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Elution of Viruses from Coastal Sediments

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Enteric viruses were eluted from estuarine sediments by using four organic mixtures; these solutions, with or without various supplements, were compared by determining their abilities to desorb virus from sediments taken from shellfish-harvesting sites. The least effective eluents consisted of glycine buffer, milk preparations, and beef extract paste. When virus type and sediment composition were taken into consideration, higher percentages of virus recovery were achieved with isoelectric casein, powdered beef extract, and nutrient broth mixtures. In addition to the type of eluent used, variations in virus recovery were due to the pH of the eluent, the composition of the sediment, and the type of virus being extracted. No clear distinction between the values of protein and inorganic ion supplements could be made.

The isolation of human enteric viruses from estuarine sediments (19, 26, 38) adds a new dimension to the overall complexity of sewage contamination of coastal waters. Enteric viruses appear to survive longer than indicator bacteria when they are attached to sediment particles rather than as free virions in seawater (23, 24, 29, 30, 37), and they exhibit type and strain dependence (15).

If the large numbers of enteric viruses that enter estuarine waters from sewage outfalls (10, 11, 17) readily attach to particulate matter, accumulate in sediments (23), persist for extended periods (29, 38, 40), and remain viable when they are attached to particulate matter (36), the potential for hydrotransportation to recreational waters or shellfish beds is increased. As demonstrated by the isolation of indigenous sediment-associated virus (6, 10, 17, 18), this type of contamination could explain the continuity of the low-level, natural viral isolations from oysters (12, 13, 16, 31, 34) observed when the animals are collected from approved growing waters.

The composition of estuarine sediment is an important factor in any study which examines viral elution, inactivation, or persistence (5, 23, 29, 30). Clays appear to play a major role in virus removal from seawater. The adsorption of viruses to sand is less efficient, and the role of silt in virus adsorption has yet to be examined.

The adsorption of viruses to clays is well documented (1, 2, 7, 14, 35), and the mechanisms by which viruses adhere to colloidal surfaces have been investigated (40, 41). Stotzky (40) has remarked that adsorption appears to be dependent on surface charge interactions wherein the charges on viruses are influenced by the PI or pK_a of the environment (ionic changes in virus surface components); the charges on crystalline clay minerals result primarily from isomorphous substitution within the clays, and the net electrical charge is negative (40). The cation exchange capacities of certain clays have been shown to affect the ability of the minerals to adsorb reovirus, but no conclusive evidence was found for T-1 phage, T-3 phage, or *Herpesvirus hominis* type 1 (41). Pretreatment of the clay with negatively charged compounds reduced the adsorption of phage to clay but did not reduce the amount of reovirus or herpes simplex virus type 1 adsorbed. These results indicate that reoviruses bind to the negatively charged sites on the clays and that other sites (or types of binding) are responsible for adsorption of these viruses (40, 41).

Demonstration of sediment-bound virus depends upon the elution and concentration procedures used (8, 18, 23), which have not been tested in collaborative efforts or compared by using sediments of varying composition or sediments from different geographic locations. Recently described extraction methods rely on alkaline-buffered eluents to desorb sediment-bound virus; virus concentration from the suspending fluid is usually accomplished by acid precipitation of soluble proteins.

In this study we examined the elution of viruses from Mississippi coastal sediments and compared previously described eluent mixtures.

