APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Aug. 1983, p. 379–385 Vol. 46, No. 2
0099-2240/83/080379-07$02.00/0

Method for Recovery of Enteric Viruses from Estuarine Sediments with Chaotropic Agents

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Received 24 January 1983/Accepted 6 June 1983

An evaluation was made of the ability of chaotropes, low-molecular-weight ionic compounds which enhance the solubilization of hydrophobic compounds in water, to improve the recovery of enteric viruses from highly organic estuarine sediments. Chaotropic agents alone were poor eluents of polioviruses from sediment but were effective when combined with 3% beef extract. Chaotropes of lower potency, $NaNO_3$, NaCl, and KCl, were more efficient eluents than the stronger chaotropes, guanidium hydrochloride or sodium trichloroacetate. The most effective eluent was 2 M $NaNO_3$ in 3% beef extract at pH 5.5, which eluted 71% of sediment-associated polioviruses. Efficient concentration of the sodium nitrate-beef extract eluate by organic flocculation required the addition of the antichaotrope $(NH_4)_2SO_4$ to a 2 M concentration and Cat-Floc T (Calgon, Pittsburgh, Pa.) a cationic polyelectrolyte, to a 0.01% concentration. Dialysis of the final concentrate was necessary to reduce salts to nontoxic levels before assay in cell cultures. Trials with highly organic estuarine sediment seeded with high or low numbers of poliovirus 1, echovirus 1, or rotavirus SA-11 demonstrated the superiority of this method over two other methods currently in use.

The discharge of domestic sewage effluent bearing pathogenic enteric viruses into estuaries and coastal waters has been demonstrated to pose a public health risk, particularly to those who bathe in or consume shellfish taken from polluted waters (4, 7, 9, 10, 21). Once introduced into coastal waters, enteric viruses tend to become associated with particulate matter and accumulate in the upper layers of marine sediments, thereby becoming concentrated in numbers significantly higher than in the overlaying water column (6, 23). Sediment-associated viruses have been shown to be inactivated more slowly than viruses suspended in seawater (16, 23; D. A. Wait and M. D. Sobsey, Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, Q94, p. 209). Resuspension of sediment-associated viruses by various natural and human activities may increase the risk of virus exposure from ingestion of contaminated water or shellfish. The concentration and persistence of enteric viruses in marine sediments, including those underlying shellfish-harvesting and bathing waters, indicate the need for a reliable method to isolate and quantify enteric viruses in sediments. Furthermore, investigations evaluating the effectiveness of indicator microorganisms as predictors of the sanitary quality of shellfish suggest that sediment samples may provide a more valid, longer-term assessment of the microbiological quality of an estuarine system than do water samples, which provide data of a more ephemeral nature (17; R. Carrick, C. Hackney, G. Lovelace, and M. D. Sobsey, Abstr. Annu. Meet. Am. Soc. Microbiol. 1981, N32, p. 178).

Chaotropic agents, low-molecular-weight ionic compounds which alter the thermodynamics of a water solution in such a way as to favor the solubilization of hydrophobic substances, have been employed in biochemical procedures to isolate membrane-bound proteins and to resolve multicomponent enzyme complexes (13). Chaotropic compounds have been shown to be effective eluents of polioviruses from membrane filters (8) and enteric viruses from aquatic sediments (3).

In the course of field studies on enteric virus contamination of estuarine waters, sediments, and shellfish, the recovery of relatively low levels of viruses from sediments in the vicinity of a sewage outfall led us to suspect that the virus recovery method employed (17) was not effective. This study was carried out to investigate the potential of chaotropic compounds to provide more efficient recovery of enteric viruses from estuarine sediments.

