THE INFLUENCE OF RELATIVE HUMIDITY ON THE SURVIVAL OF SOME AIRBORNE VIRUSES

by

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INTRODUCTION

Airborne infection has been a subject of controversy since the communicability of disease was first recognized. Its history has been especially turbulent during the last 100 years. Attitudes toward the importance of airborne infection have often been stronger, either pro or con, than the evidence at hand justified. Such a phase now exists with general underestimation of the importance of the subject.

The lack of a sound theoretical basis has been the primary reason for the lack of growth in this field.

A rebirth of interest in the epidemiology of airborne infection has been experienced during the past 20 years. Methods for generating, sampling and quantitating aerosols have been developed. Factors that account for some of the variation in host susceptability, such as the structure and function of the respiratory tract, mucocilliary function as a protective mechanism and the distribution of inhaled particles in the respiratory tract, have been elucidated.

Considerable attention has been given to the effects of environmental factors on airborne microorganisms. The purpose of the study reported in this paper was to determine the effect of relative humidity on some airborne viruses.

LITERATURE REVIEW

Airborne infection

Historically, the study of airborne infection has been cyclic and without firm foundation. To quote Langmuir (1), "Rather than developing from a series of hypotheses based on epidemological observations and tested repeatedly in the laboratory and in the field, there has been a tendency to empiricism and to overgeneralization from limited observations. The field of airborne infection has had no John Snow to lay down sound theoretical principles early in its development."

Galen (131-201 A.D.) wrote, "When many sicken and die at once, we must look at a simple common cause, the air we breathe."* Albertus Magnus (350 A.D.) regarded the cause of epidemic disease as pestilential winds and corruption of the air.* It is doubtful that either of these men conceived of airborne transmission of disease but view it as a change in the air itself. The concept of disease during this period of history was generally founded in demonology of one form or another. The belief in demons as the cause of disease held sway until the middle of the 14th century when the black plague ravaged Europe killing an estimated one-fourth to three-fourths of the population. Winslow (2) states that this occurrence of epidemic plague which marked the nearest approach to a definite break in the continuity of history that has ever occurred, produced almost universal conversion to the doctrine of communicability of disease.

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*Refers to Winslow (2) in this chapter

During this period epidemic disease was generally regarded as caused by corrupt air. The source of this corruption was considered to emanate from dead bodies, swamps, or stagnant water. The nature of this atmospheric pollution was regarded as a change in the nature of the air itself. It was referred to as dirty, vile, black and obscure.

Fracastorius (1483-1553) proposed that rather than a change in the air itself, it was something carried by the air that caused disease.* Sydenham (1624-1689) wrote, "I much doubt if the disposition of the air tho' it be pestilential is of itself able to produce the plague; but the plague, being always in some place or other; it is conveyed by pestilential particles or the coming of an infected person from some place when it rages into an uninfected one, and is not epidemic there, unless the constitution of the air favors it."*

Fracastorius in Contagion wrote, "The original germs which have adhered to the neighboring humors with which they are analogous, generate and propagate other germs precisely like themselves, and they in turn propagate others until the whole mass and bulk of humors is infected by them." From this statement it would appear that he had conceived of the germ theory; however, as Winslow points out, the bulk of his writing indicated he considered these "germs" ferments rather than living organisms.* Kircher (1598-1680) who published his Scrutinum Pestis in 1658 was the first to present the theory that living organisms were the primary cause of disease. The major defect in his theory was that these organisms were spontaneously generated by the decomposition of organic matter.*

Henle (1809-1885) in his On Miasmata and Contagia concludes, "That the matter of the contagions is not only organic, but also animate, indeed endowed with individual life, and that it stands in the relation of a parasitic organism to the diseased body." He also plainly stated, "The contagium of the miasmatic contagious disease is a matter which may float in the air" (3).

Though the theory of spontaneous generation had already received several serious setbacks, Pasteur (1822-1895), Tyndall (1820-1893), and others, by steady quantitative studies of the germ content of air, dealt it a death blow. Tyndall in his Essay on the Floating Matter of the Air in Relation to Putrefaction and Infection (1881) leaves no doubt to his views on the importance of airborne bacteria in producing disease. "Consider the woes," he says, "Which these wafted particles during historic and prehistoric ages have inflicted on mankind; consider the loss of life in hospitals from putrefying wounds; consider the loss in places where there are plenty of wounds but no hospitals, and in the ages before hospitals were anywhere founded; consider the slaughter which has hitherto followed that of the battlefield, when these bacterial destroyers are let loose, often producing a mortality far greater than that of the battle itself; add to this the other conception that in times of epidemic disease the self-same floating matter has mingled with it the special germs which produce the epidemic, being thus able to sow pestilence and death over nations and continents."*

Lister (1827-1912), convinced that airborne germs were responsible for disease, introduced the carbolic acid spray to combat the dangers of airborne infection.*

The new science of bacteriology, under the leadership of Pasteur and Koch (1843-1910) made rapid progress in establishing the relationships between organisms and disease. This increased familiarity with bacteria made it possible to study airborne infection experimentally. Villemin (1827-1892) suggested in 1868 that tuberculosis arose from the inhalation of the infectious material.* Koch's discovery of the tubercle bacillus stimulated the direct testing of this hypothesis. Some investigators believed that the expectorated sputum dried up and fragmented into fine dust which was suspended in the air and inhaled. Flügge (1847-1923) disagreed with this concept and by extending the work of Laschtschenko, he and his co-workers demonstrated that in talking, coughing, and sneezing, many minute droplets were liberated and these constituted the main danger of infection. He showed that these droplets from tuberculous persons contained tubercle bacilli and that guinea pigs could be infected by breathing them. These findings were in harmony with Koch's opinion that the main, if not the only, source of human tuberculosis was the human consumptive (4).

A major disagreement arose over the method of transmission of tuberculosis. Some asserted that transmission was primarily by the ingestion of tuberculous milk. Fidel in 1907 demonstrated that as few as 20 inhaled organisms could produce tuberculosis in dogs while in some experiments as many as six billion introduced by stomach tube failed to do so. In spite of experimental results of this type the popularly accepted view was transmission by ingestion (4).

Riley and O'Grady (4) pointed out that much of the experimental evidence at this time was contradictory and that the views of some of the adherents of airborne transmission were not easily distinguished from beliefs in miasms. Yellow fever and malaria, long thought to be airborne, were proven to be transmitted by insect vector. With water-borne and food-borne diseases accounted for, it looked as though it would ultimately be proven that no disease was transmitted by air.

Chapin (5) believed that contact was all important in the transmission of disease in the community. He fought against the practice of terminal fumigation. His influence undoubtedly contributed to the loss of interest in airborne infection which waned for more than 25 years.

Wells (6) in 1934 developed the concept of droplet nuclei and challenged the essentially exclusive dominance of contact infection which had held sway since Chapin's (5, 7) work a quarter of a century before. Robertson (8) reviewed the work on airborne infection prior to 1943. He and his group at the University of Chicago as well as Andrewes (9) made contributions in the area of controlling cross infections in hospitals, army barracks, and air raid shelters.

During the past 20 years a great deal of basic work on the precise mechanisms of contact and airborne infection has been done. Such underlying principles as aerosol sampling, natural and artificial aerosol generation, precise points of portals of entry in the respiratory tract and importance of particle size were subjects of comprehensive study.

Considerable work has been done on factors which affect the viability of airborne bacteria. While the greater part of this work has been done on the effect of relative humidity (RH) and temperature on airborne cells, considerable differences of opinion still exist. De Ome (10), Loosli et al. (11), and Williamson et al. (12) indicated that high RH was more lethal than medium or low RH. Wells et al. (13) reported opposite findings. Dunklin et al. (14), Ferry et al. (15), Shechmeister et al. (16), claimed intermediate RH level to be the most lethal. Goodlow and Leonard (17) stated that low RH (20-30%) hasten the decay rate for most cells as does very high RH (95%); optimal RH ranges from 40 to 80%.

Webb (18, 19, 20), studying factors affecting the viability of airborne bacteria, found that the death of bacterial cells aerosolized from distilled water suspensions occurred at two different rates. A rapid initial kill took place within the first second, followed by a subsequent slower death. He suggested that the death of the cell resulted from the movement of water molecules into and out of the cell in an equilibrium system, resulting in a collapse of the natural structure of cellular protein. Survival of airborne cells was enhanced by the addition of some amino acids, long chain protein degredates, some sugars, and polyhydroxycyclohexanes. He suggested that the mechanism of this action was hydrogen bonding, thus preserving the natural structure of the cellular protein. Using aerosols of S. marcescens, E. coli, Staph. albus, and B. subtilis, he also demonstrated individual variation in aerosol survival. However, high RH favored survival with each organism.

Using a continuous flow cloud chamber, Rosebury (21) found that aerosols of S. marcescens, B. suis, M. pseudomallei, M. mallei, P. tularensis, pneumonitis virus, and 3 strains of psittacosis virus were more stable at an RH above 70-80% than when sprayed into a dry atmosphere.

The literature contains few reports on the influence of RH and temperature on airborne viruses. Edward et al. (22), Loosli et al. (23), Shechmeister (24) and Hemmes et al. (25) reported studies with airborne influenza virus. However, the quantitative aspects of this work leaves something to be desired. Harper (26) tested aerosols of vaccinia, influenza, Venezuelan equine encephalitis (VEE) and polio for survival at temperatures of approximately 10° C., 20° C. and 30° C. All agents survived best at the lowest temperature tested. Aerosols of these agents were also evaluated at 20, 50 and 80% RH. Vaccinia, influenza and VEE survived best at 20% RH, while polio survived best at 80% RH.

Webb et al. (27) found pigeon pox virus to be stable in aerosols. The virus was little affected by changes in RH. Rous sarcoma virus (RSV) was extremely sensitive to changes in RH and survived best at RH above 70%. He also found that with the addition of 6.0% inositol the harmful effects of lower RH were overcome. An increase in virulence was also detected in the RSV after storage as an aerosol.

Langmuir (1) proposed the following definitions relative to the study of airborne infection. Contact can be defined as contiguous touching either directly as in kissing or indirectly as in the use of a contaminated surgical instrument, shaking hands, or the passing of a toy from child to child. Droplets emanate from the mouth and to some extent

from the nose during talking, coughing and sneezing. These droplets extend not more than about one meter from the mouth. The large droplets fall to the floor, the smaller ones dry to form small residues and remain suspended in the air for long periods of time. Thus, droplets actually pass through the air and in a literal sense are airborne; but at the same time, they exist only in the immediate vicinity of their source. Droplet nuclei are the small residues arising from the dried droplets that remain suspended and may be wafted on air currents to the far corners of the room or pass through ventilation ducts. Dust is composed of the usually large particles that exist on the floors, clothing or bedding that may be periodically suspended and resuspended in the air by activity, especially sweeping, dressing, or bed making. These terms will be used as defined by Langmuir throughout this paper.

Table 1 illustrates the evaporation time and falling distance before evaporation takes place.

Particle size is of extreme importance in the study of airborne infection. Goodlow and Leonard (17) showed that if the particle size was 1 μ the LD_{50} dose of P. tularensis for guinea pigs was 3 cells. If the particle size was increased to 12 μ the LD_{50} dose was 20,000 cells.

Table 1. Evaporation time of droplets and falling distance before evaporation (water droplets in unsaturated, still air at 22° C.)[a]

Diameter of droplet (microns)	Evaporation time (seconds)	Distance in feet droplets will fall before evaporation (at 50 per cent RH)
200	5.2	21.7
100	1.3	1.4
50	0.31	0.085
25	0.08	0.0053
12	0.02	0.00028

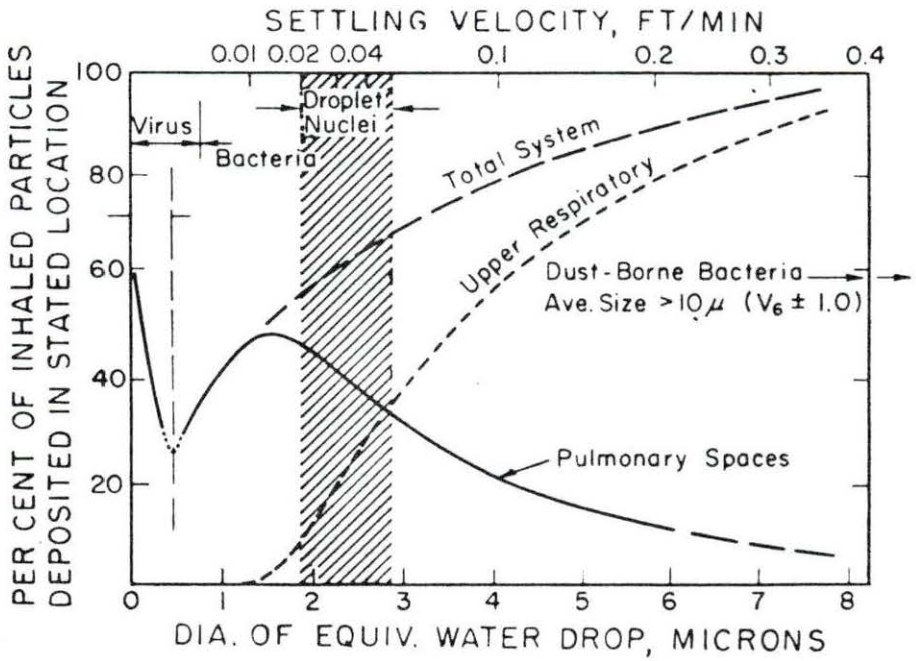
[a]Wells (28, p. 9).

Wright (29) and Bang (30) point out the importance of the structure of the upper respiratory system in removing particulate matter from the air. The shape of the air passages, the large surface area and the close proximity of the turbinates all aid in the removal of particulate matter from the air. Turbulence and eddying, produced by the torturous course further tend to precipitate matter from the inspired air.

Fig. 1 from Hatch (31) illustrates the regions of the respiratory system where deposition takes place with droplets from 1 μ to 8 μ in diameter. The optimum size range for deposition in the lower respiratory system is 2-3 μ in diameter. At a diameter of 10 μ virtually all particles are deposited in the upper respiratory system.

Nasal filtration of particles larger than 10 μ in diameter is virtually 100%; however, smaller particles tend to escape deposition. Once deposition of particulate material occurs in the nose and nasopharynx,

Fig. 1. Total and regional deposition of inhaled particles in relation to the aerodynamic particle size (Hatch 31, p. 238)


SETTLING VELOCITY, FT/MIN
0.01 0.02 0.04
0.1
0.2
0.3
0.4
PER CENT OF INHALED PARTICLES DEPOSITED IN STATED LOCATION
100
80
60
40
20
Virus
Bacteria
Droplet Nuclei
Total System
Upper Respiratory
Dust-Borne Bacteria
Ave. Size >10μ (V6 ± 1.0)
Pulmonary Spaces
0
1
2
3
4
5
6
7
8
DIA. OF EQUIV. WATER DROP, MICRONS

it is carried by ciliary action on a thin blanket of mucus toward the posterior end of the pharynx where, as further accumulation occurs, it is either expectorated or swallowed; some aspiration into the lungs has been demonstrated.

The general stability of aerosols, disregarding their biological nature, is determined by a number of factors. Several different forces influence the movement of individual particles in an aerosol resulting in their deposition. Some of these forces are brownian movement, gravitational settling, thermal forces causing movement of particles toward any object colder than its surroundings, electrical forces and acoustical forces. In addition there may be condensation causing the particles to increase in size until they fall out very rapidly. This deposition of particles is called physical decay.

