Comparative Study of Four Microporous Filters for Concentrating Viruses from Drinking Water

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Received for publication 25 March 1975

Four microporous virus-adsorbent filter media for recovering low levels of virus from 380 liters of drinking water were compared. In addition, two of the filter media were compared with 1,900 liters of drinking water. The filter media evaluated were MF nitrocellulose membranes (293 mm), AA Cox M-780 epoxy-fiberglass-asbestos disks (267 mm), K-27 yarn-wound fiberglass cartridges + AA Cox M-780 disks (127 mm), and Balston epoxy-fiberglass tubes (24.5 by 63.5 mm). The filters were used to concentrate seeded poliovirus from 380 liters of finished drinking water. Sodium thiosulfate was added to the drinking water to neutralize chlorine, and hydrochloric acid was added to adjust the pH to 3.5. Virus was eluted from the filters with glycine-NaOH buffer at pH 11.5. In terms of virus recovery efficiency, the filter media ranked Balston > Cox 267-mm > MF 293-mm \simeq K-27 + Cox 127-mm, but differences were slight. The Balston filters and holders were also superior to the other systems in terms of size, weight, cost, and handling factors. Experiments with 2- and 8-µm porosity Balston filters showed no statistically significant difference in virus recovery. Virus was readily detected by the Balston and the MF 293-mm systems at input levels of 12 to 22 PFU/1,900 liters. Preliminary experiments indicated that an elution pH lower than 11.5 may be satisfactory.

One obvious approach to the determination of the potential public health hazard of viruses in potable water is to demonstrate the presence or absence of such agents in the water. However, the need to process large volumes of water and the lack of satisfactory concentration methodology had hampered such efforts. Several methods for concentrating viruses from large volumes of water have recently been proposed (8-10, 16, 20). All of the methods utilize a virus-absorbing microporous filter medium. The sample water is continuously conditioned to enhance virus adsorption by the addition of stock solutions of hydrochloric acid and/or divalent or trivalent cations. These solutions are added to the process water either through a pressure tank system (20) or a hydraulically operated proportioner pump (7). Elution of the absorbed virus from the filter medium may be accomplished by the use of protein-containing eluants at pH 9.0 (8, 10) or a glycine buffer system at pH 11.5 (20). The current study was undertaken in an attempt to determine the comparative virus recovery efficiency of four microporous filter media by using large volumes (380 liters) of finished drinking water seeded with low levels of poliovirus.

MATERIALS AND METHODS

Virus. The LSc2ab strain of attenuated poliovirus type 1 was used. The virus stock was filtered to remove aggregates by a modification of the Ver et al. (18) technique. Seventy-five milliliters of 10% fetal calf serum in water for injection was clarified by filtration through a 47-mm membrane AP20 (Millipore) and 0.22- and 0.05-µm nitrocellulose membrane filters and then made isotonic with $10 \times Earle$ balanced salt solution. Ten milliliters of the clarified isotonic solution was used to serum coat 47-mm, 0.22-µm, and 0.05-µm membrane filters by pressure filtration. The virus stock was filtered twice through the serum-coated membranes. The virus preparation, considered to be monodispersed, was then diluted in nutrient broth to contain approximately 100 plaqueforming units (PFU)/ml and stored in screw-cap plastic tubes in 3- to 10-ml volumes at -80 C until used.

Plaque assay. HEp-2 cell monolayers grown in 25-cm³ plastic flasks were used for plaque assays. Virus was assayed by the plaque technique. The overlay medium consisted of basal medium Eagle, 2% fetal calf serum, 25 mM MgCl₂, 0.0017% neutral red, antibiotics, and 1% Difco purified agar. For assay, 1 ml of the sample concentrate was inoculated onto each monolayer and allowed to absorb for 2 h at 35 C; then the inoculum was poured off and agar overlay added. Plaques were counted daily through the fifth

day after inoculation. The entire sample concentration from each experimental run was assayed for virus.