MATERIALS AND METHODS

Viruses. Initial elution and concentration experiments were performed with poliovirus type 1 strain

LSc. Poliovirus propagation and plaque assays were carried out with buffalo green monkey kidney cells, as previously described (24). Echovirus type 1 strain V239 and simian rotavirus SA-11 were also employed in the procedure comparison experiments. Echovirus type 1 was propagated and enumerated by plaque assay in buffalo green monkey kidney cells by using techniques identical to those for poliovirus. Rotavirus SA-11 was propagated and enumerated by plaque assay with MA-104 cells. Confluent MA-104 monolayers were rinsed with minimum essential medium to remove residual serum, inoculated with SA-11 samples, and incubated at 37°C for 1 h. The first overlay consisted of Eagle minimum essential medium, 0.9% agar, 0.3% $NaHCO_3$, 15 mM HEPES buffer, 2 mM glutamine, 1× Eagle nonessential amino acids, 100 U of penicillin G ml^{-1}, 100 μg of streptomycin sulfate ml^{-1}, and 40 μg of pancreatin ml^{-1}. After incubation for 4 days at 37°C, a second overlay similar in composition to the first overlay but with 1.4% agar and 38 μg of neutral red ml^{-1} was applied. Plaques were counted 24 and 48 h after the addition of the second overlay.

Sediment elution experiments. Estuarine sediment, a black organic muck, was collected from Calico Creek, a tidal creek emptying into the Newport River estuary near Morehead City, N.C. Residual seawater was poured off after the sediment had completely settled out of suspension. The sediment was stored at 4°C.

Seawater was seeded with poliovirus to concentrations of 4×10^4 to 4×10^5 PFU per ml. A 10-ml amount of seeded seawater was thoroughly mixed with 10 ml of wet sediment on a rotary shaker for 15 min and then centrifuged at 1,850 × g for 10 min. Under these conditions, the extent of poliovirus adsorption to sediment consistently exceeded 99.9%.

Beef extract was purchased from three sources, BBL Microbiology Systems, Cockeysville, Md., Difco Laboratories, Detroit, Mich., and Oxoid Ltd. (via KC Biologicals, Lenexa, Kans.). Each lot was prescreened for adequate flocculation ability. Beef extract was sterilized by autoclaving.

Eluent formulations were added to each pelleted sediment sample at a ratio of 30 ml of eluent per 10 ml of wet sediment. The pH was adjusted to the desired value with 1 N HCl or 1 N NaOH, and each sample was mixed vigorously on a rotary shaker for 15 min. The pH of the mixture was found to remain relatively stable during this step. After centrifugation at 5,140 × g for 5 min, each eluate was sampled to determine elution efficiency. All elution experiments were conducted in duplicate.

Concentration experiments. The concentration of viruses eluted from sediment was accomplished by organic flocculation (14). The eluent developed in elution experiments, 2 M $NaNO_3$ in 3% beef extract, was seeded with 10^4 to 10^5 PFU of poliovirus per ml, mixed, sampled, and split into 10-ml aliquots. Flocculation enhancers were added, and each mixture was adjusted to pH 3.5. After slow mixing for 20 min, mixtures were centrifuged at 5,140 × g for 5 min. The pellets were suspended in 3 ml of 0.1 M Na_2HPO_4 and sampled. All concentration experiments were performed in duplicate.

Procedure comparison experiments. Experiments were conducted with poliovirus, echovirus, and SA-11 to compare recovery efficiencies of the newly developed method with recoveries for two other procedures. A 150-ml seawater sample was seeded with a high or low concentration of one virus strain and then sampled. The seawater was split into 50-ml aliquots, each of which was mixed with 50 g of wet sediment and then centrifuged. One sediment sample was taken through the elution-concentration procedure developed in this study (Fig. 1). The second sample was carried through the elution-flocculation procedure developed by Cooper et al. (5), in which viruses were eluted with 150 ml of 0.25 M glycine with 3% beef extract at pH 10.5 and then concentrated by organic flocculation. Viruses were recovered from the third sediment sample by a technique developed by LaBelle et al. (17). For this method, elution was carried out with 150 ml of 0.25 M glycine with 0.05 M EDTA at pH 11.0. After centrifuging to remove the sediment, the concentration of viruses was accomplished by adjusting the eluate to pH 3.5 with 1 M glycine (pH 2), adding 1 M $AlCl_3$ to a final 0.06 M concentration, adsorbing the viruses onto AP-25 (Millipore Corp., Bedford, Mass.) and 0.45-μm (Filterite, Timonium, Md.) filters, in series, and eluting the filters with two 30-ml volumes of pH 11.5 tryptose phosphate broth containing 10% calf serum. When SA-11 was employed as the test virus, 3% beef extract was substituted for 10% serum to prevent the loss of virus titer.