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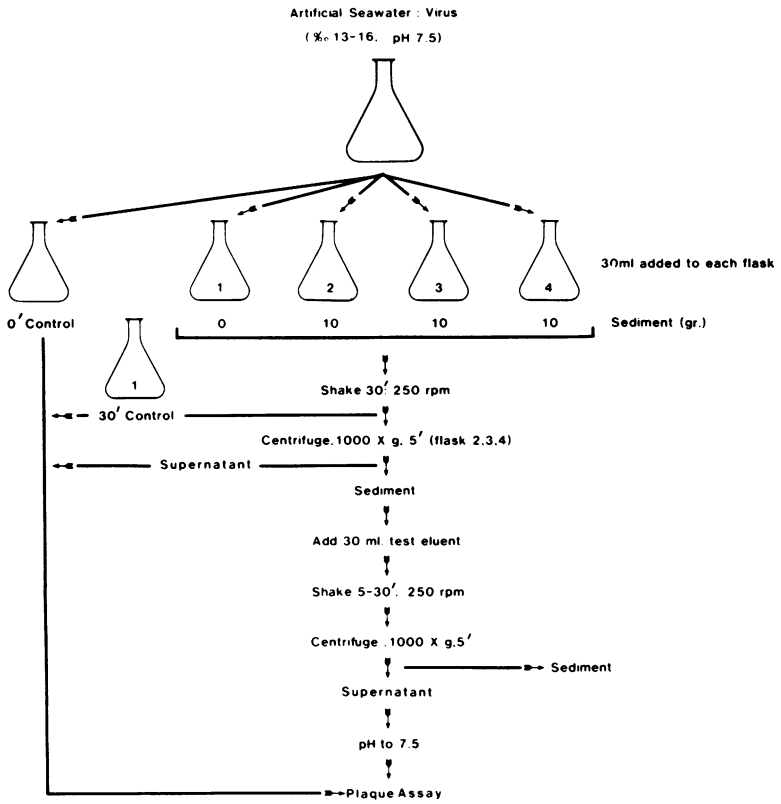

FIG. 1. Sediment extraction procedure.

The ultimate intent of this study was to examine the degree to which estuarine sediments play a role in the natural viral contamination of shellfish-growing and recreational waters.

MATERIALS AND METHODS

Sediment collection. Sediments were collected in an area (Bayou Graveline) south of Pascagoula, Miss., which was known to be contaminated with sewage. This location was selected because previous studies (12, 13) had demonstrated the presence of enteroviruses in oysters examined during a 24-month investigation. In this study, a procedure to evaluate the degree of sediment viral contamination was sought.

Sediments L2 and L3 were collected at the oyster-harvesting site used previously and at the mouth of Bayou Graveline; the average composition of sediment L2 was 10.1% sand, 48.2% silt, and 41.7% clay, and the average composition of sediment L3 was 79.2% sand, 11.8% silt, and 9.1% day. Samples were collected with a hand-held Ekman dredge (Wildco); the upper 2 to 3 cm of sediment was removed and placed into sterile plastic containers.

Sediment extraction. The procedure shown in Fig. 1 was used as the model for all experiments. Artificial seawater with a salinity range of 13 to 16 parts per thousand (13 to 16 mg/ml) and a pH of 7.5 was prepared by using Instant Ocean (Aquarium Systems, Eastlake, Ohio). A 30-ml portion of the seawater-virus mixture containing 100,000 to 1,000,000 PFU/ml was mixed with 10 g of sterile sediment. A flask containing no sediment was used as an input control. Dry sediment was prepared as previously described (19); sterile, wet sediment was prepared by autoclaving. Supernatant and control samples were collected as indicated in Fig. 1 and either plaqued immediately or frozen at −70°C.

Cell cultures and viruses. The Buffalo African green monkey kidney cell line (BGM cells) (9) (passages 120 to 200) was used in all viral experiments. Briefly, cells were grown in a minimal essential medium-medium L15 mixture (1:1) supplemented with 10% newborn calf serum, 1% 100÷ antibiotic-antimycotic mixture, 1% L-glutamine, and 1% sodium bicarbonate (7.5% solution), all of which were purchased from GIBCO Laboratories, Grand Island, N.Y. Viruses (poliovirus type 1, strain Sabin [ATCC VR-192] and coxsackievirus B3, strain Nancy [ATCC VR-30]) were purchased from the American Type Culture Collection, Rockville, Md., and were used to produce large, monodispersed pools whose volumes were sufficient to serve as inocula in all seeded experiments. The titers of the viral pools and the fluids associated with extraction studies were determined by a double-agar overlay plaque method. Cell cultures (a minimum of three replicates) were inoculated with 0.2 to 0.5 ml of sample and placed on a rocker platform for 1 h at 37°C. After the addition of overlay medium (12), the flasks were inverted and incubated at 37°C. Plaques were

