is especially true for adenovirus, since illness regularly follows inoculation with aerosol, whereas asymptomatic infection is usually produced by nasal inoculation.

Distribution of Particles in Sneezes and Coughs

The airborne transmission of respiratory viral infection depends upon the production

Table 1-2. Fifty Per Cent Human Infectious Dose (HID_{50}) in Units of 50% Tissue Culture Infectious Doses ($TCID_{50}$) for 4 Respiratory Viruses by 1.5-Micron Diameter Aerosol.

Site	(1) Nose	(2) Pharynx, Bronchi	(3) Bronchioles	(4) Alveolar Ducts	(5) Total Ret.	(6) Exhaled	(7) Total Inhaled	(8) Nasal Drops
% of Inhaled Dose	37.0	1.0	25.0	21.0	84.0	16.0	HID_{50}	HID_{50}
Rhinovirus type 15	0.24	0.007	0.170	0.14	0.56	0.12	0.68* (0.2–2.0)	0.032 (S.D. = 0)
Coxsackievirus A type 21	10.10	0.200	7.000	5.900	23.20	4.80	28.00 (15–49)	6.00 (3–13)
Adenovirus type 4	0.18	0.005	0.125	0.11	0.42	0.08	0.50 (0.2–1.4)	35.00 (8–157)
Influenza A/2/ Bethesda/10/63	1.08	0.030	0.750	0.63	2.49	0.51	3.00 (estimate)	†

*50% human infectious doses in $TCID_{50}$, with 95% confidence limits.
† Probably 5- to 10-fold greater than HID_{50} by aerosol.
Underlined figures represent doses approximately equal to or greater than HID_{50} by nasal drops.

Table 1-3. Size Distribution of Particles Expelled by Sneezes and Coughs

Diameter	Sneeze		Cough	
	No. Particles	Vol.	No. Particles	Vol.
μ		μ^3		μ^3
<1–1*	800,000	167,000	66,000	13,860
1–2*	686,000	1,209,219	21,300	37,701
2–4†	280,000	3,948,520	1,600	22,562
4–8†	134,000	15,117,197	1,290	145,531
8–16†	36,000	32,490,694	490	442,234
±22†	4,500	27,806,786	85	472,715
Total	1,940,000		90,765	

* Particle distribution determined by light-scattering effect. Data from Gerone, P. J., Couch, R. B., Keefer, G. V., Douglas, R. G., Derrenbacker, E. B., and Knight, V.: Assessment of experimental and natural viral aerosols. Bact. Rev., 30:576, 1966.

† Counts made of particles recovered on oiled slide in Bourdillon slit sampler. Data from Duguid, J. P.: The size and duration of air-carriage of respiratory droplets and droplet nuclei. J. Hyg. (Camb.), 44:471, 1945–46.

