APPLIED MICROBIOLOGY, Jan., 1967, p. 35-42

Vol. 15, No. 1
Printed in U.S.A.

Influence of Relative Humidity on the Survival of Some Airborne Viruses

JOSEPH R. SONGER

National Animal Disease Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Ames, Iowa

Received for publication 22 June 1966

ABSTRACT

A system for studying the effects of relative humidity (RH) and temperature on biological aerosols, utilizing a modified toroid for a static aerosol chamber, is described. Studies were conducted at 23 C and at three RH levels (10, 35, and 90%) with four viruses (Newcastle disease virus, infectious bovine rhinotracheitis virus, vesicular stomatitis virus, and *Escherichia coli* B T3 bacteriophage). Virus loss on aerosol generation was consistently lower at 90% than at 10 or 35% RH. When stored at 23 C, Newcastle disease virus and vesicular stomatitis virus survived best at 10% RH. Infectious bovine rhinotracheitis virus and *E. coli* B T3 bacteriophage survived storage at 23 C best at 90% RH.

A considerable amount of work has been done on the effect of relative humidity (RH) and temperature on airborne bacteria with considerable differences of opinion. De Ome (1), Loosli et al. (9), and Williamson and Gotass (21) indicated that high RH was more lethal than medium or low RH. Wells and Zappasodi (20) reported opposite findings. Dunklin and Puck (2), Ferry and Maple (4), and Schechmeister and Goldberg (14) claimed intermediate RH level to be the most lethal. Goodlow and Leonard (6) stated that low RH (20 to 30%) hastened the decay rate for most cells, as did very high RH (95%); optimal RH ranged from 40 to 80%.

Webb (17-19), 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, and this was 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, a movement which resulted 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 degradates, some sugars, and polyhydroxycyclohexanes. He suggested that hydrogen bonding was the mechanism responsible for the preservation of the natural structure of the cellular protein. using aerosols of *Serratia marcescens, Escherichia coli, Staphylococcus albus,* and *Bacillus subtilis,* he also demonstrated individual variation in aerosol survival. High RH favored survival with each organism.

Using a continuous-flow cloud chamber, Rosebury (12) found that aerosols of *S. marcescens, Brucella suis, Pseudomonas pseudomallei, Actinobacillus mallei, Pasteurella tularensis,* pneumonitis virus, and three strains of psittacosis virus were more stable at a RH above 70 to 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. (3), Loosli et al. (10), Schechmeister (13), and Hemmes et al. (8) reported studies with airborne influenza virus. Harper (7) tested aerosols of vaccinia, influenza, Venezuelan equine encephalitis (VEE), and poliovirus for survival at temperatures of approximately 10, 20, and 30 C. The lowest temperature favored survival of all of these airborne viruses. Aerosols of these agents were also evaluated at 20, 50, and 80% RH. Vaccinia, influenza, and VEE virus survived best at 20% RH, whereas poliovirus survived best at 80% RH.

Webb et al. (19) found that pigeon poxvirus was stable in aerosols and 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.

Many types of cloud or static aerosol chambers have been described, each with the purpose of

minimizing physical decay. Wolfe (24) used a cylindrical chamber with a slow-moving fan to maintain the aerosol in suspension. Goldberg et al. (5) 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 rev/min. Reduction in physical loss of an aerosol 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.

MATERIALS AND METHODS

Static aerosol chamber. The static aerosol chamber used in this study (Fig. 1) was an aluminum drum 30 inches (76.2 cm) in diameter and 15 inches (38.1 cm) deep with a capacity of 140 liters. It was powered by a Zero-Max variable-speed motor (The Zero-Max Co., Minneapolis, Minn.) with potential speeds of 0 to 400 rev/min. The speed of rotation for this series of studies was 3 rev/min. Access panels were mounted on each side of the chamber to facilitate the generation of aerosols and collection of aerosol samples, and to measure temperature and RH.