Virus-adsorbent systems. Four types of microporous filters were used as the primary virus adsorbents. In those systems containing filters of more than one porosity, the filters were assembled in the holders from top to bottom in decreasing order of porosity. The four adsorbent systems were: (1) nitrocellulose membrane filters (Millipore Corp., Bedford, Mass.), 293-mm diameter, 8-, 1.2-, and 0.45-µm porosity; (ii) AA Cox M-780 epoxy-fiberglass-asbestos microfilters (Cox Instrument, Div. of Lynch Corp., Detroit, Mich.), 267-mm diameter, 5- and 0.45-µm porosity; (iii) K-27 yarn-wound fiberglass depth filter (Commerical Filter Div., Carborundum Co., Lebanon, Ind.), 5- μ m nominal porosity, connected to a Cox filter holder with 127-mm diameter filters with porosities of 1 and 0.45 μ m; and (iv) borosilicate glass microfiber-epoxy resin filter tubes (Balston Inc., Lexington, Mass.), 25.4-mm diameter, 63.5 mm long, 8-µm nominal porosity. A three-place Balston filter tube holder was assembled in parallel with three type 92 filter units as previously described (9).

Water supply and conditioning. Cincinnati tap water was used in all experiments. Characteristics of the tap water during the period of experimentation (about 3 months) are shown in Table 1. The water was conditioned continuously during the experiments with the apparatus described by Hill et al. (7) by adding hydrochloric acid to reduce the tap water pH to 3.5 and sodium thiosulfate solution to produce a concentration of 50 mg/liter. Two proportioner pumps, adjusted to provide a dilution of 1:100 for eachadditive solution, were used to add the chemicals to

 TABLE 1. Characteristics of the tap water used in virus recovery experiments

Median

16.7

0.2

8.4

46

137

25

227

40

9.6

Parameter

Temperature (C)

units)

pН

Turbidity (formazin

Alkalinity, CaCO,

Chloride (mg/liter)

Calcium (mg/liter)

Magnesium (mg/liter)

Total dissolved solids

(mg/liter)

Total hardness

(mg/liter)

(mg/liter)

Valueª

Range 10.6-22.2

0.1 - 0.35

8.0-9.3

38 - 52

114-163

19 - 40

212-253

37 - 42

7.9 - 10

^a Temperature and turbidity values were based on 19 observations made on laboratory tap water. Data on the remaining parameters were obtained from treatment plant personnel. These values were based on 19 observations each for pH, alkalinity, total hardness, and chloride, and on monthly composite samples for dissolved solids, calcium, and magnesium. the tapwater before it passed through the virusadsorbent filter system.

Experimental. A schematic representation of the experimental system for comparing the four virusadsorbent filters under identical conditions is shown in Fig. 1. All filter units were connected to the same water supply and to common hydrochloric acid solution and sodium thiosulfate solution containers. The input virus was added to the thiosulfate solution container. The flow rate for each filter unit was controlled at 3.8 liters/min for the 380-liter runs and at 9.5 liters/min for the 1,900-liter runs with a limiting-orifice valve (Dole Valve Co., Morton Grove, Ill.). Virus was eluted from the filters by using 1 liter of glycine-sodium hydroxide buffer, pH 11.5, under positive pressure. This primary eluate was then reconcentrated by using a modification of the method of Sobsey et al. (16). The primary eluate was adjusted to pH 3.5 with pH 1.1 glycine-hydrochloride buffer, AlCl, was added to a final concentration of 0.0005 M. and the virus was readsorbed to 47-mm AA Cox M-780 filters of 5-, 1-, and 0.45-µm porosity by vacuum filtration of the eluate. Virus was eluted from the 47-mm filters with two 7-ml portions of glycinesodium hydroxide buffer, pH 11.5, the eluate was made isotonic, antibiotics were added, and the pH was adjusted to 7.4. The volume of concentrate obtained for virus assay by this procedure was about 20 ml.