TABLE 1. Summary of experiments designed to evaluate the elution of virus from sediment by glycine base eluents

Expt[a]	Sediment	Virus type[b]	Titer (PFU/ml)		% Recovery	
			Avg input	Avg recovery	Range	Mean
A	L3	P	1.2×10^6	1.6×10^4	0.4–4.3	1.3
B	L3	P	1.9×10^6	1.1×10^5	2.3–10.9	5.7
C	L3	C	6.5×10^5	2.8×10^4	3.4–4.8	4.3
D	L2	P	1.0×10^6	2.2×10^4	0.1–4.2	2.2
E	L2	C	3.6×10^5	2.8×10^4	4.2–11.1	7.7

[a] In experiment A the sediments were dried at 60°C. In experiments B through E the sediments were autoclaved.
[b] P, Poliovirus; C, coxsackievirus.

counted for 10 days or until no new plaques appeared for 2 consecutive days.

Eluent mixtures. The following four basic eluent solutions were used in this investigation: (i) 0.25% glycine buffer (Eastman Kodak, Rochester, N.Y.); (ii) milk proteins, including 0.5% skim milk (SM) and 0.5% isoelectric casein (IC; Difco Laboratories, Detroit, Mich.); (iii) beef extract (BE), including a paste preparation (Difco) and a powdered preparation (Inolex) (IBE); and (iv) nutrient broth (NB; Difco). The basic eluent solutions were modified by adding one or more of the following reagent grade substances: (i) EDTA (Sigma Chemical Co., St. Louis, Mo.); (ii) citric acid (Sigma); (iii) sodium oxalate (Sigma); (iv) fetal calf serum and newborn calf serum (GIBCO); (v) sodium dodecyl sulfate; and (vi) disodium phosphate (Fisher Scientific Co., Pittsburgh, Pa.). Unless otherwise stated, all eluents were prepared in Tris buffer (Bio-Rad Laboratories, Richmond, Calif.). The pH of each eluent was corrected with sterile 1 N NaOH or 1 N HCl.

RESULTS AND DISCUSSION

We tried methods used previously to elute enteroviruses from estuarine sediments (10, 18, 19) and similar methods used for soils and other solids (5, 22, 42), and none was suitable for extraction of virus from the estuarine sediments examined in this study. With each of these procedures we did recover sediment-bound virus, but the efficiency of recovery was low (0.1 to 1%).

Nevertheless, enteroviruses readily adsorbed to the Mississippi estuarine sediments, and the epidemiological and ecological concerns expressed previously (25) probably apply to this geographic location. The ability of a sediment to bind virus was not influenced by the sampling location or the method of sterilization of the sediment before viral addition and elution.

Poliovirus and coxsackievirus removal from seawater by each sediment type was consistent, averaging a 3-log decrease in the seawater virus titer. Virus adsorption to sediment was rapid, with an average of 92 to 95% of the viruses adsorbed during the first 1 min of shaking. When the average viral titers of the zero-time and 30-min seawater controls (no sediment) were compared, there was no significant difference between the virus levels.

Since most studies which have dealt with elution of viruses from estuarine sediments have utilized the glycine-EDTA procedure (17–19, 24–26), this method was analyzed for its ability to recover virus from sediments L2 and L3. Table 1 shows that recovery with this eluent was

TABLE 2. Summary of experiments to evaluate the elution of virus from sediments by solutions containing milk proteins

