

Effect of Prehumidification on Sampling of Selected Airborne Viruses

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Studies were undertaken to determine if a prewetting device (humidifier bulb) used in combination with an all glass impinger (AGI-30) would increase the recovery of airborne mengovirus-37A, vesicular stomatitis virus (VSV), and the S-13 coliphage. Suspensions of T3 coliphage with mengovirus-37A, VSV, or S-13 were aerosolized and collected by using the AGI-30-humidifier bulb combination to sample the aerosols before and after shifts in relative humidities (RH). These studies revealed the following. (i) At low RH values there was a 3 to 4 log increase in recovery of airborne T3 phage; (ii) concomitantly, the recovery of mengovirus-37A and VSV decreased; and (iii) only at the mid-range RH values was the recovery of S-13 enhanced. The prehumidification technique significantly increased the recovery of airborne T3 phage but decreased the recovery of the two animal viruses tested.

In most aerosol studies with either mammalian or bacterial viruses, the All Glass Impinger [AGI-30; Ace Glass Co., Vineland, N.J. (13)] has been the principal collection device employed because of its efficiency for recovering viable airborne bacteria. The AGI-30 has been used to sample a number of mammalian virus aerosols including adeno, parainfluenza 3 (7), pigeon pox, Rous sarcoma (11), Newcastle disease, infectious bovine rhinotracheitis (8), Colorado tick fever, vesicular stomatitis, neurovaccinia (12), and encephalomyocarditis (EMC) group viruses (2). However, May and Harper (6) have indicated that airborne viruses could not be effectively sampled with the AGI-30 when the aerosols contained a high proportion of very small droplets of single virions.

Hatch and Warren (5) recently reported that a prehumidification device used in combination with the AGI-30 increased recovery of airborne T3 coliphage and *Pasteurella pestis* bacteriophage by 3 logs as compared to results when the AGI-30 was used alone.

Studies were therefore undertaken to determine the effect of this aerosol-sampling technique on the recovery of two airborne animal viruses, vesicular stomatitis virus (VSV) and mengovirus-37A (an attenuated encephalomyocarditis group virus strain), and the tailless phage, S-13. VSV was selected for this study because it was nearly the same size as T3. S-13 was included because, aside from being a tailless phage, it was similar in size (30 nm) to mengovirus-37A.

MATERIALS AND METHODS

Bacteria. *Escherichia coli* C, used as host bacteria for S-13 coliphage, was grown as described by Tessman (9) and incubated at 37 C for 16 hr. *E. coli* B, used as host bacteria for T3 bacteriophage, was grown in nutrient broth (Difco) on a shaker-incubator at 31 C for 16 hr.

Viruses. The original stock for S-13 coliphage was obtained from Irwin Tessman, Purdue University. Working lysates were prepared by the method of Tessman (9). Stocks were stored at 4 C until used. T3 phage, originally obtained from Gunther Stent, University of California, Berkeley, and working lysates were prepared by the method of Worthington et al. (14). Stocks were stored at -20 C until used. Bacteriophage suspensions derived from aerosol sampling were assayed for T3 coliphage by the method of Warren and Hatch (10), and for S-13 by the method of Adams (1).

Preparation and assay procedures in L cells (1-929) for both mengovirus-37A and VSV have been previously described (2).

Aerosol equipment. Aerosols generated from a modified Wells type of reflux atomizer (3) operated at 15 psi for 15 min were contained in a Dual Aerosol Transport Apparatus (DATA) (4) maintained at 21 ± 2 C and at selected relative humidity (RH) levels. RH was controlled by mixing measured volumes of wet and dry air and was determined by the wet-dry bulb method. Aerosol samples were taken by using either the AGI-30 alone or in combination with the humidifier bulb (5). The procedure was to first sample with the AGI-30 and then immediately thereafter to sample with the AGI-30-humidifier bulb (AGI-30 + HB) combination at the same sampling port.

Airborne recovery as depicted in Fig. 1 through 4 is presented as the arithmetic mean of at least three DATA runs for the particular aerosol in question.

RESULTS

A series of experiments were conducted in which mixed suspensions of T3 and mengovirus-37A or T3 with VSV suspensions were aerosolized into the DATA system. Aerosol samples were collected at 10 sampling points along the entire length of the DATA tube with use of either the AGI-30 alone or the AGI-30 + HB combination. When the RH of a mixed T3 plus mengovirus-37A aerosol was shifted from low (approximately 25%) to high (approximately 60%) RH, and sampled with AGI-30, an increase of 2 to 4 logs in recovery of viable T3 particles was observed (Fig. 1). Mengovirus-37A recoveries were not increased as a result of the RH shift. When the AGI-30 + HB combination was used to sample the same aerosol, a 3 to 4 log increase in recovery of T3 both before and after the RH shift was observed, whereas mengovirus-37A recovery remained unchanged.