Analysis of data. Input virus for a given experiment was expressed as a mean value calculated from titration of 10 replicate 0.1-ml volumes of input virus stock. Since the actual number of PFU put into each system cannot be precisely determined and is an estimate calculated from assay of the virus stock, the observed 95% confidence limits are presented for each experiment. A comparison of the observed

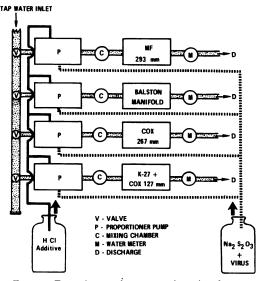


FIG. 1. Experimental system for simultaneous comparison of four virus-adsorbing filter media.

confidence limits with the expected confidence limits based on Poisson distribution showed close agreement.

The virus recovered in the eluate may be regarded as an absolute number rather than an estimate. To determine comparative efficiency, the percentage of virus recovered was calculated from the PFU recovered in the eluate versus the mean virus input for a given experiment. Though all virus-adsorbent systems were assumed to receive equal quantities of input virus in each experiment, it was recognized that the Poisson distribution of virus in the additive container may have resulted in a given virus-adsorbent system receiving more or less virus than another. With the experimental design employed, however, all systems were equally biased, and no single system should have consistently received a greater quantity of input virus with repetitive runs.

Statistical treatment of the data is presented in the following section.

RESULTS

Evaluation of four filter media for virus recovery. The results of a series of 12 experimental runs comparing the virus-adsorbing filter media simultaneously are given in Table 2. Mean virus input titers of 12 to 173 PFU/380 liters were used. Mean virus recoveries varied from 28.8% for the K-27 + Cox 127-mm combination to 45.3% for the Balston. An examination of the standard deviations shows that all systems overlap with respect to mean virus recovered. Because of the small number of experiments in which the Cox 267-mm filter was included, these data were not utilized in the following analyses. However, the values obtained with the Cox 267-mm filter suggest a recovery efficiency intermediate between the MF 293-mm and the Balston.

Application of the Spearman rank correlation coefficient (15) to the recovery data indicated little, if any, correlation of the percentage of virus recovered among any of the systems. In other words, the percentage of virus recovered by one filter medium could not be used to predict the percentage of virus recovered by another system. The correlation coefficients (r_s) obtained were: MF 293-mm versus K-27 + Cox 237-mm, -0.04; Balston versus K-27 + Cox 127-mm, 0.1; and MF 293-mm versus Balston, 0.2.

Because each experiment represented as nearly as possible a set of identical conditions for input virus and water quality, the data were analyzed nonparametrically by the Wilcoxon matched-pairs, signed-ranks test (15). In this test, the difference in the percentage of recovery between two virus adsorbents was determined for each experiment. The differences were ranked and totaled according to sign. The significance of the values thus obtained was determined by reference to appropriate probability tables. By using this test, a significant difference in virus recovery (P < 0.05) was demonstrated between the Balston and the MF 293-mm and between and Balston and the K-27 + Cox 127-mm combination. There was no significant difference (P > 0.05) in recovery between the MF 293-mm and the K-27 + Cox

Expt		s input per 380 ters (PFU)	% Virus recovered				
	X ^a	95% C.L.°	MF 293-mm	Cox 267-mm	Balston	K-27 + Cox 127-mm	
1	173	136-210	17	ND ^c	11	28	
2	153	136-170	44	ND	48	42	
3	103	87-119	50	ND	39	52	
4	95	73-117	37	ND	57	24	
5	42	31-53	52	48	74	24	
6	39	32-46	28	62	62	41	
7	36	28 - 45	33	50	19	0	
8	32	26-38	15	43	45	33	
9	28	20-36	29	ND	61	25	
10	24	17-31	17	ND	50	46	
11	22	17 - 27	32	23	27	14	
12	12	10-14	17	8	50	17	
Range			15-52	8-62	11-74	0-52	
Mean			30.9	39	45.3	28.8	
SD			± 13	±19.8	±18.5	±14.8	

TABLE 2. Recovery of poliovirus from 380 liters of tap water by using four filter media

^a Calculated from titration of 10 replicate 0.1-ml volumes of input virus stock.