Many types of cloud or static aerosol chambers have been described each with the purpose of minimizing physical decay. Wolfe (32) used a cylindrical chamber with a slow moving fan to maintain the aerosol in suspension. Goldberg et al. (33) described a static aerosol chamber which he called a Dynamic Aerosol Toroid (DAT). It consisted of a 500 liter drum which was rotated at 3 r.p.m. Reduction in physical loss of an aerosol contained in the DAT resulted from the rotation of the drum. The path of a contained particle was modified by the rotation of the drum in a circular orbit slowly spiraling toward the periphery. Aerosols with particle size less than 6 μ would remain airborne for a period of 2 days with a minimal physical loss. A modified DAT was designed for this study and is described under materials and methods.

Newcastle disease

The survival of Newcastle disease virus (NDV) under a variety of conditions has been studied by several workers. Doyle (34) found the virus active for 48 hours at 37° C. but inactive after 72 hours. Farinas (35) found that virus infected crop content in a liquid state stored in cotton stoppered tubes remained virulent for 15 days at room temperature; but if allowed to dry at room temperature on absorbent cotton, the virus was inactivated in 5 days.

Jungherr (36) reported that, on sterile strips of burlap, the virus was viable for 55 days at 22° C. and 7% RH. On non-sterile burlap strips, the virus was viable for 22 days at -1.7° C. and 45% RH, 16° C. and 51% RH and 22° C. and 7% RH.

Doyle (34) found that NDV stored in a refrigerator in a 50% solution of glycerin in normal saline was active after 197 days but not after 259 days. Farinas (35) found that refrigerated crop content from infected chickens remained infective for 5 months and 11 days. Hudson (37) observed that virus infected brain and spleen, suspended in 50% glycerin and held at 1° C. for 239 days, produced death in birds. Iyer (38) found that tissue from infected organs dried *in vacuo* over phosphorus pentoxide, and stored in the refrigerator, was virulent for about 3 years. Brandly *et al*. (39) observed that amnioallantoic fluids stored in a moist or lyophilized state at 0° and -72° C. was of unaltered infectivity after 23 months. Olesiuk (40) found that NDV survived best in fresh eggs, chicken down, and broth stored at various temperatures and conditions. She found a decrease in duration of viability at 20° C. or higher, with a

marked decrease at 37° C. The virus was viable in fresh eggs, in the incubator for 126 days; normal room temperature 235 days; hen house 255 days; refrigerator, 538 days (last test). When placed on chicken down the virus survived 87 days in the incubator, 192 days at normal room temperature, 255 days in the hen house and at least 528 days in the refrigerator and deepfreeze.

De Lay et al. (41) demonstrated that chickens suspended 4.5 ft. above the floor in pens where Newcastle disease infected chickens were housed, developed symptoms of the disease. Air samples collected at the same level were also infective for 10-day chicken embryos.

Infectious bovine rhinotracheitis

The viral agent that causes infectious bovine rhinotracheitis (IBR) has apparently existed in Europe for many years, producing a disease syndrome known as Bläschenausschlag or coital vesicular exanthema (CVE) (42). Infectious bovine rhinotracheitis, a respiratory disease of cattle, was first reported in California in 1954 by McIntyre (43), McKercher et al. (44) and Schroeder and Moys (45). A similar illness was discovered in Colorado in 1955 by Jensen et al. (46). McKercher (42) established, by cross immunity and serum neutralization tests, that CVE and IBR were caused by the same virus. He also found that CVE virus readily produced symptoms of IBR with no period of adaptation required. From this, one could conclude that the disease syndrome that developed was dependent upon the method of elimination of the agent from an infected animal and its proximity to a susceptible host. Since the respiratory tract is the site of infection in the IBR syndrome, airborne transmission is highly

favored. The shorter the survival time of airborne particles, the denser the concentration of cattle required to support the spread of the virus. Such environmental factors as sunlight, relative humidity and temperature are also factors to be considered.

Madin et al. (47) cultivated IBR virus in tissue culture using bovine kidney cells. A cytopathogenic effect was demonstrated and the disease was reproduced in calves with infected tissue culture fluids.

Vesicular stomatitis

Vesicular stomatitis, a disease of horses, cattle and swine was first reported in South Africa in 1884 (48). There are 2 immunologically distinct types, vesicular stomatitis, New Jersey and vesicular stomatitis Indiana.

The disease is spread with difficulty by indirect contact; however, is easily spread by direct contact. Biting insects have also been used experimentally for transmission of the disease. There have been no reports in the literature to indicate that vesicular stomatitis is transmitted naturally by infectious aerosol.

Vesicular stomatitis virus (VSV) is a rod-shaped particle approximately 200 mμ long and 60 mμ in diameter. It is apparently an RNA virus containing much phospholipid, is ether sensitive and resists 0.5% phenol (49).

METHODS AND MATERIALS

Static aerosol chamber

A modification of the rotating drum chamber described by Goldberg et al. (33) was designed for this study (Figs. 2, 3). It consisted of an aluminum drum 30 inches in diameter and 15 inches deep with a capacity of 140 liters. It was powered by a Zero-Max variable speed motor with potential speeds of 0-400 r.p.m. The speed of rotation for this series of studies was 3 r.p.m. Access panels were mounted on each side of the chamber to facilitate the generation of aerosols, collection of aerosol samples and for sensing temperature and RH.

Hygrometer indicator

A Universal A.C. Hygrometer Indicator,* accurate to ± 1.5% RH and ± 2° F. temperature was used to sense the temperature and RH within the aerosol chamber. It was equipped with 8 narrow range type TH elements which sensed both temperature and RH. The elements were mounted in a multiple mounting (Figs. 4, 5) with an external connection to the hygrometer and a selector switch for selection of the desired element.

Humidity controller

A variable controller was designed which activated systems to increase or decrease humidity within the chamber (Fig. 6). This unit was designed so that control was effected by preset limits on the linear scale of the hygrometer. Regardless of the element range, control was based on a

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*Hygrodynamics, Inc., Silver Spring, Md.

Fig. 2. Rotating drum aerosol chamber and accessory equipment

1. rotating drum
2. temperature and RH probes
3. hygrometer
4. recorder
5. Zero-Max motor
6. all glass impingers
7. sampling vacuum pumps
8. humidity controller

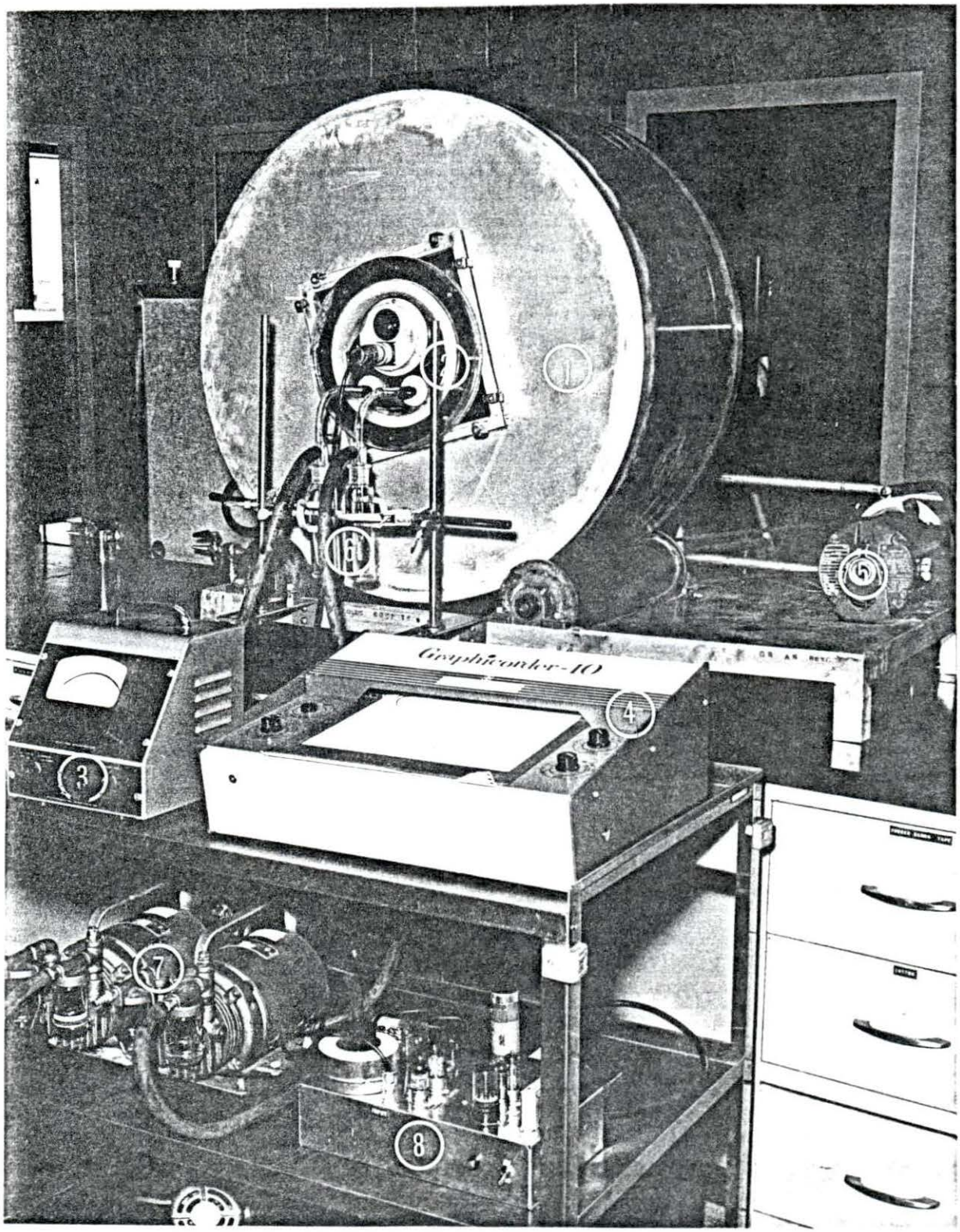

Geophysicograph-10
1
2
3
4
5
7
8

Fig. 3. Rotating drum aerosol chamber and accessory equipment

1. rotating drum
2. nebulizer
3. Zero-Max motor
4. nebulizer compressor

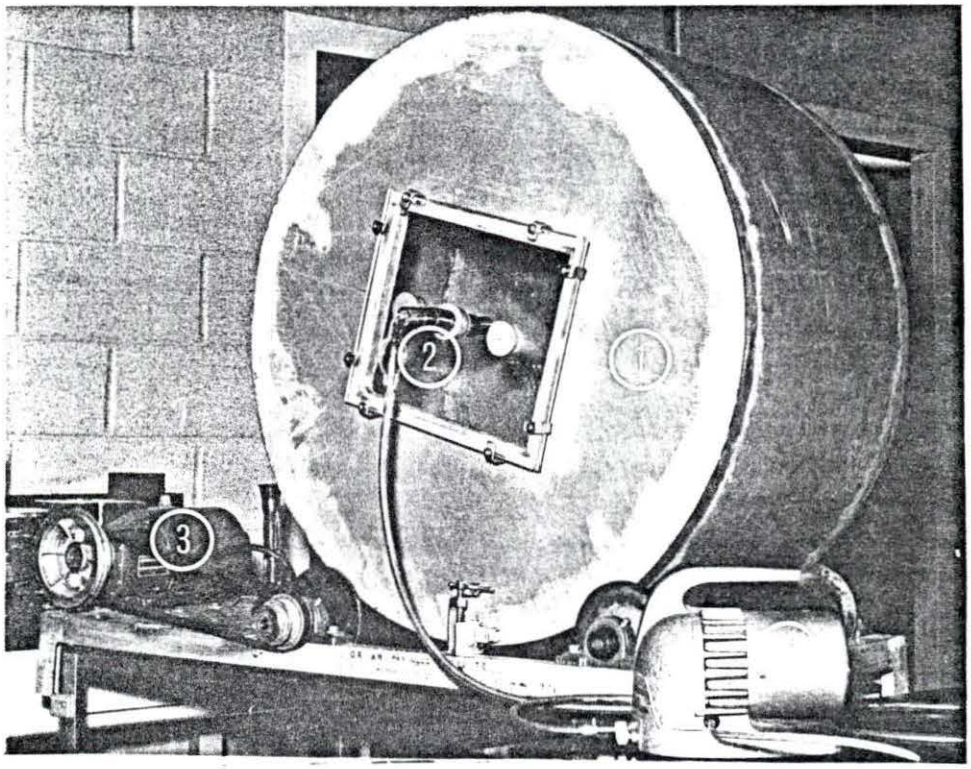
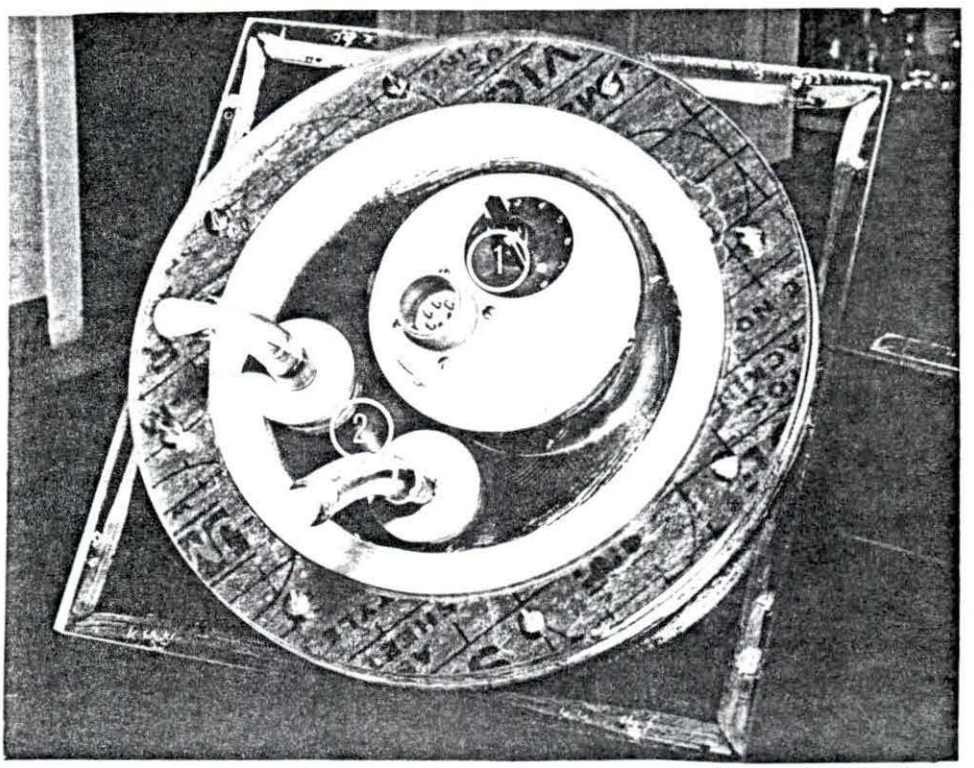

1
2
3

Fig. 4. External view chamber access port
1. multiple mounted narrow range temperature and RH sensing elements
2. aerosol sampling probes

Fig. 5. Internal view chamber access port
1. multiple mounted narrow range temperature and RH sensing elements
2. aerosol sampling probes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2

