Comparative Poliovirus Permeability of Silver, Polycarbonate, and Cellulose Membrane Filters

RICARDO G. HAHN, JACK B. HATLEN, AND GEORGE E. KENNY

Department of Preventive Medicine, University of Washington, School of Medicine, Seattle, Washington 98105

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Three types of filters, silver, polycarbonate, and cellulose, were evaluated for permeability toward poliovirus suspended in water, salt-containing, and proteinaceous solvents. The ability of virus to pass through cellulose filters depended on the suspending medium; virus did not pass through cellulose filters in either water or salt solution, whereas the use of protein solutions increased filterability. The virus permeability of both the silver and polycarbonate filters was independent of the suspending medium. Apparently, pore size alone determined their permeability toward poliovirus, and electrostatic forces between filters and the particles being filtered did not appear to play a significant role. Both the silver and polycarbonate filters appear to be promising tools for the separation of viruses from contaminating bacteria and fungi.

The utility of cellulose membrane filters for sizing viruses (8, 12) and for bacterial sterilization of virus preparations (14) has been greatly hampered by the fact that electrostatic forces bind viruses to the membrane, thus preventing passage through pores of more than adequate diameter. The degree of binding or adsorption depends upon the virus type and the suspending medium (12). Salts facilitate virus adsorption, but proteins appear to block the virus adsorption sites and permit viral passage through the filter (3, 6, 13).

Several new types of filters are commercially available: elemental silver and polycarbonate. We have investigated the viral permeability of these filters. Poliovirus filtration through both polycarbonate and elemental silver filters appeared to depend only on the pore size of the filter.

MATERIALS AND METHODS

Cell cultures. Human heteroploid cells, M HeLa (4), were grown on glass in Eagle minimal essential medium (MEM) with 10% fetal bovine serum.

Virus. Type 1 poliovirus strain LSc 2ab was propagated in M HeLa cells. Fifteen hours after infection of the cells, the medium was discarded, the cell monolayer was rinsed once with balanced salt solution (BSS), and the cells were scraped from the glass in a small volume of BSS. The cell suspension was disrupted by freeze-thawing the cells three times and centrifuged at 8,000 × g for 30 min. The supernatant fluid was treated with one-half volume of Genetron (Genesolv D), shaken for 5 min, and centrifuged at 800 × g for 15 min. The supernatant fluid was removed and frozen in 0.5-ml samples in nonwettable plastic test tubes (16 by 125 mm; Falcon Plastic). The stock virus titered 10^6 plaque-forming units (PFU) per ml.

Plaque assay. One and one-half million cells, in 5 ml of growth medium, were inoculated into a 60-mm plastic petri dish (Falcon Plastic) and used for plaque assay the following day. An inoculum of 0.2 ml of each filtrate was absorbed on duplicate monolayers for 30 min at 37°C, and 5 ml of plaque overlay medium was added to each petri dish. The overlay for plaque assay consisted of MEM plus 2% fetal bovine serum and 0.4% agarose. Petri dishes were incubated at 37°C in 2.5% CO_2 in air for about 2.5 days, fixed with 5% Formalin, stained with 1% crystal violet in 20% ethyl alcohol (after discarding overlay) for 2 to 3 min, and rinsed with water.

Filtration process. Filtrations were done in triplicate through filters 13 mm in diameter. The filters and average pore sizes (as described by manufacturer) used were: silver filters (Selas Flotronics, Spring House, Pa.), 0.2 and 0.45 μm; polycarbonate filter ("nuclepore," General Electric, Pleasonton, Calif.), 0.5 μm (only size available); and cellulose filters ("mixed ester" filter, Millipore Filter Co.), 0.22 and 0.45 μm. A 2.5-ml amount of viral suspension containing 500 PFU per ml was filtered by using plastic syringes and metal or plastic "Swinnny" filter holders (Millipore Filter Co.). The filtrates were collected in nonwettable plastic test tubes and assayed immediately. Filters were tested for breakage by pulling back on syringe plungers after filtration. An intact filter prohibited air flow.

