Infectivity-destroying Effect of Humidity for Dried Coliphage T1

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Received for publication 27 May 1968

Infectivity of dried coliphage T1 has been measured as a function of humidity, temperature, and atmospheric pressure. Loss of infectivity by a factor of 10⁴ was caused by water vapor of approximately 40 to 85% saturation when the microorganisms were kept for 3 days at 34 C in evacuated containers. At humidities below 40% and above 90% saturation, no loss of infectivity occurred. At a temperature of 24 C, the infectivity loss was 20-fold. When the virus preparation was kept at 34 C and atmospheric pressure, some loss of infectivity was also found at humidities below 40% and above 90% saturation. Damage to tail proteins or to the phage chromosome is considered as a possible explanation for the inactivation.

Dried films of coliphage T1 have been used for measurements of ionizing radiation in the laboratory (10) and of solar ultraviolet radiation in space (15). Particularly the space-borne experiments have raised the question of the influence of humidity on the survival of unirradiated transport and laboratory controls. Sampling of airborne bacteria has also shown significant variations of collected bacteria at different relative humidities (11). Humidity was described as one of the main factors of the aerial environment affecting the survival of microorganisms (12). Such observations have made more detailed studies of this phenomenon desirable and this report presents data on the infectivity of dried coliphage T1 after exposure to various humidities.

Materials and Methods

Preparation of microorganisms and assay techniques. Escherichia coli B was grown in beef extract for 5 hr on a rotating shaker at 37 C. When the optical density of the culture at 650 nm had risen to 0.85, coliphage T1 was added. The multiplicity of infection was approximately 0.1. After 3 hr of further incubation at 37 C, lysis was complete. The lysate was clarified by centrifugation at 2,500 × g for 1 hr at 4 C. Samples (10 ml) of clarified lysate were mixed with 1-ml samples of chloroform and stored at 4 C. The titer of the suspension was 10⁶ plaque-forming units (PFU) per ml as determined by plaque assay on E. coli B as follows. Serial dilutions (1:10) of the phage in beef extract were prepared. Portions (0.5 ml) of each dilution were mixed with 4 ml of a suspension agar and 0.5 ml of a diluted (1:10) suspension of E. coli B cells grown overnight in twofold concentrated beef extract at 37 C. A 2.0-ml sample from each resulting suspension was pipetted on base agar. Two 10-cm petri dishes were seeded per dilution. The petri dishes were incubated at 37 C for approximately 5 hr. Whenever possible, titers are based on at least 100 plaques per petri dish.

Composition of media. Suspension agar contained 274 × 10⁻² M NaCl, 33 × 10⁻³ M glucose, 14 × 10⁻³ M sodium citrate, 2.0 g of tryptone (Difco) per 100 ml, 1.2 g of agar (Difco) per 100 ml, adjusted to pH 6.6 to 6.8. Base agar contained 137 × 10⁻⁴ M NaCl, 7 × 10⁻³ M glucose, 7 × 10⁻³ M sodium citrate, 1.3 g of tryptone per 100 ml, 1.0 g of agar per 100 ml, adjusted to pH 6.9 to 7.0. The media were sterilized in an autoclave. F-14A beef extract was prepared as described elsewhere (23).

Drying and resuspending of microorganisms. Samples (0.01 ml) of phage lysate were seeded on small plastic-coated autoclaved aluminum plates with circular depressions (4.5 mm diameter) by use of an AGLA micrometer syringe (Burroughs Wellcome & Co., London). Three aluminum plates were placed in each of three to four desiccators, with volumes of 9,300 ml, which served as containers for the experiments. The desiccators also contained individual membrane hygrometers (Serdex B relative humidity indicator, Bacharach Industrial Instrument Co., Pittsburgh, Pa.) and thermometers, in the control experiments, petri dishes filled with P₂O₅ powder. The phage droplets were dried by evacuation at pressures of 0.02 mm of Hg at room temperature as measured before injection of water (see below). After the experiments, each aluminum plate was immersed in a 1-ml sample of beef extract and agitated for 30 sec on a Vortex Jr. mixer (Scientific Industries, Inc., New York). The infectivity of eluted phage was determined as described above.

Adjustment of humidity. Membrane hygrometers were adjusted at room temperature and atmospheric
pressure to humidity levels measured with a dew-point ether hygrometer. Small amounts of water were injected with tuberculin syringes through pressure hoses into the sealed containers to give specific saturation levels of water vapor at the temperatures chosen. Various humidity levels were rapidly established by this method; the average exposure period was 71.5 ± 1.2 hr. Temperature and humidity were frequently recorded during this time. The "expected" humidity levels were calculated according to the measured temperatures. In some cases, variation between measured and expected humidity levels was considerable. Reasons for this may have been inaccuracy of the hygrometers as well as difficulty of precise dosage of very small amounts of water. The averages between expected and measured values were accepted as "real."

In the cases of the 0 and 100% levels there was no doubt, since the presence of P₂O₅ powder guaranteed dryness, and excessive amounts of water injected into the containers resulted in visible condensation on the samples. For experiments at atmospheric pressure, air was dried with P₂O₅-cotton filters and filled into the containers prior to injection of water. Two temperature-control experiments at 0% saturation were performed for each humidity survival test. One of these was kept at the same temperature as the humidity test. The other control set was kept at 4 C when the humidity experiment was done at 34.1 ± 1.3 C and at 4 C when the experiment was done at 24.2 ± 0.3 C.

RESULTS

Results are expressed as average titers plus or minus standard deviations obtained from three to four identical experiments. After drying and storage for approximately 3 days at various temperatures, close to 1% of the PFU seeded was recovered from the controls (see Table 1). Some variation of the controls is apparent, and the differences of the average titers are within a factor of 4. The experiments were performed during the course of 1 year, and a slight decrease of the titer of the phage suspension may account for the variation.