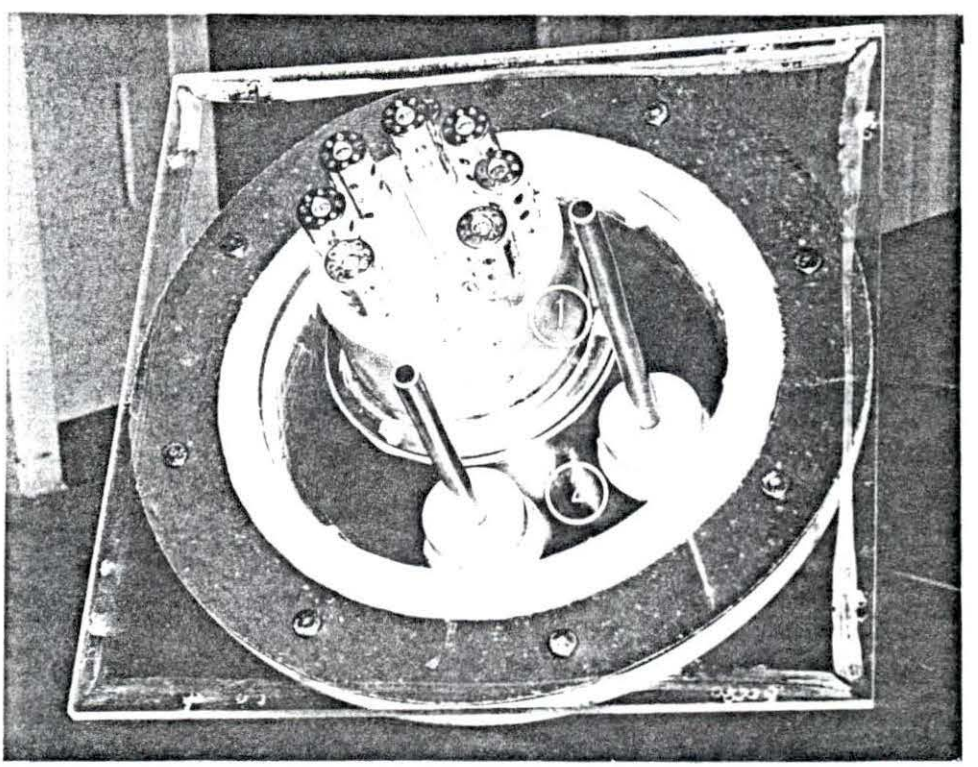
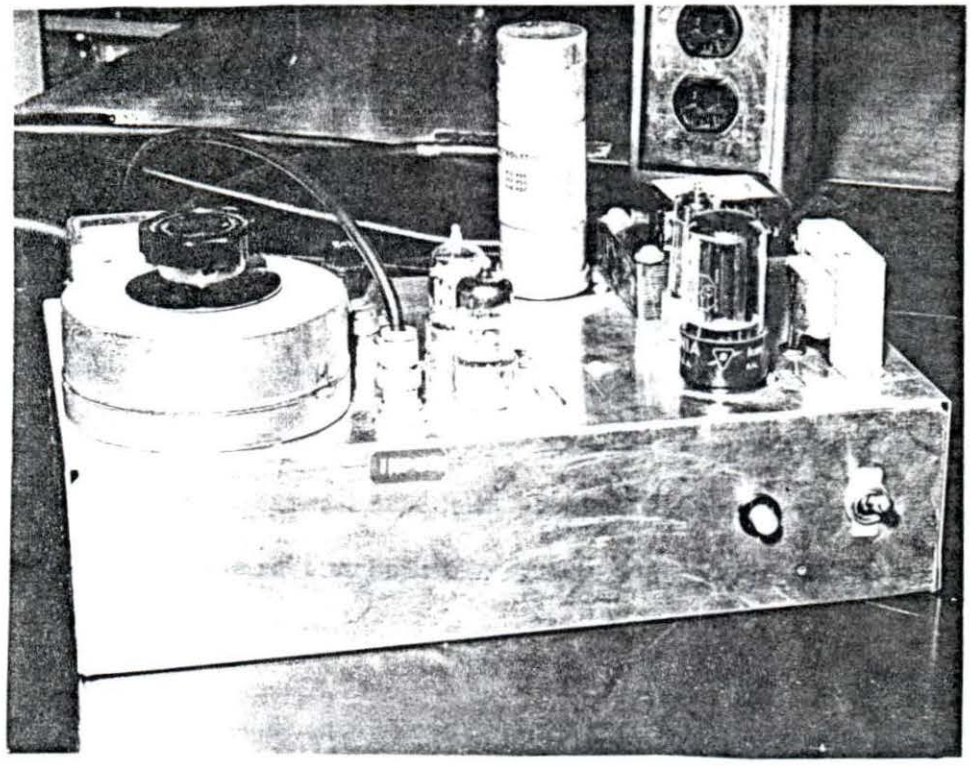
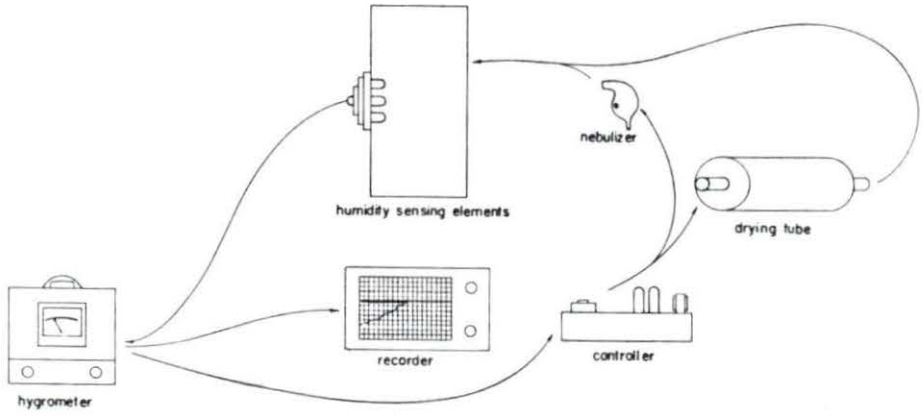

1
2

Fig. 6. Relative humidity controller

Fig. 7. Relative humidity sensing recording, and control system




humidity sensing elements
nebulizer
drying tube
recorder
controller
hygrometer

linear scale reading. Thus, it was possible to control the humidity over narrow ranges. The humidity control system is illustrated in Fig. 7.

Recorder

A Graphicorder 10* (Fig. 2) was used to continuously record the temperature and humidity during a test period. This recorder had a straight linear scale from 0-10 which coincided with the scale on the hygrometer. Calibration curves were used to find the humidity and temperature from these linear scales. With this recorder it was possible to follow the temperature and humidity throughout the experiment.

Aerosol generator

Aerosols were generated with a De Vilbiss #40 nebulizer.** This nebulizer had a capacity of 0.2 ml per minute and operated at a pressure of 5 pounds per square inch (Fig. 3).

Aerosol samplers

All-glass impingers,*** described by Wolf et al. (50), were used to collect all aerosol samples. Each sampler contained 20 ml of 1% peptone solution containing 500 µgm of streptomycin and 500 units of penicillin per ml for the collection of NDV aerosols. These samplers had a constant flow rate of 12 liters per minute by virtue of a limiting orifice located in the

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*Dynatronic Instruments Corp., Melrose Park, Ill.

**De Vilbiss Co., Somerset, Pa.

***Ace Glass Co., Vineland, N. J.

sampling tube. As long as a vacuum of 15 inches of water was maintained, the flow rate was constant.

Fluorometer

A Turner 110 fluorometer* was used to measure the fluorescence of Rhodamine B dye used in the physical decay system. Rhodamine B could be detected with this instrument in quantities as low as 0.1 part per billion. The fluorometer (Fig. 8) was equipped with #110-814 and #110-822 primary filters and #110-819 secondary filters.

Rhodamine B

Rhodamine B dye is non-toxic and is almost unique in being excited to fluorescence by the green line of mercury, 546 mμ. A standard 0.1% solution was prepared and used throughout the series of studies.

Newcastle disease virus

The GB-Texas strain of NDV used in this study was produced in 10-day chicken embryos. Sixty dozen 10-day embryonated chicken eggs were inoculated with 0.1 ml of 10-fold dilution of GB-Texas strain of NDV. Allantoic and amnionic fluids were harvested aseptically after 48 hours incubation. These fluids were centrifuged, bottled, and stored at -40° C. This pool of virus was sufficient for the entire series of studies. The titer of this virus pool was $1 \times 10^{8.38}$ ELD_{50}/ml.**

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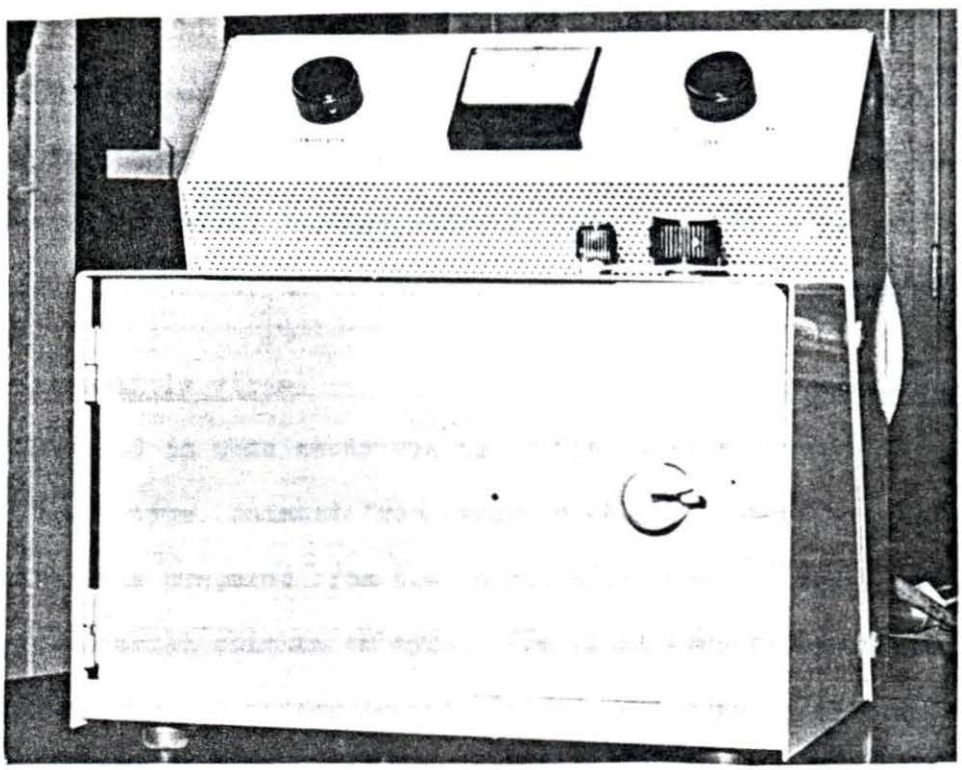
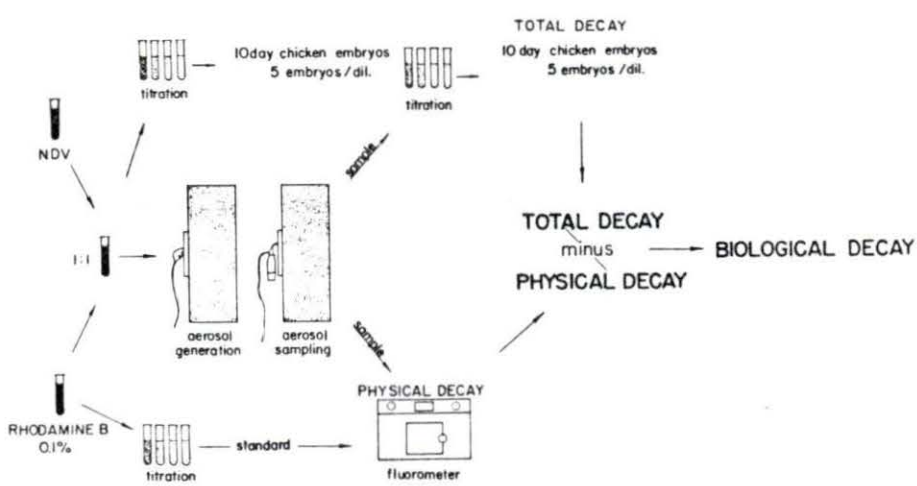
*G. K. Turner Associates, 2425 Pulgas Ave., Palo Alto, Calif.

**Fifty per cent embryo lethal dose per ml.

Fig. 8. Fluorometer

Fig. 9. Determination of physical, biological and total decay of aerosols in the rotating drum chamber




TOTAL DECAY
10day chicken embryos
5 embryos / dil.
titration
10day chicken embryos
5 embryos / dil.
titration
NDV
sample
1:1
aerosol generation
aerosol sampling
TOTAL DECAY
minus
PHYSICAL DECAY
BIOLOGICAL DECAY
sample
PHYSICAL DECAY
RHODAMINE B
0.1%
standard
titration
fluorometer

Infectious bovine rhinotracheitis virus

The IBR virus used in this study was the 14th tissue culture passage of a virus isolated from an infected bovine animal.* Primary bovine kidney cell cultures were used for the titration and production of this virus. It was stored at -40° C. until time of use.

Vesicular stomatitis virus

The VSV used in this study was the fifth passage in chicken embryos of a New Jersey type isolated from swine in Atlanta, Georgia.** A pool of this virus was prepared from the chorio-allantoic and amnionic membranes from infected chicken embryos. The final preparation was the supernatant from a 20% suspension of blended membranes. Titrations were conducted with primary swine kidney cell cultures.

Bacteriophage T_3

Bacteriophage T_3 (T_3 phage) used in this study was previously described by Songer et al. (51). All tests were conducted with virus from the same pool.

Chicken embryos

Ten-day chicken embryos from non-vaccinated, Newcastle disease free flocks were used for the titration of NDV.

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*Received from Dr. D. Baldwin, NADL, Ames, Iowa.

**Received from Dr. E. W. Jenney, NADL, Ames, Iowa.

Tissue cultures

Primary embryonic bovine kidney cell cultures were used for titrating IBR virus. The culture medium consisted of Earl's balanced salt solution with 0.5% lactalbumin hydrolysate, 5% SPF calf serum and 200 µgm of streptomycin per ml of fluid.

Primary swine kidney cell cultures were used for titrating VSV. The culture medium consisted of Earl's balanced salt solution with 0.5% lactalbumin hydrolysate, 5% calf serum and 200 units of penicillin and 200 µgm of streptomycin per ml of fluid.

Test procedure

Aerosols of the test agent were generated with a De Vilbiss #40 nebulizer connected to the access port of the chamber. To facilitate drum rotation the connection was made with a tube within a tube sealed with stopcock grease (Fig. 2). Generation time was 5 minutes, followed by a 2-minute stabilization period.

Two air samples of 3 liters each were collected in 20 ml of 1% peptone at each predetermined time interval. Time interval determination was based on the temperature and humidity of the chamber during the test period. Studies with NDV were conducted at one of 3 different temperatures: 4° C. (walk-in refrigerator), 23° C. (room temperature), or 37° C. (walk-in incubator). Studies with IBR, VSV and T_3 phage were conducted at 23° C.

Before an aerosol was generated in the chamber, the approximate RH desired was established. If a high RH was desired, water was nebulized

into the chamber. If a low RH was desired, the chamber air was replaced with pre-dried air. In the lowest RH range the aerosol was generated across a layer of $CaSO_4$ into the pre-dried chamber. Diluting air that replaced the sample air at the time of sampling was also preconditioned. All experiments were conducted at 10, 35 or 90% RH with an average maximum deviation of $\pm$ 5%.

The humidity-temperature sensing and control system is illustrated in Fig. 7. The probe most sensitive for the desired range was selected. Temperature and RH were sensed by the same probe. Hygrometer readings were linear from 0-100. Actual temperature and RH were determined by the use of calibration curves. This data was also recorded, providing a constant record of the temperature and RH throughout the test.

Aerosol samples were titrated using tenfold dilutions prepared in 1% peptone containing 500 units of penicillin and 500 µgm of streptomycin per ml. When titrating T_3 phage, antibiotics were deleted from the medium.