^bObserved confidence limits.

^c ND, Not done.

127-mm. Of the 12 experiments, the Balston recovered more virus than the MF 293-mm in eight trials, and more than the K-27 + Cox 127-mm in 10 trials. The percentage of recovery data were subjected to a one-way analysis of variance that produced the same results. In terms of virus recovery efficiency, the four filter media may be ranked as follows: Balston tubes $> \text{Cox } 267\text{-mm} > \text{MF } 293\text{-mm} \simeq \text{K-}27 + \text{Cox } 127\text{-mm}$.

Comparison of size, weight, and cost factors. Although statistically significant, the differences in recovery efficiency among the four adsorbent media were minor. Other factors could therefore become more important in the selection of a particular adsorbent system. The adsorbent systems were compared with regard to size, weight, and cost (Table 3). For purposes of this comparison. "virus-adsorbent system" refers to the filter holder required for each system. The conditioning apparatus, hose, tubing, water meter, and additive containers did not enter into the comparisons because these items were common to all systems. The Balston system had the lowest values for all parameters, so it was used as the denominator in the calculation of ratios. With respect to size and initial cost, the systems ranked as follows: MF $293\text{-mm} > \text{Cox } 267\text{-mm} > \text{K} \cdot 27 + \text{Cox } 127\text{-mm}$ > Balston. For weight, the order was Cox $267\text{-mm} > \text{MF} 293\text{-mm} > \text{K} \cdot 27 + \text{Cox} 127\text{-mm}$ > Balston; and for operating cost, the order was K-27 + Cox 127-mm > MF 293-mm > Cox267-mm > Balston.

System characteristics. In the course of accumulating the virus recovery efficiency data presented in Table 2, a large number of 380-liter samples were processed with the various filter media. The total number of 380-liter samples examined with each system was 26 for the MF 293-mm, 6 for the Cox 267-mm, 31 for the Balston manifold, and 12 for the K-27 + Cox 127-mm. Consequently, a considerable amount of technical experience was gained in handling each system. Various objective and subjective observations were made (Table 4).

The weight of the Cox 267-mm (29.5 kg) and the MF 193-mm (20.9 kg) made the handling of these units difficult and cumbersome. These holders were also the most difficult to seal, and they commonly leaked. All of the units are autoclavable, but fragility of the membrane filters could result in breaks in the membrane. All of the holders could accommodate flow rates up to 9.5 liters/min, with the exception of the K-27 + Cox 127-mm combination. The maximum flow rate obtained with the latter system

 TABLE 3. Comparison of size, weight, and cost of virus-adsorbent systems

	Ratios ^a					
Virus-adsorbent	Size		Cost ^c			
system		Wt	Initial	Oper- ating		
Membrane filter holder, 293 mm ^d	6.1	7.9	6.96	1.5		
Cox filter holder, 267 mm ^e	6.0	11.2	4.64	1.2		
K-27 + Cox 127-mm ⁷	1.3	5.3	2.66	1.6		
Balston 3-place manifold ^g	1.0	1.0	1.0	1.0		

^a Virus-adsorbent system/Balston system.

^bBased on length \times height \times width.

^c Based on August 1974 prices. Initial cost is that of holders and associated hardware; operating cost is that of filters per sample.

^d Catalog no. YY3029316, Millipore Filter Corp. ^e Cox series 710.

Catalog no. SSB10-3/4, Commercial Filter Corp.,
 + Cox series 705.

[#] Type 92, Balston Co.

was about 8 liters/min, with the limiting factor apparently being the small diameter and porosity of the Cox 127-mm filters. The Balston was the only system allowing visual inspection of the filters during the sample collection and elution phases of the procedure. This characteristic has proved useful in determining whether the integrity of the filter medium was maintained throughout sample collection and in ensuring satisfactory contact of the eluting medium with the entire filter surface.

