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Virus in Water

II. Evaluation of Membrane Cartridge Filters for Recovering Low Multiplicities of Poliovirus from Water

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The efficiency of a Millitube MF cartridge filter, a membrane filter, for recovery of poliovirus from 100-gal volumes of both fresh (tap) and estuarine water was determined. In the high multiplicity of virus input-output experiments, recovery of 97% or greater of input virus was achieved in both types of water when the final concentration of divalent cation as Mg^{2+} was 1,200 μg/ml and the *p*H was 4.5. Virus was effectively eluted from the membrane cartridge with 5× nutrient broth in 0.05 M carbonate-bicarbonate buffer at *p*H 9.0. Four elutions of 250 ml each were used. In the low multiplicity of virus input-output experiments under the same cationic and *p*H conditions, up to 67% of the input virus was recovered when the virus was further concentrated from the eluates by the aqueous polymer two-phase separation technique. The volume reduction was 126,000-190,000 to 1. The use of the combined techniques, i.e., membrane adsorption followed by aqueous polymer two-phase separation, provided a highly sensitive, simple, and remarkably reliable sequential methodology for the quantitative recovery of poliovirus occurring at multiplicities as low as 1 to 2 plaque-forming units per 5 gal of water.

The occurrence and distribution of viruses in water are of major concern to environmental health officials. The enigma of the virus-in-water problem is directly related to the technical deficit of a reliable method for recovering viruses that occur at low multiplicities in water. Consequently, an unequivocal need exists for a standard method of concentrating and recovering low multiplicities of virus from very large quantities of water. This is particularly relevant if viral surveillance of potable water supplies is to be implemented. The most promising methods for the recovery of low multiplicities of virus from surface waters and finished waters are those that rely on virus adsorption or retention within a flow-through sampling system, or both. In this regard, a number of methods have been shown experimentally to be good candidates for assessing the occurrence of viruses in various types of water. The most promising virus recovery methods are: membrane adsorption technique (2, 4, 6, 7, 15, 24); adsorption to iron oxide (18) or polyelectrolytes (22, 25, 26); flow-through gauze sampler (14); and soluble alginate filter technique (8). Most of these methods have shown good-to-excellent virus recovery efficiencies in controlled laboratory experiments. Some of the advantages and disadvantages of the above methods have recently been reviewed by Hill et al. (10). With two exceptions (14, 25), the major drawback of most of the methods mentioned is that they have not been evaluated with large volumes of water. In fact, volumes of 10 ml up to 19 liters have been most frequently used for the evaluation studies. The merits of any method remain questionable until the method is tested using low multiplicities of virus and volumes of water of at least 100 gal (ca. 378.5 liters).

Of the number of methods available, it appears that membrane adsorption may hold the greatest potential for satisfying the basic requirements of a method for recovering low multiplicities of virus in large quantities of water. In recent years, a number of papers have appeared in the literature that indicate that the membrane adsorption technique efficiently concentrates enteric viruses from various types of environmental waters (16, 17, 23). One of the cardinal features of the membrane adsorption technique that has practical appeal

is that the size of the water sample may be increased to quantities of several orders of magnitude. Thus, the probability of recovering low multiplicities of virus from contaminated waters is proportionately increased.

At the present time, there is a collaborative effort being undertaken among four laboratories to evaluate various methods scaled-up to examine at least 100-gal volumes of water for virus concentration and recovery efficiency. These efforts are oriented toward developing standard methods for viral assessment of all types of water including waters used for recreation, drinking, and shellfish production. This report, which represents the second of a series (14), describes the evaluation of the Millitube MF cartridge filter (Millipore Corp.), a membrane filter, for quantitatively recovering poliovirus from 100-gal volumes of both fresh and estuarine waters.

MATERIALS AND METHODS

Virus. The LSc2ab strain of poliovirus type 1 was used throughout the study. The virus was kindly supplied by the Lederle Research Laboratories, American Cyanamid Co., Inc. The virus was propagated in HEp-2 cells. The virus was put up in 1-ml quantities and stored at −70 C until used.

Cell culture. HEp-2 cells were used for plaque assays. The procedures for cultivating the cells, preparing cell monolayers, and handling the cell overlay have been described previously (11).