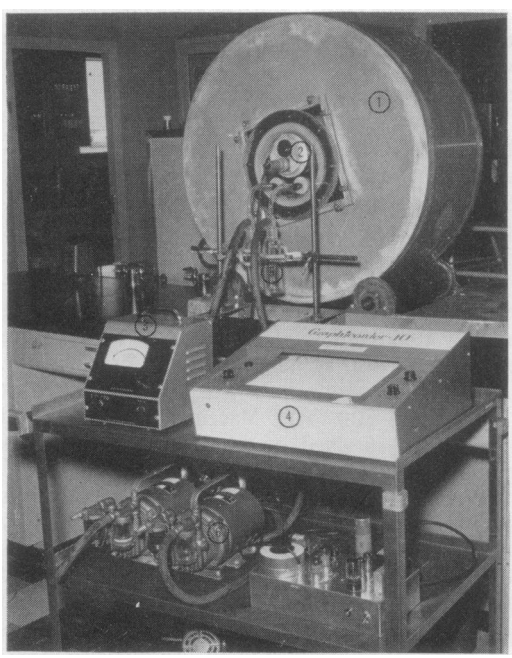

FIG. 1. *Rotating-drum aerosol chamber and accessory equipment. (1) Rotating drum, (2) temperature and RH probe, (3) hygrometer, (4) recorder, (5) all-glass impingers, and (6) sampling vacuum pumps.*

Hygrometer indicator. A Universal A.C. Hygrometer Indicator (Hygrodynamics, Inc., Silver Spring, Md.), accurate to $\pm 1.5\%$ RH and ± 2 F (± 1.1 C), was used to sense the temperature and RH within the aerosol chamber. It was equipped with eight narrow range type TH elements which sensed both temperature and RH. The elements were mounted in a multiple mounting (Fig. 1) with an external connection to the hygrometer and a selector switch for selection of the desired element.

Recorder. A Graphicorder 10 (Dynatronic Instruments Corp., Melrose Park, Ill.) was used to record the temperature and humidity throughout each test period (Fig. 1). This recorder had a straight linear scale from 0 to 10 which coincided with the scale on the hygrometer. Calibration curves were used to find the humidity and temperature from these linear scales.

Aerosol generator. Aerosols were generated with a De Vilbiss no. 40 nebulizer (De Vilbiss Co., Somerset, Pa.). This nebulizer had a capacity of 0.2 ml/min and operated at a pressure of 5 psi.

Aerosol samplers. All-glass impingers (Ace Glass, Inc., Vineland, N.J.) described by Wolf et al. (23) were used to collect all aerosol samples. These samplers had a constant flow rate of 12 liters per min by virtue of a limiting orifice located in the sampling tube. As long as a vacuum in excess of 15 inches of water was maintained, the flow rate was constant.

Fluorometer. A Turner 110 fluorometer (Turner, G. K., Associates, Palo Alto, Calif.) 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 was equipped with no. 110-814 and no. 110-822 primary filters and no. 110-819 secondary filters.

Rhodamine B. Rhodamine B dye is nontoxic and is excited to fluorescence by the green line of mercury, 546 mμ. A standard 0.1% solution was prepared in 1% peptone and was used throughout the series of studies.

Newcastle disease virus (NDV). The GB-Texas strain of NDV was used in this study (received from R. A. Packer, Iowa State University, Ames). A pool of this virus was prepared from the amnionic and allantoic fluids from infected chicken embryos. The titer of this pool was $10^{8.38}$ ELD_{50}/ml.

Infectious bovine rhinotracheitis (IBR) virus. The IBR virus used in this study was the 14th tissue culture passage of a virus (received from D. Baldwin, National Animal Disease Laboratoy, Ames, Iowa) isolated from an infected cow. Primary bovine kidney cell cultures were used for the production of a pool of this virus. The titer of this pool was $10^{7.68}$ $TCID_{50}$/ml.

Vesicular stomatitis virus (VSV). The VSV used in this study (received from E. W. Jenney, National Animal Disease Laboratory, Ames, Iowa) was the fifth chicken embryo passage of a New Jersey type isolated from swine in Atlanta, Ga. A pool of this virus was prepared from the chorioallantoic and amnionic membranes from infected chicken embryos. The final preparation was the supernatant fluid from a 20% suspension of blended membranes. The titer of this pool was $10^{6.64}$ $TCID_{50}$/ml.

E. coli B T3 bacteriophage (T3 phage). The T3 phage used in this study was grown in a 3-hr tryptose phosphate broth culture of *E. coli* B (received from R. A. Packer, Iowa State University, Ames). The titer of this pool was $10^{9.41}$ plaques per milliliter.