When titrating NDV, 0.1 ml of each dilution was inoculated into five 10-day embryonated chicken eggs. Eggs were candled daily for 5 days and questionable deaths were verified by the hemagglutination test.

Infectious bovine rhinotracheitis virus was titrated in primary bovine kidney cultures. Each tube contained 0.9 ml of tissue culture fluid and was inoculated with 0.1 ml of the appropriate virus dilution.

Five tubes were inoculated with each dilution. Cultures were checked daily for a period of 5 days for cytopathic effect.

Vesicular stomatitis virus was titrated in primary swine kidney cultures. Each tube contained 0.9 ml of tissue culture fluid and was inoculated with 0.1 ml of the appropriate virus dilution. Five tubes were inoculated with each dilution. Cultures were checked daily for a period of 5 days for cytopathic effect.

Bacteriophage T_3 was titrated by the method previously described by Songer et al. (51). Two-tenths ml of the appropriate dilution was added to 3 ml of melted soft agar which had previously been inoculated with one drop of a 3-hour culture of E. coli B. After mixing it was poured into a pre-poured agar plate and allowed to solidify. Following 6 to 10 hours incubation, plaques were counted and the number of plaques per liter of aerosol calculated.

The Reed-Muench (52) method of calculating 50% endpoint was used to establish the titration endpoints for NDV, VSV and IBR virus.

PRELIMINARY STUDIES

Aerosol sampling fluids

Preliminary studies were conducted to determine the value of various sampling fluids. Tryptose-saline, buffered saline, 1% peptone, tryptose broth and Hanks' solution were evaluated.

An aerosol of GB-Texas NDV was generated for a period of 5 minutes into the roller drum chamber. Following a 2-minute stabilization period, 10 aerosol samples of 3 liters each were collected, 2 with each test fluid. All samples were then titrated each in its own type of fluid. Five 10-day embryonated chicken eggs were inoculated with 0.1 ml of each dilution. From the results shown in Table 2, 1% peptone was selected as the appropriate sampling fluid.

Table 2. Evaluation of aerosol sampling fluids

Samples per interval	Sampling fluid	NDV ELD_{50}/ml fluid
2	Tryptose-saline	$10^{3.52}$
2	Buffered saline	$10^{3.50}$
2	1% peptone	$10^{4.03}$
2	Tryptose broth	$10^{3.74}$
2	Hanks' solution	$10^{3.50}$

Titration of NDV in the presence of the use concentration of rhodamine B

Tenfold dilutions of the GB-Texas strain of NDV were prepared in 2 dilution fluids. One fluid, 1% peptone containing 200 µgm streptomycin and 200 units of penicillin per ml; the other, 1:1000 rhodamine B suspended in 1% peptone containing 200 µgm streptomycin and 200 units of penicillin per ml. Five 10-day chicken embryos were inoculated with 0.1 ml of each dilution. The results are shown in Table 3. No detectable toxic effect of 1:1000 rhodamine B on NDV could be detected.

Table 3. Titration of NDV in 1% peptone and 1% peptone containing 1:1000 rhodamine B

Titration fluid	NDV ELD_{50}/ml
1% peptone	$10^{8.63}$
1:1000 rhodamine B in 1% peptone	$10^{8.63}$

Aerosol decay determination

Preliminary studies were conducted to evaluate the roller drum as an aerosol chamber. Physical decay has been a serious problem in aerosol studies in the past; however, Goldberg et al. (33) stated that with the roller drum, minimal physical decay was experienced. Physical decay can be simply stated as a loss of the organisms from the aerosol contrasted with biological decay which is a loss of biological activity. A further

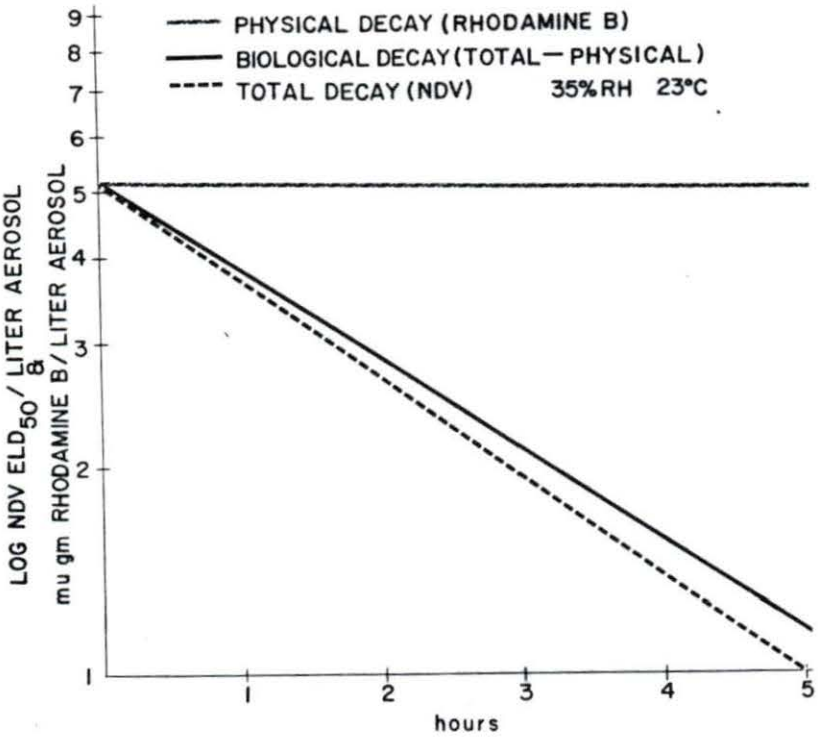
step was taken to show the relationship between total decay, biological decay and physical decay with this system.

Rhodamine B, a fluorescent dye detectable in concentrations as low as 0.1 part per billion was selected for physical decay studies.

Fig. 9 diagrammatically illustrates the test procedure. Equal parts of 0.1% rhodamine B and undiluted allantoic-amnionic fluid containing GB-Texas NDV were mixed. This mixture was titrated in 10-day chicken embryos and used for aerosol generation in the roller drum chamber. Following a 2-minute stabilization period, 2 initial samples of 3 liters each were collected. At appropriate intervals, two 3-liter samples were collected and all samples assayed for NDV and rhodamine B.

Tenfold dilutions of each air sample were made in 1% peptone containing 500 units of penicillin and 500 µgm of streptomycin. Five 10-day chicken embryos were inoculated with each dilution. An aliquot of each sample was assayed for fluorescence and the concentration of rhodamine B was calculated. From the baseline established by the initial samples, the total and physical decays were established for each time interval. By subtracting physical decay from total decay, biological decay was determined. Comparison of the physical, biological and total decay are graphically illustrated in Fig. 10.

Fig. 10. Comparison of physical, biological and total aerosol decay in the rotating drum chamber


PHYSICAL DECAY (RHODAMINE B)
BIOLOGICAL DECAY (TOTAL — PHYSICAL)
TOTAL DECAY (NDV) 35%RH 23°C
LOG NDV ELD50 / LITER AEROSOL
& mu gm RHODAMINE B / LITER AEROSOL
9
8
7
6
5
4
3
2
1
1
2
3
4
5
hours

RESULTS

Survival of airborne Newcastle disease virus

The results of NDV aerosol survival tests are presented in the graphs in Figs. 11, 12, 13 and 14. Data from which these graphs were drawn appears in Tables 6 through 17 of the appendix.

From the graphs in Fig. 11 it can be seen that at 23° C. survival of airborne NDV was favored at 10% RH. Survival also appeared to be slightly higher at 90% than at 35% RH.

In Fig. 12 it is observed that at 37° C. survival of airborne NDV was also favored at 10% RH. The difference in survival rates at 90% and 35% RH was greater at this temperature than at 23° C.

In Fig. 13 it should be noted that the time intervals were changed from minutes to hours. The reason for this change was that airborne NDV survived much better at 4° C. than it did at either 23° C. or 37° C. This increased survival was best determined by extending the storage period. At 4° C. survival was still better at 10% RH than at either of the other levels of RH. The lines in graph 3 compare favorably with the physical decay line in Fig. 10. This suggests that under these conditions of temperature and RH aerosol decay was due, primarily, to physical decay or evanescence.

Another observation that should be made from the graphs in Fig. 13 is that survival was better at 35% than at 90% RH. In tests conducted at 23° C. and 37° C. the reverse was observed.

For the purpose of comparison the results of all NDV aerosol survival tests conducted at the same temperature and RH were averaged and plotted

in the graphs in Fig. 14. Tests conducted at 4° C. extended over a 24-hour period and were plotted accordingly in graph 1. However, tests conducted at 23° C. and 37° C. were for considerably shorter periods of time. To provide a basis of comparison, these lines were extrapolated to 24 hours. From these condensed results, several observations should be made. Airborne NDV survived storage better at 4° C. than at 23° C. or 37° C. regardless of the RH of the chamber. This finding was anticipated and was consistent with Harper's (26) results with other viruses. An observation that was not anticipated, however, was that at 10% RH, airborne NDV survived equally as well at 37° C. as it did at 23° C. At 35% and 90% RH survival was considerably greater at 23° C. than at 37° C.

Survival of airborne infectious bovine rhinotracheitis virus

The results of IBR virus aerosol survival tests are presented in the graphs in Figs. 15 and 16. Data from which these graphs were drawn appears in Tables 18 through 21 of the appendix.

From the graphs in Figs. 15 and 16 it can be seen that at 23° C. airborne IBR virus survived best at 90% RH. It should also be noted that the initial level of virus was greater at 90% than at either of the other RH levels. The deviation between individual tests shown in Fig. 15 was considerably greater at 35% and 10% than at 90% RH. In Fig. 16 it should also be noted that survival was better at 10% than at 35% RH.

Fig. 11. Decay of NDV aerosols at 23° C. and 35, 90 and 10% RH. Each line represents a single test

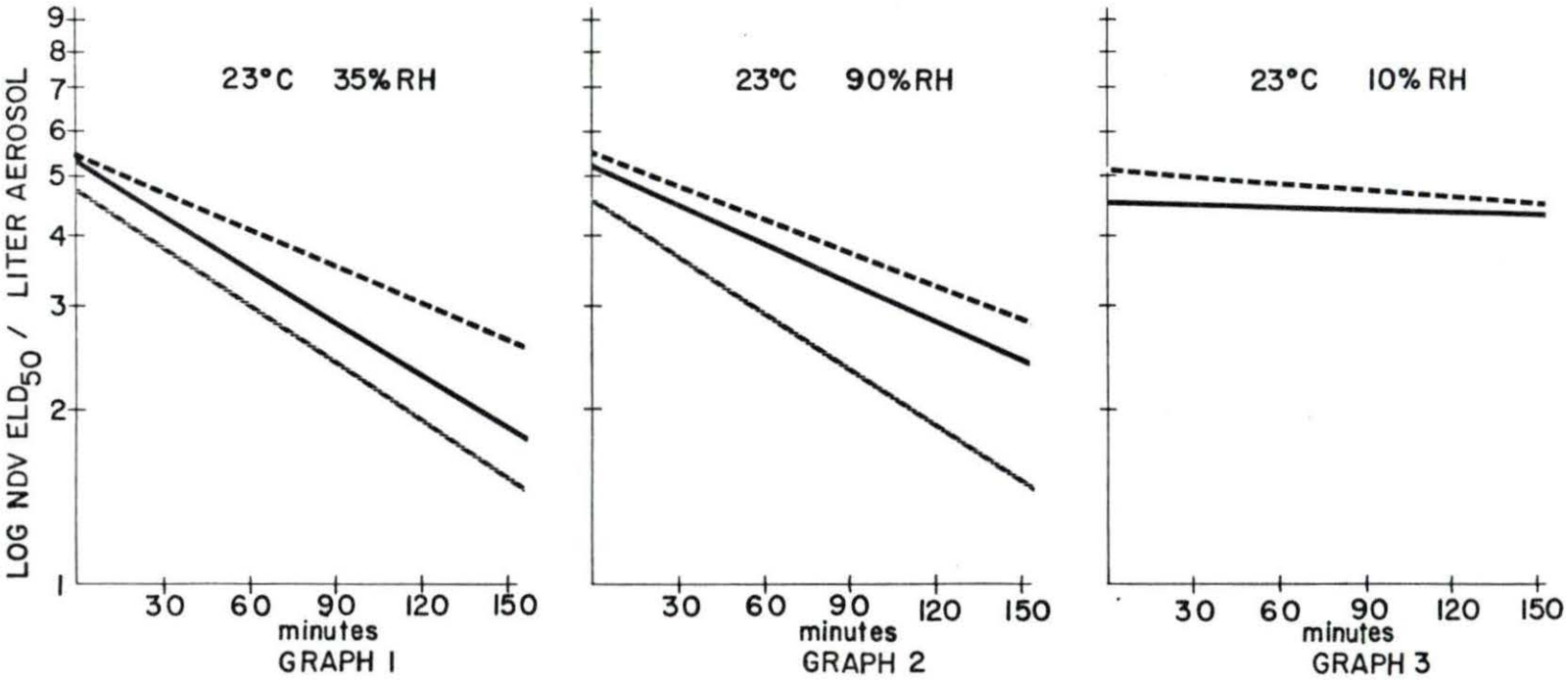
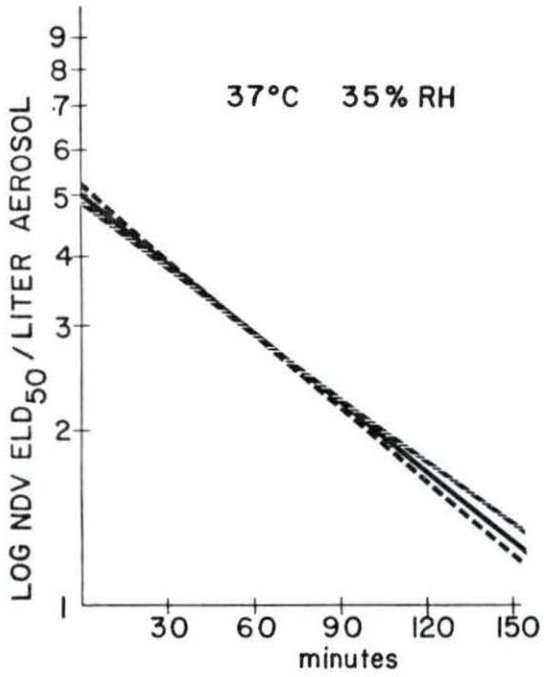
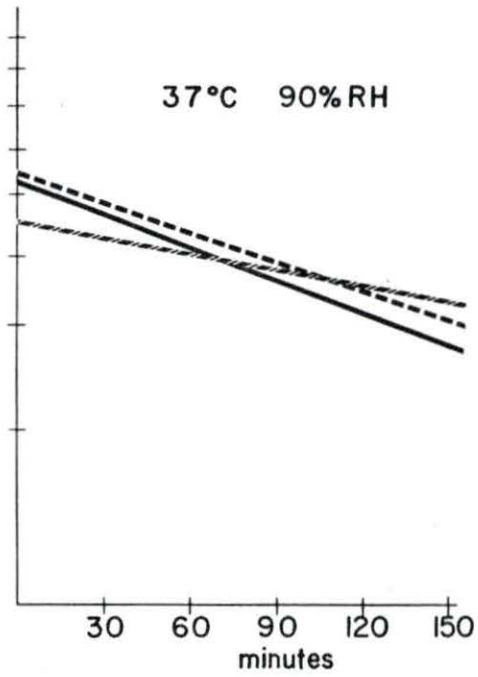

