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Polyethylene Glycol Precipitation for Recovery of Pathogenic Viruses, Including Hepatitis A Virus and Human Rotavirus, from Oyster, Water, and Sediment Samples

GILLIAN D. LEWIS† AND THEODORE G. METCALF*

Department of Virology and Epidemiology, Baylor College of Medicine, Houston, Texas 77030

Received 8 February 1988/Accepted 2 May 1988

Polyethylene glycol 6000 precipitation was found to be an effective concentration method that enhanced the chances for detecting human virus pathogens in environmental samples. Percent recoveries from eluates of fresh and estuarine waters with 8% polyethylene glycol 6000 averaged 86 for hepatitis A virus, 77 for human rotavirus Wa, 87 for simian rotavirus SA11, and 68 for poliovirus. Percent recoveries of 97, 40, 97 and 105, respectively, for the same viruses were obtained from oyster eluates by the same procedure. Percent recoveries of 97 for hepatitis A virus and 78 for human rotavirus Wa were obtained from sediment eluates containing 2 M $NaNO_3$ with a final concentration of 15% polyethylene glycol 6000. The polyethylene glycol method was shown to be more effective than the organic flocculation method for recovery of hepatitis A virus and rotaviruses Wa and SA11, but not of poliovirus 1 in laboratory studies. In field trials, hepatitis A virus or rotavirus or both were recovered from 12 of 18 eluates by polyethylene glycol, compared with recovery from 9 of 18 eluates by organic flocculation from fresh and estuarine waters subject to pollution.

Many methods have been suggested in recent years for reconcentrating viruses from the various eluants used to recover human viruses from shellfish, sediments, cartridge filters used in water testing, and other environmental samples. Most methods are based on acid precipitation as described by Sobsey et al. (32) and the organic flocculation (OF) procedure of Katzenelson et al. (13). Although good recoveries of many enteric viruses have been reported, both methods have some disadvantages that may detract from their usefulness and impair virus recovery effectiveness (13, 21, 32, 36). First, effective elution of virus from tissues, filters, or solids is generally considered to require high eluant conductivity (≥8,000 ppm of NaCl) (32, 34, 36), while flocculation of virus by organic floc formation is more effective at a lower conductivity (≤1,500 ppm of NaCl). The necessary dilution may increase the sample volume as much as 10-fold, severely limiting sample processing effectiveness. Second, a pH in the range of 3.0 to 4.5 is generally used in these procedures and may be maintained for 1 h or more, depending on the time required for centrifugation. This pH may compromise the viability of some viruses, notably human rotavirus (10). Last, effective flocculation and precipitation of viruses require the presence of proteins or organic matter. Adequate organic material is already present in shellfish samples, and no further addition is needed, but it may be necessary to add organics to water or sediment eluates in the form of beef extract (BE) or skim milk. Residuals of these materials may later compromise viral assays, including plaque assay (27), antigen detection immunoassays (22), and gene probe hybridization (11).

Polyethylene glycol (PEG) has been used in aqueous polymer two-phase systems developed by Albertsson that were helpful for concentrating and isolating viruses from a variety of environmental samples (2). Philipson et al., who applied these systems to the purification and concentration of viruses, found PEG-based phase separation methods to be rapid, inexpensive, and nondestructive of virus (24). Two-phase virus separation systems have been used successfully by a number of investigators for the concentration and detection of viruses from sewage and water (17, 23, 29).

The use of PEG to precipitate rather than to partition viruses between two immiscible aqueous polymer phases was reported by Hebert, who successfully concentrated both rod-shaped and spherical plant viruses with a solution of PEG and sodium chloride (8). Since then, PEG 6000 precipitation methods have been successfully utilized for concentrating other plant viruses (14), bacterial viruses (37), and a number of animal viruses. Quantitative recoveries were obtained with vesicular stomatitis virus (18), polyomavirus and simian virus 40 (7), and several myxoviruses (9, 12). Labile viruses such as the Epstein-Barr virus (1) and the foot-and-mouth disease viruses (35) have been successfully concentrated, with recoveries of ≥65%. Maximum recovery of Rous sarcoma virus from cell culture fluids was obtained by treatment of PEG-precipitated virus followed by pronase treatment to release virus entrapped in proteinaceous debris (4). Effective PEG concentration of viruses from pond water (28) and aquatic sediment eluates (16) has also been obtained. Advantages of the PEG concentration method, in addition to its gentle effect on viruses, include the ability to obtain precipitation at neutral pH and high ionic concentrations as well as in the absence of other organic materials. PEG concentrations may also be manipulated to remove particles within a narrow size range (3, 25).