Eluent	Expt[a]	Sediment	Virus type[b]	Titer (PFU/ml)		% Recovery	
				Avg input	Avg recovery	Range	Mean
SM	A	L3	P	8.8×10^5	9.7×10^3	0.1–4.3	1.1
	B	L3	P	2.0×10^6	8.9×10^4	0.2–11.0	4.5
	C	L3	C	6.5×10^5	5.4×10^4	6.0–10.7	8.3
	D	L2	P	1.2×10^6	5.6×10^3	0.1–0.8	0.5
	E	L2	C	3.0×10^5	2.3×10^4	6.7–11.7	7.7
IC	F	L3	P	3.4×10^5	2.0×10^5	58.8	
	G	L3	C	2.8×10^5	5.3×10^4	18.9	

[a] In experiment A the sediment was dried at 60°C. In experiments B through G the sediment was autoclaved.
[b] P, Poliovirus; C, coxsackievirus.

TABLE 3. Elution of poliovirus from autoclaved sediments with BE paste

Sediment	BE concn (%)	Titer (PFU/ml)		% Recovery	
		Avg input	Avg recovery	Range	Mean
L3	3	1.1×10^6	4.6×10^4	2.5–6.6	4.2
L3	10	2.5×10^6	7.5×10^4	2.8–3.2	3.0
L3	10[a]	8.0×10^5	9.5×10^4	11.8	
L2	3	1.0×10^6	1.3×10^5	0.8–25.0	13.0

[a] Prepared in McIlvaine buffer (pH 7.0).

low but consistent (less than 12%). In experiment A, 0.25 M glycine buffer alone resulted in the lowest recovery from dry sediments (mean, 0.6%). Glycine–0.05 M EDTA, glycine–10% fetal calf serum, and glycine-EDTA-fetal calf serum mixtures gave mean recoveries of 1.6, 4.3, and 2.5%, respectively. When glycine eluents were used to extract coxsackievirus from autoclaved sediment L3 (Table 1, experiment C), the efficiencies of recovery were approximately the same as those observed in the poliovirus experiments. Similar trends were noted when each virus was eluted from sediment L2 (Table 1, experiments D and E). The highest percentage of recovery (11.1%) when glycine-EDTA was used as the eluent was observed when coxsackievirus was eluted from sediment L2. These experiments confirm the observations of Bitton and co-workers (6) concerning the use of glycine-EDTA as an eluent and indicate the need for eluent selection with regard to each sediment type under consideration.

Eluents containing milk proteins have been used to recover viruses from soils (5) and marine sandy sediments (6). Table 2 summarizes the results of experiments designed to test the efficiency of elution of viruses from coastal sediments with SM eluents. A mixture containing 0.5% SM (pH 9.0) did not appear to enhance the elution of virus from sediment L3 by SM mixtures. The recovery of poliovirus from autoclaved sediment L2 (Table 2, experiment D) by SM mixtures was poor (mean, 0.5%). Although the recovery of poliovirus was usually higher from sediment L3, the mean recoveries of coxsackievirus from sediments L3 and L2 were similar (Table 2, experiments C and E) (means, 8.3 and 7.7%, respectively).

IC eluent recovered greater amounts of virus from both types of sediment (Table 2) but appeared to favor the desorption of poliovirus.

The use of BE to recover viruses from soils (5, 27), river water solids (3), sludge (22, 43), wastewater (28), and sediments (6) is well documented. In this investigation, two types of BE (paste and powdered) were tested; the results of the experiments with Difco paste BE are shown in Table 3. We observed no significant difference when virus was eluted from sediment L3 with 3 and 10% BE solutions (pH 9.0). In addition, 10% BE did not produce higher recoveries when it was supplemented with either 10% newborn calf serum or 0.25% sodium dodecyl sulfate (3.0 versus 3.2%); however, when 0.05 M Na_2HPO_4 and 1.2 g of citric acid per liter (McIlvaine buffer; pH 7.0) were added to 10% BE, 11.8% of the seeded virus was recovered. Berg and Dahling (3) reported recoveries of poliovirus from river water solids by this method which ranged from 17.4 to 63%. When sediment L2 was eluted with similar mixtures, the highest recovery (25%) was associated with the use of 3% Difco BE containing 10% newborn calf serum.