Plaque assay. The plaque assay procedure as modified by Hsiung and Melnick (12) was used throughout the study. Plaques were permanently marked and counted daily. Final counts were made on the 5th day of incubation. Cell monolayer bottles exhibiting the greatest number of plaques short of overcrowding were recorded and used for computations. Counts were expressed as plaque-forming units (PFU). For purposes of assay, serial 10-fold dilutions of virus were made in nutrient broth (9), and 1 ml of virus at each dilution level was inoculated onto cell monolayers. In the high multiplicity of virus input-output experiments, all samples were assayed for virus by conducting five replicate titrations.

Membrane filter equipment. The membrane filters used in the study were manufactured by the Millipore Corp., Bedford, Mass. They consisted of: (i) Millitube MF cartridge, 0.45-μm porosity, 22-inch (ca. 55.88 cm) length (CFHA02220); and (ii) MF-Lifegard 11 cartridge prefilters (CP14002203). All filters were housed in a special Teflon-lined stainless-steel filter holder designated as type ST by the manufacturer. The Millitube MF cartridge filter and filter holder are shown in Fig. 1. The pump used to pressure-filter the virus-seeded water was a Vanton Flex-i-Liner pump, type XB-P60A (Vanton Pump

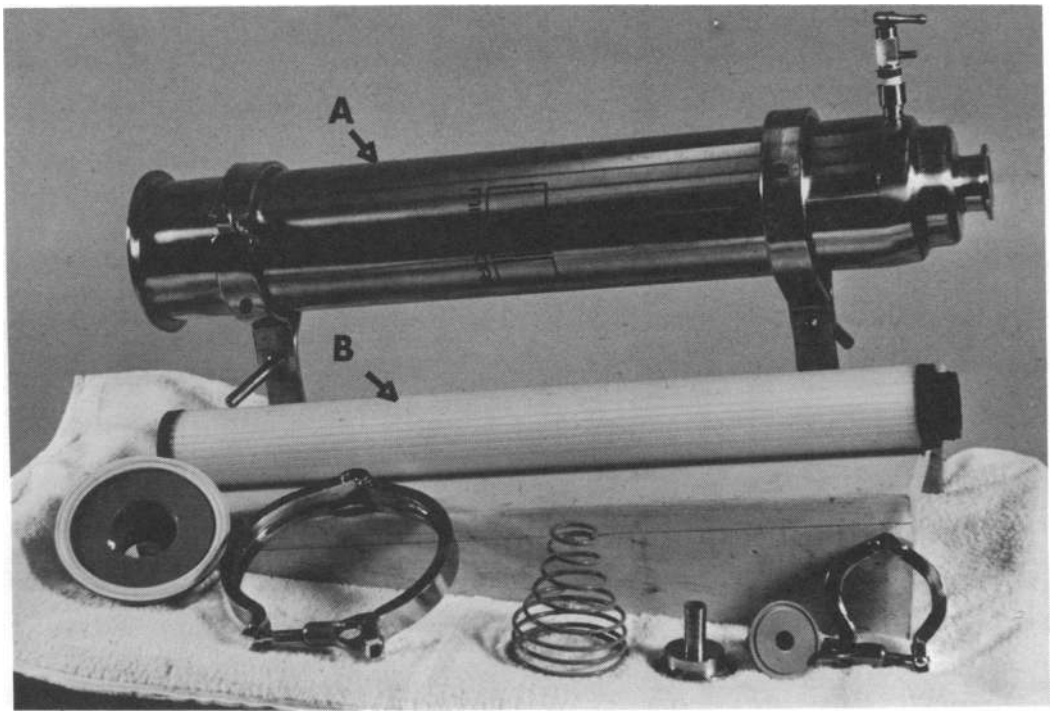

FIG. 1. *Millitube MF cartridge filter system. (A) The stainless-steel, Teflon-lined filter holder, (B) the 0.45-μm porosity membrane-cartridge filter.*

and Equipment Corp., Hillside, N.J.) operating with a 0.5 hp motor at a maximum speed of 1,750 rev/min. When pumping at a rate of 2.5 gal (ca. 9.5 liters) per min, the operating pressure was 35 psig.

Water supply. The estuarine water used in the experiments was pumped into the laboratory from Dauphin Island Bay, Ala. The fiberglass intake lines extended 21 m into the Bay and reached a maximal depth of 0.9 m below sea level. All estuarine water contact surfaces in the supply system were nonmetallic. Artificial seawater was prepared according to the method of Kester et al. (13), and then diluted to the desired salinity similar to estuarine water. Salinity of all saline waters was determined hydrometrically (27). Dauphin Island drinking water was used in the tap water runs. The tap water was tested for residual chlorine by the orthotolidine test; if chlorine was present, it was neutralized with sodium thiosulfate. All waters used throughout the reported experiments were prefiltered, and, except for certain preliminary test runs, the acidity was adjusted to *p*H 4.5 ± 0.1 with 12 N HCl before virus seeding unless otherwise indicated.