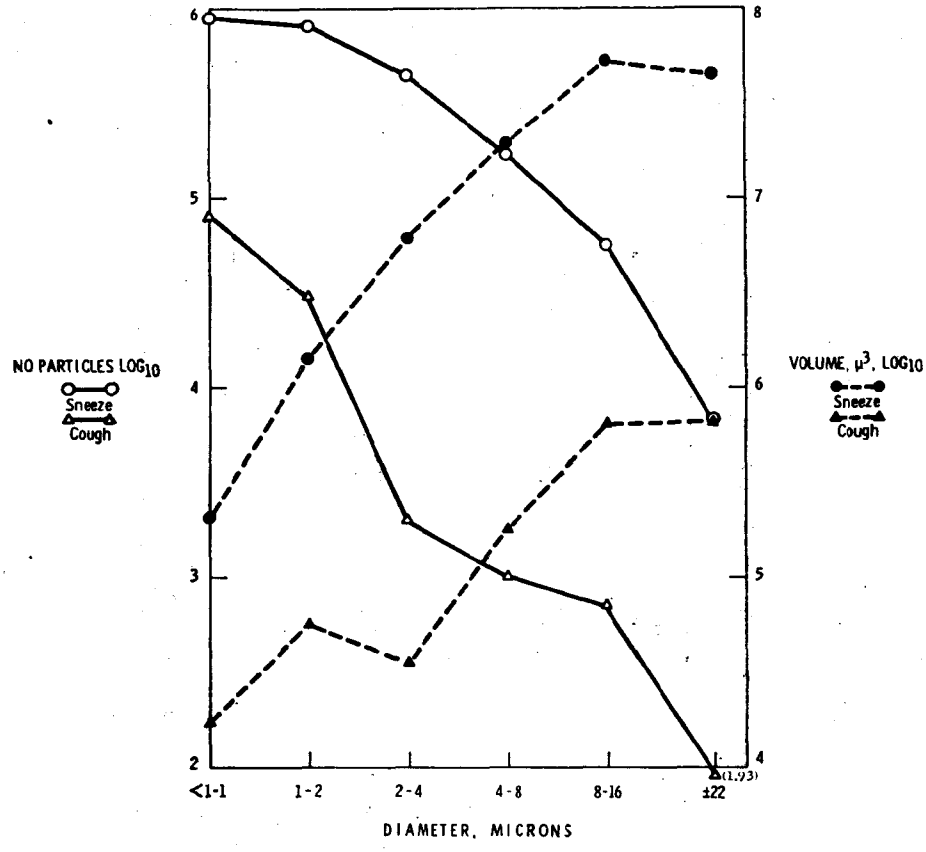

Fig. 1–2. Distribution of particles by size in coughs and sneezes. (Data from Duguid, J. P.: The size and the duration of air-carriage of respiratory droplets and droplet-nuclei. J. Hyg. [Camb.], 44:471, 1946; and Gerone, P. J., et al.: Assessment of experimental and natural viral aerosols. Bact. Rev., 30:576, 1966.)

by infected persons of aerosols containing viruses in doses large enough to initiate infection. The dose varies with the microorganism involved and the site of deposition, which is dependent on the size of the particle. A number of studies have been made of the number and size of particles expelled by sneezes, coughs, talking, or singing. The results of these studies vary considerably, reflecting variation in the methodology and also a considerable variation in the numbers of droplets expelled by any one event.

Size and Number. Table 1–3 and Figure 1–2 illustrate examples of the size distribution of particles contained in sneezes and coughs. Measurements of diameters below 2 microns were based on results from a particle size analyzer using the principle of light scattering. Results above this size were based on visual measurement of particles collected on an oiled slide in the Bourdillon slit sampler. A small number of particles larger than 22 microns were omitted from consideration because of their high settling velocities. Measurement of particle size by light scattering is most accurate below 2 microns in diameter, and visual measurement is more accurate with larger particles. The data show a parallelism in the pattern of distribution of particles of the various sizes, with a 20-fold greater number of particles in the sneeze. The largest number of particles with both events was in the < 1- to 2-micron range, with a steady fall thereafter. The total volume of particles in the various size categories, also shown, increases with the size of particles, despite the diminishing frequency of occurrence of larger particles. This finding is important, since a study of coxsackievirus A type 21 in aerosol revealed the concentration of virus more closely related to the volume than to the numbers of particles (Fig. 1–3).

Occurrence of Virus. Gerone and Couch and their associates detected coxsackievirus A type 21 in the air phase and from mucous droplets sedimented on the wall of weather balloons that had been used as receptacles for sneezes and coughs.[5] The amount of virus recovered was variable, and recoveries were

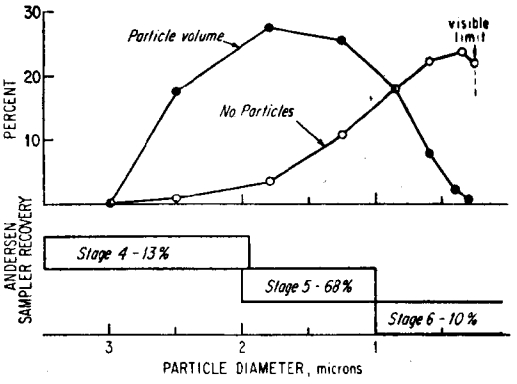

Fig. 1-3. Distribution in aerosol of particles, particle volume, and virus according to particle diameter. (From Couch, R. B., et al.: Preparation and properties of a small-particle aerosol of coxsackie A21. Proc. Soc. Exp. Biol. Med., 118:818, 1965.)

more frequent from coughs than from sneezes despite the larger number of particles in sneezes. Concomitant titrations of oral secretions showed virus regularly present at the time virus was recovered from droplets in sneezes and coughs. The size of particles in the air phase was not determined, but it was noted that they remained suspended in a small volume for several minutes after sampling. This would indicate that a large proportion of the small particles previously described in sneezes and coughs were in the air phase. The wall phase would contain the larger droplets that impinged by inertia from the sneeze or cough and those with settling velocities great enough to settle out in the brief period before sampling.

