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Method for Recovering Viruses from River Water Solids

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Small numbers of virions (poliovirus 1) that had been adsorbed to river water solids were eluted by mixing the solids for 30 min with a 10% solution of beef extract that contained sufficient Na_2HPO_4 to bring the molarity of the salt to 0.05 and sufficient citric acid to bring the pH to 7. The virions were recovered by inoculating the beef extract onto cell cultures. With this method, 39 to 63% of the poliovirions that had been adsorbed onto the river water solids were recovered.

Enteroviruses adsorb readily to many solids in rivers and in other waters. The numbers of virions recovered from the solids in river waters often exceed the numbers of virions recovered from the waters (1, 2, 4, 7, 8). If the viruses in the water environment tend to concentrate in and on the solids in the water, good techniques for recovering viruses from those solids must be developed.

This paper describes a method for recovering viruses from the solids in water.

MATERIALS AND METHODS

Virus. Two preparations of poliovirus 1 (Mahoney) were used in these studies. One had undergone 42 passages in cynomolgus monkey kidney and African green monkey kidney cell cultures. The other preparation had undergone one fewer passage in African green kidney cells and then a final passage in rhesus kidney cells.

Virion assays. Virions were assayed by the plaque technique. BGM cells in passages 222 to 227 were used for the assays. The method for preparing BGM cell cultures and the method for the plaque assay were described earlier (5).

Beef extract. Powdered beef extract (Lab-Lemco) was dissolved in distilled water, and the solution was autoclaved at 121°C for 15 min. In some studies, $Na_2HPO_4 \cdot 7H_2O$ and citric acid, in appropriate quantities, were dissolved with the beef extract powder into the water. The eluting capacity of each new lot of beef extract was compared with that of earlier lots to ascertain that its capacity to elute poliovirus 1 was maximal (3).

River water solids. Water from the Ohio River was collected in 50-gallon (188-liter) drums, and the solids in the water were settled for 4 days. The water above the settled solids was pumped from the drums, and the sediments were centrifuged in 250-ml round-bottom centrifuge bottles at 1,250 × *g* for 20 min. The water in the centrifuge bottles was decanted and replaced with sediments from the drums. The centrifugation was repeated. This process was continued until the centrifuge bottles were two-thirds full of packed sediments. The sediments were then stored under a thin layer of water at 4°C.

Elution of poliovirus 1 from river water solids. A 15-g sample of the wet river water solids was mixed with 200 ml of distilled water and inoculated with the poliovirions. The virions were mixed with the suspended solids for 30 min on a magnetic stirrer, and the suspension was filtered under suction through a Millipore AP20 fiber glass prefilter pad (diameter, 124 mm) that had been placed in a Büchner funnel. The solids were scraped from the pad. To elute the virions from the solids, the solids were mixed with beef extract or with beef extract with buffer on a magnetic stirrer. Unless otherwise noted, the volume of beef extract or beef extract with buffer used for eluting the virions from the solids was 40 ml, and the time of mixing was 30 min. The mixture was centrifuged at 4°C for 15 min at 1,250 × *g*. The supernatant was recovered and centrifuged at 4°C for 30 min at 15,000 × *g*. In one series of tests (Table 2, series 2), this second centrifugation was done at 140,000 × *g*. To remove contaminating bacteria, the supernatant was filtered through a Swinnex filter apparatus that contained a filter with a diameter of 47 mm and a pore size of 0.45 μm. The virions in the filtrate were assayed by the plaque technique on BGM cells. Unless otherwise noted, the numbers of virions seeded into each test were determined from assays of seed virus preparations diluted into the eluent used for the test (controls).

RESULTS

Elution of poliovirus from river water solids. (i) Elution with beef extract. A 3% solution of beef extract under sonication eluted all of the enterovirions adsorbed to cellulose nitrate membranes. Without sonication, lesser recoveries resulted (3). In repeated tests, a 10% solution of beef extract consistently eluted more of the poliovirions adsorbed to river water solids than a 3% solution of beef extract did (Table 1). Sonication did not improve elution (Berg and Dahling, unpublished data). The eluting effectiveness of the beef extract began to diminish when its concentration was somewhere in the 10 to 20% range (Table 1, series 3, 4, and 5).

Although the volumes of beef extract varied in series 5 because of some losses that occurred when the eluents were prepared, the data in that series suggest strongly that the numbers of viri-

TABLE 1. *Elution with beef extract of poliovirus 1 from river water solids*

Series no.	Test no.	Beef extract		Virions	
		Concn (%)	Vol (ml)	Seeded (PFU)[a]	Recovered (PFU)
1	1	3	40	102	24
	2	3	40		11
	3	10	40		37
2	1	3	40	82	0
	2	10	40		25
3	1	3	40	78	6
	2	5	40		16
	3	8	40		21
	4	10	40		24
	5	15	40		26
	6	20	40		22
4	1	10	40	216	71
	2	15	40		54
5	1	10	40	121	29
	2	20	25		16
	3	30	35		8
	4	40	30		3
	5	50	30		2

[a] PFU, Plaque-forming units.

ons recovered in elution diminished markedly with beef extract concentrations in excess of 20%. The consistency of beef extract solutions at the 40 and 50% levels was soupy, suggesting that osmotic pressure and possibly other physical factors may have destroyed virions or interfered with their recovery. It is also possible that in 40 and 50% solutions of beef extract, inhibitors reached concentrations that produced an observable effect.