In reference to Fig. 1, the initial T3 recovery differences at zero min for aerosols sampled with or without the humidifier bulb were due to the fact that aerosol transit time from the atomizer to the first sampling port was approximately 10 sec. During this short transit time, the effect of a

low RH (23%) on viable recovery was manifested when using the AGI-30. On the other hand, the humidifier bulb increased the RH of the aerosol to 95% prior to entrance into the AGI-30

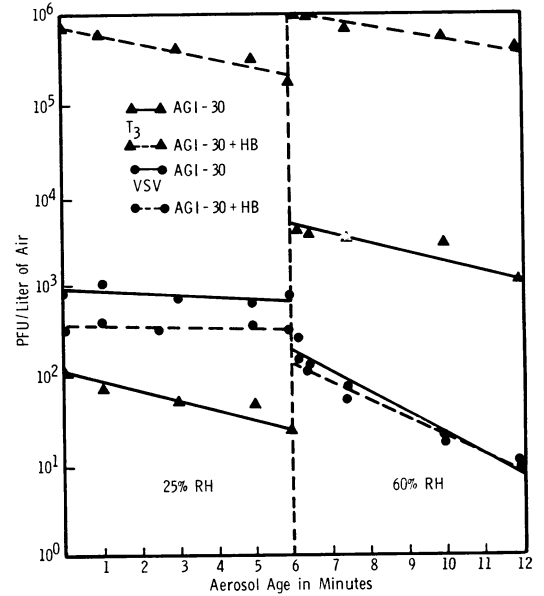


FIG. 2. Effect of low (25%) to high (60%) RH shift on the recovery of mixed VSV and T3 aerosols.

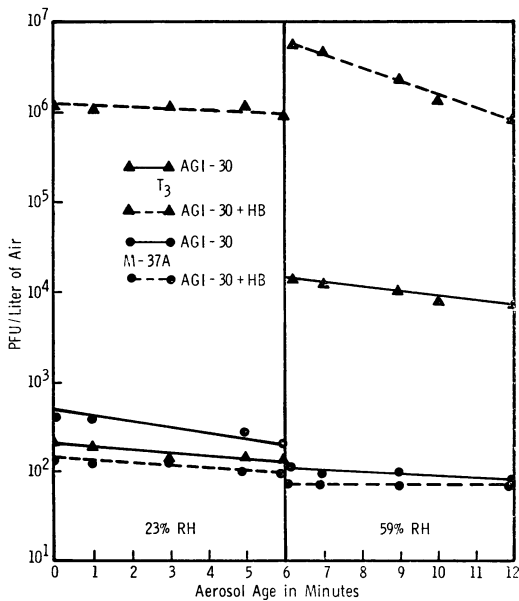


FIG. 1. Effect of low (23%) to high (59%) RH shift on the recovery of mixed mengovirus-37A (M-37A) and T3 aerosols.

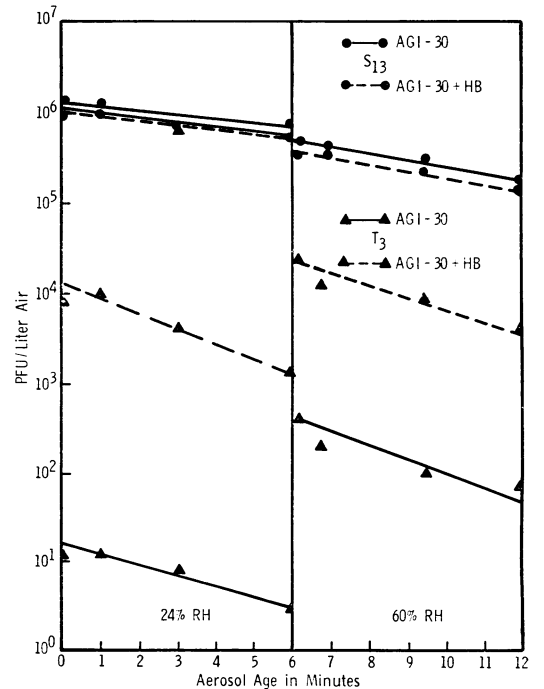


FIG. 3. Effect of low (24%) to high (60%) RH shift on the recovery of mixed T3 and S-13 aerosols.