This study reports the effective use of PEG precipitation for reconcentrating hepatitis A virus (HAV), rotavirus, and poliovirus in primary eluates of oyster, water, and sediment samples.

MATERIALS AND METHODS

Virus culture and assay. HAV was prepared in persistently infected AGMK cells as described by Simmonds et al. (30). HAV was purified from cell culture lysates by chloroform extraction and CsCl gradient centrifugation and was assayed

* Corresponding author.
† Present address: Department of Microbiology, University of Otago, Box 56, Dunedin, New Zealand.

TABLE 1. Eluant effectiveness for recovery of rotavirus SA11 from oyster tissue[a]

Eluant	pH	Conductivity ($\mu\Omega^{-1}$)	Virus recovery	
			PFU	%
0.5% Tricine–0.01 M PBS	9.0	6,500	4.67×10^6	68.2
10% TPB–0.05 M glycine	9.0	18,000	6.50×10^6	94.9
3% BE (Oxoid powder)	9.0	6,200[b]	5.80×10^6	84.7
6% BE (Oxoid powder)	9.0	6,700	4.67×10^6	68.2
0.5 M glycine–0.01 M PBS	9.0	6,300	4.81×10^6	70.2
5% Trypticase broth–0.01 M PBS	9.0	6,900	5.51×10^6	80.4
2% Skim milk–0.01 M PBS	9.0	7,000	7.30×10^3	0.1
3% BE–2 M $NaNO_3$	5.5	57,000	7.59×10^6	110.8
2% Casein–0.01 M PBS	9.0	6,400	5.10×10^6	74.5

[a] A total of 6.85×10^6 PFU of SA11 added exogenously to each 10-g sample of oyster tissue was used with a test eluant.
[b] Conductivity was adjusted to 6,200 from 2,600 $\mu\Omega^{-1}$ by adding 10 ml of 1 M NaCl.

by solid-phase radioimmunoassay, using ^{125}I-labeled human anti-HAV immunoglobulin G (IgG) (30). Physical particle counts were made by direct electron microscopy (31).

Rotaviruses Wa (human, serotype 1, subgroup 2) and SA11 (simian, serotype 3, subgroup 1) were cultured in fetal rhesus monkey kidney cells (MA-104) in serum-free media in the presence of acetylated trypsin (7 to 10 μg/ml) or pancreatin (25 μg/ml), respectively (6). Plaque assay in MA-104 cells was used for virus enumeration. Poliovirus type 1 (P1) (LSc 1 strain) was cultured and assayed by plaquing on BGM cells with media as recommended by Dahling and Wright (5).

Environmental samples. (i) Shellfish. Oysters from nonpolluted areas of Galveston Bay were shucked and stored at −70°C until used. Oysters for controlled experiments were seeded with virus by direct tissue injection followed by 30 to 60 min of equilibration time at room temperature. Oyster tissue was homogenized in a Waring blender for 30 s at high speed with 1:7 (wt/vol) 10% tryptose phosphate broth (TPB) in 0.05 M glycine (pH 9.0) for 30 min, shaken at 250 rpm at room temperature, and centrifuged at 10,000 × *g* for 30 min at 4°C; the pellet was then discarded, and the supernatant was used in subsequent experiments.

(ii) Water. Natural fresh or estuarine water (10 to 50 liters) was seeded with virus, mixed, and adjusted to pH 3.5. $AlCl_3$ was added to a final concentration of 0.5 mM before filtration through assemblies of 3.0- and 0.45-μm-pore-size Filterite cartridge filters. Virus was eluted from the filters by 1 liter of a 10% TPB–3%BE–0.05 M glycine (pH 9.5) eluant. The eluate-containing virus was assayed directly and used in reconcentration experiments.

One-hundred-gallon samples (378 liters) of bayou or estuary water were assayed for natural virus following collection and concentration as described above. Samples were divided into two aliquots, with one reconcentrated by PEG and the other reconcentrated by OF.