The recovery of virus from sediment with powdered BE (IBE) was generally higher than the recovery observed when BE paste was used (Table 4). The elution of poliovirus by 3% IBE from sediments L2 and L3 ranged from 11 to 46% (mean, 35%); the levels of coxsackievirus recovery with this eluent were lower (sediment L3, 14%; sediment L2, 29%). The highest eluent pH used, 11.0, appeared to decrease the efficiency of virus recovery from sediment L3 and may have inactivated the virus during the 30-min elution period. The average levels of recovery of poliovirus from sediment L3 at pH values of 9, 10, and 11 were 41, 46, and 11%, respectively, and the average recovery from sediment L2 at pH 9 was 40%. Coxsackievirus recovery from

TABLE 4. Elution of poliovirus and coxsackievirus with 3% BE powder

Sediment	Virus type[a]	Eluent pH	Titer (PFU/ml)		% Recovery	
			Avg input	Avg recovery	Range	Mean
L3	P	9	1.3×10^6	5.3×10^5	25.0–52.0	41
		10	1.1×10^6	5.1×10^5	37.1–65.0	46
		11	1.0×10^6	1.1×10^5	10.1–12.0	11
L2	P	9	9.7×10^5	3.9×10^5	25.0–42.1	40
L3	C	9	4.4×10^5	6.0×10^4	12.1–15.0	14
L2	C	9	3.2×10^5	9.3×10^4		29

[a] P, Poliovirus; C, coxsackievirus.

TABLE 5. Effects of buffer, concentration, and pH on the elution of poliovirus and coxsackievirus from sediment by powdered BE mixtures

Sediment	Virus type[a]	Buffer	Eluting pH	IBE concn (%)	Titer (PFU/ml)		% Recovery (mean)
					Avg input	Avg recovery	
L3	P	Tris (0.2 M)	7	3	4.8×10^4	1.2×10^4	25
			8	3	4.8×10^4	1.5×10^4	31
			9	3	4.8×10^4	2.0×10^4	42
			11	3	4.8×10^4	1.7×10^3	4
		Glycine (0.25 M)	7	3	4.8×10^4	1.2×10^4	25
			9	3	4.8×10^4	1.6×10^4	33
		McIlvaine	7	3	6.2×10^4	2.9×10^4	32
			7	10	8.0×10^5	2.5×10^5	31
L3	C	Tris	9	3	3.3×10^5	4.3×10^4	13
L2	C	Tris	9	3	3.2×10^5	8.3×10^4	26

[a] P, Poliovirus; C, coxsackievirus.

sediments L3 and L2 by 3% IBE averaged 14 and 29%, respectively. When 3% IBE mixtures were prepared in other buffers, no major differences in the elution of poliovirus from sediment L3 were observed (Table 5). Tris and glycine buffers containing 3% IBE resulted in increases in virus recovery as the pH was increased to 9.0, with Tris-IBE producing the highest recovery (42%). McIlvaine buffer was as efficient as the Tris and glycine mixtures.

The addition of various supplements to IBE eluents did not produce dramatic increases in the recovery of either poliovirus or coxsackievirus from sediment L2 or L3 (Table 6); 3% IBE containing 0.025 M sodium dodecyl sulfate recovered 38% of the seeded virus, whereas the same eluent (at the same pH) containing 10% newborn calf serum recovered 18%. Overall, the presence of eluent supplements seemed to decrease the recovery of poliovirus from either sediment; coxsackievirus recovery remained at the same level for sediment L3, and recoveries were higher (26 versus 19%) from sediment L2 when IBE was used alone.