Experimental setup. The experimental setup is shown in Fig. 2. Virus suspensions were thawed and diluted in nutrient broth to the desired multiplicity immediately before use. Virus was then added to the 100-gal water reservoir. In the high multiplicity of virus input-output experiments, the virus-seeded water was stirred for 10 min before samples were collected for input virus multiplicity determinations and before starting the filtration. During the filtration process, samples of filtrate were collected at 10-gal (ca. 37.8 liters) intervals to assess virus penetration of the membrane filter. After filtration, the cartridge filter holder was drained of residual water. The filter was then removed and transferred aseptically to a sterile elution tube (Wheaton Glass Co., Millville, N.J.). Eluent was added (250 or 500 ml) to the elution tube and the tube was placed on the elution roller apparatus. The apparatus was rotated at approximately 60 rev/min to facilitate virus elution. Elution was conducted in the cold at 4 C. Multiple eluates were collected and assayed for virus content at different elution time periods throughout the course of the study depending upon the experimental conditions. The final elution time period consisted of four 30-min elutions. The elution tube and roller apparatus are shown in Fig. 3.

Elution fluids. During the initial high multiplicity of virus input experiments, a number of different solutions were used for virus elution. Eluents used were (i) 1× nutrient broth in 0.15 M phosphate-buffered saline (PBS), *p*H 8.5; (ii) 5% agamma calf

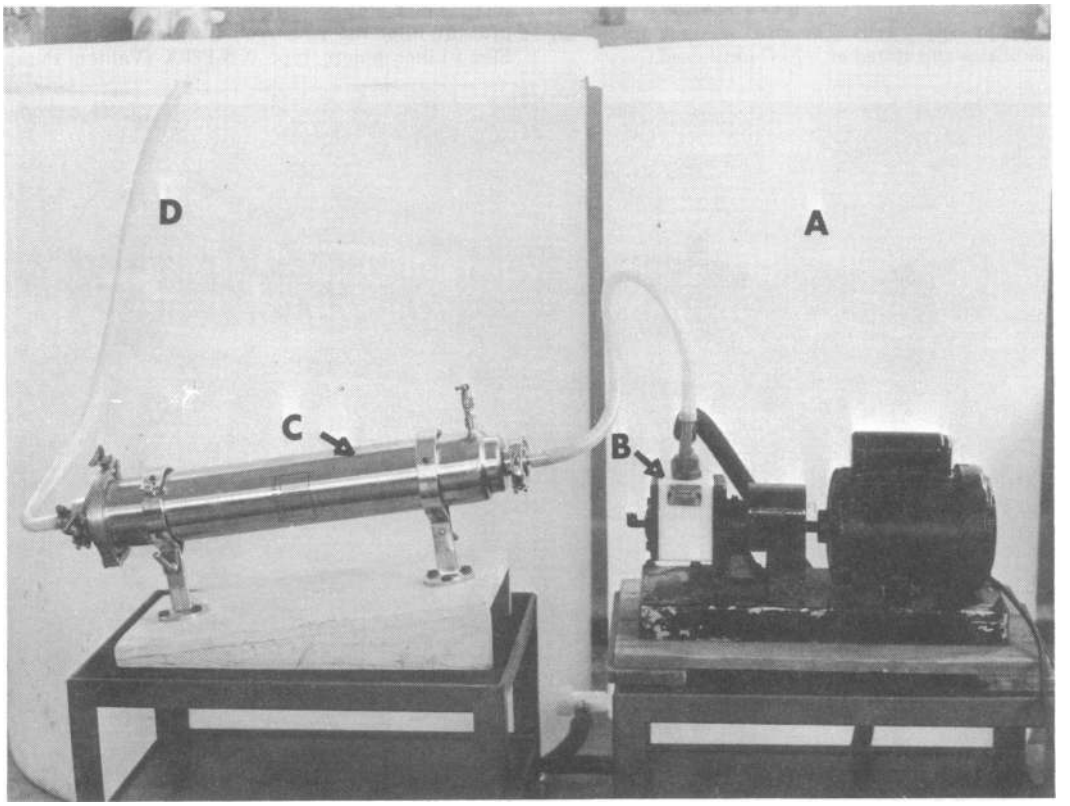

FIG. 2. *Experimental setup. (A) The 100-gal water tank, (B) the water pump, (C) the Millitube MF cartridge filter system, and (D) the water collection tank used for disinfecting the filtrate water.*