(iii) Sediment. Freshwater sediment samples were mixed with 3% BE–2 M $NaNO_3$ eluant (pH 5.5) (36) for 30 min, and the solids were removed by centrifugation at 10,000 × *g* for 10 min. The eluate was then adjusted to pH 7.5 before it was seeded with virus.

Reconcentration of virus. (i) PEG precipitation. Oyster sample eluates and eluates of filters through which water samples had been passed were adjusted to pH 7.5, and PEG 6000 (BDH Chemicals, Gallard-Schlesinger, Carle Place, N.Y.) was added to a final concentration of 8% (wt/vol). The resulting suspension was stirred for 1.5 to 2 h at 4°C and centrifuged at 10,000 × *g* for 20 min. The PEG-containing supernatant was discarded, and the pellet was suspended in 0.15 M Na_2HPO_4 (pH 9.0), sonicated for 30 s, shaken for 20 min at 250 rpm, and recentrifuged at 10,000 × *g* for 30 min. The supernatant was adjusted to pH 7.4; treated with penicillin (150 μg/ml), streptomycin (250 μg/ml), mycostatin (5 μg/ml), neomycin (50 μg/ml), gentamicin (5 μg/ml), and kanamycin (100 μg/ml); and stored at −70°C until assay. Sediment sample eluates were treated in the same way except that PEG 6000 was added to a 15% final concentration.

(ii) Organic flocculation. Sample eluates were diluted with distilled water to give a conductivity of ≤1,500 ppm of NaCl and were adjusted to pH 3.5 with 1 N HCl. The solution was stirred slowly for 15 min to allow floc formation and centrifuged at 10,000 × *g* for 20 min. The supernatant was discarded, and the pellet was suspended, clarified, treated with antibiotics, and stored as described above for PEG precipitation.

RESULTS

Four techniques were compared for their effectiveness in promoting recovery of virus from oyster tissue. No significant difference was shown between maceration in a stomacher for 2 min (76%) and homogenization at high speed in a Waring blender for 30 s (87.5%) or twice for 30 s each time (85.3%) or four times for 30 s, each time with a 30-s sonication (89.6%). Blending for 30 s was used in subsequent experiments because it required less time and generated less heat than other methods. A number of eluants were tested to determine their relative effectiveness for elution of SA11 from macerated oyster tissue. SA11 was used as the test virus due to its having greater lability than either P1 or HAV. Results of trials are given in Table 1. Recovery using 2% skim milk–0.01 M phosphate-buffered saline (PBS; pH 9.0) was very poor (0.1%), while 0.5% Tricine–0.01 M PBS (pH 9.0), 3 and 6% BE (Oxoid powder) (pH 9.0), 0.5 M glycine–0.01 M PBS (pH 9.0), and 2% casein–0.01 M PBS (pH 9.0) returned 68 to 84% of input virus. Greatest SA11 recovery was achieved with 10% TPB–0.05 M glycine (pH 9.0; conductivity, 18,000 μΩ/cm) (94.9%) and 3% BE–2 M $NaNO_3$ (pH 5.5; conductivity, 57,000 μΩ/cm) (110.8%). Of these, 10% TPB–0.05 M glycine was selected for use in further experiments because eluates containing $NaNO_3$ are very difficult to reconcentrate and tend to exhibit greater toxicity than TPB concentrates (36).

To confirm the usefulness of the 10% TPB eluant, 20-g oyster samples seeded with HAV, Wa, SA11, or P1 by injection were homogenized, and elution was performed (Table 2). Elution was very efficient for each virus type, with 97 to 109% of the virus present in an eluate. Virus elution

TABLE 2. Elution of test viruses from oyster tissue by 10% TPB and 0.05 M glycine[a]

Virus	Virus input (PFU)[b]	Virus recovery (%)[c]
P1	4.0×10^5	97 ± 18
Rotavirus SA11	2.4×10^5	109 ± 24
Rotavirus Wa	1.2×10^5	99 ± 7
HAV	4.6×10^8	98 ± 6

[a] Test viruses added exogenously to 20-g samples of oyster tissue. Elution of virus was by 10% TPB (pH 9.0) made as described in Materials and Methods.
[b] HAV physical particles established from radioimmunoassay and electron microscopy.
[c] Mean recovery for three trials.

from sediments and cartridge filters has been reported previously and is not discussed here (20, 26, 36).