Chicken embryos. Ten-day chicken embryos from nonvaccinated, NDV-free flocks were used for the titration of NDV.

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

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

Test procedure. Aerosols of the test agents were generated with a De Vilbiss no. 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. Generation time was 5 min and was followed by a 2-min 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 three different temperatures: 4 C (walk-in refrigerator), 23 C (room temperature), or 37 C (walk-in incubator). Studies with IBR, VSV, and T3 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, and if a low RH was desired, the chamber air was replaced with predried air. In the lowest RH range, the aerosol was generated across a layer of $CaSO_4$ into the predried chamber. Diluting air that replaced the sample air at the time of sampling was also preconditioned. Temperature and RH were sensed by the same probe. Hygrometer readings were linear from 0 to 100, and actual temperature and RH were determined by the use of calibration curves. These data were recorded and provided a constant record of the temperature and RH throughout the test.

Aerosol samples were titrated using 10-fold dilutions prepared in 1% peptone containing 500 units of penicillin and 500 μg of streptomycin per ml. When T3 phage was titrated, antibiotics were deleted from the medium.

When NDV was titrated, 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.

IBR virus was titrated in primary bovine kidney cultures, and VSV, 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.

E. coli B T3 bacteriophage was titrated by the method previously described by Songer et al. (15). We added 0.2 ml of the appropriate dilution to 3 ml of melted soft agar which had previously been inoculated with one drop of a 3-hr culture of *E. coli* B. After mixing, it was poured into a prepoured agar plate and allowed to solidify. After 6 to 10 hr of incubation, plaques were counted, and the number of plaques per liter of aerosol was calculated.

The Reed and Muench (11) method of calculating 50% end point was used to establish the titration end points for NDV, VSV, and IBR virus.

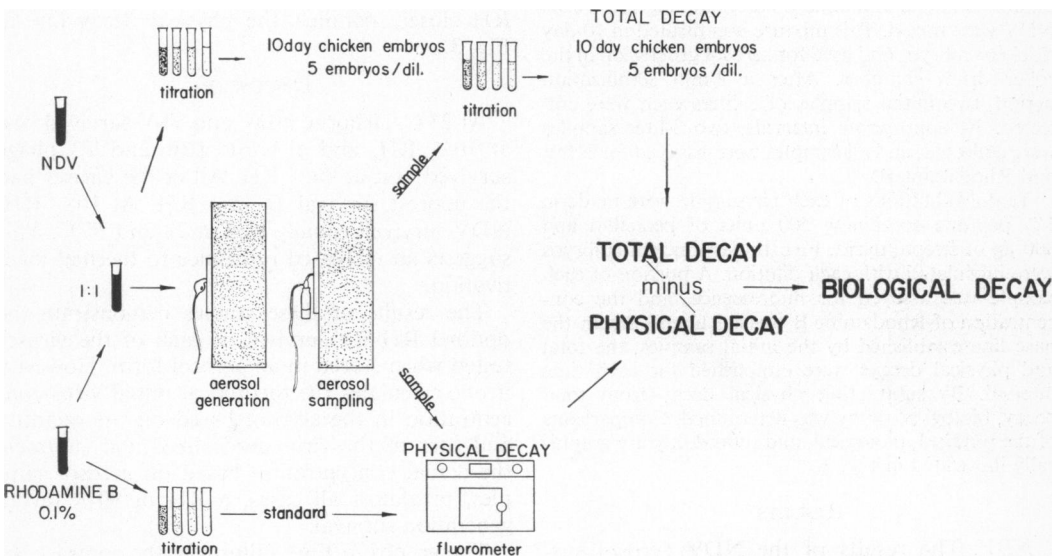

FIG. 2. *Determination of physical, biological, and total decay of aerosols in the rotating drum chamber.*

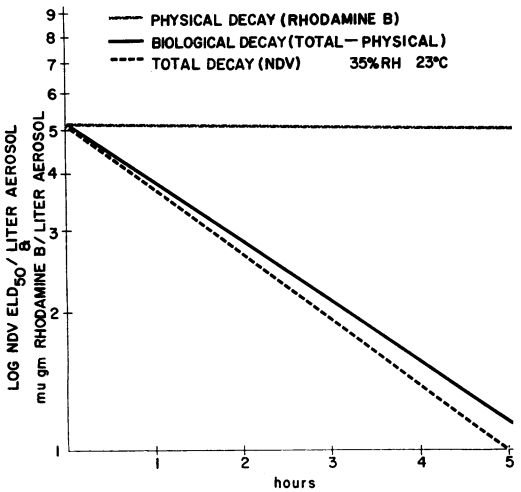

FIG. 3. *Comparison of physical, biological, and total aerosol decay in the rotating drum chamber.*

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. (5) 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, in contrast to biological decay which is a loss of biological activity. A further step was taken to show the relationship of total decay, biological decay, and physical decay with this system.