Effect of filter porosity on virus recovery. The virus adsorption efficiency of membrane filters has been reported to drop with increasing pore size (2, 5). The virus-adsorbent systems used in the present study all contained filters with a porosity of $0.45 \,\mu\text{m}$, with the exception of the Balston system, which used $8 \,\mu\text{m}$ porosity filters. The $8 \,\mu\text{m}$ porosity filter had originally been selected for virus concentration methodology to serve as a holder for the insoluble polyelectrolyte PE-60 (N. A. Clarke et al., J. Am. Water Works Assoc., in press). Subsequent investigation at this laboratory showed the filter itself to be equal to or better than the PE-60coated filter for virus recovery.

A number of experiments were conducted to determine if the virus recovery efficiency of the Balston-type filter was affected by porosity. The results, comparing $2-\mu m$ and $8-\mu m$ porosity filters, are shown in Table 5. Although more

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Adsorbent system	Advantages	Disadvantages		
Membrane filter, 293 mm	 Has been studied extensively; body of literature is large 	 Membrane filter is fragile and sometimes breaks on autoclaving Weight of unit makes handling difficult Plugs easily Has highest acquisition cost Has highest space requirement Has second highest operating cost 		
Cox 267-mm	 Filter is not fragile; readily auto- clavable Has high flow rate and is relatively resistant to plugging 	 Is the heaviest of the filter holders Has second highest acquisition cost Has second highest space requirement Holder is designed for one filter, but method utilizes two, leaks are common 		
K-27 + Cox 127-mm	 Removes substances that interfere with reconcentration step Is resistant to plugging Has second lowest initial cost and space requirement 	 Cox holder frequently leaks Has lowest flow rate, apparently because of small size of Cox holder Fiberglass cartridges are difficult to obtain Failed to detect viruses on two occasions when other systems have Has highest operating cost 		
Balston manifold	 Has lowest size, weight, initial cost, and operating cost Has high flow rates and is resistant to plugging Visual inspection of filters is possible during sampling and elution steps; it is not possible with other systems 	 Filter tubes cannot take differential pressures greater than 25 lb/in* on inside to outside mode Cadmium-coated center posts corrode rapidly under acid sampling conditions Corrosion of aluminum head is common around center post threads 		

TABLE 4. Advantages and disadvantages of virus-adsorbent systems

TABLE 5. Recovery of poliovirus from 380 liters of tap water by using 2- and 8-µm porosity Balston filters

	Virus input per 380 liters (PFU)		Virus recovered			
Expt			2-µm filter		8-µm filter	
	X ^a	95% C.L.*	PFU	%	PFU	%
1	104	74-134	53	51	28	27
2	104	74-134	18	17	14	13
3	80	61-99	61	76	23	29
4	80	61-99	50	63	21	26
5	42	31-53	23	55	31	74
6	39	32-46	28	72	24	62
7	36	28-45	9	25	7	19
8	32	26-38	24	75	18	56
9	22	17-27	7	32	6	27
10	12	10-14	5	42	6	50

^aCalculated from titration of 10 replicate 0.1-ml volumes of input virus stock.

^o Observed confidence limits.

virus was recovered with the $2-\mu m$ porosity filters in 8 of the 10 trials, this difference was not statistically significant when analyzed by the Wilcoxon test.

Filters with a nominal porosity of 0.9 μ m were also used in several of these trials. These filters tended to rupture, however, apparently because of plugging of the filter with particulates. Nevertheless, in two trials where the filter

did not rupture, virus recovery with the $0.9 \ \mu m$ filters was no better than that observed with the 2- and $8 \ \mu m$ filters. Preliminary experiments with 25- μm porosity filters indicated virus recoveries equivalent to those obtained with the $8 \ \mu m$ porosity filters.