The following suspending media were used: (i) Hanks BSS; (ii) sterile distilled water; (iii) 1% bovine serum albumin (BSA) in distilled water; (iv) 0.3% beef extract (Difco) in BSS; (v) 0.03% beef extract (Difco) in BSS; (vi) 0.003% beef extract
Table 1. Comparison of filterability of poliovirus LSC/2ab as a function of filter type and suspending medium

| Suspending medium                  | Filter type     | Nominal pore size (μm) | Control (PFU/dish) | Filter (PFU/dish) | Per cent recovery | Filtrate (CFU/plate of Serratia marcescens) | Per cent recovery of S. marcescens |
|-----------------------------------|-----------------|-------------------------|-------------------|------------------|------------------|---------------------------------------------|----------------------------------|
| BSS                               | Silver          | 0.2                     | 77                | 23               | 22-24            | 30                                          | 0                                | <5                               |
| BSS                               | Silver          | 0.45                    | 77                | 61               | 59-62            | 79                                          | 99                               | 10                              |
| BSS                               | Cellulose       | 0.22                    | 77                | 0                | 0                | <5                                          | 0                                | <5                               |
| BSS                               | Cellulose       | 0.45                    | 77                | 0                | 0                | <5                                          | 0                                | <5                               |
| BSS                               | Polycarbonate   | 0.5                     | 77 (74-80)        | 56               | 54-59            | 73                                          | 0                                | <5                               |
| Sterile distilled water           | Silver          | 0.2                     | 73                | 44               | 43-46            | 60                                          | 0                                | <5                               |
| Sterile distilled water           | Silver          | 0.45                    | 73                | 58               | 56-59            | 79                                          | 97                               | 10                              |
| Sterile distilled water           | Cellulose       | 0.22                    | 73                | 0                | 0                | <5                                          | 0                                | <5                               |
| Sterile distilled water           | Cellulose       | 0.45                    | 73                | 0                | 0                | <5                                          | 0                                | <5                               |
| Sterile distilled water           | Polycarbonate   | 0.5                     | 73 (72-74)        | 58               | 57-60            | 80                                          | 0                                | <5                               |
| 1% BSA in distilled water         | Silver          | 0.2                     | 82                | 49               | 42-58            | 60                                          | 0                                | <5                               |
| 1% BSA in distilled water         | Silver          | 0.45                    | 82                | 62               | 60-65            | 78                                          | 98                               | 10                              |
| 1% BSA in distilled water         | Cellulose       | 0.22                    | 82                | 0                | 0                | <5                                          | 0                                | <5                               |
| 1% BSA in distilled water         | Cellulose       | 0.45                    | 82                | 2                | 0-4              | 31                                          | 0                                | <5                               |
| 1% BSA in distilled water         | Polycarbonate   | 0.5                     | 82 (81-84)        | 57               | 55-60            | 70                                          | 0                                | <5                               |
| 0.003% BE in BSS                  | Cellulose       | 0.2                     | 146               | 11               | 10-12            | 7                                           | 0                                | <5                               |
| 0.003% BE in BSS                  | Silver          | 0.2                     | 146               | 110              | 108-111          | 75                                          | 0                                | <5                               |
| 0.003% BE in BSS                  | Cellulose       | 0.22                    | 146               | 98               | 96-100           | 67                                          | 0                                | <5                               |
| 0.003% BE in BSS                  | Silver          | 0.2                     | 146 (145-148)     | 142              | 137-146          | 98                                          | 0                                | <5                               |
| 0.3% BE in BSS                    | Cellulose       | 0.22                    | 166               | 127              | 122-131          | 76                                          | 0                                | <5                               |
| 0.3% BE in BSS                    | Silver          | 0.2                     | 166 (165-167)     | 142              | 132-150          | 86                                          | 0                                | <5                               |
| 0.003% BE in BSS                  | Polycarbonate   | 0.5                     | 100 (99-100)      | 80               | 80               | 80                                          | 0                                | <5                               |

a Average of unfiltered viruses in three experiments; duplicate plates for each experiment; values in parentheses indicate range of three experiments.

b Average of three filtration experiments; duplicate plates used for each experiment.

c Range of values obtained in the three experiments.

d Per cent recovery = (average PFU per dish of filtrate/average PFU of respective control) × 100.

BE = colony forming units.

f Bovine serum albumin.

g Beef extract.

(Difo) in BSS. Virus was diluted approximately 1:10,000 into each medium.

Marker. As suggested by Bowman et al. (1), Serratia marcescens (ATCC 14756) was used as a marker for all filtrations to detect any filter breakdown and to determine maximum pore size of the filters. (The average size of S. marcescens is 0.5 μm in diameter by 2 to 3 μm in length.) S. marcescens was grown on nutrient agar, washed off the surface, centrifuged, and resuspended; this suspension was stored in BSS at 4°C. For use as a marker, 10⁶ bacteria per ml were added to the viral suspension by diluting the stored suspension 1:10,000 in the specified diluent.

Nutrient agar pour plates containing 0.1 ml of each filtrate were prepared with the plaque assays, incubated at room temperature, and observed for colonies at 48 hr.

RESULTS

It has been shown (3, 6, 13) that the suspending medium for poliovirus has a considerable effect on its filterability through cellulose filters. A comparison of the three filter types showed that, although this is true for cellulose filters, neither the polycarbonate nor the silver filter showed large effects of suspending medium.