RESULTS

Elution of poliovirus from estuarine sediments. Experiments were first conducted to test the ability of different chaotropic agents to elute poliovirus from estuarine sediment (Table 1). Trichloroacetate possesses relatively large chaotropic capacity, whereas guanidium hydrochloride is a moderately potent chaotrope, and nitrate is a weak chaotrope (12). None of these

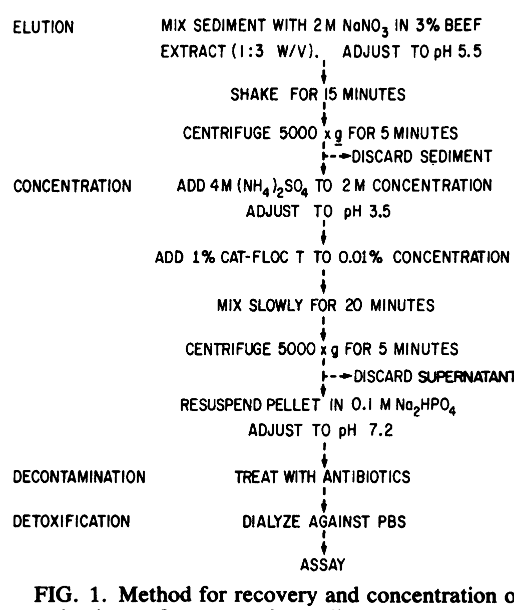

FIG. 1. Method for recovery and concentration of enteric viruses from estuarine sediments.

TABLE 1. Elution of poliovirus from sediment by chaotropes

Eluent	Concn (M)	Elution efficiency (%)[a] at pH:	
		7.5	9.5
Sodium trichloroacetate	1.0	0.04	0.02
Guanidium hydrochloride	0.1	0.08	0.08
Sodium nitrate	1.0	0.05	0.06

[a] Mean percentage of adsorbed viruses eluted.

chaotropes eluted poliovirus efficiently from estuarine sediment at pH 7.5 or 9.5.

In the search for effective virus eluents, substances besides chaotropes were also tested. Beef extract, alone and supplemented with glycine and disodium EDTA, a combination proposed as a virus eluent for marine sediments (5), and citrate, used as a buffer with beef extract to elute viruses from river water solids (1) alone and supplemented with beef extract, were evaluated for their ability to elute polioviruses from estuarine sediment (Table 2). Beef extract eluted polioviruses more efficiently than any of the chaotropes, a 3% concentration being most effective. The addition of glycine and disodium EDTA did not improve the elution efficiency of beef extract. Sodium citrate alone was a poor eluent, but 2 M sodium citrate in 3% beef extract at pH 7.5 eluted 40% of sediment-associated polioviruses in small-scale experiments. However, when subsequently tested with larger sediment samples, the citrate-beef extract combination eluted less than 1% of adsorbed polioviruses (data not shown).