(ii) Elution with beef extract in McIlvaine buffer. A 10% solution of beef extract in McIlvaine buffer of appropriate strength had a greater capacity than a 10% solution of beef extract alone to elute poliovirus 1 from the river water solids to which poliovirions had been adsorbed (Table 2). A 10% solution of beef extract that contained sufficient Na_2HPO_4 to bring the molarity of the salt to only 0.005 M and sufficient citric acid to bring the pH to about 7 did not elute more poliovirions than a 10% solution of beef extract alone did. However, a 10% solution of beef extract that contained sufficient Na_2HPO_4 to bring the molarity of the salt to 0.05 and sufficient citric acid to maintain the pH at about 7 consistently eluted more virions than a 10% solution of beef extract alone did. Since the pH levels of both solutions of beef extract were the same, it would seem that improved elution with the addition of the buffer reflected an effect of one or both of the buffer components.

A 10% solution of beef extract with sufficient Na_2HPO_4 to bring the molarity of the salt to 0.087 and sufficient citric acid to maintain the pH of the solution at 7.1 consistently eluted more virions than a 10% solution of beef extract alone did (Table 3).

Effect of elution interval on yield of poliovirions adsorbed to river water solids. A 10% solution of beef extract (pH 7.1) eluted at least as many poliovirions from river water solids to which the virions had been adsorbed in 30 min as in 60 or 90 min. Similar results were obtained when a 10% solution of beef extract in McIlvaine buffer (pH 7.1) was used as the eluent (Table 3). Studies were not done with shorter elution intervals, and it is possible that maximum elution may be achievable in less than 30 min.

Effect of pH on the elution with beef extract of poliovirions adsorbed to river water solids. In some situations, elution of virions from adsorbents increases at high pH levels. Therefore, studies were undertaken to determine whether elevated pH levels would increase the elution of poliovirions adsorbed to river water solids. Elution with a 10% solution of beef extract at about pH 7 yielded considerably greater numbers of virions than elution at pH levels of about 10 and 11 (Table 4). This was the case whether the 10% solution of beef extract was used alone or in McIlvaine buffer, which had a molarity of 0.13 with respect to Na_2HPO_4. The beef extract in McIlvaine buffer was again a more effective eluent than the beef extract alone.

Effectiveness of 10% beef extract in McIlvaine buffer for eluting poliovirus 1 from river water solids. In 30 min of mixing, 15 g of river water solids in 200 ml of distilled water adsorbed all or almost all of the poliovirus 1 added to such suspensions (unpublished data). Therefore, in determining the effectiveness of a 10% solution of beef extract in McIlvaine buffer for eluting poliovirions, all of the virions added to suspensions of river water solids were assumed to adsorb on the solids. Equal volumes of a suspension of virions were added to a volume of the eluent (control) and to the test system as described in Materials and Methods. The number of virions counted in the control was taken as the number of virions adsorbed to the river water solids in the test system. On this basis, the 10% beef extract in McIlvaine buffer recovered 39 to 63% of the seed poliovirus from the solids (Table 5). The strength of the buffer within the range tested did not affect viral recovery. If less than all of the poliovirions had adsorbed to the solids, then the effectiveness of the eluent would have been proportionately greater than that shown in Table 5.

TABLE 2. *Elution of poliovirus 1 from river water solids with 10% beef extract in McIlvaine buffer*

Series no.	Test no.	pH	Strength of buffer in eluent[a]	Virions	
				Seeded (PFU)[b]	Recovered (PFU)
1	1	7.0	None	178	68
	2	7.0	Na_2HPO_4 (0.05 M) Citric acid (1.2 g/liter)	204	128
	3	7.1	Na_2HPO_4 (0.005 M) Citric acid (0.12 g/liter)	204	55
2	1	7.1	None	210	26
	2	7.2	Na_2HPO_4 (0.05 M) Citric acid (1.2 g/liter)	178	31
	3	7.2	Na_2HPO_4 (0.005 M) Citric acid (0.12 g/liter)	194	30
3	1	7.1	None	216	71
	2	7.1	Na_2HPO_4 (0.05 M) Citric acid (1.2 g/liter)	242	107
	3	7.1	Na_2HPO_4 (0.005 M) Citric acid (0.12 g/liter)	229	57

[a] The volume of eluent in all tests was 40 ml.
[b] PFU, Plaque-forming units.