LOG NDV ELD50 / LITER AEROSOL
9
8
7
6
5
4
3
2
1
23°C 35% RH
30 60 90 120 150
minutes
GRAPH 1
23°C 90% RH
30 60 90 120 150
minutes
GRAPH 2
23°C 10% RH
30 60 90 120 150
minutes
GRAPH 3

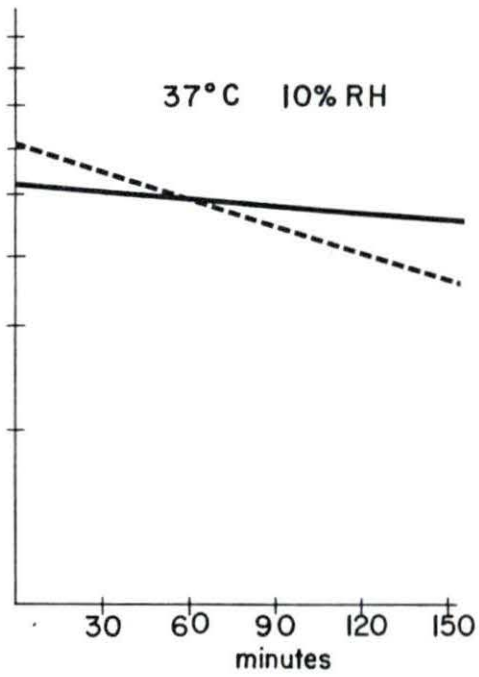
Fig. 12. Decay of NDV aerosols at 37° C. and 35, 90 and 10% RH.
Each line represents a single test



GRAPH 1



GRAPH 2



GRAPH 3

Fig. 13. Decay of NDV aerosols at 4° C. and 35, 90, 10% RH.
Each line represents a single test

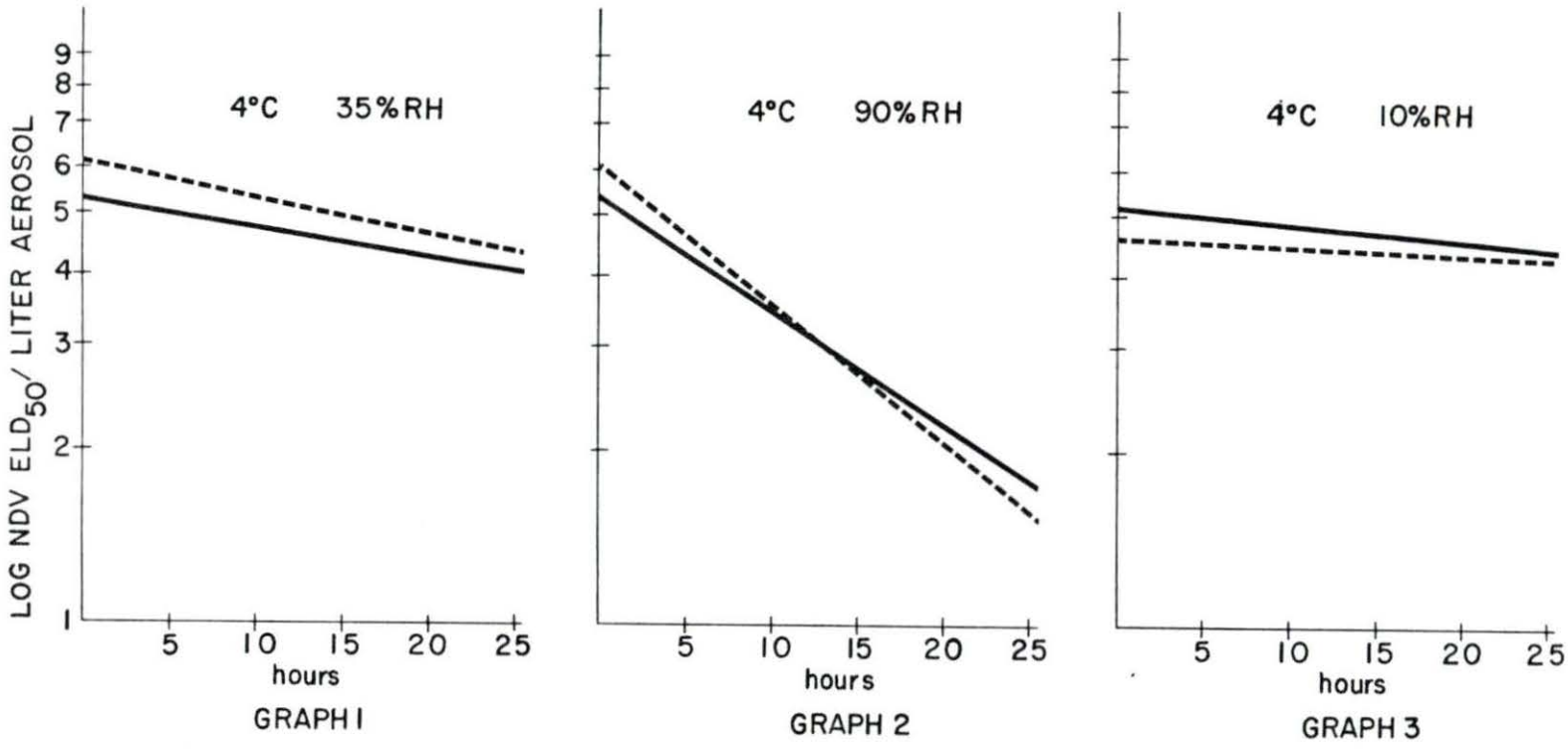

LOG NDV ELD50/ LITER AEROSOL
9
8
7
6
5
4
3
2
1
4°C 35%RH
5 10 15 20 25
hours
GRAPH I
4°C 90%RH
5 10 15 20 25
hours
GRAPH 2
4°C 10%RH
5 10 15 20 25
hours
GRAPH 3

Fig. 14. Averaged results of all aerosol decay studies with NDV at 3 temperatures and 3 RH levels

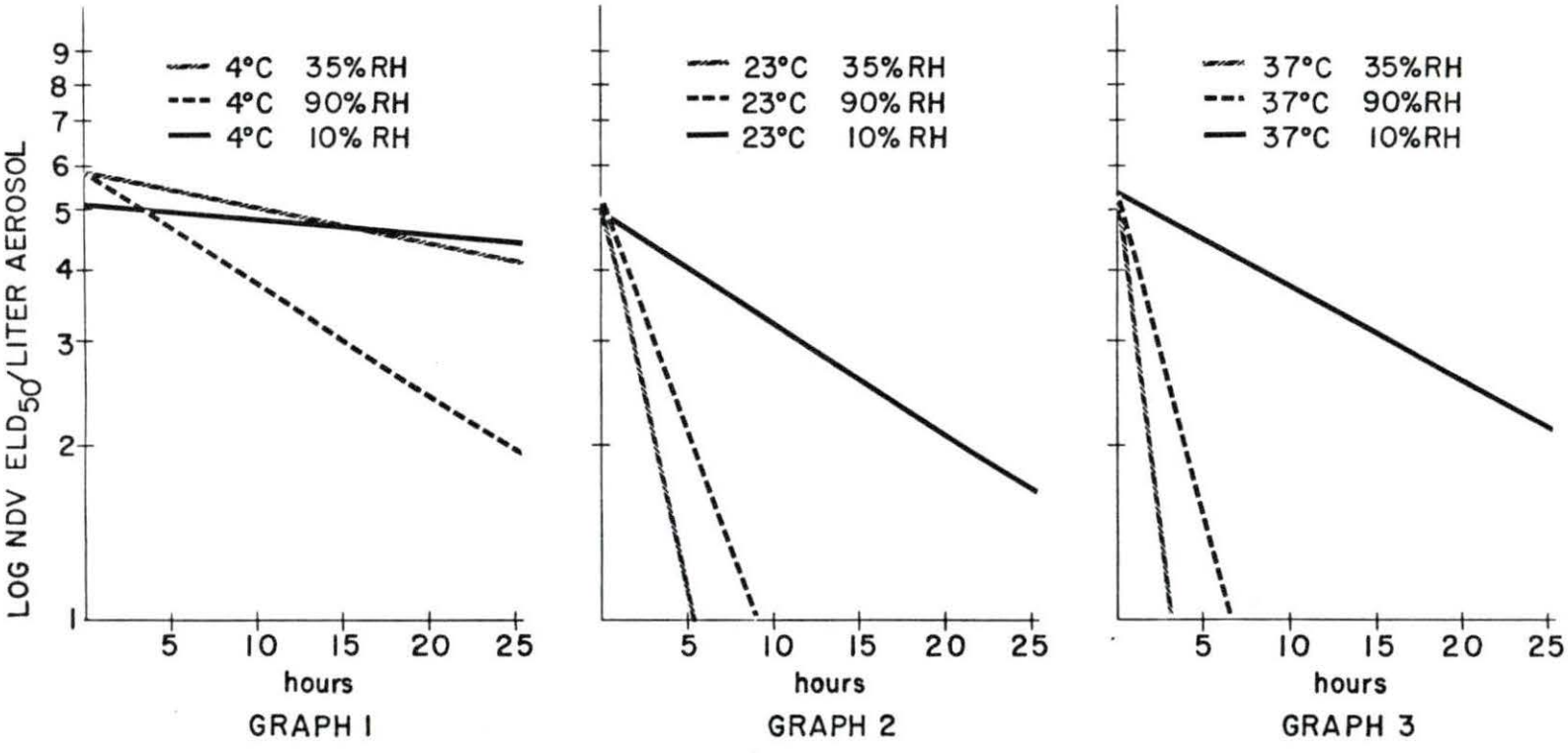

LOG NDV ELD50/LITER AEROSOL
9
8
7
6
5
4
3
2
1
4°C 35%RH
4°C 90%RH
4°C 10% RH
5
10
15
20
25
hours
GRAPH 1
23°C 35%RH
23°C 90% RH
23°C 10% RH
5
10
15
20
25
hours
GRAPH 2
37°C 35%RH
37°C 90%RH
37°C 10%RH
5
10
15
20
25
hours
GRAPH 3

Fig. 15. Decay of IBR virus aerosols at 23° C. and 35, 90 and 10% RH. Each line represents a single test

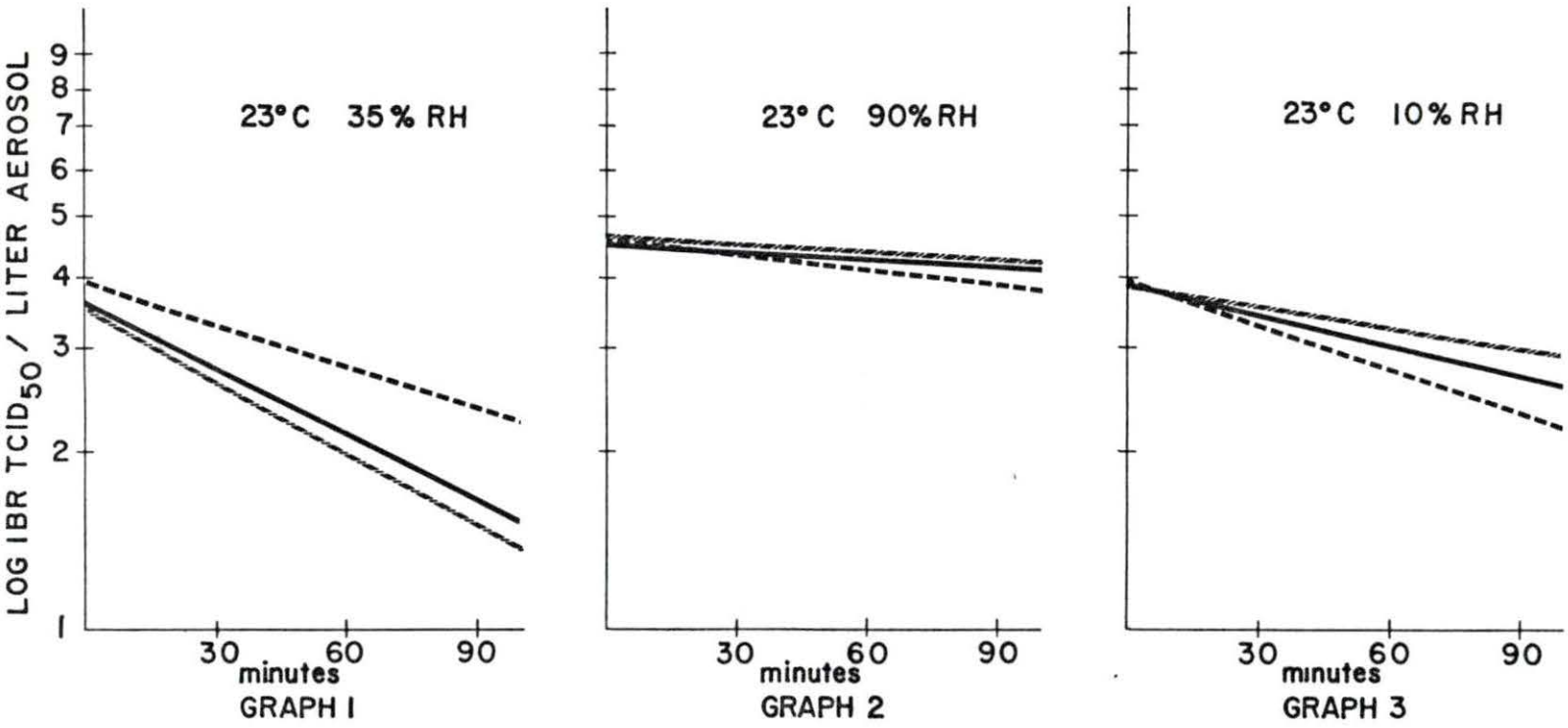
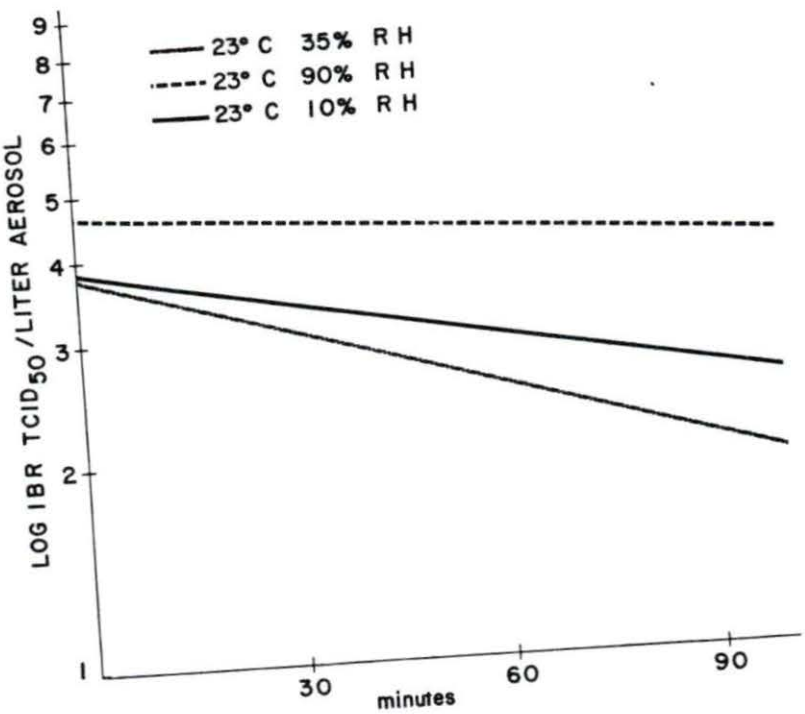

LOG IBR TCID50 / LITER AEROSOL
9
8
7
6
5
4
3
2
1
23° C 35% RH
30
60
90
minutes
GRAPH 1
23° C 90% RH
30
60
90
minutes
GRAPH 2
23° C 10% RH
30
60
90
minutes
GRAPH 3

Fig. 16. Averaged results of all aerosol decay studies with IBR at 23° C. and 35, 90 and 10% RH


23° C 35% R H
23° C 90% R H
23° C 10% R H
LOG IBR TCID50/LITER AEROSOL
9
8
7
6
5
4
3
2
1
30
60
90
minutes

Survival of airborne vesicular stomatitis virus

The results of VSV aerosol survival tests are presented in Figs. 17 and 18. Data from which these graphs were drawn appears in Tables 22 through 25 of the appendix.