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

Figure 2 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. After a 2-min stabilization period, two initial samples of 3 liters each were collected. At appropriate intervals, two 3-liter samples were collected, and all samples were assayed for NDV and Rhodamine B.

Tenfold dilutions of each air sample were made in 1% peptone containing 500 units of penicillin and 500 μg of streptomycin. Five 10-day chicken embryos were inoculated with each dilution. A portion of each sample was assayed for fluorescence, and the concentration of Rhodamine B was calculated. From the base line 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. Comparisons of the physical, biological, and total decay are graphically illustrated in Fig. 3.

RESULTS

NDV. The results of the NDV aerosol survival tests are presented in graph 1, Fig. 4. From this graph it can be seen that at 23 C airborne NDV survived better at 10% than at 35 or 90% RH.

Similar studies were conducted with NDV at 4 and 37 C, and lower temperatures favored survival of airborne NDV. However, at 10% RH, airborne NDV survived equally well at 37 and 23 C. At 4 C and 10% RH, the NDV aerosol decay curve closely paralleled the physical decay curve in Fig. 3.

IBR virus. The results of the IBR virus aerosol survival tests are presented in graph 2, Fig. 4. At 23 C, IBR virus aerosol survived best at 90% RH. The initial level of virus was also greater at 90% RH than at either of the other RH levels. Survival was better at 10% than at 35% RH.

VSV. Aerosol survival test results with VSV are presented in graph 3, Fig. 4. At 23 C, VSV survived best at 10% RH. Survival was also greater at 90% than at 35% RH. The difference in survival at the three RH levels was not as marked with VSV as it was with the other viruses. The initial survival was approximately 1 log higher at 90% than at either 35 or 10% RH.

Bacteriophage T3. The results of this series of tests with T3 phage were more disparate than with any of the previous viruses tested. From graph 4 in Fig. 4, it is obvious that high humidity favors survival of airborne T3 phage. At 35% RH, survival was extremely poor; however, at 10% RH survival was considerably better. The initial virus concentration in the aerosol generated at 10% 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 that the line representing 90% RH closely parallels the physical decay line in Fig. 3.

DISCUSSION

At 23 C, airborne NDV and VSV survived best at 10% RH, and airborne IBR and T3 phage survived best at 90% RH. All of the viruses had the poorest survival at 35% RH. At 10% RH, NDV survived equally well at 23 and 37 C. This suggests an increased resistance to thermal inactivation.

The results of these studies demonstrate 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, in almost all cases, a RH of 90% favors generation survival.

The graphs in Fig. 5 illustrate the contrast between generation and storage loss. Each of the