TABLE 2. Elution of poliovirus from sediment with various eluents

Eluent	Elution efficiency (%)[a] at pH:			
	5.5	7.5	9.5	10.5
3% Beef extract	5.0	20	1.6	22
6% Beef extract	NT[b]	9.4	2.0	NT
10% Beef extract	NT	8.6	1.5	NT
3% Beef extract–0.25 M glycine	NT	18	5.7	14
3% Beef extract–0.05 M EDTA[c]	NT	12	7.4	16
3% Beef extract–0.25 M glycine–0.05 M EDTA	NT	20	7.1	17
0.05 M Sodium citrate	NT	4.4	4.9	NT
3% Beef extract–0.05 M citrate	NT	16	12	NT
3% Beef extract–2.0 M citrate	NT	40	NT	NT
3% Beef extract–3.0 M citrate	NT	0.9	NT	NT

[a] Mean percentage of adsorbed viruses eluted.
[b] NT, Not tested.
[c] Disodium EDTA.

TABLE 3. Elution of poliovirus from sediment with chaotrope-beef extract mixtures

3% Beef extract plus the following chaotrope (M):	Elution efficiency (%)[a] at pH:			
	5.5	6.5	7.5	9.5
Sodium trichloroacetate				
0.005	NT[b]	NT	14	NT
0.01	NT	NT	15	NT
0.05	NT	NT	17	NT
0.10	NT	NT	26	8.3
1.00	NT	NT	0.7	6.0
Guanidium hydrochloride				
0.1	NT	NT	0.6	NT
1.0	NT	NT	<0.1	NT
Sodium nitrate				
0.1	NT	NT	11	NT
0.5	NT	NT	21	NT
1.0	36	NT	29	19
2.0	71	42	46	NT
3.0	53	NT	58	NT
4.0	52	NT	58	NT
Sodium chloride (2.9)	49	NT	NT	NT
Potassium chloride (2.3)	42	NT	NT	NT

[a] Mean percentage of adsorbed viruses eluted.
[b] NT, Not tested.

In view of the initial significant increase in elution efficiency of citrate when mixed with beef extract, the chaotropic agents were retested for their ability to elute polioviruses from estuarine sediment when supplemented with 3% beef extract (Table 3). The presence of beef extract improved the elution efficiency of all chaotropes tested, with relatively weak chaotropes, nitrate and chloride, possessing the highest elution efficiencies. The most effective eluent, 2 M $NaNO_3$ with 3% beef extract, eluted 71% of polioviruses at pH 5.5.

Concentration of virus eluates by organic flocculation. To reduce final eluate volumes to minimize cell culture requirements, a concentration step employing organic flocculation (14) was investigated. A simple procedure, in which the 2 M $NaNO_3$–3% beef extract eluate was adjusted to pH 3.5 to flocculate beef extract proteins, resulted in the recovery of only 2% of polioviruses (Table 4), with the rest of the viruses remaining unflocculated in the supernatant. Increasing the beef extract concentration to as high as 6% or adding $MgCl_2$ to a 0.05 M concentration or DEAE dextran to 1,000 mg liter^{-1} to enhance virus adsorption to the floc failed to reduce the loss of viruses during concentration

TABLE 4. Effect of antichaotropes upon poliovirus concentration from eluate by organic flocculation

Antichaotrope concn[a]	Recovery efficiency (%)[b]
None	2.4
1 M sodium citrate	2.0
0.5 M Na_3PO_4	<0.1
2.0 M $MgSO_4$	15
0.5 M $(NH_4)_2SO_4$	1.8
1.0 M $(NH_4)_2SO_4$	9.1
2.0 M $(NH_4)_2SO_4$	24

[a] Final antichaotrope concentration in 2 M $NaNO_3$–3% beef extract eluent.
[b] Percentage of poliovirus in eluate recovered in concentrate.

(data not shown).

A second approach to improving flocculation efficiency involved the addition of antichaotropes (11), compounds which reduce the solubility of hydrophobic substances in water. The addition of these compounds to the eluate visibly increased protein flocculation. Virus recovery efficiency was modestly improved, with 2 M ammonium sulfate yielding the highest mean recovery efficiency of 24% (Table 4). The addition of $MgCl_2$ to 0.5 M in the presence of 2 M $(NH_4)_2SO_4$ resulted in the recovery of 36% of polioviruses during organic flocculation (data not shown).