It will be noted from Figs. 17 and 18 that 10% RH favors survival of airborne VSV. Survival was also greater at 90% than at 35% RH. The differences in survival at the 3 RH levels were not as marked with VSV as was observed with the other agents. The initial survival was approximately one log higher at 90% than at either 35% or 10% RH.

Survival of airborne bacteriophage T_3

The results of T_3 phage aerosol survival tests are presented in Figs. 19 and 20. Data from which these graphs were drawn appear in Tables 26 through 29 of the appendix.

The results of this series of tests with T_3 phage were more dramatic than with any of the previous viruses tested. From graph 2, Fig. 19 and the graph in Fig. 20 it can readily be seen that 90% RH favors survival of airborne T_3 phage. Note the small amount of deviation between tests in graph 2, Fig. 19. At 35% RH survival was extremely poor and marked deviation between tests occurred. However, at 10% RH survival was considerably better. The initial virus concentration in the aerosol generated at 10% RH was approximately 4.5 logs below that generated at 90% RH. Once this initial loss had occurred, considerable stability was observed.

It should be noted in Fig. 20 that the line representing 90% RH closely parallels the physical decay line in Fig. 10. As in the case with

Fig. 17. Decay of VSV aerosols at 23° C. and 35, 90 and 10% RH.
Each line represents a single test

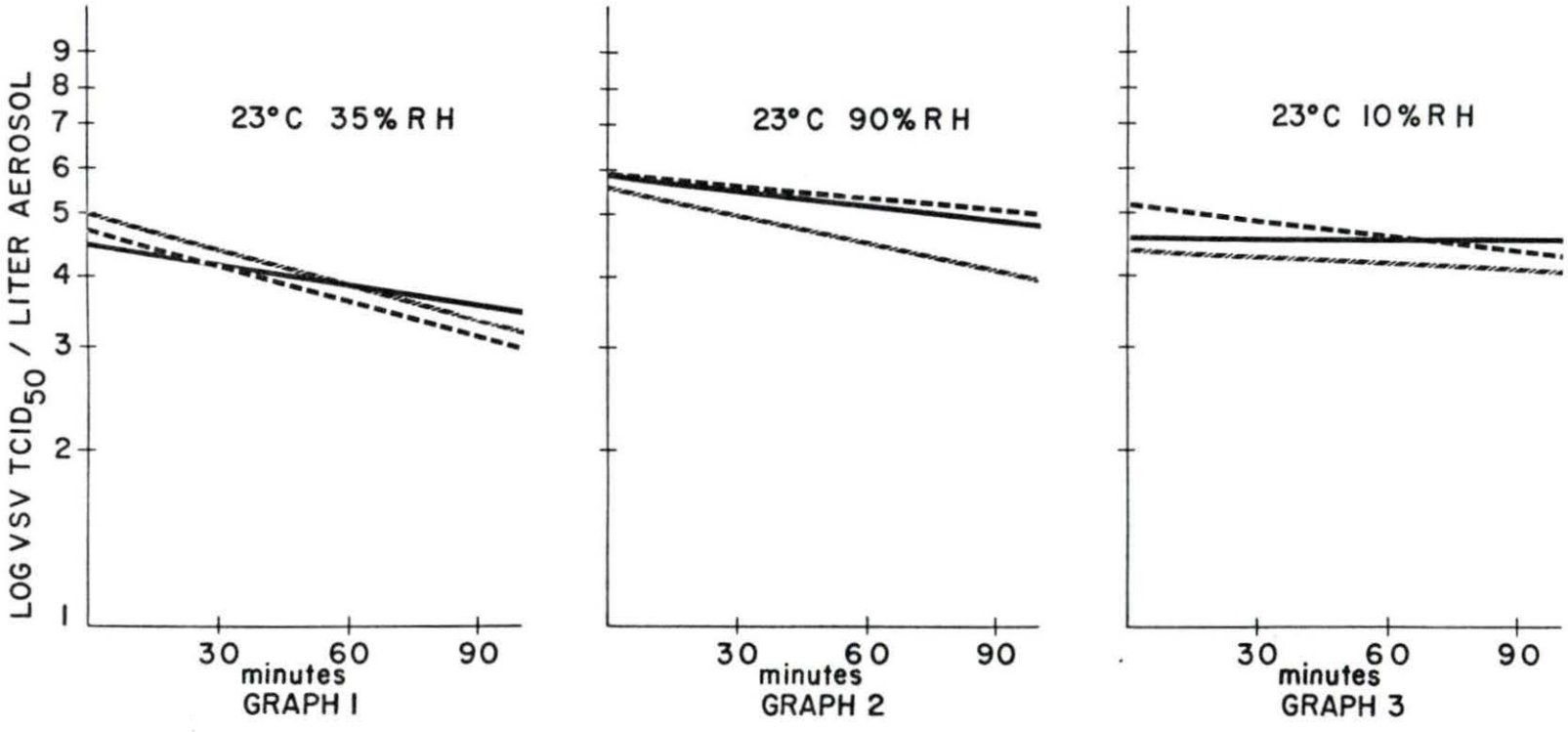

LOG VSV TCID50 / LITER AEROSOL
9
8
7
6
5
4
3
2
1
23°C 35% R H
30
60
90
minutes
GRAPH I
23°C 90% R H
30
60
90
minutes
GRAPH 2
23°C 10% R H
30
60
90
minutes
GRAPH 3

Fig. 18. Averaged results of all aerosol studies with VSV at 23° C. and 35, 90 and 10% RH

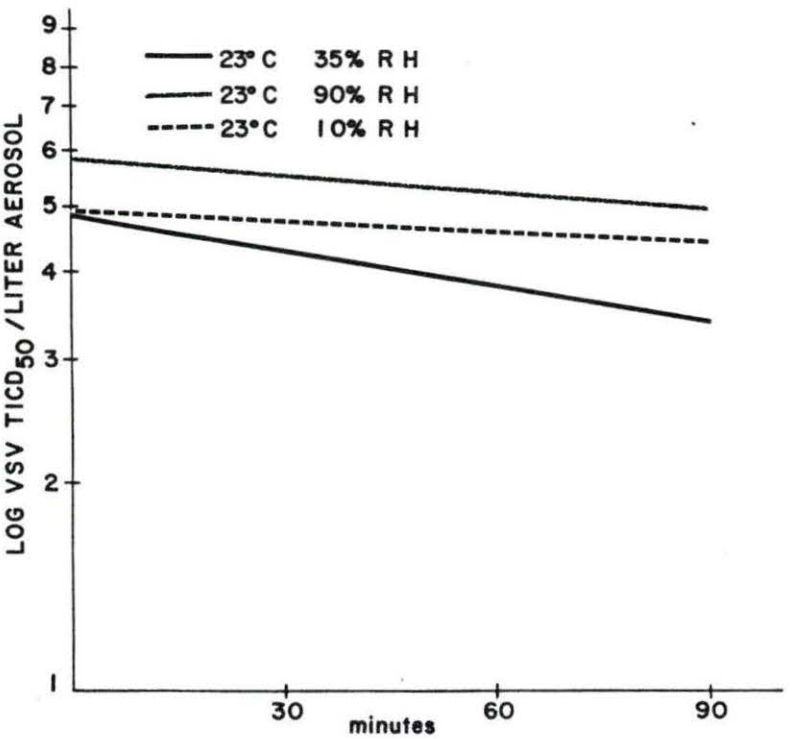

23° C 35% R H
23° C 90% R H
23° C 10% R H
LOG VSV TICD50/LITER AEROSOL
9
8
7
6
5
4
3
2
1
30
60
90
minutes

Fig. 19. Decay of T_3 phage aerosols at 23° C. and 35, 90 and 10% RH. Each line represents a single test

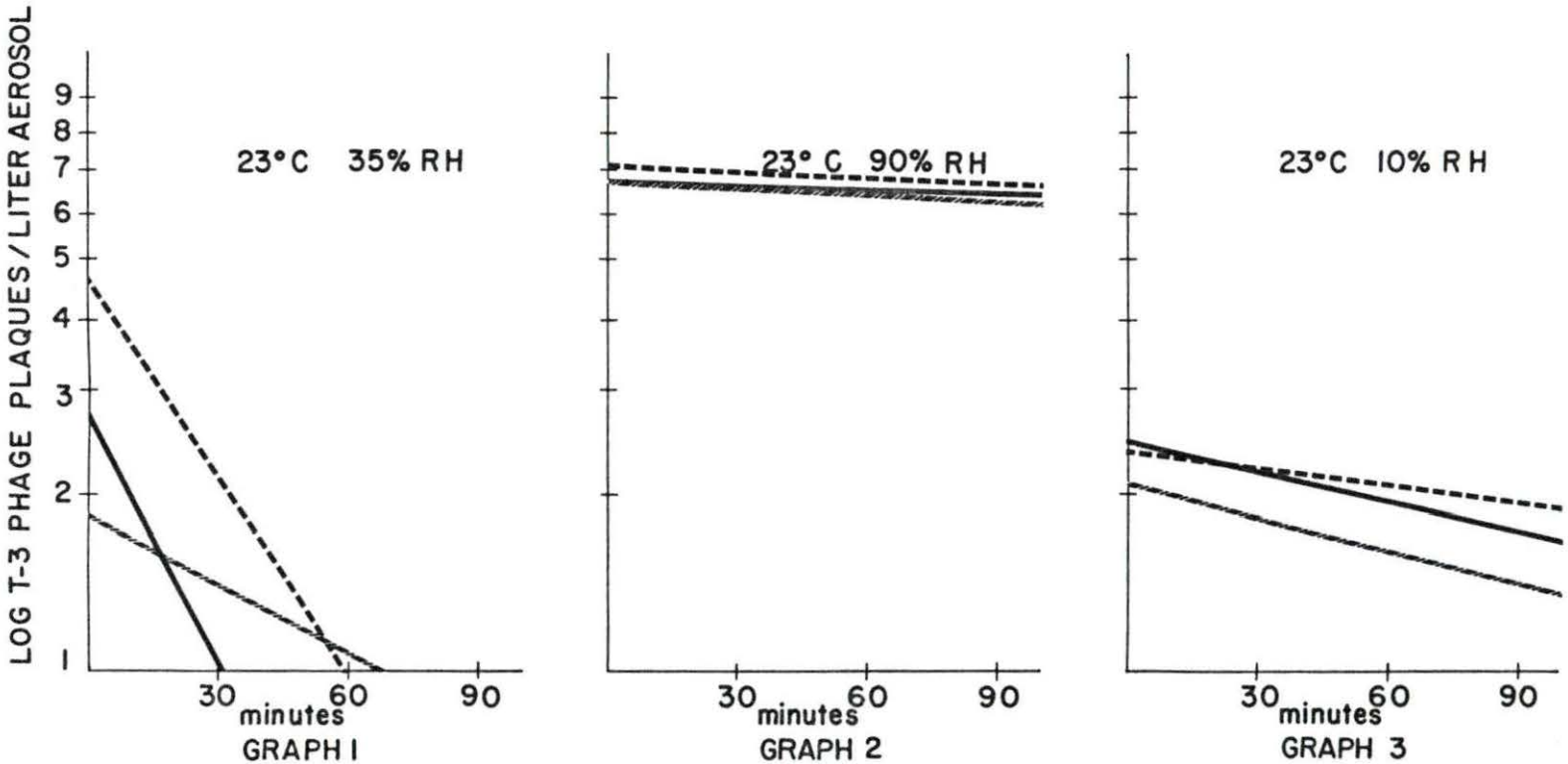
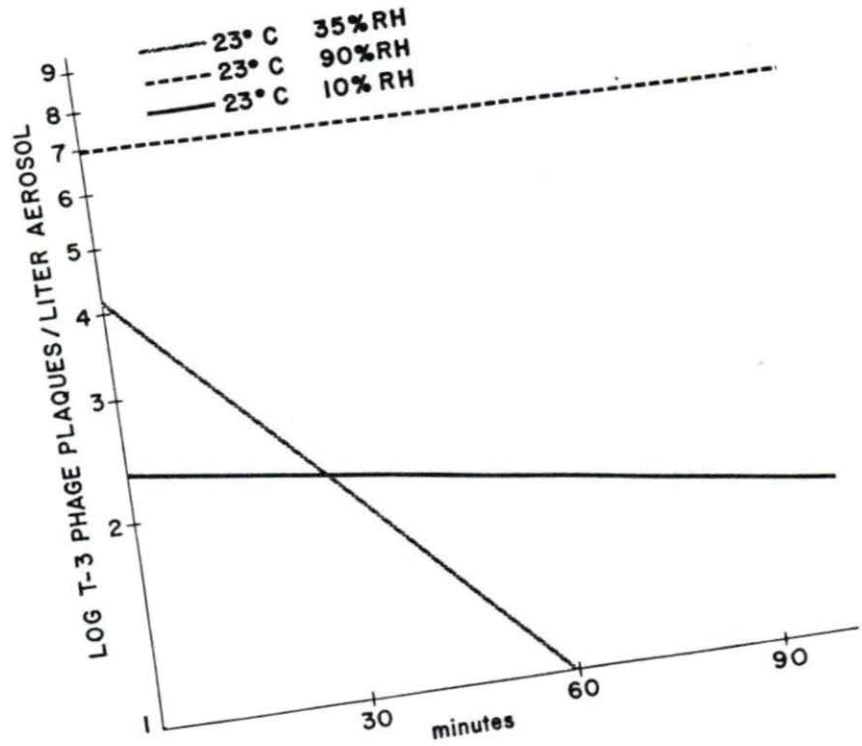

LOG T-3 PHAGE PLAQUES / LITER AEROSOL
9
8
7
6
5
4
3
2
1
23°C 35% RH
30
60
90
minutes
GRAPH I
23° C 90% RH
30
60
90
minutes
GRAPH 2
23°C 10% RH
30
60
90
minutes
GRAPH 3

Fig. 20. Averaged results of all aerosol studies with T_3 phage at 23° C. and 35, 90 and 10% RH


23° C 35%RH
23° C 90%RH
23° C 10% RH
LOG T-3 PHAGE PLAQUES/LITER AEROSOL
9
8
7
6
5
4
3
2
1
30
60
90
minutes

NDV at 4° C. and 10% RH this suggests that aerosol decay was due, primarily, to evanescence.