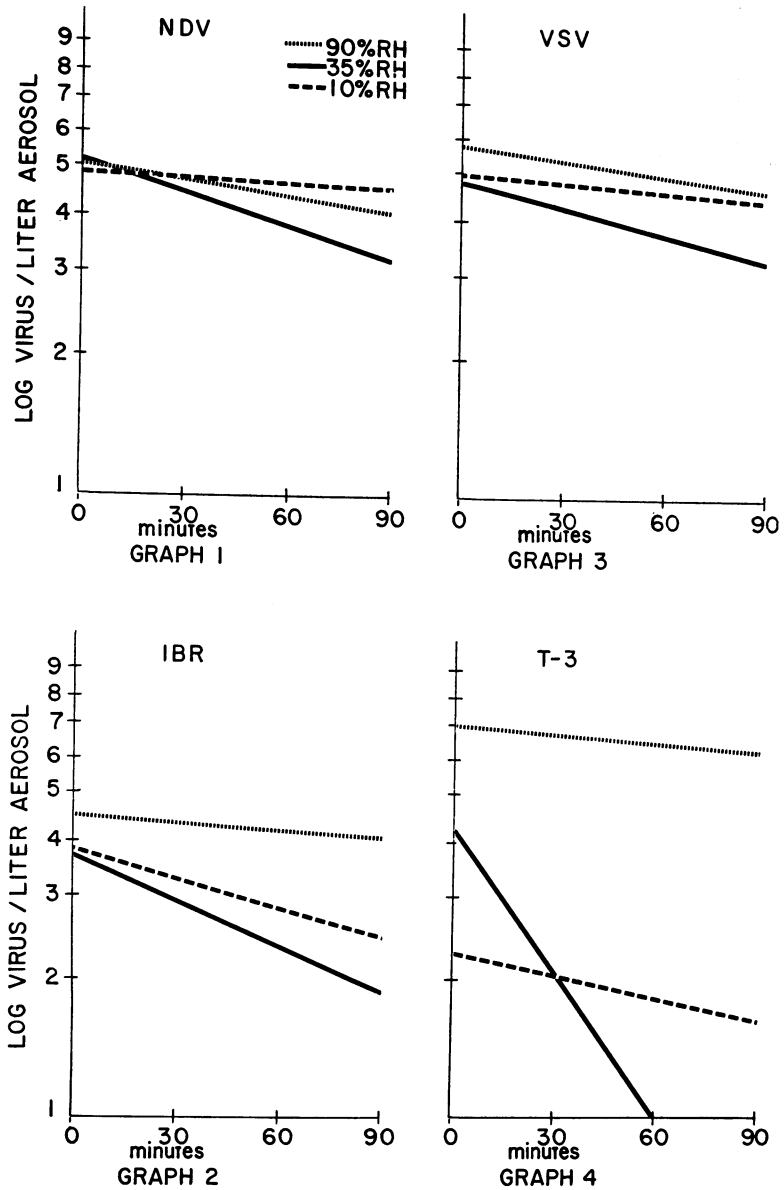

FIG. 4. *Decay of NDV, IBR, VSV, and T3 phage aerosols at 23 C and at 35, 90, and 10% RH. Each line represents three tests.*

four viruses was plotted separately at the three RH levels used. The initial virus concentration was calculated from the total volume nebulized. The first interval of the graph represents the 5-min generation time and the 2-min stabilization period. The remainder represents storage time. In graph 1, it can be seen that the initial loss was greatest at 10% with only a slight difference between 90 and 35% RH. However, on storage, 10% RH favored maximal survival. 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 virus 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 oc-