Recovery of virus from 1,900 liters of tap water. A virus standard of one infectious unit per 380 liters for drinking water has recently been suggested (11). Obviously, to detect one virus unit in 380 liters of water with a method that is less than 100% efficient, more than 380 liters would have to be sampled. The MF 293-mm with 8- and 1.2-µm porosity filters and the Balston with 8- μ m porosity filters were used to concentrate poliovirus from 1,900-liter volumes of tap water. The samples were processed at a flow rate of 9.5 liters/min. The results are shown in Table 6. Both systems readily detected virus at input levels of 1 PFU/86 to 158 liters (12 to 22 PFU/1,900 liters). The percentages of virus recovered were equivalent to those observed with the 380-liter samples. It is interesting to note that there was no apparent decrease in virus recovery because of elimination of the 0.45-µm membrane filter.

Elution of virus at pH 9.8 and 11.5. Experience in our laboratory has demonstrated a decrease in poliovirus titer of approximately 50% after exposure to pH 11.5 glycine-sodium

D		input per ters (PFU)	% Virus recovered		
Expt	<u>X</u> ª	95% C.L.*	MF 293-mm	Balston	
1	22	18-26	36	32	
2	19	13-25	26	26	
3	15	12-19	ND ^c	80	
4	12	9-16	25	42	
5	12	8-16	50	25	

 TABLE 6. Recovery of poliovirus from 1,900 liters of tap water by using MF and Balston filter media

^aCalculated from titration of 10 replicate 0.1-ml volumes of input virus stock.

^bObserved confidence limits.

^c ND, Not done.

hydroxide buffer for 3 min (unpublished data). The effect of a lower eluant pH on virus recovery was investigated with the MF 293-mm and Balston filters by using 380-liter samples of tap water. A pH of 9.8 was chosen because glycine has a dissociation constant at this value; consequently, the maximum buffering capacity would be available at this pH.

The results of four experiments are shown in Table 7. More virus was recovered in each trial with the MF 293-mm filter at pH 11.5 than at pH 9.8. With the Balston filters, pH 11.5 elution recovered more virus in two of the four trials. The differences, however, were not great with either filter adsorbent system.

DISCUSSION

Many of the earlier studies performed in the development of virus concentration methodology necessarily used high levels of input virus (>1 PFU/liter) and small volumes (<20 liters) of distilled, deionized, or prefiltered waters (6). These studies were required to identify candidate techniques and elucidate factors affecting virus recovery efficiency. The extrapolation of these results to natural waters may not be appropriate, however, because of the presence of various dissolved and suspended substances that may affect virus adsorption and elution (12). In the present study, the four virus-adsorbing filter media were compared simultaneously by using low input levels and the same tap water source. Although more virus was recovered in a greater number of trials with the Balston filters, there was no clear-cut distinction in terms of mean percentage of virus recovered among the four filter media. The lack of correlation of virus recovered by one system with virus recovered by the other systems may have been a function of the random distribution of input virus in the additive container, a factor that can cause one system to receive more virus

than another system. The wide range of recoveries observed with all systems could have been due to variations in water quality from one experiment to the next. Since differences in average virus recovery among the four filter media were not great, the selection of one over another for a particular application may be dependent on other factors. The Balston system was more advantageous than the others in regard to size, weight, cost, and handling characteristics.

Preliminary experiments with Balston filters of 2- and 8- μ m porosity showed no significant difference in virus recovery. Wallis (19) has reported the same degree of virus adsorption with 0.45- and $8-\mu m$ porosity membrane filters when filtration proceeds by gravity, but viruses passed the 8- μ m filters when pressure filtered. Viruses passing cellulose nitrate membrane filters went from none at 0.45- μ m porosity to 60% at 8- μ m porosity in a study conducted by Cliver (5). The virus suspensions were filtered with a syringe-type holder. Scutt (13) reported that the adsorption of poliovirus by 0.45-µm porosity cellulose nitrate membranes was filtration-rate dependent. Virus recovered in the filtrates increased sharply with increasing filtration rates. When no significant loss of virus recovery efficiency is encountered, the selection of a large porosity filter for concentrating viruses from water is desirable from the standpoint of facilitating sampling and obviating plugging difficulties. However, it should be borne in mind that viruses may be attached to particles of suspended matter in water. Selecting a filter porosity large enough to allow passage of these particles would negate the reason for sampling. Further studies are indicated on the occurrence of viruses in water (as free particles or attached to larger particulates) and on procedures for eluting viruses from particulates.