The same investigators tested the air of rooms occupied by volunteers with coxsackievirus A type 21 infection during the time when they were coughing and sneezing. By use of a large-volume air sampler, an estimated 82 per cent of the air of a 70,000 liter hospital room was sampled in 12 minutes. In rooms occupied by two or three volunteers, 5 to 185 $TCID_{50}$ of virus were recovered from 6 of 16 samples. The sampler was found to be about 10 per cent efficient, thus the rooms contained 10 times more virus than indicated. Samples were obtained at two- to

4-hour intervals. The virus recovered could have been introduced just before sampling or could represent an accumulation for a longer period. The decay rate of viruses in aerosol is relatively rapid, and it is probable that the results obtained represent virus expelled not long before sampling.

Artenstein and Miller recovered adenovirus type 4 from coughs of military recruits naturally ill with the disease and from air of rooms occupied by them, using methods similar to those of Gerone and Couch and their associates.[1]

Experimental Airborne Transmission of Coxsackievirus A Type 21 Infection

Couch and his associates studied natural airborne transmission of respiratory infection with coxsackievirus by exposing antibody-free adult volunteers to other volunteers previously infected by inhalation of viral aerosol.[4] Non-infected were separated from infected men by a double-walled wire screen four feet in width. This would prevent passage of large airborne droplets, yet allow smaller particles to disseminate freely. The air in the room was gently circulated by large fans. Infection and illness began in men inoculated by aerosol on days 2 to 4 (Fig. 1–4). On day 6, 2 to 4 days later, a wave of infection was detected in men on the opposite side of the room. This also followed by 3 days the recovery of coxsackievirus A type 21 from the air of the room. The agitation of air by fans could lead to continued airborne suspension of particles 20 microns in diameter or larger, which in still air would sediment in a brief period. These particles would almost exclusively sediment on the nasopharynx, the site of greatest susceptibility to infection with coxsackie virus A type 21.

A second major wave of infection occurred

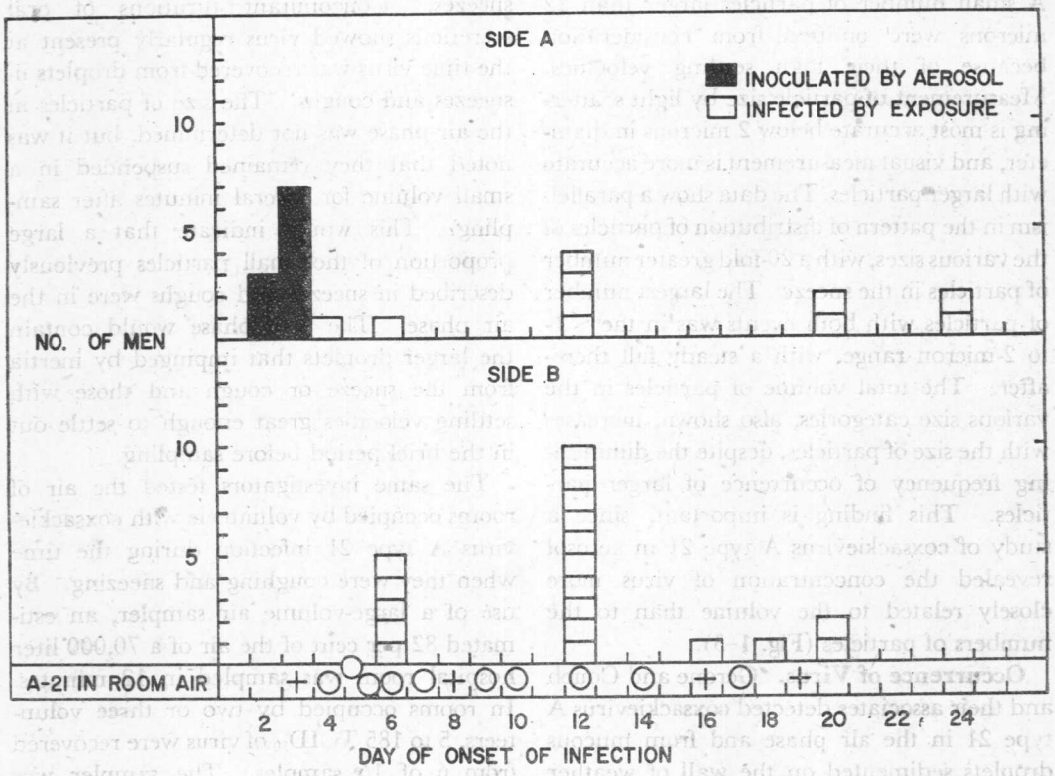

Fig. 1–4. Number and location of volunteers with onset of shedding of A21 on indicated day after inoculation of 10 volunteers on side A. Time of collection and results of air sampling are indicated. (From Couch, R. B., et al.[4])