The Infectivity of the Nucleic Acid of Aerosol-inactivated Poliovirus

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Poliovirus is inactivated rapidly in aerosols at low to moderate relative humidity (r.h.) (Hemmes, Winkler & Kool, 1960; Harper, 1961) and it is of biological and epidemiological importance to know whether the nucleic acid remains infective during this process. De Jong & Winkler (1968) have already reported that whole virus and virus RNA are inactivated in parallel.

However, during similar studies with the virus of encephalomyocarditis of mice (EMC-virus) it was found that the RNA was not inactivated in aerosols even when the infectivity of the whole virus decreased rapidly (de Jong, 1969). This difference is remarkable because of the resemblance between these viruses in structure and aerosol survival. Moreover, Dubovi (1971) showed that for the structurally related bacteriophage MS2 the infectivity of the RNA, but not the intact virus, was stable in aerosols. We have reinvestigated this problem for poliovirus.

Poliovirus type 1 strain Lsc2ab was used. The virus was grown in HEp-2 cells maintained as a monolayer in Eagle’s minimal essential medium, modified according to Yamane, Matsuya & Jimbo (1968) and supplied with a buffer solution of 10 mM-HEPES. The virus suspension obtained was clarified by centrifuging at low-speed and the virus concentrated 200 × by ultracentrifugation for 1.5 h. at 40000 rev/min. The pellet was resuspended in Hanks’s balanced salt solution and showed an infectivity of 10¹¹ p.f.u./ml.

Aerosols were sprayed and stored as described previously (de Jong & Winkler, 1968). Using an FK8 direct-type nebulizer, 1 ml of virus suspension was sprayed in 6 s into a static air cabinet of 2000 l volume which was kept at 20 °C and homogenized by a fan. Air samples were taken with the lower stage of a multistage liquid impinger (May, 1966), filled with 10 ml of phosphate buffered saline pH 7.2 (PBS). A volume of 275 l of air was drawn through the impinger in 5 min. The sampling efficiency and the loss through physical fall-out were determined in separate experiments with fluorescein as a tracer.

The impinger fluids were titrated for the infectivity of complete virus by conventional plaque assay on HEp-2 monolayers. The infectivity of RNA was determined by the method of Tovell & Colter (1967). Virus suspensions in PBS supplemented with EDTA 0.01 % and sodium deoxycholate were shaken vigorously by hand for 4 min with an equal volume of phenol previously saturated with a solution of EDTA 0.01 % in PBS. The extraction was repeated twice by shaking for 1 min with fresh phenol. The phenol was then removed by shaking 4 times for 20 s with a double volume of ether. The ether was removed by bubbling nitrogen gas through the solution for 5 min. Thereafter the phenol extract was supplied with 100 μg/ml of DEAE-dextran (Pharmacia, Uppsala; mol. wt. 2 × 10⁶) and 2 ml of this solution were mixed with 20 × 10⁶ HEp-2 cells and incubated for 2 min at 37 °C. Dilutions of this cell suspension were then plated on monolayers of HEp-2 cells for plaque assay as above. With this method the infectivity of the extracted RNA was 0.03 to 0.3 % of the original virus suspension.

Experiments were made on aerosols held at 35 and 55 % r.h. At 35 % r.h. a considerable inactivation of infectivity of intact virus occurred during spraying and equilibration (Fig. 1). This was followed by a slower loss of infectivity on storage of the aerosol. The plaque-form-
Fig. 1. Inactivation of the infectivity of poliovirus and its RNA in aerosols at 35 % r.h. ○—○, for intact virus; △—△, for RNA extracted from virus in aerosol; ▲—▲, for extracted RNA in aerosol.

Fig. 2. Inactivation of the infectivity of poliovirus and its RNA in aerosols at 55 % r.h. Details as in Fig. 1.

The infectivity of the intact virus was reduced to 5 % immediately after spraying and to 2.5 % over the next 60 min. In contrast, the infectivity of the RNA extracted from sprayed virus was unchanged throughout the experimental period.

The results for aerosols held at 55 % r.h. are shown in Fig. 2. At this humidity the infectivity of intact virus fell to 25 % during spraying and to 1.5 % during storage in aerosol for 1 h. Despite this difference between results at 35 and 55 % r.h., the RNA extracted from sprayed virus again retained its infectivity. When extracted RNA was sprayed and held in aerosols at 35 % r.h. (Fig. 1) or 55 % r.h. (Fig. 2) the infectivity of this RNA was not significantly inactivated in the aerosol; Akers & Hatch (1968) reported similarly for mengovirus RNA in aerosols.

In earlier experiments with poliovirus de Jong & Winkler (1968) found no infective RNA in aerosol-inactivated virus. No RNase activity was detected in the spray-medium (Hanks's balanced salt solution with lactalbumin hydrolysate 0.25 %, calf serum 2.5 % and peptone 0.5 %) or in collecting fluid (PBS with peptone 1 % and antifoam 0.1 %). In control tests the infectivity of poliovirus-RNA was stable in these solutions for 4 h at 37 °C.

These experiments were repeated and yielded the same results. However, this is not conclusive, because the medium is concentrated a 100-fold during spraying as the droplets equilibrate by evaporation with the ambient air. Therefore, the stability of the infectivity of free RNA in medium with the concentrated components was also determined. In undiluted calf serum the infectivity of free RNA fell to 1 % or less on storage for 4 h at 37 °C (Table 1). It is probable that the failure in the earlier study to recover infective RNA from aerosol-inactivated virus must be ascribed to the composition of the spray-fluid. With EMC-virus in
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Table 1. Stability of infectivity of poliovirus RNA in different media

<table>
<thead>
<tr>
<th>Medium</th>
<th>Incubation at 37 °C</th>
<th>Infectivity log (p.f.u./ml)</th>
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<tbody>
<tr>
<td>PBS None</td>
<td>2.21</td>
<td></td>
</tr>
<tr>
<td>PBS 4 h</td>
<td>2.28</td>
<td></td>
</tr>
<tr>
<td>Spray fluid*</td>
<td>4 h</td>
<td>2.03</td>
</tr>
<tr>
<td>Collecting fluid†</td>
<td>4 h</td>
<td>2.06</td>
</tr>
<tr>
<td>Calf serum</td>
<td>4 h</td>
<td>&lt; 0.30</td>
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</tbody>
</table>

* Spray fluid: Hank’s balanced salt solution with lactalbumin hydrolysate 0.25 %, calf serum 2.5 % and peptone 0.5 %.
† Collecting fluid: PBS with peptone 1 % and antifoam 0.1 %.

aerosols the RNA becomes accessible to inactivation by environmental substances such as ribonuclease (J. C. de Jong, M. Harmsen & T. Trouwborst, in preparation).

Our present results show that the infectivity of the subsequently extracted nucleic acid of poliovirus is stable in aerosols under conditions in which the whole virus quickly loses infectivity. This suggests that in aerosols the protein coat is the target for inactivation. The infectivity of RNA in the free state was also stable in aerosols. These results are in accordance with those for EMC-virus (de Jong, 1969) and phage MS2 (Dubovi, 1971). A similar behaviour was found for the thermal inactivation of poliovirus (Dimmock, 1967). We are investigating whether the detailed mechanisms of inactivation of EMC-virus are similar in these cases.

Akers et al. (1966) reported that mice were infected as efficiently by the free RNA of EMC-virus in aerosol infected as by intact virus, when the same number of p.f.u. was administered. Nevertheless, our results are of doubtful epidemiological significance since the infectivity of RNA for cells is barely 0.1 % of that of complete virus, and virus-containing secretions from infected animals may contain RNA-inactivating substances.

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