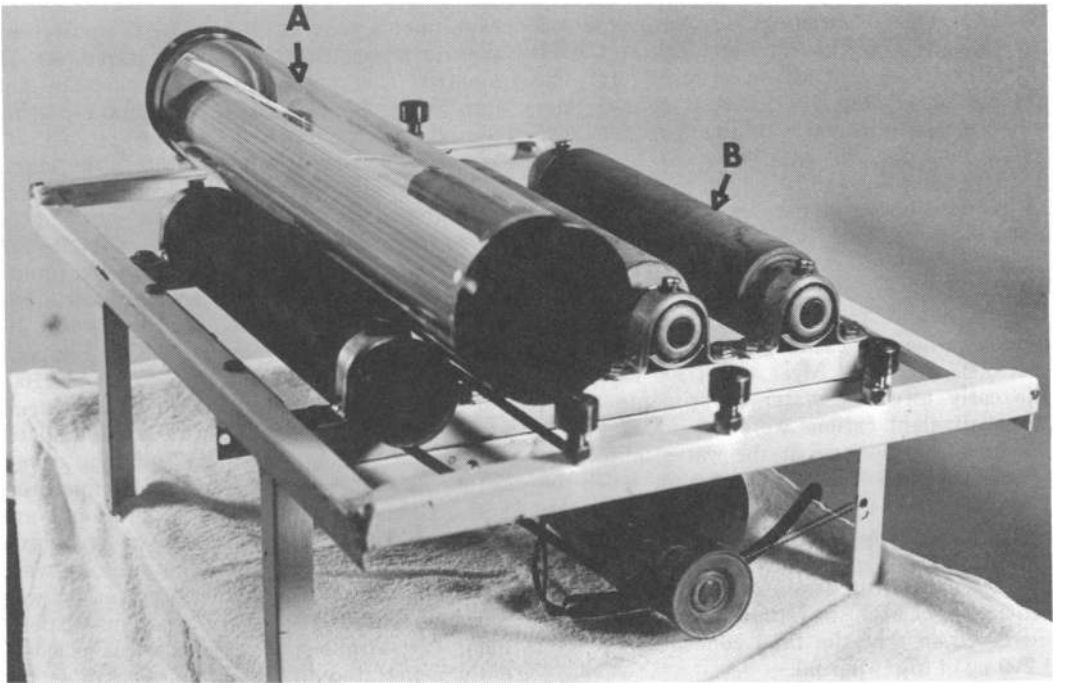

FIG. 3. *(A) Elution tube and (B) roller apparatus used for elution of virus from the membrane cartridge filters.*

serum in PBS; (iii) 30% agamma calf serum in PBS; (iv) 10× nutrient broth in PBS; (v) 3% beef extract (Difco), *p*H 8.5; and (vi) 5× nutrient broth in 0.05 M carbonate-bicarbonate buffer, *p*H 9.0. These eluents were not critically compared. However, our observations suggested that all six eluents were equally effective in eluting poliovirus from the filters. Nutrient broth (5× concentration) buffered to *p*H 9 was selected as the preferred eluent for all subsequent experiments because it was convenient, inexpensive, and easy to handle.

Concentration of virus in the eluates. When low multiplicities of input virus were tested, the virus was further concentrated in the eluates by the aqueous polymer two-phase separation technique (slightly modified) as described by Shuval et al. (20). Briefly, this consisted of adding 2 g of sodium dextran sulfate 2000 (Sigma Chemical Co.), 64.5 g of polyethylene glycol (Carbowax 4000), and 17.53 g of NaCl to each liter of pooled eluate (*p*H adjusted to 7.2). The mixture was then stirred on a magnetic stirrer until the chemicals were dissolved (approximately 20 min). The solution was transferred to a 2,000-ml separatory funnel and held overnight at 4 C to allow the two phases to develop. The top phase was carefully removed by suction and transferred to a second 2,000-ml separatory funnel. To this phase, again was added 2 g of sodium dextran sulfate and 17.53 g of NaCl, and the liquid partitioning process was repeated (H. Seraichekas, *personal communication*). The bottom phase and the interphase recovered from both of the first-stage liquid partitioning procedures were pooled (ca. 12 to 14 ml) and used in the second-stage liquid partitioning procedure. This was done by adding NaCl to a final concentration of 1.0 M to the pooled phases. The sample was then shaken vigorously and held overnight at 4 C to allow the two phases to develop. The sample was then centrifuged (desk-top model clinical centrifuge) for 20 min at maximum speed. The top phase (ca. 3 ml) was withdrawn and diluted (i.e., 1:6) with distilled water to achieve isotonicity for direct virus assay.

