Inactivation of Bacteriophage T₃ in Aerosols: Effect of Prehumidification on Survival After Spraying from Solutions of Salt, Peptone, and Saliva

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Coliphage T₃ was inactivated by a factor of 10³ to 10⁴ within 30 min after spraying from solutions of NaCl. Addition of peptone to the spray medium protected against inactivation at high relative humidity (RH), presumably by preventing surface inactivation. Prehumidification of the sample before collection had no effect on recovery if sprayed from solutions of NaCl, with or without peptone. If only peptone was present in the spray medium, prehumidification of the aerosol sample increased the recovery by a factor of 1,000 at low RH and by a factor of 5 at high RH. In aerosols sprayed from saliva, inactivation was nearly equal to that in peptone, with an increase of recovery after prehumidification by a factor of 1,000.

Inactivation of viruses in aerosols is strongly dependent on the composition of the spray medium and on the method of aerosol collection. If viruses are sprayed from solutions of salt at a high relative humidity (RH), surface inactivation may occur, as shown for bacteriophage T₁ and MS₂ (5, 6). Addition of peptone to the virus suspension protects against aerosol inactivation at high RH and against surface inactivation (7). Prehumidification of the aerosol sample before collection can alter the recovery by a factor of 1,000 in both directions. Prehumidification reduces the recovery of phage MS₂ sprayed from a NaCl solution without peptone (3), whereas the recovery of phage T₁ and Pasteurella pestis bacteriophage is increased (4, 6). The reduction of recovery of phage MS₂ is probably caused by increased surface inactivation during prehumidification (5). The reason for the increase of recovery is still unknown. Therefore the interaction of the spray medium with the effect of prehumidification was studied with phage T₃.

Prehumidification has some practical significance for natural transmission of diseases since prehumidification occurs in the upper respiratory tract. To make the situation more realistic, inactivation of phage T₃ was also studied after spraying from saliva.

MATERIALS AND METHODS

Propagation, purification, and assay of virus. Bacteriophage T₃ was propagated on Escherichia coli K-12. The virus suspension protects against aerosol inactivation of MS₂, and T₃, was inactivated by a factor of 10³ to 10⁴ within 30 min after spraying from solutions of NaCl. Addition of peptone to the spray medium protected against inactivation at high relative humidity (RH), presumably by preventing surface inactivation. Prehumidification of the sample before collection had no effect on recovery if sprayed from solutions of NaCl, with or without peptone. If only peptone was present in the spray medium, prehumidification of the aerosol sample increased the recovery by a factor of 1,000 at low RH and by a factor of 5 at high RH. In aerosols sprayed from saliva, inactivation was nearly equal to that in peptone, with an increase of recovery after prehumidification by a factor of 1,000.

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RESULTS

Bacteriophage T₃ was diluted 1:1000 in 0.1 M NaCl, and survival after spraying was studied at various relative humidities. At any RH, inactivation was most rapid immediately after
INACTIVATION OF BACTERIOPHAGE T₃ IN AEROSOLS

Evaporation (Fig. 1A). At t = 0.5 min, \( \log N_t / N_0 \) was 10² at low RH and 10³ at 80% RH, where \( N_t \) is amount of PFU at time \( t \) and \( N_0 \) at time zero. Between \( t = 10 \) and 30 min, \( \Delta \log \) PFU at low RH was approximately equal to decay by physical loss, which amounts to 0.15 at low RH (T. Trouwborst, Ph.D. thesis, State University, Utrecht, 1971). If 0.1% (wt/vol) peptone was added to the spray medium with 0.1 M NaCl, inactivation was less at high RH at \( t = 0.5 \) min and 30 min, but the recovery at low RH remained the same (Fig. 1B). At high RH, surface inactivation might occur (5, 6), which could be prevented by peptone. Therefore, the sensitivity to surface inactivation was tested by bubbling air through the virus suspension. Phage T₃ was rapidly inactivated in solutions of 2.6 M NaCl by aeration (Fig. 2). Without aeration no inactivation was observed, excluding the possibility of toxicity of this concentrated NaCl solution. These results suggest that the air/water