Two organic polymers, polyethylene glycol (Carbowax PEG 20,000; Dow Chemical Co., Midland, Mich.) and Cat-Floc T (Calgon, Pittsburgh, Pa.) were also tested for their ability to improve virus recovery efficiency by enhancing flocculation during the concentration step. Both Carbowax PEG and Cat-Floc T greatly improved poliovirus recovery in the presence of 2 M $(NH_4)_2SO_4$ (Table 5). Cat-Floc T, when used at a 0.01% concentration in the absence of Mg^{2+}, yielded a mean virus recovery efficiency of 138%. Virus recovery in excess of 100% may be due to the ability of Cat-Floc T to enhance virus adsorption to cell monolayers during plaque assay (unpublished observation).

Final virus concentrate samples resulting from this procedure were found to contain high residual concentrations of salts, causing cell death during assay unless the samples were diluted. Dialysis of concentrate samples against phosphate-buffered saline for 18 to 24 h at 4°C eliminated this problem. The complete virus concentration procedure is outlined in Fig. 1.

Procedure comparison experiments. The virus recovery efficiency of the method developed in this study was compared with recoveries obtained by two other techniques currently in use. One method (5) involves the elution of sediment with a 3% beef extract–0.25 M glycine mixture (pH 10.5) followed by concentration by organic flocculation. The second method (17) employs a 0.2 M glycine–0.05 M EDTA eluent (pH 11) followed by adsorption of viruses onto a filter (Filterite) and subsequent elution of viruses from the filter. The virus recovery efficiency of the method using a chaotrope in combination with beef extract as the eluent was higher than either of the two current techniques for high and low input levels of all three virus types (Table 6). Comparison of the mean recovery efficiencies with a two-tailed t test demonstrated that the recovery efficiency of the chaotrope method was significantly higher than either of the alternate techniques at the 5% level for poliovirus 1 and echovirus 1 and at the 20% level for rotavirus SA-11.

DISCUSSION

Enteric viruses, because of their size, behave as colloidal particles in natural waters. Viruses are generally considered to be hydrophilic in nature, possessing a strong affinity for water.

Under conditions associated with natural waters, most enteric viruses possess a net negative surface charge due primarily to the ionization of carboxyl groups present on the external surfaces of viral capsid proteins. Adsorption of enteric viruses to sediment particles, also generally possessing net negative charges in natural waters, is currently explained in terms of the physical double-layer model for colloidal behavior (26). Adsorption of a virion to a sediment particle, according to this theory, requires that the two particles overcome their mutually opposing negative charges and approach one another closely enough for attractive London-van der Waals

TABLE 5. Effect of organic polymers on poliovirus concentration from eluate by organic flocculation

Polymer concn[a]	Recovery efficiency (%)[b]
PEG (30,000 mg liter^{-1})–0.5 M $MgCl_2$	5.6
PEG (6,000 mg liter^{-1})–0.5 M $MgCl_2$	72
PEG (600 mg liter^{-1})–0.5 M $MgCl_2$	45
PEG (60 mg liter^{-1})–0.5 M $MgCl_2$	45
0.3% Cat-Floc T–0.5 M $MgCl_2$	49
0.2% Cat-Floc T–0.5 M $MgCl_2$	66
0.1% Cat-Floc T–0.5 M $MgCl_2$	105
0.1% Cat-Floc T–0.25 M $MgCl_2$	93
0.1% Cat-Floc T–0.10 M $MgCl_2$	93
0.1% Cat-Floc T	111
0.01% Cat-Floc T	138
0.001% Cat-Floc T	127

[a] Final polymer concentration in 2 M $NaNO_3$–3% beef extract eluent supplemented with 2 M $(NH_4)_2SO_4$.
[b] Percentage of total poliovirus in eluate recovered in concentrate.