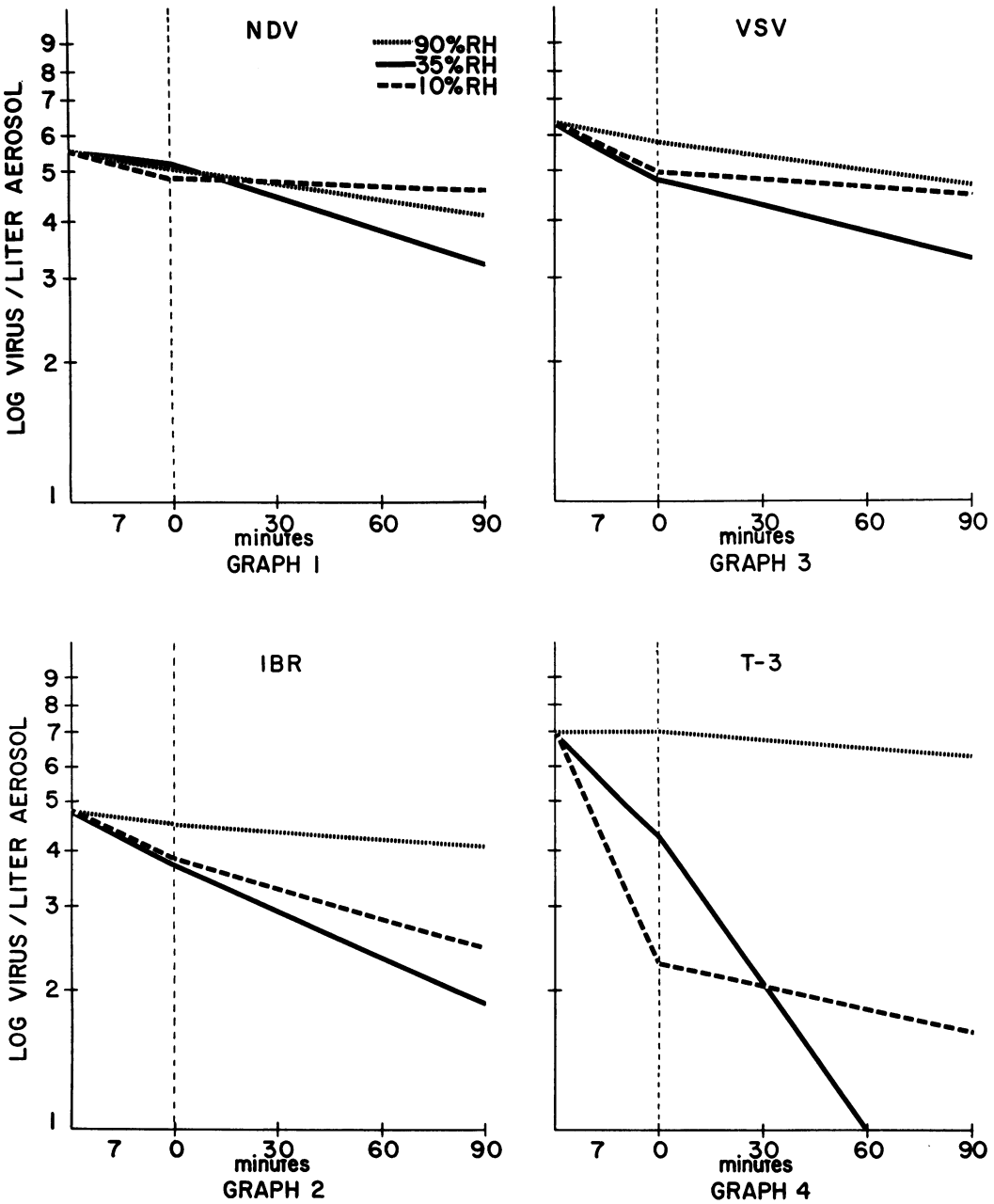

FIG. 5. *Comparison of generation and storage aerosol decay with NDV, IBR, VSV, and T3 phage at 23 C and three RH levels. The first interval represents the 7-min generation and stabilization period and is followed by a 90-min storage period.*

curred at 10% RH. However, once this initial loss occurred, the virus survived better at this RH than at 35%.

From these observations, one may 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,

TABLE 1. *Comparison of the sensitivity of 11 viruses to aerosol generation and storage at three relative humidity (RH) levels*

Virus	RH level favoring survival			Nucleic acid core	Ether sensitivity	Classification by Wilner	Reference
	Low	Medium	High				
Rous sarcoma			+	RNA	+	Myxovirus	19
Influenza A	+			RNA	+	Myxovirus	7
Newcastle disease	+			RNA	+	Myxovirus	Author
Pigeon pox	+	+	+	DNA	+	Poxvirus	19
Vaccinia	+			DNA	Chloroform+	Poxvirus	7
Poliomyelitis			+	RNA	−	Picornavirus	7
VEE	+			RNA	+	Arborvirus	7
IBR			+	DNA	+	Herpesvirus	Author
Vesicular stomatitis	+			RNA	+	Unclassified	Author
Psittacosis (6 BC)			+	RNA/DNA	+	Not classified as virus	12
Bacteriophage T3			+	DNA	−	Bacteriophage	Author

aids in establishing the animal exposure dose. The results of these experiments with four viruses indicate that 90% RH favors maximum survival in a dynamic system.

An attempt was made to show some relationship between sensitivity to RH and other characteristics of viruses. In Table 1, 11 viruses are listed with their sensitivity to RH, their nucleic acid core, their sensitivity to ether, and their classification by Wilner (22). Although these data are incomplete, they suggest that simple generalizations cannot be made regarding the sensitivity of classification groups of viruses to RH. Some ribonucleic acid (RNA) viruses survived best at low RH, and others at high. Some viruses that survived best at high RH were ether-sensitive, and others were not. One of the myxoviruses survived best at high RH, the other two at low RH. Sensitivity to RH appears to be an individual characteristic of a virus. With the evolution of a classification system based on the physical structure and chemical nature of viruses, generalizations on their sensitivity to RH will no doubt be possible.