In the past many investigators have used 3% BE, but no specific concentration has been characterized as standard. Landry et al. (28) reported that powdered BE concentrations of less than 3% appeared to be as effective as 3% BE for virus reconcentration from wastewater effluent samples. The results of studies to determine the effect of IBE concentration on poliovirus elution are shown in Table 7. As the level of IBE increased from 1 to 15%, a gradual increase in the efficiency of recovery occurred. Concentrations of IBE greater than 15% were viscous, and the resultant lower percentages of virus recovery may have been due to interference with virus desorption from sediment or attachment of virus to BGM cells. A similar pattern of recovery was reported by Berg and Dahling (3) when 10 to 20% BE eluents were used.

The effect of time of elution and the use of different lots of IBE on the recovery of poliovi-

TABLE 6. Effects of supplements on the elution of viruses from sediments by powdered BE mixtures

Sediment	Virus type[a]	Eluent pH	IBE concn (%)	Supplement(s)[b]	Titer (PFU/ml)		% Recovery (mean)
					Avg input	Avg recovery	
L3	P	9	3	10% NCS	8.5×10^5	1.5×10^5	18
		10	3	10% NCS	8.5×10^5	2.3×10^5	29
		11	3	10% NCS	8.5×10^5	2.0×10^5	25
		9	3	0.25% SDS	2.5×10^6	3.3×10^5	13
		9	3	0.025% SDS	9.0×10^5	3.5×10^5	38
		9	3	10% NCS + 0.025% SDS	9.0×10^5	3.0×10^5	33
		9	10	0.25% SDS	2.5×10^5	3.8×10^5	15
L2	P	9	3	10% NCS	1.0×10^6	3.0×10^5	30
L3	C	9	3	10% NCS	3.3×10^5	4.2×10^4	13
L2	C	9	3	10% NCS	3.2×10^5	6.0×10^4	19

[a] P, Poliovirus; C, coxsackievirus.
[b] NCS, Newborn calf serum; SDS, sodium dodecyl sulfate.

TABLE 7. Effect of concentration of IBE at pH 9.0 on the elution of poliovirus from autoclaved sediment L3

IBE concn (%)	Virus input (PFU/ml)	Virus recovery (PFU/ml)	% Recovery
1	1.8×10^6	3.5×10^5	19.4
2	1.8×10^6	5.0×10^5	27.8
3	1.3×10^6	5.7×10^5	43.8
5	4.8×10^4	2.0×10^4	42.0
8	4.6×10^4	2.1×10^4	46.0
10	4.5×10^4	2.2×10^4	48.0
15	9.2×10^5	4.4×10^5	47.8
20	9.2×10^5	1.4×10^5	15.2
25	1.8×10^6	3.0×10^5	16.6
30	1.2×10^6	1.5×10^5	12.5
40	4.6×10^4	1.0×10^4	21.7

rus from sediment L3 were examined. Hurst et al. (22) reported that 30-s and 1-min mixing times could yield similar or higher results than longer periods of time (for example, 15 min) when they conducted studies on activated sludge. In this study, 3% IBE recovered the greatest amount of virus after 30 min of elution (43.8%). When the time of elution was increased to 45 min, the recovery decreased (34.2%). Elution times of 5 and 15 min produced poliovirus recoveries of 31.5 and 34.2%, respectively. The recovery of coxsackievirus from sediment L3 with 3% IBE averaged 13% after 30 min of elution. The results obtained with elution times of 5, 15, and 45 min did not vary from this average by more than 2%. It is possible that the low recoveries of virus by eluent mixtures other than IBE could be improved by varying the time of elution.

Berg and Dahling (3) reported that different lots of BE could have different eluting capacities, but six lots tested in this study recovered approximately the same quantities of poliovirus (average of all lots, 30%; range, 22.7 to 39.1%).

Elution of viruses with NB (Table 8) favored the recovery of poliovirus. The average recovery of poliovirus from sediment L3 was 53% (range, 25 to 63.2%). The lower recovery of poliovirus from sediment L2 (40.0%) was higher than the recovery of coxsackievirus from either sediment (sediment L3, 15.4%; sediment L2, 25.9%).