TABLE 3. *Effect of time on elution of poliovirus 1 from river water solids with 10% beef extract in McIlvaine buffer*

Test no.	Elution time (min)	Virions seeded (PFU)	Virions recovered (PFU) with eluent:[a]	
			Beef extract (pH 7.1)	Beef extract in McIlvaine buffer (pH 7.1)
1	30	202	67	
2	30	196		118
3	60	202	67	
4	60	196		119
5	120	202	60	
6	120	196		77

[a] The volume of eluent in all tests was 40 ml. Beef extract in McIlvaine buffer consisted of 10% beef extract and sufficient Na_2HPO_4 to bring the molarity of the salt to 0.087 and sufficient citric acid (1.25 g/liter) to bring the pH to 7.1. PFU, Plaque-forming units.

TABLE 4. *Effect of pH on elution with 10% beef extract of poliovirus 1 adsorbed to river water solids*[a]

Test no.	pH	pH of eluent adjusted with:	Virions seeded (PFU)[b]	Virions recovered (PFU)
1	7.0	Unadjusted	81	25
2	6.9	McIlvaine buffer[c]		36
3	9.8	Borate buffer[c]		2
4	11.1	NaOH		12

[a] The volume of eluent in all tests was 40 ml.
[b] The numbers of virions seeded were determined from assays of the seed virus preparation diluted into 3% beef extract. PFU, Plaque-forming units.
[c] McIlvaine buffer comprised 0.13 M Na_2HPO_4 and 3.0 g of citric acid per liter. Borate buffer comprised 0.016 M boric acid, 15 g of KCl per liter, and 8 g of NaOH per liter.

DISCUSSION

Commercially produced beef extracts are by their nature a variable commodity, and this has been reflected in the variable efficiency with which different lots elute viruses (3). Therefore, for seeding experiments, we test each lot of beef extract for its eluting efficiency with the virus under study. For field samples, it has been our practice to determine the eluting effectiveness of beef extract preparations for three different viruses of the family or families targeted for recovery and to use only lots with high recovery efficiencies. It is not clear yet whether the relative eluting ineffectiveness of many lots of beef extract reflects a lack of eluting factors in those lots or whether there is present in those lots materials that are virucidal or inhibitory to the multiplication of viruses. In any event, there is

TABLE 5. *Effectiveness of a 10% solution of beef extract in McIlvaine buffer (pH 7 ± 0.2) for eluting poliovirus 1 from river water solids*

Series no.	Strength of buffer	Virions (PFU)[a]		Elution interval (min)	PFU recovered (%)
		Adsorbed	Eluted		
1	Na_2HPO_4 (0.05 M) Citric acid (1.2 g/liter)	204	128	30	63
2	Na_2HPO_4 (0.05 M) Citric acid (1.2 g/liter)	242	107	30	44
3	Na_2HPO_4 (0.13 M) Citric acid (3 g/liter)	82	36	30	44
4	Na_2HPO_4 (0.087 M) Citric acid (1.25 g/liter)	196	118	30	60
5	Na_2HPO_4 (0.087 M) Citric acid (1.25 g/liter)	196	119	60	61
6	Na_2HPO_4 (0.087 M) Citric acid (1.25 g/liter)	196	77	120	39

[a] PFU, Plaque-forming units.

no guarantee that a lot of beef extract that yields high recoveries of viruses under one set of conditions will do so under others. Certainly, the recoveries of viruses from solids are not as good as they are from membrane filters (3). Moreover a lot of beef extract with high eluting effectiveness for some viruses may not be equally effective for others. And, of course, we should not expect that all viruses within the solids (some of which may be fecal material) in river water are even reached by the eluent.

In experimental laboratory studies, the effectiveness of the recovery technique may also be affected by the length of time that the viruses adsorb to the solids. We have found that the longer adsorption proceeds, the less of the seed virus we are able to recover (unpublished data).

Adsorption of viruses to solids is often best achieved at low pH levels, and elution is often practiced at high pH levels. In our studies, elution was more effective at pH 7 than at pH 10 to 11 (Table 4). It is not clear why. It may be that inhibitors or virucides were produced in the beef extract or potentiated at the higher pH levels.

The presence, in sufficient concentrations, of McIlvaine buffer (Na_2HPO_4 and citric acid) in a 10% solution of beef extract increased the virus-eluting capability of the extract. The phosphate in the buffer was not the effective agent (unpublished data). Whether the citric acid in the buffer increased the eluting effectiveness of the beef extract solution, and, if so, whether the citric acid alone is a good eluent, is yet to be determined. McIlvaine buffer, in the same strength used in the studies reported here, facilitates the adsorption of enteroviruses to cellulose nitrate membrane filters (3).

The technique described here, at least in laboratory studies, is an effective one. With this technique, we have recovered many different enteroviruses from river water solids. The method is not as practical as it needs to be when small numbers of viruses are to be recovered (especially for laboratories that must purchase cell cultures), because it requires large numbers of cell cultures for assay. At some cost in the recovery efficiency of viruses, the economic problem has been resolved by incorporating as a reconcentration procedure the organic flocculation technique of Katzenelson et al. (6). The results of those studies will be reported elsewhere.

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