Table 1. Average survival of 0% saturation controls

<table>
<thead>
<tr>
<th>Environment during storage</th>
<th>Infectivityb</th>
<th>Fractionc</th>
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<tbody>
<tr>
<td>Temp (C)</td>
<td>Pressure (mm of Hg)a</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.02</td>
<td>3.0 × 10⁴</td>
</tr>
<tr>
<td>34</td>
<td>0.02</td>
<td>1.3 × 10⁴</td>
</tr>
<tr>
<td>24</td>
<td>0.02</td>
<td>7.8 × 10⁴</td>
</tr>
<tr>
<td>34</td>
<td>Atmospheric</td>
<td>2.0 × 10⁴</td>
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</table>

a Measured with a McLeod gauge at the end of drying period.

b Given in PFU; each unit was seeded with 10⁶ PFU.

c Expressed relative to controls kept at 4 C.

![Figure 1](http://aem.asm.org/)

**Figure 1.** Infectivity of coliphage T1 as function of humidity, temperature, and pressure. Dried phage was kept for 3 days at 34.1 ± 1.3 C in vacuo (curve A), at 24.2 ± 0.3 C in vacuo (curve B), and at 34.1 ± 1.3 C and atmospheric pressure (curve C). Horizontal T bars indicate range between expected and measured humidities, vertical T bars show standard deviations of phage infectivity.

Figure 1 shows phage inactivation depending on humidity, temperature, and atmospheric pressure. The infectivity at 0% saturation of each humidity survival curve represents the total of all corresponding temperature controls. In vacuo, infectivity was not reduced by humidity from approximately 0 to 45% saturation (Fig. 1, curves A and B). In the range from approximately 45 to 85% saturation, a sharp reduction of infectivity by a maximum factor of approximately 10³ was observed in vacuo at 34 C. Little inactivation was found when the humidity was further increased. Curve B represents data from experiments done at 24 C in vacuo and shows a pattern similar to curve A. Maximal loss of infectivity by a factor of 10¹ was observed at 70% saturation. When the humidity was further increased, there was no loss of infectivity. Admission of air to the containers prior to injection of water resulted in loss of infectivity at 30% saturation, as shown by curve C. Showing a steady loss of infectivity with increasing humidity, curve C joins the down-
ward slope of curve A near 65% saturation. With further increase of humidity, the residual infectivity was approximately 10-fold higher.

DISCUSSION

Inactivation of airborne viruses at various relative humidity (RH) levels has been described (2, 7, 13, 17, 21). Comparing the survival at 10 and 90% RH, Newcastle disease virus and vesicular stomatitis virus survived better at 10% RH, whereas coliphage T3 showed higher survival at 90% RH (17). Survival of coliphage T3 in aerosols was significantly reduced by RH below 70% (7). Airborne coliphage T1 was rapidly inactivated at 85% RH, whereas survival was relatively high at 50 and 55% RH (13). These results agree with the data described in this report. At lower humidities, coliphage T1 was found more stable than coliphage T3 in air (13). Aerosols of pigeon pox and Rous sarcoma viruses showed rapid inactivation of Rous sarcoma virus by RH below 80% (21). Survival of pigeon pox virus was only slightly affected by humidity. A lethal effect of various ranges of RH for several bacterial species has also been described (3, 16, 19, 20, 22, 24). E. coli JEP was rapidly inactivated in aerosols by 90 to 82% RH (5). When aerosols of E. coli B were kept at low RH levels in a nitrogen atmosphere, survival was higher than when the cells were suspended in air (6). These data indicate that the lethal effect of humidity for microorganisms is a complex phenomenon. Survival depends on the species of the microorganism(s), the temperature, and the composition of the environmental atmosphere.

The mechanism of this inactivation is unknown and its explanation invites speculation. Surface denaturation of enzymes, toxins, and T-coliphages by exposure to "unbalanced forces" existing at gas-liquid interphases has been described (1). Such undefined forces, which are possibly enhanced by air at normal atmospheric pressure at humidities below 40% and above 90% saturation, may denature tail proteins necessary for the attachment of the virus to the host-cell surface. It has been suggested that loss of hydrogen-bonded water from proteins and nucleoproteins below a critical level may be the cause of death of aerosolized bacteria (20). The fastest inactivation rates were observed between 30 and 70% RH. Since the coliphage T1 chromosome is deoxyribonucleic acid (DNA), the effect of humidity on the architecture of lithium and sodium salts of DNA demonstrated by infrared absorption studies (4, 8, 9, 18) may be of interest. Significant protection of coliphage T5 against heat by certain readily dissociable complexes with lithium, sodium, and other cations has earlier been described (14), and a possible effect of humidity on cations associated with viruses should be considered. The spectroscopic studies mentioned above showed characteristic shifts of absorption bands with RH as well as dichroism of the DNA-salts at RH above 90%. At this humidity level, the bases are arranged perpendicular to the DNA axis. With decreasing humidity, transition to an increasingly disordered form concurred with decrease of dichroism. Total loss of dichroism was found at an RH of 49%. This may indicate an important influence of humidity on viral DNA structure and infectivity. However, all spectroscopical changes were reversible, whereas the loss of infectivity appeared to be irreversible. Additional studies are therefore needed to throw more light on the mechanism of the lethal effect of humidity for microorganisms.

ACKNOWLEDGMENTS

This work was supported by NASA grant NSG 155-61. I acknowledge the helpful advice of G. W. Fuhs and J. Barlow, and the technical assistance of M. Cordato, K. Stinner, and J. Reidl.

LITERATURE CITED