TABLE 6. Comparison of methods for recovery of enteric viruses from estuarine sediment

Virus	Virus input (PFU/50 g of sediment)	No. of trials	Mean virus recovery (%)[a]		
			$BE\text{-}NO_3$	BE-GLY	GLY-EDTA
Poliovirus 1	10^4	2	39	9.7	0.1
	218	2	44	18	0
Echovirus 1	10^4	2	43	4.4	0.1
	615	2	16	2.6	1.5
Rotavirus SA-11	370	2	23	0	0

[a] $BE\text{-}NO_3$, Beef extract-sodium nitrate method; BE-GLY, beef extract-glycine method (5); GLY-EDTA, glycine-EDTA method (17).

forces to predominate. The pH and ionic strength of the system exert a controlling influence upon adsorption, since pH determines the surface charge of the colloidal particles by regulating the type of surface groups which are ionized and the degree of ionization, whereas ionic strength controls the depth of the double layer around each particle.

Analysis of soluble proteins whose structures are well characterized has demonstrated that nonpolar residues make up 40 to 50% of the surface area accessible to solvent molecules (18). Determinations of the amino acid composition of picornavirus capsid proteins have revealed that 40 to 50% of the amino acid residues are nonpolar in nature (22). These findings suggest that enteric viruses possess considerable hydrophobic character and that hydrophobic interactions may also play a significant role in the process of adsorption of enteric viruses to surfaces.

The solubilization of a hydrophobic substance in water is accompanied by an entropy decrease. This entropy decrease is produced by the reordering of the loose hydrogen bond network of liquid water around nonpolar solute particles to maximize hydrogen bonding among water molecules in the solvation layer surrounding the particles (25). Entropy reduction is a result of the limited configuration possibilities allowing the maximum amount of hydrogen bonding when solute particles are present. The association of one hydrophobic particle with another or the preferential adsorption of a virion to a sediment particle surface is favored thermodynamically because less reordering of water molecules is required than when a virion remains in suspension in the liquid phase. This hydrophobic bonding is a consequence of the thermodynamically unfavorable interaction of hydrophobic substances with water molecules and is not due to interactions among hydrophobic particles themselves (15).

Chaotropic compounds are low-molecular-weight ionic compounds which enhance the solubilization of hydrophobic substances in water. The mechanism for the action of chaotropes is in dispute but is generally believed to be due to the ability of certain anions to decrease the ordered structure of water, thereby decreasing the entropy barrier which inhibits the solubilization of hydrophobic substances in water (12). Chaotropic anions increase in potency with decreasing charge density, the strongest chaotropes possessing a single charge and a large ionic radius.

The inability of a number of chaotropic agents of varying potencies to elute poliovirus from sediment when employed alone suggests that hydrophobic interactions are not the predominant forces involved in preferential adsorption of viruses to sediments. When employed in conjunction with a proteinaceous material, however, chaotropic agents become powerful virus eluents. The action of beef extract, by which the elution efficiency of a chaotrope is enhanced, may result from the phenomenon through which soluble organic substances reduce the extent of adsorption of viruses to surfaces (2). This phenomenon is hypothesized to consist of competition between soluble organic compounds and viruses for specific adsorption sites on surfaces. Smaller protein molecules present in beef extract may intercalate between the larger virions and their adsorption sites on sediment particles and reduce the strength of their mutual attractive electrostatic forces. Chaotropic ions may further encourage desorption of virions by reducing the thermodynamic barrier inhibiting the movement of partially hydrophobic virions into the liquid phase. Therefore, both electrostatic and hydrophobic interactions are hypothesized to play a role in the adsorption and desorption of enteric viruses.

The ability of relatively weak chaotropic agents such as nitrate or chloride to be more effective than stronger chaotropes in certain systems has been noted by others (19). Weak chaotropes are hypothesized to enhance the solubility of hydrophobic substances more efficiently when both hydrophobic and electrostatic interactions are significant.