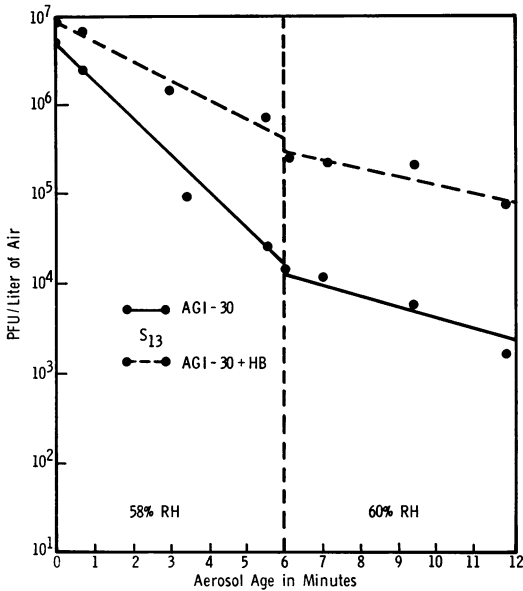


FIG. 4. Increased recovery from *S-13* aerosols (58 to 60% RH) by means of the humidifier bulb technique.

and this avoided the effect of low RH values on airborne T3 recovery.

An RH shift from low to high (Fig. 2) was deleterious to airborne VSV survival, whereas T3 responded again as when mixed and aerosolized with mengovirus-37A. Moreover, it was apparent that airborne VSV was rapidly inactivated at 60% RH. When aerosols of T3 and VSV were initially maintained at 60% RH and the RH was then shifted to 66% RH, inactivation continued (*not shown*). In view of the sensitivity of VSV, one can conclude that prehumidification actually lowers recovery of infectious VSV particles.

When the recovery of *S-13* (a tailless phage) was compared with that of T3 (possessing a stubby tail), it was found that airborne *S-13* was stable during a shift from low to high RH, and that use of the humidifier bulb in combination with the AGI-30 had little effect on recovery (Fig. 3). However, further experiments demonstrated that *S-13* is sensitive to mid-range humidity values and that prehumidification significantly increases recovery of viable particles in this sensitive region (Fig. 4).

DISCUSSION

Use of the prehumidification device of Hatch and Warren (5) increased viable recovery of airborne T3 coliphage, but not of VSV or mengovirus-37A. It was postulated that this difference

might be attributable to the tail structure of T3. However, *S-13*, a tailless phage, showed increased recovery with prehumidification at mid-range humidity values. It was therefore evident that the possession of tail fibers was not the sole characteristic which permitted increased recovery through prehumidification.

It was also suspected that use of the humidifier bulb would not enhance recovery of airborne VSV. VSV is a lipid-containing virus, and Webb, et al. (12) reported that such viruses are inactivated rapidly at high RH values, whereas lipid-free viruses decay at a higher rate in dry air. We found the rate of decay of VSV to increase as RH increased and the use of the humidifier bulb at the same time further enhanced decay. Although optimal survival of EMC group viruses is at 70 to 90% RH (2) and it was therefore expected that prehumidification would increase recovery of viable mengovirus-37A from the aerosol, this was not observed. Recovery of mengovirus-37A decreased slightly when sampled with the AGI-30 + HB combination.

There are at least two possible explanations for the effects of prehumidification. Increases in RH within the DATA system resulted in increased droplet size within the aerosol (4), and with the use of the humidifier bulb there is also an increase in RH and in droplet size immediately before impingement (Warren, J.C., 1969, *unpublished data*). In both of these instances there was a dramatic increase in recovery of T3. This phenomenon may be due to increased efficiency of the AGI-30, resulting from larger airborne droplets. However, no evidence yet exists to support this idea. To the contrary, the data in Fig. 1 argues against this idea since no increase in recovery of mengovirus-37A was observed with mixed aerosols.

The second possible explanation is that prehumidification reverses the detrimental effects of low humidity. This is not difficult to imagine when one considers the many examples of reversible denaturation of protein molecules. The quaternary and tertiary structure of protein molecules are the result of a delicate balance of opposing forces. A physical stress such as drying could easily lead to the denaturation of the protein. The reversibility of the denaturation depends on the nature of the molecule and the extent of the stress. Each virus will react to a stress in a way that is as unique as its capsid protein. Therefore, as the stress of drying on the viruses studied is different, the effect of prehumidification is also different. In the case of T3 and *S-13*, the effects of RH may be reversible and prehumidification causes this reversal. Conversely, the detrimental

effects of RH on mengovirus-37A and VSV may be irreversible.

From the evidence compiled to date, no generalizations can be drawn concerning the effect of prehumidification. In those instances where high RH are detrimental to certain airborne viruses, prehumidification will probably give lower recoveries. However, in those cases where low RH decreases airborne recovery, such viruses will have to be individually studied to determine if the prehumidification technique will be beneficial or deleterious to viable recovery.

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