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\log \frac{N_t}{N_0} = \log \frac{10^2}{10^3} = 1.0 \text{ at low RH,}
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\log \frac{N_t}{N_0} = \log \frac{10^3}{10^3} = 0.0 \text{ at 80% RH.}
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Fig. 2. Inactivation of phage T₃ by aeration of the phage suspension. Phage T₃ was diluted 1:10⁴ in 2.6 M NaCl (×) or 2.6 M NaCl + 0.1% (wt/vol) peptone (○). Thereafter air was bubbled through the solution.

Fig. 1. (A) Inactivation of bacteriophage T₃ in aerosols. Samples were collected at \( t = 0.5, 10, \) and 30 min after spraying from 0.1 M NaCl. (B) Inactivation of bacteriophage T₃ in aerosols after spraying from 0.1 M NaCl + 0.1% (wt/vol) peptone.
interface is an important factor for inactivation of phage T₃. Peptone protected against inactivation by aeration (Fig. 2) as well as against aerosol inactivation at high RH (Fig. 1b).

The effect of prehumidification was tested after spraying from solutions with 0.1 M NaCl + 0.1% (wt/vol) peptone. Prehumidification had no effect on the recovery either at high or low RH, regardless of sampling time (Table 1).

**TABLE 1. Effect of prehumidification on inactivation of bacteriophage T₃ after spraying from 0.1 M NaCl + 0.1% (wt/vol) peptone**

| RH   | t (min) | With prehumidification | Without prehumidification |
|------|---------|-------------------------|----------------------------|
| 23%  | 0.5     | -2.02                   | -2.06                      |
|      | 10      | -2.16                   | -2.26                      |
|      | 30      | -2.45                   | -2.72                      |
| 88%  | 0.5     | -0.74                   | -0.67                      |
|      | 10      | -1.12                   | -0.95                      |
|      | 30      | -1.42                   | -1.12                      |

The results are at variance with those of Hatch and Warren (4) who reported a strong effect of prehumidification on recovery of phage T₃. Therefore we tested whether the spray medium was important. Phage T₃ was sprayed from a solution with 0.1% (wt/vol) peptone without salt. In this case, the effect of prehumidification was very pronounced (Fig. 3). The prehumidification effect was most significant at low RH, increasing the recovery by a factor of 10³ at all sampling times. From these results it is concluded that salt reduces the effect of prehumidification. At high RH the recovery was about the same with or without salt, providing that peptone was present in the spray medium (Fig. 1b and 3).

After spraying from saliva (Fig. 4), inactivation was rapid at low RH (about a factor 10⁴) but was less at high RH. Also in this case prehumidification had an important effect at low RH, but no effect was observed at high RH.

**DISCUSSION**

Recovery of phage T₃ after spraying from salt without peptone was low over the entire range of RH. If peptone was added protection occurred...
at high RH, but inactivation at low RH remained about the same. These results suggest at least two mechanisms of inactivation: (i) surface inactivation at high RH, which was prevented by addition of peptone (5-7) and (ii) inactivation at low RH presumably by removal of structural water molecules. Prehumidification of aerosols containing peptone increased the recovery at low RH if no salt was present. This suggests that in salt-deficient aerosols containing peptone the inactivation obtained at low RH may occur at collection. The presence of salt in the spray medium abolished the prehumidification effect, suggesting an irreversible inactivation in the presence of salt.

After spraying from saliva, prehumidification had an important effect on the recovery of viable phage T₃. Some constituents in saliva seem to counteract the effect of the sodium (80 meq) and chloride (~40 meq) in saliva. The beneficial effect of prehumidification on survival of phage in saliva suggests the importance of prehumidification for retention of viable viruses in the respiratory tract.

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