TABLE 8. Elution of viruses from autoclaved sediments by 4% NB (pH 7.5)

Sediment	Virus type[a]	Titer (PFU/ml)		% Recovery (mean)
		Avg input	Avg recovery	
L3	P	1.7×10^6	9.0×10^5	53
L2	P	1.0×10^6	4.0×10^5	40
L3	C	3.3×10^5	3.5×10^4	15.4
L2	C	3.2×10^5	8.3×10^4	25.9

[a] P, Poliovirus; C, coxsackievirus.

A comparison of all of the experimental eluents used to desorb virus from sediments L2 and L3 demonstrated that higher recoveries were achieved with organic eluents. The highest recoveries obtained in individual experiments were 63.2% (recovery of poliovirus from sediment L3 with 4% NB at pH 7.5) and 65% (recovery of poliovirus from sediment L3 with 3% IBE, pH 10.0). Glycine and SM solutions removed higher percentages of coxsackieviruses than polioviruses from both types of sediment but in an overall sense were not effective for virus removal.

As shown in Table 9, IC, NB, and IBE eluents resulted in the highest recoveries of poliovirus from sediment L3. The recoveries with BE paste, glycine, and SM mixtures were consistently low. The same trend was evident in the sediment L3 and coxsackievirus experiments and in the sediment L2 experiments. Overall, the recovery of coxsackievirus from either sediment was lower than the recovery of poliovirus. This finding was not consistent with the data which established the effect of eluent composition on virus viability (Table 10). The lowest percent loss of poliovirus (7%) occurred with 4% NB (pH 7.5); the highest losses of virus occurred in 0.25 M glycine (pH 11.0) and 0.5% IC (pH 9.0). The titer of coxsackievirus suspended in 4% NB (pH 9.0) dropped 10%, but no virus loss

TABLE 9. Eluent comparisons based on virus and sediment types

Virus type[a]	Sediment	Eluent	% Recovery (mean)
P	L3	IC	58.8
		NB	53.0
		IBE	32.7
		BE	4.0
		Glycine	3.5
		SM	2.7
	L2	IBE	40.0
		NB	40.0
		BE	9.3
		Glycine	2.2
		SM	0.5
C	L3	IC	18.9
		NB	15.4
		IBE	13.0
		SM	8.3
		Glycine	4.3
	L2	IBE	29.0
		NB	25.9
		SM	9.2
		Glycine	7.7

[a] P, Poliovirus; C, coxsackievirus.

TABLE 10. Effect of eluent composition on virus titer during a 30-min shaking period

Virus type[a]	Eluent	Avg recovery (PFU/ml)	Avg % reduction in titer
P	0.25 M glycine, pH 11.0	2.5×10^5	42
	3.0% IBE, pH 9.0	3.6×10^5	17
	4% NB, pH 7.5	4.0×10^5	7
	4% NB, pH 9.0	3.8×10^5	12
	0.5% IC, pH 9.0	2.7×10^5	37
C	3% IBE, pH 9.0	6.2×10^5	0
	4% NB, pH 7.5	6.8×10^5	—[b]
	4% NB, pH 9.0	5.6×10^5	10

[a] P, Poliovirus; C, coxsackievirus. The poliovirus inoculum contained 4.3×10^5 PFU/ml, and the coxsackievirus inoculum contained 6.2×10^5 PFU/ml.

[b] There was a 9% increase in titer.

occurred in either 3% IBE (pH 9.0) or 4% NB (pH 7.5).

The results of this investigation indicate the need to understand the nature of virus-sediment complexes. There is no doubt that differences in virus adsorption and elution (6, 39) are associated with differences in virus composition, but in the study of soil (4) and sediment virology, we will need a more complete understanding of the composition of the sediment matrix, not only the percentages of sand, silt, and clay. For example, the type of clay (1, 39–41), the presence of organic matter (20, 21, 23) and inorganic salts (25, 33), and the physiochemical conditions of adsorption and elution all affect the recovery of virus. These factors will be more important as future investigators attempt to determine the levels and persistence of viruses in contaminated soils and sediments.

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