Reconcentration. Initial experiments to establish the appropriate concentration of PEG 6000 for good virus recovery were carried out with oyster supernatants. Virus-seeded eluates were divided into portions, and 50% PEG 6000 solution prepared in 10% TPB was added to give a range of final concentrations of from 0 to 10%. Optical density at 600 nm of a suspension was measured before and after PEG precipitation; the mass of the resulting pellet was recorded, and suspended pellets were assayed for virus. The results (Fig. 1) show that solids were rapidly precipitated, with more than 90% in a pellet with 5% PEG concentration, while viruses required higher concentrations for precipitation (6% for HAV and 7% for Wa). Because of these results, 8% PEG was selected as the concentration for further study.

OF is most efficient in solutions with high organic content and low conductivity. Diluted shellfish eluates with their heavy organic content provide a good basis for comparison of OF and PEG procedures. A number of trials were carried out on eluates prepared from oysters seeded with HAV, Wa, SA11, or P1. The results are shown in Table 3.

The differences in the two methods are striking, especially with regard to virus type. Concentration of P1 was not significantly different with either method. HAV and SA11 were concentrated equally well by PEG (97%) but significantly less effectively by OF (49 versus 12% for HAV and

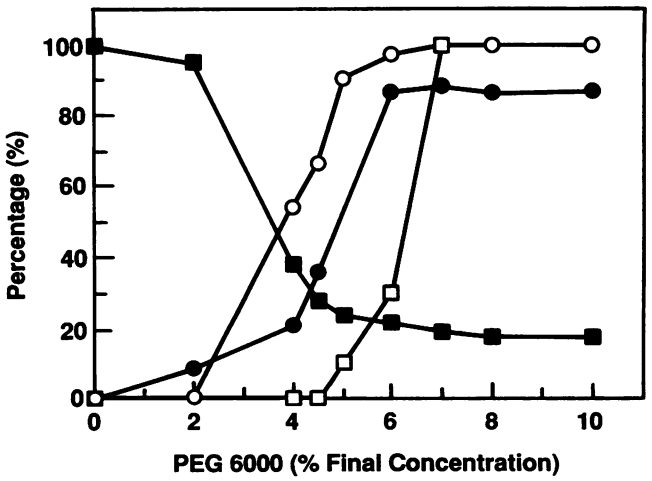

FIG. 1. Protein and test virus precipitation at different PEG 6000 concentrations. Symbols: ○, pellet mass; ■, optical density of supernatant; ●, HAV; □, rotavirus Wa.

TABLE 3. Reconcentration of viruses from oyster eluates by PEG and OF methods[a]

Reconcentration method	Virus (% concentrated)			
	SA11	Wa	HAV	P1
PEG precipitation[b]	110	51	84	128
	91	31	118	105
	102	44	76	126
	90	39	89	80
	70	34	120	120
	128	52	75	70
	99	52	85	
	86	49	90	
Mean	97 ± 17	40 ± 8	97 ± 20	105 ± 25
OF[c]	9	1	48	60
	15	2	48	85
	11	1	50	100
Mean[d]	12 ± 3	1.3 ± 0.6	49 ± 1	82 ± 20

[a] Oyster tissues (20 g) were inoculated with virus and blended for 30 s, and virus was eluted with 10% TPB–0.05 M glycine (pH 9.0).
[b] Eluate (pH 7.5) was adjusted to 8% PEG 6000, stirred for 2 h at 4°C, and centrifuged at 10,000 × *g* for 20 min. Virus recovery was assessed by assay of the suspended pellet.
[c] Eluate was adjusted to pH 3.5 with 1 N HCl, stirred slowly for 15 min, and centrifuged at 10,000 × *g* for 20 min. Virus recovery was assessed by assay of the suspended pellet.
[d] Unpaired *t* test showed that SA11, Wa, and HAV were recovered significantly better by PEG than by OF ($P < 0.001$). No significant difference was found for P1.

SA11, respectively; $P < 0.001$), possibly a result of the greater acid stability of HAV. Wa was recovered at a much lower level by both methods (40% by PEG and 1% by OF). Wa is known to be acid sensitive, and this could account for the small amount (1%) recovered by OF. Recovery of only 40% of the virus by PEG was surprising and indicated unexpected behavioral differences between Wa and SA11.