The potency of the nitrate-beef extract eluent was demonstrated by the difficulty encountered when the concentration of eluted viruses by organic flocculation was attempted. The presence of a chaotrope apparently reduced the extent of denaturation and subsequent flocculation of beef extract proteins at pH 3.5 and may also have inhibited virus adsorption to the floc that does form. The addition of divalent cations and DEAE dextran or an increase in beef extract concentration did not improve virus recovery. The addition of ammonium sulfate, an antichaotrope which alters water structure in such a way as to reduce the solubility of hydrophobic substances (11), countered the action of sodium nitrate and increased both flocculation and virus recovery. Virus recovery efficiency was further increased by the addition of a cationic polyelectrolyte in the presence of ammonium sulfate. The polyelectrolyte improved floc formation and probably enhanced virus adsorption to both the floc (as demonstrated by assay of supernatant fluids) and to cell monolayers (data not shown).

The poor recovery efficiencies of two alternative methods for virus recovery from estuarine sediments when tested in parallel with the procedure developed in this study may be due in part to the fact that each method was developed for sediments found at different geographical locations and possibly differing considerably in composition and adsorptive characteristics.

The ability of low potency chaotropic agents to act as efficient eluents of enteric viruses from estuarine sediments further demonstrates the utility of these agents in virus recovery procedures. Current techniques for the recovery of enteric viruses from shellfish (20, 24) include a step in which viruses are eluted from shellfish homogenates by using sodium chloride as an eluent. The mechanism for this step may involve the chaotropic action of the chloride ion. Studies in this (R. S. Moore, D. A. Wait, and E. H. Stokes, Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, Q55, p. 219) and other laboratories (8) indicate that chaotropic agents act as efficient virus eluents from membrane filters. The effectiveness of weak chaotropic agents as eluents of enteric viruses demonstrates the benefit that can be derived by recognizing both the hydrophobic and the hydrophilic character of enteric viruses and exploiting this dual character in virus recovery procedures.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant AI17277 from the National Institute of Allergy and Infectious Disease, by the Office of Sea Grant, National Oceanographic and Atmospheric Administration, U.S. Department of Commerce grant NA79AA-D-00048, and by the North Carolina Department of Administration. M.D.S. was a recipient of a Research Career Development Award from the National Institute of Environmental Health Sciences (5 KO4 ES00026).