LITERATURE CITED

1. DE OME, K. B. 1944. Effect of temperature and humidity and glycol vapour on viability of airborne bacteria. Am. J. Hyg. **40**:239–250.
2. DUNKLIN, E. W., AND T. T. PUCK. 1948. The lethal effect of relative humidity on airborne bacteria. J. Exptl. Med. **87**:87–101.
3. EDWARD, D. G., W. J. ELFORD, AND P. P. LAIDLAW. 1943. Studies on airborne virus infections. J. Hyg. **43**:1–15.
4. FERRY, R. M., AND T. G. MAPLE. 1954. Studies of the loss of viability of stored bacterial aerosols. J. Infect. Diseases **95**:142–159.
5. GOLDBERG, L. J., H. M. S. WATKINS, E. E. BOERKE, AND M. A. CHATIGNY. 1958. The use of a rotating drum for the study of aerosols over extended periods of time. Am. J. Hyg. **68**:85–93.
6. GOODLOW, R. J., AND F. A. LEONARD. 1961. Viability and infectivity of microorganisms in experimental airborne infection. Bacteriol. Rev. **25**:182–187.
7. HARPER, G. T. 1961. Airborne microorganisms: Survival tests with four viruses. J. Hyg. **59**: 479–486.
8. HEMMES, J. H., K. C. WINKLER, AND S. M. KOOL. 1960. Virus survival as a seasonal factor in influenza and poliomyelitis. Nature **188**:430–431.
9. LOOSLI, C. G., H. M. LEMON, O. H. ROBERTSON, AND E. APPEL. 1943. Experimental airborne influenza infection. Proc. Soc. Exptl. Biol. Med. **53**:205–210.
10. LOOSLI, C. G., O. H. ROBERTSON, AND T. T. PUCK. 1943. The production of experimental influenza in mice by inhalation of atmospheres containing influenza virus dispersed as fine droplets. J. Infect. Diseases **72**:142–153.
11. REED, L. J., AND H. MUENCH. 1938. A simple method of estimating fifty per cent endpoints. Am. J. Hyg. **27**:493–497.
12. ROSEBURY, T. 1947. Experimental airborne infection. The Williams & Wilkins Co., Baltimore.
13. SCHECHMEISTER, I. L. 1950. Studies on the experimental epidemiology of respiratory infections. J. Infect. Diseases **87**:128–132.
14. SCHECHMEISTER, I. L., AND L. J. GOLDBERG. 1950. Studies on the epidemiology of respiratory infections. J. Infect. Diseases **87**:116–127.
15. SONGER, J. R., J. F. SULLIVAN, AND J. W. HURD. 1963. Testing airfiltering systems. Appl. Microbiol. **11**:394–397.
16. WEBB, S. J. 1959. Factors affecting the viability of airborne bacteria. I. Bacteria aerosolized from distilled water. Can. J. Microbiol. **5**:649–669.
17. WEBB, S. J. 1960. Factors affecting the viability

of airborne bacteria. II. The effect of chemical additives on the behavior of airborne cells. Can. J. Microbiol. **6**:71–87.

18. WEBBS, S. J. 1960. Factors affecting the viability of airborne bacteria. III. The role of bonded water and protein structure in the death of airborne cells. Can. J. Microbiol. **6**:89–105.
19. WEBB, S. J., R. BATHER, AND R. W. HODGES. 1963. The effect of relative humidity and inositol on airborne viruses. Can. J. Microbiol. **9**:87–92.
20. WELLS, W. F., AND P. ZAPPASODI. 1948. The effect of humidity on B streptococci atomized in air. Science **96**:277–278.
21. WILLIAMSON, A. E., AND H. B. GOTASS. 1942. Aerosol sterilization of airborne bacteria. Ind. Med. Surg. **11**:40–45.
22. WILNER, B. I. 1965. A classification of the major groups of human and lower animal viruses, 2nd ed. Cutter Laboratories, Berkeley, Calif.
23. WOLF, H. W., P. SKALEY, L. D. HALL, M. M. HARRIS, H. M. DECKER, L. H. BUCHANAN, AND C. M. DAHLGREN. 1959. Sampling microbiological aerosols. U.S. Public Health Monograph 60.
24. WOLFE, E. K., JR. 1961. Quantitative characterization of aerosols. Bacteriol. Rev. **25**:194–202.