RESULTS

Effects of divalent cations on poliovirus recovery from 100 gal of fresh water. It has been reported that salts, particularly the divalent cationic types, enhance the adsorption of virus to membranes (16, 17, 23, 24). The type of cation and optimal concentration used by other investigators (16, 17, 23) varied, however. Consequently, in order to exercise a value judgment as to type and concentration of cation to use in our 100-gal volume experiments, we conducted preliminary experiments. We examined two concentrations of Ca^{2+}, (200 and 500 μg/ml) and one concentration of Mg^{2+}, (1,200 μg/ml). Final concentration of cations in the 100-gal volumes was determined by atomic absorption spectrophotometry. The initial acidity of all waters was adjusted to *p*H 4.5 before filtration. The results are shown in

Table 1. Total per cent of virus recovered was 18, 56, and 233% in the 200, 500, and 1,200 μg/ml salt concentrations, respectively. The precise reason for the observed greater than 100% recovery in one experiment remains obscure but may be attributed to biological random-sampling error. In high multiplicity of virus input-output experiments, however, it is not infrequent that "virus recovered" exceeds 100% of the virus input. This observation has been reported by others (7, 18, 20). Noteworthy was the failure to detect virus in the filtrate samples when the cation concentration of Ca^{2+} was 500 μg/ml and Mg^{2+} was 1,200 μg/ml. In the early estuarine water experiments, additional divalent cations were not added to the saline waters because at the water salinity at which we were working minimum levels of both Mg^{2+} and Ca^{2+} occurred naturally in the water (i.e., at a salinity of 20 g/kg, the concentration was 740 μg of Mg^{2+} per ml and 236 μg of Ca^{2+} per ml). In later experiments, the concentration of Mg^{2+} in estuarine water was also increased so that the final concentration was 1,200 μg of Mg^{2+} per ml.

Recovery of high multiplicity of poliovirus from 100-gal volumes of estuarine waters. A total of eight experiments were conducted using saline water. In two of these experiments, artificial seawater (13) was used. Four experiments were carried out with natural estuarine water that had been stored for 11 months. This water was used to gain insight into the problem of MCC (membrane-coating components) as reported by others (16, 23, 24) and its effect on virus adsorption to the membrane cartridge filters. It should be noted that natural estuarine water contains a multitude of living organisms at all levels of biological complexity. These organisms, of course, proliferate and go through their normal growth cycles and in the process release a variety of ill-defined substances not unlike MCC. For convenience, the recoveries of poliovirus from 100-gal volumes in natural (unstored) and artificial seawater (salinity adjusted with demineralized water) were tabled separately from the stored estuarine water experiments. The unstored and artificial estuarine water experiments are shown in Table 2. The recovery of poliovirus ranged from 42 to 97%. The best results were observed when the final concentration was adjusted to 1,200 μg of Mg^{2+} per ml (experiment 4). With one exception, virus consistently penetrated the membrane cartridge filter, as evidenced by finding virus in the filtrate samples, and may be attributed to the presence of MCC. Despite the loss of virus to the filtrate, three out of four recovery efficiencies were 62% or greater. These results were

TABLE 1. *Effect of divalent cations on poliovirus recovery from 100 gal of fresh water*[a]

Expt no.	Cation concn (μg/ml)	Virus input[b] (PFU/ml)	Total virus input (PFU × 10^6)	Virus in filtrate[b] (PFU/ml)	Virus recovered in eluate (PFU/ml)	Total virus recovered (PFU × 10^6)	Total virus recovered (%)
1	Ca^{2+}, 200	15.4 ± 3.3	5.83	3.1 ± 0.5	425.2	1.06	18
2	Ca^{2+}, 500	13.8 ± 2.2	5.22	0	1,941.3	2.91	56
3	Mg^{2+}, 1,200	10.8 ± 1.2	4.09	0	9,521.5	9.52	233

[a] Demineralized tap water.
[b] Mean virus plaque counts of five replicate subsamples ± standard error. PFU = plaque-forming units.