The final PEG precipitation method developed for recovering viruses from oysters is given in Fig. 2. Separation of mantle fluid is easily accomplished with sieves having mesh openings of 4 to 2 mm. Conductivity values of ≥10,000 may vary widely without influencing virus recovery effectiveness. Microbial contaminants are eliminated, preferably by the addition of antibiotic mixtures, but membrane filtration methods can also be used.

Filter eluates from large-volume water samples do not generally present the same complex organic-protein solution as do shellfish and, as such, are ideal candidates for reconcentration by the PEG method. Filter eluates prepared from both virus-seeded natural fresh and estuarine water samples were reconcentrated with 8% PEG, and virus recovery was assessed as the percentage of virus eluted from the filters. Results (Table 4) showed good virus recovery in all cases (>61%). Although a more effective reconcentration was obtained with eluates of freshwater samples other than HAV, a significant difference was found only with the human rotavirus Wa ($P < 0.001$).

Reconcentration of sediment eluates containing the chaotropic agent $NaNO_3$ required a modification of the procedure described above. An 8% PEG final concentration was ineffective, so sediment eluates using 3% BE–2 M $NaNO_3$ (pH 5.5) were seeded with HAV and Wa test viruses, and recoveries with PEG concentrations greater than 8% were determined. The results of trials using final concentrations of

Recovery

1. Separate tissue (100 g) from mantle fluid

Tissue — Mantle Fluid

Blend 30 seconds in 7 x volume (700 ml) 10% TPB, pH 9.0, conductivity 18,000 ppm NaCl

Adjust to pH 9.0, shake 30 minutes at 250 rpm at room temperature, centrifuge 10,000 x g for 30 minutes.

Reconcentration

1. Pool supernate and mantle fluid, add PEG-6000, final concentration - 8% w/v, stir 1.5 hours at 4°C, centrifuge 10,000 x g for 20 minutes.
2. Resuspend pellet in minimal volume 0.15 M Na_2HPO_4, pH 9.5 (ca 10 ml), adjust to pH 9.0, sonicate 30 seconds, shake at 250 rpm for 30 minutes at room temperature, centrifuge 10,000 x g for 30 minutes.
3. Adjust supernatant to pH 7.2 - 7.5, treat with antibiotics for filter through serum treated 0.22 um low protein binding filter.
4. Assay directly or store at -70°C.

FIG. 2. Outline of methods of recovery and reconcentration of viruses from oysters.

up to 20% are shown in Fig. 3. Increasing recoveries of both test viruses were obtained, reaching maximum values at a 15% PEG concentration. Thereafter, recoveries dropped off rapidly.

Humic acid is known to interfere in many assay systems and has been shown to be concentrated along with viruses in

TABLE 4. Reconcentration of viruses from cartridge filter eluates by PEG precipitation

Virus input[a]	% Recovery of eluate from[b]:	
	Freshwater sample	Estuarine sample
SA11	118	79
	75	89
	95	68
Mean ± SE	96 ± 21	79 ± 11
Wa	98	53
	90	60
	90	69
Mean ± SE	93 ± 5	61 ± 8
HAV	112	123
	63	74
	58	87
Mean ± SE	78 ± 30	95 ± 25
P1	71	60
	79	66
	69	66
Mean ± SE	73 ± 5	64 ± 3

[a] Input PFU per 100 ml was 7×10^3 for SA11, 2.9×10^5 for Wa, and 3.35 for P1. P/N (counts per minute test sample divided by counts per minute negative control sample) was 68.65 for HAV.

[b] Eluant was 10% TPB–3% BE–0.05 M glycine (pH 9.5).