DISCUSSION

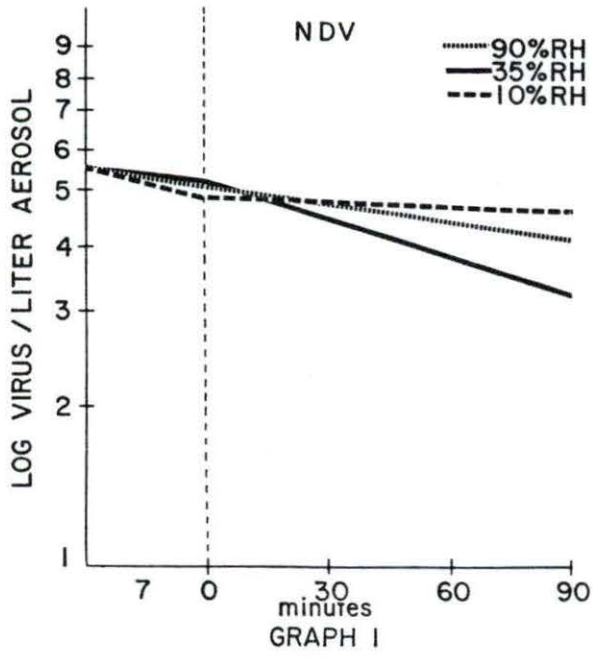
In general, it can be stated that at 23° C. airborne NDV and VSV survived best at 10% RH and that airborne IBR and T_3 phage survived best at 90% RH. It is interesting to note that all of the viruses survived poorest at 35% RH. The only exception was NDV at 4° C., where 35% favored survival over 90% RH. This decrease in airborne virus at low temperature and high RH could be of a physical rather than a biological nature. These studies were conducted in a walk-in refrigerator and a slight rise in temperature occurred at the time of entry for sample collection. If the chamber air approached the dew point during the temperature fluctuation that followed, droplet nuclei would tend to increase in size and gravitate more rapidly. Time did not permit the investigation of this possibility.

Another observation of interest is that at 10% RH, NDV survived equally well at 23° C. and 37° C. This suggests an increased resistance to physical inactivation.

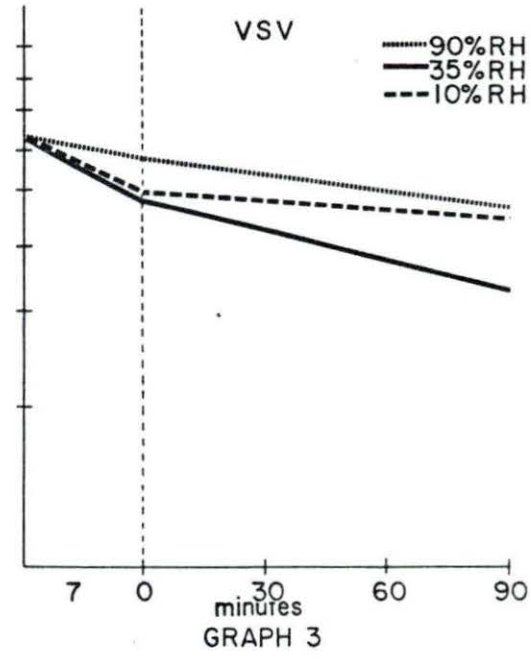
The results of these studies indicate the optimal RH for survival of each of the viruses tested when stored in an aerosol form. However, if one calculates the theoretical initial virus concentration in the aerosol based on the quantity and titer of the virus aerosolized and subtracts the actual concentration based on aerosol samples, we see that in almost all cases a RH of 90% favors generation survival.

The graphs in Fig. 21 illustrate the contrast between generation and storage loss. The data from which these graphs were drawn appears in Table 30 of the appendix. Each of the 4 viruses were plotted in

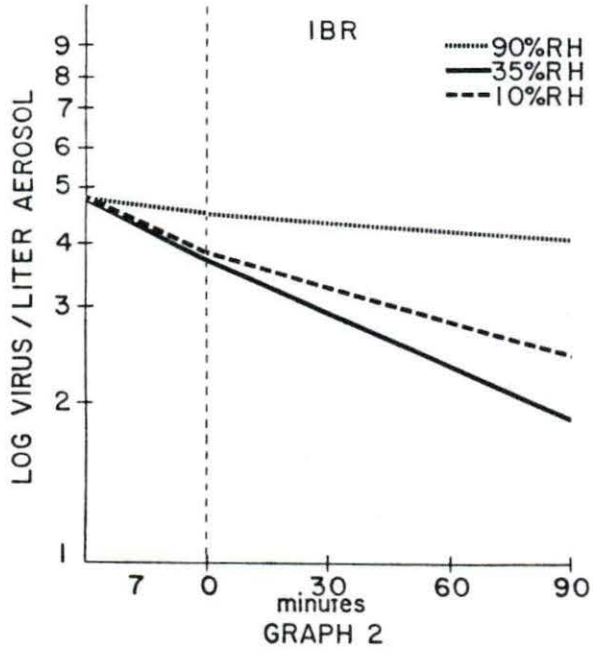
Fig. 21. Comparison of generation and storage aerosol decay with NDV, IBR, VSV and T_3 phage at 23° C. and 3 RH levels. Seven minute generation and stabilization period followed by 90-minute storage period



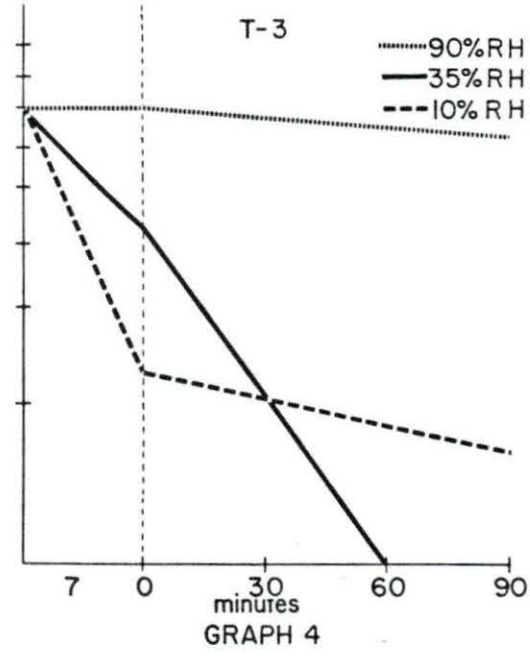
GRAPH 1



GRAPH 3



GRAPH 2



GRAPH 4

separate graphs at the 3 RH levels used. The first interval of the graph represents the 5-minute generation time and the 2-minute stabilization period. The remainder represents storage time. In graph one it can be seen that the initial loss was greatest at 10% with only a slight difference between 90% and 35% RH. The picture was reversed on storage. If graphs 2 and 3 are compared, it is seen that the initial losses compare quite closely. In this case 90% RH favored initial survival with both agents. On storage IBR survived best at 90% RH and VSV at 10% RH. The most dramatic difference between generation and storage loss was seen with T_3 phage in graph 4. Generation loss at 90% was extremely low and storage loss approached that which would be expected from physical decay alone. The greatest loss on generation occurred at 10% RH; however, once this initial loss occurred the virus survived better at this RH than at 35%.

From these observations one would conclude that generation loss as well as storage loss must be considered when evaluating the effects of RH on airborne viruses. When using a dynamic aerosol system for the exposure of animals it is customary to determine the spray factor. This factor, which is the ratio of the number of organisms in the aerosol to the number of organisms in the suspension from which the aerosol was produced aids in establishing the animal exposure dose. From the results of these experiments, it would appear that RH would greatly influence the spray factor. From the results with the 4 viruses tested, 90% RH would appear to give maximum survival in a dynamic system.

Why does one virus survive better at low RH while another survives equally well at high and yet another is unaffected at all RH levels?

Table 5 was prepared in an attempt to establish some relationship between groups of viruses and their response to aerosol storage at low, medium and high RH. Eleven viruses are listed. The RH favoring survival, nucleic acid core, ether sensitivity and Wilner's (53) classification are also listed. When one examines these results it is obvious that generalization regarding the sensitivity of groups of viruses to different levels of RH is impossible. Sensitivity to RH appears to be an individual characteristic of the virus.

There are undoubtedly several factors that account for inactivation of airborne viruses at various RH levels. Webb et al. (27) suggested that the death of airborne viruses was a direct result of a loss of water molecules bound to the nucleoprotein. He also demonstrated that the adverse effect of low RH on RSV could be prevented by the incorporation of inositol in the virus suspension nebulized. He theorized that the inositol molecule filled the critical spaces in the protein structure when the bound water was lost at low RH. This is only a partial explanation since this theory doesn't account for the increased survival of some viruses at low RH.

Pollard (54) in his "Theory of the physical means of the inactivation of viruses" states that there are 6 aspects of virus action that could be influenced by physical agents. These 6 actions are schematically illustrated in Fig. 22. The virus is shown as a coiled nucleic acid center which is formed about a protein molecule of specific size called the form preservative. The protein coat which is formed about the nucleic acid is divided into 2 parts, a protective part concerned with protecting the virus when it is outside the cell and a part which aids in attachment.

Table 5. A comparison of the sensitivity of 11 viruses to aerosol generation and storage at 3 RH levels

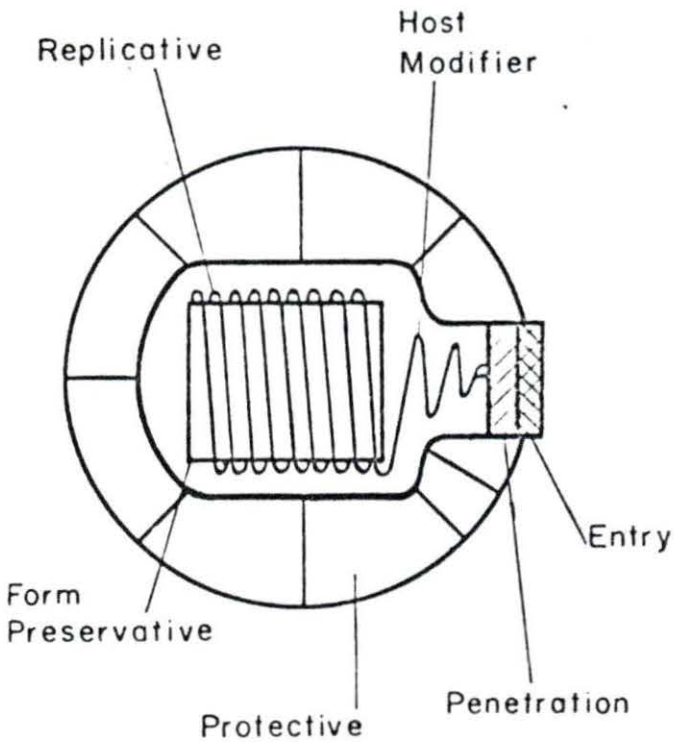
Virus	RH favoring survival			Nucleic acid core	Ether sensitivity	Classification by Wilner	Reference
	Low	Med	High				
Rous sarcoma			+	RNA	+	Myxovirus	Webb[a]
Influenza A	+			RNA	+	Myxovirus	Harper[b]
Newcastle disease	+			RNA	+	Myxovirus	Songer
Pigeon pox	+	+	+	DNA	+	Poxvirus	Webb[a]
Vaccinia	+			DNA	Chloroform+	Poxvirus	Harper[b]
Poliomyelitis			+	RNA	-	Picornavirus	Harper[b]
VEE	+			RNA	+	Arborvirus	Harper[b]
IBR			+	DNA	+	Herpes virus	Songer
Vesicular stomatitis	+			RNA	+	Unclassified	Songer
Psittacosis (6 BC)			+	$\frac{\text{RNA}}{\text{DNA}}$	+	Not classified as virus	Rosebury[c]
Bacteriophage T_3			+	DNA	-	Bacteriophage	Songer

[a]Webb et al. (27)

[b]Harper (26)

[c]Rosebury (21)

Fig. 22. Schematic drawing of animal virus illustrating the functional units (Pollard 54, p. 665)


Replicative
Host
Modifier
Entry
Form
Preservative
Protective
Penetration

A specific organ, probably concerned with penetration, is also shown. The final aspect of virus action, an auxillary function of the nucleic acid, is referred to as modification of the host.

It is unlikely that RH affects every virus in the same manner. In one case instability at a given RH level may be due to an effect on the penetration mechanism; in another case an effect on the protective coat, or form preservative. Interference with any one or any combination of these aspects of virus action could account for inactivation. Further studies are needed to establish the nature of inactivation with each virus.

SUMMARY

A static aerosol chamber system was developed for airborne microorganism survival studies. A procedure for determining biological, physical and total aerosol decay is described. Newcastle disease virus, infectious bovine rhinotracheitis virus, vesicular stomatitis virus and bacteriophage T_3 were evaluated for their survival in aerosol form. In summary:

1. Airborne NDV survived best at 4° C., the lowest temperature tested.
2. Airborne NDV survived best at 10% RH at all temperatures tested.
3. Airborne IBR virus survived best at 90% RH when held at 23° C.
4. Airborne VSV survived best at 10% RH when held at 23° C.
5. Airborne T_3 phage survived best at 90% RH when held at 23° C.

The significance of these results in relation to the existing literature on the survival of airborne microorganisms is discussed.

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Assistance in photography by Mr. R. M. Glazier was greatly appreciated.

APPENDIX

Table 6. The survival of NDV in an aerosol at 23° C. and 35% RH

Tests	Temp.	RH	Post generation time in minutes	Samples per interval	Log ELD_{50}/liter aerosol
			0	2	$10^{4.68}$
			30	2	$10^{3.83}$
1	23°	35%	60	2	$10^{3.03}$
			90	2	$10^{2.30}$
			120	2	$10^{2.15}$
			0	2	$10^{5.35}$
			30	2	$10^{4.67}$
2	23°	35%	60	2	$10^{3.48}$
			90	2	$10^{2.85}$
			120	2	$10^{2.29}$
			0	2	$10^{5.39}$
			30	2	$10^{4.72}$
3	23°	35%	60	2	$10^{3.88}$
			90	2	$10^{3.80}$
			120	2	$10^{3.11}$

Table 7. The survival of NDV in an aerosol at 23° C. and 90% RH

Tests	Temp.	RH	Post generation time in minutes	Samples per interval	Log ELD_{50}/liter aerosol
			0	2	$10^{4.58}$
			30	2	$10^{3.68}$
4	23° C.	90%	60	2	$10^{2.94}$
			90	2	$10^{2.20}$
			120	2	$10^{1.99}$
			0	2	$10^{5.25}$
			30	2	$10^{4.99}$
5	23° C.	90%	60	2	$10^{4.35}$
			90	2	$10^{3.67}$
			120	2	$10^{2.47}$
			0	2	$10^{5.45}$
			30	2	$10^{5.13}$
6	23° C.	90%	60	2	$10^{4.29}$
			90	2	$10^{4.21}$
			120	2	$10^{2.50}$

Table 8. The survival of NDV in an aerosol at 23° C. and 10% RH

Tests	Temp.	RH	Post generation time in minutes	Samples per interval	Log ELD_{50}/liter aerosol
7	23° C.	10%	0	2	$10^{4.59}$
			90	2	$10^{4.59}$
			180	2	$10^{4.42}$
			270	2	$10^{4.04}$
			360	2	$10^{4.02}$
8	23° C.	10%	0	2	$10^{5.07}$
			90	2	$10^{4.35}$
			180	2	$10^{4.08}$
			270	2	$10^{3.77}$
			360	2	$10^{4.15}$

Table 9. The survival of NDV in an aerosol at 37° C. and 35% RH

Tests	Temp.	RH	Post generation time in minutes	Samples per interval	Log ELD_{50}/liter aerosol
9	37° C.	35%	0	2	$10^{5.37}$
			30	2	$10^{4.39}$
			60	2	$10^{2.99}$
			90	2	$10^{2.65}$
			120	2	$10^{1.48}$
10	37° C.	35%	0	2	$10^{5.23}$
			30	2	$10^{4.30}$
			60	2	$10^{3.23}$
			90	2	$10^{2.29}$
			120	2	$10^{1.62}$
11	37° C.	35%	0	2	$10^{5.01}$
			30	2	$10^{3.50}$
			60	2	$10^{3.10}$
			90	2	$10^{2.55}$
			120	2	$10^{1.60}$