TABLE 2. *Recovery of poliovirus from 100 gal of estuarine water*

Expt no.	Salinity (g/kg)	Virus input[a] (PFU/ml)	Total virus input (PFU × 10^6)	Virus in filtrate[a] (PFU/ml)	Virus recovered in eluate (PFU/ml)	Total virus recovered (PFU × 10^6)	Total virus recovered (%)
1	20.8[b]	18.8 ± 2.4	7.12	0	1,978.7	2.97	42
2	17.5[b]	16.8 ± 1.4	6.36	4.2 ± 0.7	1,314.7	3.94	62
3	25.1[c]	11.4 ± 0.7	4.75	1.2 ± 0.3	2,485.3	3.73	79
4	19.1[d]	12.6 ± 2.9	4.77	4.0 ± 0.6	4,611.5	4.61	97

[a] Mean virus plaque counts of five replicate subsamples ± standard error.
[b] Artificial seawater.
[c] 110 gal (ca. 416.35 liters).
[d] $MgCl_2 \cdot 6H_2O$ was added to give a final concentration of 1,200 μg/ml as Mg^{2+}.

therefore considered good-to-excellent as far as total per cent of virus recovered. The recovery of poliovirus from stored estuarine water is shown in Table 3 and ranged from 20 to 51%. With the exception of experiment 4, the total per cent of virus recovered was considered unsatisfactory from a quantitative virus input-output viewpoint. We consider a virus recovery of 50 to 100% the satisfactory efficiency range for an acceptable method. Consequently, waters containing virus-adsorption interfering substances (MCC) must be "cleaned-up" before membrane filtration or the results may be unsatisfactory. It should be noted that the estuarine water was prefiltered with an MF-Lifegard 11 cartridge prefilter which obviously did not remove MCC effectively. Admittedly, the use of stored estuarine water subjected the membrane adsorption technique to rather severe conditions and further demonstrated one of the limitations of the membrane filter system for concentrating and recovering viruses effectively from raw surface waters.

Recovery of low multiplicities of poliovirus from 100-gal volumes of water. The paramount need of a standard method for concentrating and recovering viruses from waters identifies with a reliable method for recovering viruses at extremely low multiplicities in very large volumes of water. Consequently, the application and efficacy of the membrane cartridge filter to satisfy the above requirement was investigated. In the experiments to be described, the eluates were further processed by the aqueous polymer two-phase technique as a final step for virus concentration and recovery (*see above*). In the experiments, stock poliovirus was initially diluted in 5× nutrient broth to a desired multiplicity of virus input. A portion of the input virus suspension was immediately frozen for subsequent assay. The virus inoculum was then added to the 100-gal test water and filtration was started immediately. Both estuarine and tap water were used as test waters. Following filtration, the membrane cartridge was handled as previously described. The final working volume for virus assay was 2 to 3 ml. This represented a volume reduction of approximately 126,000–190,000 to 1. The input multiplicity of virus varied from 24 PFU to 194 PFU per 100 gal of water. The results are shown in Table 4. At an input multiplicity of virus as low as 24 PFU per 100 gal of tap water, a total of 16 PFU were recovered representing 67% of the original input virus multiplicity. In the estuarine water test system conducted at the same time, a total of 9 PFU were recovered representing 38% of the original input virus multiplicity. With virus input multiplicities of 29.6, 62.4, and 194 PFU

TABLE 3. *Recovery of poliovirus from 100 gal of stored estuarine water*

Expt no.	Salinity (g/kg)	Virus input[a] (PFU/ml)	Total virus input (PFU × 10^9)	Virus in filtrate[a] (PFU/ml)	Virus recovered in eluate (PFU/ml)	Total virus recovered (PFU × 10^9)	Total virus recovered (%)
1	19.4	30.8 ± 8.2	11.66	10.2 ± 1.9	2,370.5	2.37	20
2	21.0	15.2 ± 1.8	5.75	6.5 ± 1.1	881.3	1.32	23
3	21.0	18.6 ± 3.1	7.04	7.1 ± 0.9	1,236.0	2.47	35
4	19.4[b]	13.4 ± 1.3	5.07	5.6 ± 1.5	2,577.2	2.58	51

[a] Mean virus plaque count of five replicate subsamples ± standard error.
[b] Acidity was adjusted to *p*H 3.0 in virus input tank.