Four different suspending media were employed: BSS, water, 1% BSA in water, and beef extract. The relative filtering efficiency of the polycarbonate filter was unrelated to suspending medium, 70 to 80% of poliovirus being passed through the filters without bacterial marker. (Complete data are shown in Table 1; a summary of the data is shown in Table 2.) Similarly, no appreciable effect of suspending medium was observed with the 0.45-μm silver filter; 79% of the virus passed through the filters with 10% of the bacterial marker. A slight effect of suspending medium was observed with the 0.2-μm silver filter; 30% of the virus passed through the filter in BSS, whereas 60 to 98% passed through this filter in other diluents.

The relatively ready filterability of poliovirus through the polycarbonate and silver filters was
Table 2. Relationship of per cent recovery of poliovirus through various filters to diluent

| Filter         | Size (μm) | BSS  | Water | BSA (1%) | BE (0.03%) | BE (0.03%) | BE (0.3%) |
|----------------|-----------|------|-------|----------|------------|------------|-----------|
| Silver         | .2        | 30   | 60    | 60       | 75         | 98         | 86        |
| Silver         | .45       | 79   | 79    | 78       | 7          | 67         | 76        |
| Cellulose      | .22       | <5   | <5    | <5       | 3          | 80         |           |
| Cellulose      | .45       | <5   | <5    | 3        | 80         |            |           |
| Polycarbonate  | .5        | 73   | 80    | 70       |            |            |           |

* BSS, Hanks balanced salt solution; BSA, bovine albumin in distilled water; BE, beef extract in BSS.

in sharp contrast to the results with cellulose filters. No detectable poliovirus was recovered through either the 0.45-μm or the 0.22-μm cellulose filter when either BSS or water was used as the diluent. Increasing poliovirus quantity to 10⁸ per ml in BSS did not result in detection of any virus (<0.02%) after filtration through the 0.45-μm cellulose filter, whereas 20% of the virus but no bacteria passed through the 0.2-μm silver filter. When 1% BSA was added to distilled water, 3% of the virus was recovered through the 0.45-μm filter, whereas none passed through the 0.22-μm filter.

The addition of beef extract to BSS greatly enhanced the filterability of poliovirus through 0.22-μm cellulose filters. The largest increase in viral filterability was observed between concentrations of 0.003% (7% filterability) and 0.03% beef extract (67% filterability). Only a slight enhancing effect of beef extract was observed with the 0.2-μm silver filter.

**DISCUSSION**

Whereas the passage of attenuated poliovirus through cellulose membrane filters (not pre-treated) was only possible when the medium contained protein or protein extracts, poliovirus readily passed through both silver and polycarbonate filters without regard to composition of suspending medium. In our investigations, no permeability enhancement was noted with cellulose filters upon removal of salts from the medium, whereas Ver et al. (12) recovered virtually all of the Mahoney strain of poliovirus type I under similar conditions. The LSc/2a6 strain of poliovirus is known to be bound more firmly to diethylaminoethyl cellulose than the Mahoney strain (7), a finding which may explain the differences between our study and that by Ver et al. In the study of virus filterability, the vaccine strain appears to be a more rigid test of membrane binding.

The 0.45-μm silver filter retained only 90% of the marker organisms suggesting variation in pore size; however, little is known about its ultrastructure. The polycarbonate and cellulose filters did not exhibit this characteristic. The ultrastructure of the two latter filters has been studied in detail. Whereas Spurny et al. (11) and Cornell (2) showed that the polycarbonate filter has a narrow range of pore sizes and uniform pore structure, Friedman et al. (5) described the wide range in the pore-size distribution of cellulose filters as well as the many tortuous paths and blind pockets.Entrapment of small-sized particles by the cellulose filter would be possible but retention of virus is presumably due to electrostatic binding.

If the silver and polycarbonate filters are indeed functioning in a strictly mechanical fashion, their use in future work would greatly simplify processes which presently are difficult to perform. Clearly these filters are superior in their filtering capabilities, at least for poliovirus. Most important, these filters may have great utility for the separation of viruses from contaminating microorganisms in specimen materials. This would be of particular use in the isolation of viruses from feces and sewage. Further investigations are called for to determine the general utility of these filters for various virus groups. The polycarbonate filter is presently available only in a relatively large pore size, which limits its utility to fungi and larger bacteria. The availability of smaller pore sizes in either polycarbonate or silver filters would aid greatly in the sizing of different viruses, in test methods for the certification of sterile heat-labile filtrates as described by Portner et al. (10), and in microbiological control methods in the brewing industry (9).

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