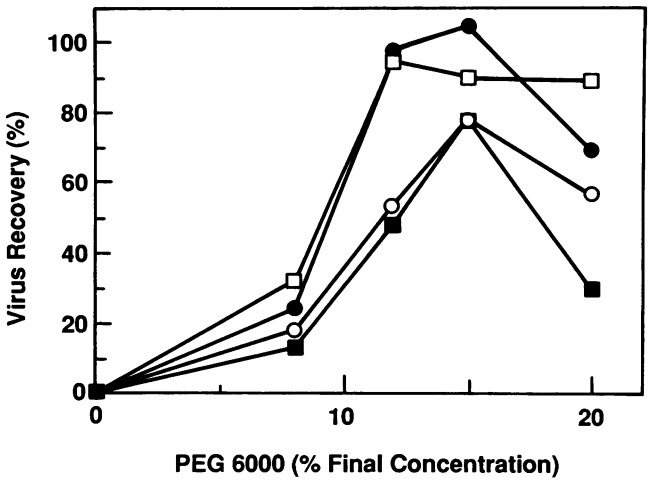

FIG. 3. Recovery of HAV and rotavirus Wa from sediment eluates containing 2 M $NaNO_3$ by PEG precipitation. Symbols: □, HAV and eluant only; ○, HAV, eluant, and sediment solids; ●, rotavirus Wa and eluant only; ■, rotavirus Wa, eluant, and sediment solids.

many of the commonly used techniques (33). To assess the effect of humic acid on PEG precipitation of viruses, eluates of cartridge filters were seeded with HAV, and humic acid was added in concentrations of from 0 to 1,000 mg/liter. Recovery of HAV by PEG precipitation was unaffected by humic acid in concentrations many times those found in natural waters (Table 5). Optical density and pellet mass figures showed that humic acids were precipitated by PEG but did not compromise virus assays.

The effectiveness of PEG and OF reconcentration methods in promoting recovery of naturally occurring HAV and rotavirus was evaluated in field trials in which 378-liter samples from bayou and estuarine waters known to be sewage polluted were collected. Samples were passed through collecting cartridge filters, and TPB-BE-glycine eluates were obtained. One of each pair of filter eluates was reconcentrated by PEG, the other was concentrated by OF, and the concentrates were assayed for rotaviruses by immunofluorescence staining and for HAV by gene probe hybridization. The results of tests made on 18 paired samples are given in Table 6.

The PEG method resulted in greater recoveries of rotavirus from both estuarine and bayou water samples. All 8 estuarine samples and 4 of 10 bayou samples were positive, compared with 8 of 18 samples positive by the OF method.

TABLE 5. Recovery of HAV from humic acid-containing eluates by PEG precipitation[a]

Humic acid concn (mg/liter)	Optical density (600 nm)		Pellet mass (mg)	HAV recovery (%)
	Before centrifugation	After centrifugation		
0	0.163	0.157	1	93.3
10	0.210	0.180	5	83.3
50	0.252	0.201	50	97.5
100	0.348	0.234	85	100.6
1,000	0.788	0.496	95	106.4
5,000	1.1255	0.715	125	106.0

[a] A total of 10^7 physical particles of HAV were added to 20-ml samples of 10% TPB–3% BE–0.05 M glycine (pH 7.5), containing from 0 to 5,000 mg of humic acid per liter.

TABLE 6. Detection of wild strains of HAV and rotavirus in paired bayou and estuarine water samples reconcentrated by PEG and OF methods[a]

Virus and reconcentration method	No. of samples[b]				Percent positive
	Estuarine water (n^b = 8)		Bayou water (n = 10)		
	+	−	+	−	
Rotavirus					
PEG 6000	8	0	4	6	66%
OF	5	3	3	7	44%
HAV					
PEG 6000	2	6	7	3	50%
OF	2	6	2	8	22%

[a] Paired samples prepared from an initial 378-liter volume of water and reconcentrated by the PEG 6000 or OF method were assayed for rotavirus by immunofluorescence and for HAV by gene probe hybridization.
[b] +, Virus detected; −, no virus detected.

No differences between the two methods were found for recovery of HAV from estuarine samples, but 7 of 10 PEG-concentrated bayou samples were positive for HAV, compared with 2 of 10 OF-concentrated samples. Comparison of field trial results by the χ^2 test showed PEG to be significantly more effective than OF for recovering HAV and Wa ($P < 0.05$) from water samples.

DISCUSSION

Study results showed PEG precipitation to be an efficient and conservative method for reconcentration of viruses from water, sediments, and oyster samples. A measure of the significance of the method is its applicability not only to differing kinds of environmental samples, but also to viruses of demonstrated pathogenic potential that are transmitted via water or shellfish routes. Viruses that may be transmitted through water or associated routes may include not only HAV, rotavirus, and Norwalk virus, but also caliciviruses, adenoviruses, astroviruses, and other gastroenteritis viruses (19).

