APPLIED MICROBIOLOGY, Apr. 1971, p. 761-762

Vol. 21, No. 4
Printed in U.S.A.

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

EDWARD J. DUBOVI

Naval Biomedical Research Laboratory, School of Public Health, University of California, Berkeley, California 94625

Received for publication 13 October 1970

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.

TABLE 1. *Comparison of viable particles and infectious deoxyribonucleic acid (DNA) from collected aerosols of φX-174*[a]

Relative humidity (%)	Viable particles/ml of impinger fluid	Infectious DNA/ml of impinger fluid[b]	
1 ± 1	1.3×10^4	7.1×10^4	3.8×10^5
13 ± 2	9.9×10^4	7.1×10^4	3.0×10^5
25 ± 2	1.5×10^5	9.5×10^4	2.6×10^5
40 ± 2	2.1×10^5	1.2×10^5	3.0×10^5
54 ± 2	6.7×10^6	9.3×10^4	1.6×10^5
64 ± 2	2.6×10^8	1.5×10^5	4.0×10^5
80 ± 2	5.3×10^8	1.5×10^5	3.2×10^5
93 ± 2	7.1×10^8	1.8×10^5	4.4×10^5

[a] Bacteriophage φX-174 atomized and collected in 0.07 M sodium borate, *p*H 9.1, and 0.002 M ethylenediaminetetraacetic acid.

[b] Duplicate assays.

There was no indication (Table 2) that the infectivity of MS-2 RNA, extracted from samples containing 10^9 infective particles, differed significantly from those containing 10^7 infective

TABLE 2. *Comparison of viable particles and infectious ribonucleic acid (RNA) from collected aerosols of MS-2*[a]

Relative humidity (%)	Tryptone, 1.5%	Viable particles/ml of impinger fluid	Infectious RNA/ml of impinger fluid[b]	
Extraction without dextran sulfate				
2 ± 1	+	9.1×10^8	3.5×10^2	1.2×10^3
65 ± 2	−	1.6×10^7	9.5×10^2	2.6×10^3
65 ± 2	+	1.1×10^9	5.1×10^2	1.2×10^3
Extraction with dextran sulfate				
1 ± 1	+	2.2×10^9	3.5×10^2	1.4×10^3
55 ± 2	−	2.3×10^7	2.2×10^2	1.1×10^3
55 ± 2	+	8.3×10^8	4.2×10^2	1.8×10^3

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

[b] Duplicate assays.

particles. RNA from samples containing tryptone consistently gave fewer infective centers (Table 2), suggesting that ribonuclease may have been introduced with the tryptone. Table 2 also shows the infectivity of RNA extracted in the presence of 2 μg of dextran sulfate per ml. If ribonuclease were present in the tryptone samples, its effect on RNA infectivity can be considered negligible.

This study shows that the nucleic acids of two bacterial viruses were not damaged with respect to biological activity by atomization, aerosol storage, or collection. The viability differences (Tables 1 and 2) must therefore be associated primarily with damage to the protein component of the virion. However, the possibility of damage to the nucleic acid through severe distortions of the viral protein(s) cannot be excluded.

If, in the aerosolized state, the nucleic acid of a viral particle is undamaged, then the potential for infectivity remains. The recovery of this infectivity 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.

This investigation was supported by the Office of Naval Research and the Bureau of Medicine and Surgery, U.S. Navy, under a contract between the Office of Naval Research and the Regents of the University of California.

I thank L. J. Goldberg and Irving Ford for their technical assistance.

LITERATURE CITED

1. Akers, T. G., and M. T. Hatch. 1968. Survival of a picornavirus and its infectious ribonucleic acid after aerosolization. Appl. Microbiol. 16:1811–1813.
2. De Jong, J. G., and K. C. Winkler. 1968. The inactivation of poliovirus aerosols. J. Hyg. 66:557–566.
3. DeOme, K. B., and the Personnel, U.S. Naval Medical Research Unit No. 1, Berkeley, Calif. Amer. J. Hyg. 40:239–250.
4. Dimmick, R. L., and M. T. Hatch. 1969. Dynamic aerosols and dynamic aerosol chambers, p. 177–193. *In* R. L. Dimmick and A. B. Akers (ed.), An introduction to experimental aerobiology. Wiley-Interscience, New York.
5. Dubovi, E. J., and T. G. Akers. 1970. Airborne stability of tailless bacterial viruses S-13 and MS-2. Appl. Microbiol. 19:624–628.
6. Girard, M. 1967. Isolation of ribonucleic acids from mammalian cells and animal viruses, p. 581–588. *In* L. Grossman and K. Moldave (ed.), Methods in enzymology, vol. 12, Part A. Academic Press Inc., New York.
7. Guthrie, G. D., and R. L. Sinsheimer. 1963. Observations on the infection of bacterial protoplasts with the deoxyribonucleic acid of bacteriophage φX-174. Biochim. Biophys. Acta. 72:290–297.
8. Hatch, M. T., and J. C. Warren. 1969. Enhanced recovery of airborne T_1 coliphage and *Pasteurella pestis* bacteriophage by means of a presampling humidification technique. Appl. Microbiol. 17:685–689.
9. Sinsheimer, R. L. 1968. Spheroplast assay of φX-174 DNA, p. 850–858. *In* L. Grossman and K. Moldave (ed.), Methods in enzymology, vol. 12, Part B. Academic Press Inc., New York.
10. Warren, J. C., T. G. Akers, and E. J. Dubovi. 1969. Effect of prehumidification on sampling of selected airborne viruses. Appl. Microbiol. 18:893–896.