The 1,900-liter experiments with the mem-

TABLE 7. Effect of pH 9.8 and 11.5 elution on recovery of poliovirus from 380 liters of tap water by using MF and Balston filter media

	Virus input per 380 liters (PFU)		% Virus recovered			
Expt			MF 293-mm		Balston	
	⊼ª	95% C.L.°	рН 9.8	рН 11.5	рН 9.8	рН 11.5
1 2 3 4	173 153 103 95	136–210 136–170 87–119 73–117	17 42 37 14	23 44 50 37	13 39 60 28	11 48 39 57

^a Calculated from titration of 10 replicate 0.1-ml volumes of input virus stock.

^bObserved confidence limits.

brane filter and Balston systems demonstrated (i) the effectiveness of the procedures and adsorbents in recovering virus from finished drinking waters at levels as low at 1 PFU in 158 liters, (ii) no apparent decrease in recovery from processing samples at a flow rate of 9.5 liters per min as opposed to 3.8 liters per min, (iii) satisfactory recovery of virus by the membrane filter system by using only the 8.0- and $1.2 \mu m$ porosity membranes, and (iv) the adaptability of the technique and adsorbents to processing volumes of water larger than 380 liters.

The requirement for sampling large volumes of water necessitates the use of physically large filter holders. Consequently, the elution procedure requires one liter or more of eluant. The primary eluate must therefore by concentrated by some technique to reduce the volume to allow assay in a reasonable number of tissue cultures. Before the development of the glycine elution procedure by Wallis and co-workers (20), protein-containing eluants were commonly used (6). Candidate reconcentration techniques included two-phase separation (8, 14), hydroextraction (4), electro-osmosis and forced-flow electrophoresis (17), and continuous-flow ultracentrifugation (1, 3). These procedures are time consuming and tedious. The glycine elution procedure permits rapid reconcentration of the primary eulate on smaller diameter microporous filters with subsequent re-elution into a final volume of 10 to 20 ml. In experiments employing 10-ml volumes of virus suspended in saline, Wallis et al. (20) found that poliovirus recovered from nitrocellulose membranes decreased sharply when the eluant pH was less than 11.0. In our experiments with 380-liter volumes of tap water, more virus was generally recovered at pH 11.5. However, the difference in virus recovery from Balston and nitrocellulose membrane filters by using pH 9.8 and 11.5 elution was very slight. Elution of virus at pH values lower than 11.5 may be advantageous with regard to viral inactivation under highly alkaline conditions. Metcalf et al. (12) reported no loss of poliovirus after 10 min of contact time at pH 11.5, 5% loss after 20 min, and 10% after 30 min. However, studies with other members of the human enteric virus group are necessary to determine viral inactivation with extreme variations in pH as well as recovery efficiency of the adsorbents with these viruses. Although each of the systems investigated in the present study has certain disadvantages, all were capable of concentrating low levels of poliovirus from 380 liters or more of finished drinking water. Our current studies are now directed toward determining the sensitivity of microporous filter

media for recovering viruses from drinking water. Preliminary data indicate that virus can be recovered at inputs as low as 1 PFU/380 liters when 1,900 liters are sampled. A detailed analysis of these data will be forthcoming.

ACKNOWLEDGMENTS

We are grateful to Ralph J. Cipolla, Robert J. Yust, and Andrew A. Heyward for their technical assistance. We also thank William H. Benton for supplying the cell cultures used in this investigation.

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