Biological Activity of the Nucleic Acids Extracted from Two Aerosolized Bacterial Viruses

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The infectivity of the nucleic acids of φX-174 and MS-2 was examined after aerosolization. It was found that the nucleic acids were undamaged by aerosolization.

There are numerous reports describing the characteristics of aerosolized viruses, but only a few have considered the nature of their inactivation in the airborne state. De Jong and Winkler (2) ascribed the inactivation of aerosolized poliovirus to "denaturation" of the ribonucleic acid (RNA) moiety, but Akers and Hatch (1) showed that aerosolized mengovirus RNA was not adversely affected by relative humidities that inactivated the intact virus. In the present study, I have explored the biological integrity of the nucleic acids of two bacteriophages (φX-174 and MS-2) after aerosolization. This was accomplished by determining the capacity of the extracted nucleic acid to infect Escherichia coli spheroplasts.

High-titered phage suspensions were obtained from Miles Laboratories, Inc., Elkhart, Ind., and aerosolized with a modified Wells atomizer (3). The aerosols were conducted into an aerosol transport apparatus (4) with an aerosol transit time of 7.5 min. Two AGI-30 impingers (Ace Glass Co., Vineland, N.J.) were used in parallel to collect the aerosol in toto. After a 15-min collection period, samples of the pooled impinger fluid were taken for viability assays and nucleic acid extractions. The extracted nucleic acids were stored at −70 C.

The method of Guthrie and Sinsheimer (7) was used to obtain infectious deoxyribonucleic acid (DNA) from φX-174 which was assayed by the method of Sinsheimer (9). Infectious RNA was extracted using the method of Girard (6). The RNA was suspended in 3 mm ethylenediaminetetraacetic acid and assayed by admixing 0.1 ml with 0.5 ml of E. coli AB301 spheroplasts at room temperature for 25 min. Three milliliters of agar were added and the suspension plated to enumerate infected centers.

Table 1 shows that there was approximately a 4-log difference in the recovery of φX-174 infectious units over the range of relative humidity values explored, but infectious DNA assays showed no significant differences. In several samples the infectious DNA gave titers higher than the intact particle.

Similar comparisons with MS-2 phage were precluded by the relative uniformity of viability of MS-2 as supplied in salt suspension. As previously reported (5), the stability of MS-2 aerosols could be increased with the addition of 1.5% tryptone to the suspension prior to aerosolization. Therefore, aerosols were generated from spray suspensions containing only salt or salt with 1.5% tryptone. Impinger samples that showed the widest differences in the numbers of infective particles were also analyzed for infectious RNA content.

There was no indication (Table 2) that the infectivity of MS-2 RNA, extracted from samples containing 10⁶ infective particles, differed significantly from those containing 10⁷ infective particles.
TABLE 2. Comparison of viable particles and infectious ribonucleic acid (RNA) from collected aerosols of MS-2

| Relative humidity (%) | Trichomes | Viable particles/ml of impinger fluid | Infectious RNA/ml of impinger fluid |
|-----------------------|-----------|--------------------------------------|------------------------------------|
| Extraction with dextran sulfate | 2 ± 1     | + 9.1 × 10^8                          | 3.5 × 10^6                         |
|                       | 65 ± 2    | - 1.6 × 10^7                          | 9.5 × 10^6                         |
|                       | 65 ± 2    | + 1.1 × 10^8                          | 5.1 × 10^6                         |
| Extraction with dex-  | 1 ± 1     | + 2.2 × 10^9                          | 3.5 × 10^6                         |
| tran sulfate          | 55 ± 2    | - 2.3 × 10^8                          | 2.2 × 10^6                         |
|                       | 55 ± 2    | + 8.3 × 10^9                          | 4.2 × 10^6                         |

a Bacteriophage MS-2 atomized and collected in 0.1 M NaCl, 0.05 M tris (hydroxymethyl)aminomethane, pH 7.6, and 0.001 M ethylenediaminetetraacetic acid.

b Duplicate assays.

tivity by means of humidification prior to sampling has been shown to occur with phage (5, 8). If these results can be repeated with mammalian viruses, then a serious flaw exists in the present methods of sampling airborne viruses. There has been only one study which has examined the effect of humidification on the sampling of aerosolized animal viruses (10). Humidification as encountered in the passage of a particle to the throat and lung areas might actually reverse damage attending aerosolization, with an increase in the likelihood of a successful infection.

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