Table 10. The survival of NDV in an aerosol at 37° C. and 90% RH

Tests	Temp.	RH	Post generation time in minutes	Samples per interval	Log ELD_{50}/liter aerosol
12	37° C.	90%	0	2	$10^{5.34}$
			30	2	$10^{4.35}$
			60	2	$10^{4.23}$
			90	2	$10^{3.29}$
			120	2	$10^{3.29}$
13	37° C.	90%	0	2	$10^{5.46}$
			30	2	$10^{4.61}$
			60	2	$10^{4.23}$
			90	2	$10^{3.88}$
			120	2	$10^{3.27}$
14	37° C.	90%	0	2	$10^{4.68}$
			30	2	$10^{4.13}$
			60	2	$10^{3.22}$
			90	2	$10^{3.61}$
			120	2	$10^{3.59}$

Table 11. The survival of NDV in an aerosol at 37° C. and 10% RH

Tests	Temp.	RH	Post generation time in minutes	Samples per interval	Log ELD_{50}/liter aerosol
			0	2	$10^{6.05}$
			90	2	$10^{4.51}$
15	37° C.	10%	180	2	$10^{4.35}$
			270	2	$10^{4.54}$
			360	2	$10^{4.29}$
			0	2	$10^{5.35}$
			90	2	$10^{4.82}$
16	37° C.	10%	180	2	$10^{4.54}$
			270	2	$10^{4.44}$
			360	2	$10^{4.35}$

Table 12. The survival of NDV in an aerosol at 4° C. and 35% RH

Tests	Temp.	RH	Post generation time in minutes	Samples per interval	Log ELD_{50}/liter aerosol
			0	2	$10^{5.26}$
			2	2	$10^{5.34}$
17	4° C.	35%	4	2	$10^{5.22}$
			21	2	$10^{4.34}$
			$22\frac{1}{2}$	2	$10^{4.08}$
			24	2	$10^{3.76}$
			0	2	$10^{6.13}$
			2	2	$10^{5.29}$
18	4° C.	35%	4	2	$10^{5.06}$
			21	2	$10^{4.06}$
			$22\frac{1}{2}$	2	$10^{4.34}$
			24	2	$10^{4.41}$

Table 13. The survival of NDV in an aerosol at 4° C. and 90% RH

Tests	Temp.	RH	Post generation time in minutes	Samples per interval	Log ELD_{50}/liter aerosol
			0	2	$10^{6.08}$
			3	2	$10^{5.29}$
19	4° C.	90%	6	2	$10^{4.34}$
			9	2	$10^{3.59}$
			13	2	$10^{3.03}$
			24	2	$10^{1.61}$
			0	2	$10^{5.42}$
			3	2	$10^{4.77}$
20	4° C.	90%	6	2	$10^{4.18}$
			9	2	$10^{3.59}$
			13	2	---
			24	2	$10^{2.58}$

Table 14. The survival of NDV in an aerosol at 4° C. and 10% RH

Tests	Temp.	RH	Post generation time in minutes	Samples per interval	Log ELD_{50}/liter aerosol
21	4° C.	10%	0	2	$10^{4.61}$
			3	2	$10^{4.49}$
			6	2	$10^{3.98}$
			18	2	$10^{4.09}$
			21	2	$10^{3.99}$
			24	2	$10^{4.22}$
22	4° C.	10%	0	2	$10^{5.23}$
			3	2	$10^{5.02}$
			6	2	$10^{5.02}$
			18	2	$10^{4.68}$
			21	2	$10^{4.77}$
			24	2	$10^{4.20}$

Table 15. Average NDV survival per liter of aerosol for tests conducted at 23° C. and 35%, 10% and 90% RH

Tests	Temp.	RH	Post generation time in minutes	Samples per interval	Log ELD_{50}/liter aerosol
1,2,3	23° C.	35%	0	2	$10^{5.14}$
			30	2	$10^{4.40}$
			60	2	$10^{3.46}$
			90	2	$10^{2.98}$
			120	2	$10^{2.51}$
4,5,6	23° C.	90%	0	2	$10^{5.09}$
			30	2	$10^{4.60}$
			60	2	$10^{3.86}$
			90	2	$10^{3.36}$
			120	2	$10^{2.32}$
7,8	23° C.	10%	0	2	$10^{4.83}$
			90	2	$10^{4.47}$
			180	2	$10^{4.25}$
			270	2	$10^{3.90}$
			360	2	$10^{4.08}$

Table 16. Average NDV survival per liter of aerosol for tests conducted at 37° C. and 10%, 35% and 90% RH

Tests	Temp.	RH	Post generation time in minutes	Samples per interval	Log ELD_{50}/liter aerosol
9,10,11	37° C.	35%	0	2	$10^{5.20}$
			30	2	$10^{4.06}$
			60	2	$10^{3.10}$
			90	2	$10^{2.49}$
			120	2	$10^{1.57}$
12,13,14	37° C.	90%	0	2	$10^{5.16}$
			30	2	$10^{4.36}$
			60	2	$10^{3.89}$
			90	2	$10^{3.59}$
			120	2	$10^{3.38}$
15,16	37° C.	10%	0	2	$10^{5.70}$
			90	2	$10^{4.66}$
			180	2	$10^{4.44}$
			270	2	$10^{4.49}$
			360	2	$10^{4.32}$

Table 17. Average NDV survival per liter of aerosol for tests conducted at 4° C. and 35%, 10% and 90% RH

Tests	Temp.	RH	Post generation time in minutes	Samples per interval	Log ELD_{50}/liter aerosol
			0	2	$10^{5.69}$
			2	2	$10^{5.31}$
17,18	4° C.	35%	4	2	$10^{5.14}$
			21	2	$10^{4.20}$
			22½	2	$10^{4.21}$
			24	2	$10^{4.08}$
			0	2	$10^{5.75}$
			3	2	$10^{5.01}$
19,20	4° C.	90%	6	2	$10^{4.26}$
			9	2	$10^{3.59}$
			13	2	$10^{3.03}$
			24	2	$10^{2.09}$
			0	2	$10^{4.92}$
			3	2	$10^{4.75}$
21,22	4° C.	10%	6	2	$10^{4.50}$
			18	2	$10^{4.38}$
			21	2	$10^{4.38}$
			24	2	$10^{4.21}$

Table 18. The survival of IBR virus in an aerosol at 23° C. and 35% RH

Tests	Temp.	RH	Post generation time in minutes	Samples per interval	Log $TCID_{50}$/liter aerosol
1	23° C.	35%	0	2	$10^{3.99}$
			30	2	$10^{3.22}$
			60	2	$10^{2.78}$
			90	2	$10^{2.42}$
2	23° C.	35%	0	2	$10^{3.54}$
			30	2	$10^{3.04}$
			60	2	$10^{2.21}$
			90	2	$10^{1.18}$
3	23° C.	35%	0	2	$10^{3.57}$
			30	2	$10^{2.45}$
			60	2	$10^{2.33}$
			90	2	$10^{1.46}$

Table 19. The survival of IBR virus in an aerosol at 23° C. and 10% RH

Tests	Temp.	RH	Post generation time in minutes	Samples per interval	Log $TCID_{50}$/liter aerosol
4	23° C.	10%	0	2	$10^{3.80}$
			30	2	$10^{3.35}$
			60	2	$10^{2.98}$
			90	2	$10^{2.68}$
5	23° C.	10%	0	2	$10^{3.82}$
			30	2	$10^{3.35}$
			60	2	$10^{3.35}$
			90	2	$10^{2.84}$
6	23° C.	10%	0	2	$10^{3.94}$
			30	2	$10^{3.03}$
			60	2	$10^{2.54}$
			90	2	$10^{2.35}$

Table 20. The survival of IBR virus in an aerosol at 23° C. and 90% RH

Tests	Temp.	RH	Post generation time in minutes	Samples per interval	Log $TCID_{50}$/liter aerosol
7	23° C.	90%	0	2	$10^{4.66}$
			30	2	$10^{4.13}$
			60	2	$10^{4.03}$
			90	2	$10^{3.93}$
8	23° C.	90%	0	2	$10^{4.48}$
			30	2	$10^{4.38}$
			60	2	$10^{4.14}$
			90	2	$10^{4.13}$
9	23° C.	90%	0	2	$10^{4.67}$
			30	2	$10^{4.42}$
			60	2	$10^{4.29}$
			90	2	$10^{4.10}$

Table 21. Average IBR virus survival per liter of aerosol for tests conducted at 23° C. and 35%, 10% and 90% RH

Tests	Temp.	RH	Post generation time in minutes	Samples per interval	Log $TCID_{50}$/liter aerosol
1,2,3	23° C.	35%	0	2	$10^{3.75}$
			30	2	$10^{3.01}$
			60	2	$10^{2.53}$
			90	2	$10^{2.01}$
4,5,6	23° C.	10%	0	2	$10^{3.80}$
			30	2	$10^{3.26}$
			60	2	$10^{3.06}$
			90	2	$10^{2.52}$
7,8,9	23° C.	90%	0	2	$10^{4.61}$
			30	2	$10^{4.32}$
			60	2	$10^{4.17}$
			90	2	$10^{4.06}$

Table 22. The survival of VSV in an aerosol at 23° C. and 35% RH

Tests	Temp.	RH	Post generation time in minutes	Samples per interval	Log $TCID_{50}$/liter aerosol
1	23° C.	35%	0	2	$10^{4.99}$
			30	2	$10^{4.02}$
			60	2	$10^{3.88}$
			90	2	$10^{3.59}$
2	23° C.	35%	0	2	$10^{4.48}$
			30	2	$10^{4.21}$
			60	2	$10^{3.80}$
			90	2	$10^{3.54}$
3	23° C.	35%	0	2	$10^{4.77}$
			30	2	$10^{3.88}$
			60	2	$10^{3.54}$
			90	2	$10^{3.42}$

Table 23. The survival of VSV in an aerosol at 23° C. and 10% RH

Tests	Temp.	RH	Post generation time in minutes	Samples per interval	Log $TCID_{50}$/liter aerosol
4	23° C.	10%	0	2	$10^{4.58}$
			30	2	$10^{4.57}$
			60	2	$10^{4.95}$
			90	2	$10^{4.27}$
5	23° C.	10%	0	2	$10^{5.21}$
			30	2	$10^{4.61}$
			60	2	$10^{4.41}$
			90	2	$10^{4.66}$
6	23° C.	10%	0	2	$10^{4.35}$
			30	2	$10^{4.13}$
			60	2	$10^{4.16}$
			90	2	$10^{4.13}$

Table 24. The survival of VSV in an aerosol at 23° C. and 90% RH

Tests	Temp.	RH	Post generation time in minutes	Samples per interval	Log $TCID_{50}$/liter aerosol
7	23° C.	90%	0	2	$10^{5.88}$
			30	2	$10^{5.80}$
			60	2	$10^{5.27}$
			90	2	$10^{4.70}$
8	23° C.	90%	0	2	$10^{5.88}$
			30	2	$10^{5.45}$
			60	2	$10^{5.03}$
			90	2	$10^{5.13}$
9	23° C.	90%	0	2	$10^{5.54}$
			30	2	$10^{5.21}$
			60	2	$10^{4.60}$
			90	2	$10^{3.68}$

Table 25. Average VSV survival per liter of aerosol for tests conducted at 23° C. and 35%, 10% and 90% RH

Tests	Temp.	RH	Post generation time in minutes	Samples per interval	Log $TCID_{50}$/liter aerosol
1,2,3	23° C.	35%	0	2	$10^{4.80}$
			30	2	$10^{4.06}$
			60	2	$10^{3.76}$
			90	2	$10^{3.52}$
4,5,6	23° C.	10%	0	2	$10^{4.87}$
			30	2	$10^{4.48}$
			60	2	$10^{4.67}$
			90	2	$10^{4.42}$
7,8,9	23° C.	90%	0	2	$10^{5.79}$
			30	2	$10^{5.55}$
			60	2	$10^{5.05}$
			90	2	$10^{4.80}$

Table 26. Survival of T_3 phage in an aerosol at 23° C. and 35% RH

Tests	Temp.	RH	Post generation time in minutes	Samples per interval	Log plaques/liter aerosol
1	23° C.	35%	0	2	$10^{2.75}$
			30	2	$10^{1.02}$
			60	2	0
			90	2	0
2	23° C.	35%	0	2	$10^{4.64}$
			30	2	$10^{2.15}$
			60	2	0
			90	2	0
3	23° C.	35%	0	2	$10^{1.85}$
			30	2	$10^{1.45}$
			60	2	0
			90	2	0

Table 27. Survival of T_3 phage in an aerosol at 23° C. and 10% RH

Tests	Temp.	RH	Post generation time in minutes	Samples per interval	Log plaques/liter aerosol
4	23° C.	10%	0	2	$10^{2.32}$
			30	2	$10^{2.15}$
			60	2	$10^{2.09}$
			90	2	$10^{1.85}$
5	23° C.	10%	0	2	$10^{2.09}$
			30	2	$10^{1.94}$
			60	2	$10^{1.72}$
			90	2	$10^{1.24}$
6	23° C.	10%	0	2	$10^{2.45}$
			30	2	$10^{1.94}$
			60	2	$10^{1.94}$
			90	2	$10^{1.85}$

Table 28. Survival of T_3 phage in an aerosol at 23° C. and 90% RH

Tests	Temp.	RH	Post generation time in minutes	Samples per interval	Log plaques/liter aerosol
7	23° C.	90%	0	2	$10^{6.72}$
			30	2	$10^{6.49}$
			60	2	$10^{6.27}$
			90	2	$10^{6.29}$
8	23° C.	90%	0	2	$10^{6.72}$
			30	2	$10^{6.49}$
			60	2	$10^{6.54}$
			90	2	$10^{6.41}$
9	23° C.	90%	0	2	$10^{7.06}$
			30	2	$10^{6.85}$
			60	2	$10^{6.81}$
			90	2	$10^{6.79}$

Table 29. Average T_3 phage survival per liter of aerosol for tests conducted at 23° C. and 35%, 10% and 90% RH

Tests	Temp.	RH	Post generation time in minutes	Samples per interval	Log plaques/liter aerosol
1,2,3	23° C.	35%	0	2	$10^{4.17}$
			30	2	$10^{2.59}$
			60	2	0
			90	2	0
4,5,6	23° C.	10%	0	2	$10^{2.31}$
			30	2	$10^{2.02}$
			60	2	$10^{1.95}$
			90	2	$10^{1.72}$
7,8,9	23° C.	90%	0	2	$10^{6.89}$
			30	2	$10^{6.64}$
			60	2	$10^{6.60}$
			90	2	$10^{6.55}$

Table 30. The comparative loss of airborne viruses during generation and storage at 3 RH levels

Virus	RH %	Calculated generation loss	Loss after 90 min. storage	Combined loss	Optimum RH		
					Generation	Storage	Total
NDV	35	$10^{0.36}$	$10^{1.94}$	$10^{2.30}$	+		
	90	$10^{0.47}$	$10^{0.99}$	$10^{1.40}$			
	10	$10^{0.67}$	$10^{0.23}$	$10^{0.90}$		+	+
IBR	35	$10^{1.08}$	$10^{1.85}$	$10^{2.93}$			
	90	$10^{0.22}$	$10^{0.51}$	$10^{0.73}$	+	+	+
	10	$10^{1.03}$	$10^{1.30}$	$10^{2.33}$			
VSV	35	$10^{1.50}$	$10^{1.50}$	$10^{3.0}$			
	90	$10^{0.51}$	$10^{1.14}$	$10^{1.65}$	+		+
	10	$10^{1.43}$	$10^{0.42}$	$10^{1.85}$		+	
T_3 phage	35	$10^{2.80}$	$10^{4.2}$	$10^{7.0}$			
	90	$10^{0.0}$	$10^{0.75}$	$10^{0.75}$	+		+
	10	$10^{4.70}$	$10^{0.60}$	$10^{5.30}$		+	

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