The effectiveness of the PEG method was shown by both the percent virus recoveries achieved and the number of viruses recovered, compared with those achieved with the widely used OF method. Results obtained with poliovirus provided an interesting insight into the two methods. Although recovery of poliovirus by the PEG method was greater than that obtained by OF, the difference was not significant. A similar result was not obtained with the other test viruses, however. The lower recoveries by OF were considered the result of the use of poliovirus as the basic model around which the method was developed and its efficiency maximized. Study data indicate the inappropriateness of OF for a number of viruses that are of increasing interest as candidate water- or shellfish-transmitted pathogens. These data also show that a virus concentration method such as OF, which is designed to select for viruses with similar properties such as isoelectric point and pH stability, probably will preclude recovery of a number, possibly a majority, of significant pathogenic viruses present in a sample. Development of a single method that will prove efficient for the widest variety of viruses possible is needed. The PEG 6000 precipitation procedure appears to be such a method.

The uniqueness of the PEG method lies in its application as a procedure for secondary concentration of viruses found in eluates of environmental samples. PEG precipitation per se enjoys widespread use among biochemists, pharmacologists, and virologists (3, 8, 25). PEG is a chemically inert, nontoxic, water-soluble synthetic polymer known to precipitate a number of proteins. Two mechanisms have been suggested to account for its effect. The first and possibly primary mechanism is discussed by Atha and Ingham (3). It is suggested that PEG acts as an "inert solvent sponge" that sterically excludes proteins from a solvent, effectively increasing their concentration until solubility is exceeded and precipitation occurs. The dynamics of this process are very similar to salting out, and precipitate formation will be affected by such factors as protein size, concentration, charge, and the initial ionic strength of the solute.

A second mechanism which emphasizes the importance of protein charge has been suggested and is discussed in detail by Lee and Lee (15). In this case, protein surface charges produce an unfavorable thermodynamic effect on the solubilized PEG, causing it to be excluded from the "protein zone"; therefore, at appropriately high concentrations of polymer, protein precipitation or crystallization occurs. More highly charged or hydrophobic proteins will be more easily precipitated than those of lesser charge.

Which of these mechanisms is preeminent in virus precipitation is not clear. Various facets of the study indicate that both mechanisms are of various degrees of importance depending on the virus under consideration. Figure 1 shows the concentration of PEG 6000 required to precipitate HAV and rotavirus Wa from an oyster supernatant. In the procedure used, both viruses were suspended in the same sample of eluate; therefore, all conditions other than the individual properties of each virus were identical. Rotavirus Wa is considerably larger (65- to 75-nm diameter) than HAV (27- to 32-nm diameter) and by simple volume considerations should be precipitated at lower PEG concentrations. Since HAV was precipitated at lower PEG concentrations, size-volume considerations did not seem to be of primary or sole importance. Although the results suggested that a highly charged or hydrophobic surface was responsible, apart from knowledge of the number of VP3 and VP7 structures in double-shelled rotavirus Wa, not enough is known about surface characteristics and charges of either virus to comment further about such a possibility.

The effective recovery of virus achieved from eluates of sediments in the presence of the highly chaotropic $NaNO_3$ was surprising. The results showed both that the PEG method could be adapted to effective concentration of HAV and rotavirus Wa in the presence of an inhibitory substance and that it was at least as effective as the method for recovery of virus from sediments reported by Wait and Sobsey (36).

Since PEG 6000 precipitation is equally effective with all eluates investigated to date (data not shown), the use of tryptose phosphate for elution of oyster tissues was adopted only because it is a simple eluant capable of maximizing the total recovery potential for rotavirus and HAV.

Although evaluations with laboratory strains of viruses showed the PEG method to be superior to the OF method, the recovery of greater numbers of wild strains of HAV and rotaviruses from natural water samples furnished more compelling evidence of the greater effectiveness of the PEG method. Field trial data also offered indirect support for the laboratory-based conclusion that PEG effectiveness would not be affected by the humic acid content of natural waters.

Study results have shown PEG precipitation to be a rapid, technically simple, and effective method for reconcentration

of virus in eluates from water, sediment, and oyster samples. The method is flexible and inexpensive and can be used without extensive pH treatment or use of multiple reagents. Furthermore, it can be used in a minimally equipped laboratory and by staff with a minimal level of training.

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