TABLE 4. *Recovery of low multiplicities of poliovirus from 100 gal of water*

Expt no.	Type of water[a]	Total virus input[b] (PFU/100 gal)	Calculated virus input after dilution (PFU/5 gal)	Total virus recovered (PFU)	Total virus recovered (%)
1	Tap	29.6 ± 2.5	1.5	7	24
2	Tap	194.0 ± 13.0	9.7	68	35
3	Estuarine[c]	24.0 ± 6.8	1.2	9	38
4	Tap	62.4 ± 3.2	3.1	33	53
5	Tap	24.0 ± 6.8	1.2	16	67

[a] $MgCl_2 \cdot 6H_2O$ was added to all waters to give a final concentration of 1,200 μg/ml as Mg^{2+}.
[b] Mean virus plaque counts of five replicate subsamples ± standard error.
[c] Salinity: 19.1 g/kg.

per 100 gal of water, a total of 7, 33, and 68 PFU were recovered, respectively. These virus recoveries represented 24, 35, and 53% of the original input virus multiplicities, respectively. The results indicated that the use of the membrane cartridge as a primary method for virus adsorption, combined with a secondary method such as the aqueous polymer two-phase separation technique, permitted the recovery of poliovirus occurring in water with as low as 1 to 2 PFU per 5 gal of water. A method or combination of methods manifesting this degree of recovery and sensitivity had not heretofore been reported.

DISCUSSION

A number of high-multiplicity virus input-output experiments and low-multiplicity virus input-output experiments have been conducted, described and evaluated. The present study concerned the successful adaptation of the membrane adsorption technique reported by Cliver (4), Wallis and Melnick (24), and Rao and Labzoffsky (17) to large quantities of water. In the low multiplicity of virus input-output experiments, we used the aqueous polymer two-phase separation technique (20) as a secondary virus concentration and recovery procedure as originally suggested by Schäfer and Borneff (evaluation of the $FeCl_3$ flocculation method combined with the two-phase system for concentrating viruses, *personal communication*). The major departure of our evaluation concerned (i) scaling up the method to handle 100 gal or more of water sample, (ii) testing the cylindrical-shaped membrane cartridge configuration, and (iii) applying a secondary procedure such as the aqueous polymer two-phase separation technique (20). Perspicuously, many of the conditions shown by other investigators to increase the efficiency of the membrane adsorption technique were capitalized on by our group. When discrepancies occurred in the literature, preliminary experiments were set up and designed to provide us with data for making appropriate value judgments, for example, the concentration and type of divalent cation to use for enhancing virus adsorption to membranes. Rao and Labzoffsky (17) recommended 200 μg of Ca^{2+} per ml, Wallis and Melnick (23) used an equivalent of 1,216 μg of Mg^{2+} per ml, and Moore et al. (16) used an equivalent of 2,432 μg of Mg^{2+} per ml. The results of our preliminary experiments suggested that, of those tested, the concentration of Mg^{2+} as reported by Wallis and Melnick (23) was best under the conditions of our experiments. Consequently, we used 1,200 μg of Mg^{2+} per ml as the choice for our experiments.

A variety of eluents have been used for virus elution from membranes. Cliver (4) used 30 to 50% agamma chicken serum. Wallis and Melnick (24) used several eluents in their studies, including whole bovine fetal serum and 10% bovine serum. Rao and Labzoffsky (17) used 3% beef extract (Difco). We also examined various eluents in the initial experiments of this investigation and observed essentially no difference in virus elution efficiency among nutrient broth, 5% agamma calf serum, 30% agamma calf serum, or 3% beef extract (Difco). We chose 5× nutrient broth buffered to *p*H 9.0 (0.05 M carbonate-bicarbonate buffer) as the eluent for all the low-multiplicity virus input experiments. This eluent effectively incorporates a protein solution and a highly alkaline solution, both of which reputedly enhance viral desorption.