LITERATURE CITED

1. **Berg, G., and D. R. Dahling.** 1980. Method for recovering viruses from river water solids. Appl. Environ. Microbiol. **39**:850-853.
2. **Bitton, G.** 1980. Adsorption of viruses to surfaces: technological and ecological implications, p. 332–373. *In* G. Bitton and K. C. Marshall (ed.), Adsorption of microorganisms to surfaces. John Wiley & Sons, Inc., New York.
3. **Bitton, G., Y. Chou, and S. R. Farrah.** 1982. Techniques for virus detection in aquatic sediments. J. Virol. Methods **4**:1–8.
4. **Cabelli, V. J.** 1980. Health effects quality criteria for marine recreational waters. EPA/600/1-80-031. U.S. Environmental Protection Agency, Cincinnati, Ohio.
5. **Cooper, R. C., K. M. Johnson, D. C. Straube, L. A. Brown, and D. Lysmer.** 1980. Development and evaluation of methods for the detection of enteric viruses in San Francisco Bay shellfish, water, and sediment. Sanitary engineering research laboratory report UCB/SERL 79-3. School of Public Health and College of Engineering, University of California, Berkeley.
6. **DeFlora, S., G. P. DeRenzi, and G. Badolati.** 1975. Detection of animal viruses in coastal seawater and sediments. Appl. Microbiol. **30**:472–475.
7. **Dougherty, W. J., and R. Altman.** 1962. Viral hepatitis in New Jersey. Am. J. Med. **32**:704–716.
8. **Farrah, S. R., D. O. Shah, and L. O. Ingram.** 1981. Effect of chaotropic and antichaotropic agents on the elution of poliovirus adsorbed to membrane filters. Proc. Natl. Acad. Sci. U.S.A. **78**:1229–1232.
9. **Goldfield, M.** 1976. Epidemiological indicators for transmission of viruses in water, p. 70–85. *In* G. Berg, H. L. Bodily, E. H. Lennette, J. L. Melnick, and T. G. Metcalf (ed.), Viruses in water, American Public Health Association, Washington, D.C.
10. **Goyal, S. M., and C. P. Gerba.** 1978. Detection and occurrence of enteric viruses in shellfish: a review. J. Food Prot. **41**:743–754.
11. **Hanstein, W. G.** 1979. Chaotropic ions and their interactions with proteins. J. Solid-Phase Biochem. **4**:189–206.
12. **Hatefi, Y., and W. G. Hanstein.** 1974. Destabilization of membranes with chaotropic ions. Methods Enzymol. **31**:770–790.
13. **Hatefi, Y., and W. G. Hanstein.** 1969. Solubilization of particulate proteins and nonelectrolytes by chaotropic agents. Proc. Natl. Acad. Sci. U.S.A. **62**:1129–1136.
14. **Katzenelson, E., B. Fattal, and T. Hostovesky.** 1976. Organic flocculation: an efficient second-step concentration method for the detection of viruses in tap water. Appl. Environ. Microbiol. **32**:638–639.
15. **Kauzmann, A.** 1959. Some factors in the interpretation of protein denaturation. Adv. Protein Chem. **14**:1–65.
16. **LaBelle, R. L., and C. P. Gerba.** 1980. Influence of estuarine sediment on virus survival under field conditions. Appl. Environ. Microbiol. **39**:749–755.
17. **LaBelle, R. L., C. P. Gerba, S. M. Goyal, J. L. Melnick, I. Cech, and G. F. Bogden.** 1980. Relationship between environmental factors, bacterial indicators, and the occurrence of enteric viruses in estuarine sediments. Appl. Environ. Microbiol. **39**:588–596.
18. **Lee, B., and F. M. Richards.** 1971. The interpretation of protein structures: estimation of static accessibility. J. Mol. Biol. **55**:379–400.
19. **Marguardt, H., C. B. Wilson, and F. J. Dixon.** 1973. Human glomerular basement membrane. Selective solubilization with chaotropes and chemical and immunological characterization of its compounds. Biochemistry **12**:3260–3266.
20. **Metcalf, T. G., E. Moulton, and D. Eckerson.** 1980. Improved method and test strategy for recovery of enteric viruses from shellfish. Appl. Environ. Microbiol. **39**:141–152.

21. **Portnoy, B. C., P. A. Mackowiak, C. T. Caraway, J. A. Walker, T. W. McKinely, and C. A. Klein, Jr.** 1975. Oyster-associated hepatitis: failure of shellfish certification program to prevent outbreaks. J. Am. Med. Assoc. **233**:1065–1068.
22. **Scraba, D. G.** 1979. The picornavirion: structure and assembly, p. 1–23. *In* R. Perez-Bercoff (ed.), The molecular biology of picornaviruses. Plenum Publishing Corp., New York.
23. **Smith, E. M., C. P. Gerba, and J. L. Melnick.** 1978. Role of sediment in the persistence of enteroviruses in the estuarine environment. Appl. Environ. Microbiol. **35**:685–689.
24. **Sobsey, M. D., H. R. Jensen, and R. J. Carrick.** 1978. Improved methods for detecting enteric viruses in oysters. Appl. Environ. Microbiol. **37**:121–128.
25. **Stillinger, F. H.** 1980. Water revisited. Science **209**:451–457.
26. **Stumm, W., and J. J. Morgan.** 1981. Aquatic chemistry, 2nd ed. Wiley-Interscience, New York.