The effect of *p*H on virus adsorption to the membrane cartridge filters was not studied. However, from an empirical physical-chemical viewpoint, it has been well established that proteins adsorb most strongly to a variety of adsorbents at their isoelectric points. Consequently, we adjusted the acidity of our test water systems to *p*H 4.5, the established isoelectric point of poliovirus type 1. Wallis and Melnick (24) in their report indicated that maximal retention of poliovirus by membranes occurred at *p*H 5. They tested virus adsorption efficiency from virus cell harvest samples having adjusted acidities of *p*H 3 to 7. More recently, Wallis et al. (26) have reported that maximum virus adsorption to polyelectrolyte (PE60) was *p*H-dependent under certain conditions. In these experiments, a *p*H of 3.5 to 4.5 was observed to be best for enteric viruses (poliovirus, coxsackievirus, echovirus, reovirus, and adenovirus). Rao and Labzoffsky (17) noted that they observed maximum virus (poliovirus) adsorption to membranes at *p*H 7.0. Interestingly, it has been reported (B. Mendel, Bacteriol. Proc., p. 164, 1970) that poliovirus has two isoelectric points, *p*H 4.5 and 7.0. In our preliminary experiments with estuarine water, the acidity was not adjusted and ranged from *p*H 8.0 to 8.5. The recovery of virus was also observed to be considerably less than 50% (*data not shown*). These findings were consistent with adsorption theories.

Flow rate was not critically studied as to its effect on virus adsorption to membranes. Most investigators apparently use the pump that is available to them or, particularly in bench

model experiments, simply allow the filtration process to proceed by gravity. Recently, however, a limited study was reported (19) which indicated that flow rate could be important. In this report flow rates ranging from 0.028 to 4.25 ml/sec were evaluated with the 47-mm HA, 0.45-μm pore size membrane filters. The investigator stated that penetration of virus through the filters was clearly rate-dependent because the least amount of virus penetrated the filter at the slowest flow rate used. Volumes filtered were 20-ml quantities except for the slowest rate (0.028 ml/sec) when the volume was 5 ml. The effect of pressure on filtration for virus recovery has also been reported (2). When pressures up to 40 psig were tested with 4-liter volumes, complete recovery of poliovirus was observed. In our experiments, of course (*see above*), the flow rate was 2.5 gal per min, and the pressure was 35 psig.

Examination of raw waters containing either MCC or particulate material (turbidity), or both, by the membrane adsorption method presents recognized problems associated with clogging of the filter surfaces and possible loss of virus. Excessive turbidity or MCC (or both) would require pretreatment of the sample. In regard to MCC, Wallis and Melnick (24) have used an anion exchange resin [Dowex 1-×8 (Cl^-) 100 to 200 mesh] to remove MCC successfully. Moore et al. (16) have also reported success by use of the Dowex 1-×8 anion resin to remove MCC from wastewater influent. To our knowledge, prefiltration of 100-gal volumes of water through an anion exchange resin has not been done, and the precise influence of this additional step on virus recovery has not been determined.

We do not contend that the membrane adsorption method is the best method for concentrating and recovering viruses occurring at low multiplicities in large volumes of water (at least 100-gal quantities), but the data would certainly indicate that for clean raw waters or finished water this method has exhibited a good-to-excellent degree of recovery and sensitivity.

From the outset, we have advanced and propounded the concept of a sequential methods approach to resolve the immediate problem of concentrating and recovering low multiplicities of virus from water. In order to process very large volumes of water of 100 gal or more, a flow-through sampling method would have priority as the primary virus concentration and recovery method, the method of choice being one that relies on virus adsorption and retention (either or both) such as microporous membranes or "sandwiched" layers of iron oxide or polyelectrolyte. These primary procedures, of course, require that the virus be eluted (desorbed) from the adsorbent. When the adsorbent surface areas are large, volumes of eluent up to at least a liter conceivably would be required. Consequently, a secondary virus concentration and recovery procedure would also be necessary to provide a reasonable working volume for virus isolation or assay. In practice, it is desirable to reduce the working volume so that the total amount can be distributed to cell cultures. A number of methods would seem to be potential candidates for the secondary virus concentration and recovery procedure; for example, electroosmosis and forced-flow electrophoresis (21), continuous-flow ultracentrifugation (1, 3), and hydroextraction (5), to name a few. However, to our knowledge, these methods have not been applied as secondary procedures. Presently, the above methods are limited as primary virus concentration and recovery procedures because of the diminutive volume of water that can be processed, i.e., 1 to 2 liters up to a few gallons.

Because the distribution and extent of virus contamination of water, particular drinking water, is presently unknown, a highly sensitive and reliable method is needed at the outset for field trials to increase the likelihood for success. We believe that the membrane cartridge filter system (membrane adsorption technique) combined with the aqueous polymer two-phase separation technique tentatively satisfies the criteria of sensitivity and reliability because poliovirus occurring at multiplicities of 1 to 2 PFU per 5 gal (ca. 18.9 liters) was readily detected.

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