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Protamine Precipitation of Two Reovirus Particle Types from Polluted Waters†

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Two forms of virus particle are released from reovirus-infected cell cultures, infectious reovirus and potentially infectious reovirus (PIV). PIV particle forms have a complete outer coat and are not infectious until the outer coat is altered or removed. The PIV concentration in polluted waters, however, has not been determined. Protamine sulfate precipitation, using 0.25% fetal bovine serum and 0.005% protamine sulfate for the first precipitation of the sample and 0.0025% for the second, was employed to concentrate infectious reovirus and PIV from water and sewage. Infectious reovirus and PIV particles were concentrated over 500-fold from river water inoculated with virus, and virus recoveries of between 80 and 100% were achieved. Virus precipitates stored at −20°C as a protamine-virus concentrate showed a 5% loss of PIV after 14 days. Virus preparations were assayed, before and after treatment, with 200 μg of chymotrypsin per ml, using a fluorescent-antibody procedure. Protamine sulfate precipitation and fluorescent-antibody detection are effective ways to recover and assay reoviruses present in raw sewage.

Extensive reuse of sewage effluent and other waters is inevitable. Procedures that effectively detect viruses in water are therefore becoming increasingly important. Since the methods used to concentrate and detect viruses in water tend to be specific for certain groups of viruses, it is the viruses most consistently and abundantly present in fecally polluted waters that must be recovered and identified.

In various parts of the world, reoviruses have been reported to occur as frequently as picornaviruses in waste waters and sewage (2, 4, 6, 8–10). Reoviruses can be recovered from lower animals as well as from humans (11), so fecal pollution from nonhuman sources can also be encountered. In 1972, England (2) developed a protamine sulfate method that effectively precipitates reoviruses and adenoviruses from sewage effluents and found reoviruses to be the most frequently present and abundant animal virus in sewage.

Infected cells release two types of reovirus particles: (i) infectious virus (IV) with a loose coat or no outer coat and (ii) potentially infectious virus (PIV), which has a complete outer coat and is not infectious (15). The PIV can be converted to IV by altering or removing the outer coat of the PIV with proteolytic enzymes. This enzymatically enhanceable reovirus fraction often accounts for over 90% of the total reovirus particles in infectious tissue culture fluids (14, 15).

The intact outer coat of PIV appears to make it more resistant to heat inactivation than is IV (16) and may account for its survival when it contacts viricidal agents in wastewater (1, 12, 18). Resistance to inactivation is a desirable characteristic of a virus to be used in water quality control testing. Nevertheless, reoviruses have received relatively little attention in water quality studies, and in none of these investigations have the PIV particles been considered.

In this study we modified the protamine sulfate concentration method of England (2) so IV and PIV particles were precipitated under conditions that permitted both viral forms to be assayed by an immunofluorescent cell-counting procedure. Immunofluorescent staining and counting of each infected cell circumvents the problem of overgrowth of reoviruses by other enteric viruses, as can occur in the plaque assay procedure (7). This assay technique also overcomes the problem of false plaque formation (K. F. Fannin, S. H. Abid, J. J. Bertucci, J. R. Reed, S. C. Vana, and C. Lue-hing, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, Q31, p. 200; and L. Y. C. Leong, S. J. Barrett, and R. R. Trussell, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, Q32, p. 200). Our procedure

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will enable the potential usefulness of reoviruses in water quality testing to be more precisely evaluated.

MATERIALS AND METHODS

Virus and cells. Plaque-purified type 1 (Lang strain) reovirus was used in all of the experiments. Virus stocks were prepared in roller bottle cultures of mouse L929 cells. Dense cell layers were incubated for 1 h at 37°C, with approximately 20 viral particles per cell. The basal medium of Eagle (GIBCO) containing antibiotics, and 2% fetal bovine serum (FBS), adjusted to pH 6.8 to 7.0 by using sodium bicarbonate, was added to infected cells. After being incubated for 24 h at 37°C, the infected cells were frozen and thawed six times and centrifuged to remove cell debris. The supernatant fluids were stored at −20°C in 2-ml aliquots.

Enzyme treatment and virus assay. Enhancement of the infectivity of reoviruses with chymotrypsin (Worthington Diagnostics) has been described (15, 16). Viral preparations were exposed to 20 to 200 μg of chymotrypsin (α-chymotrypsin from bovine pancreas, 3× crystallized; Worthington Diagnostics) per ml at 37°C for 1 h. Viral assays were performed both before and after chymotrypsin treatment, using Maden-Darby bovine kidney cells. To assay viral infectivity, we inoculated confluent cover slip cultures (15 mm diameter) with 0.02 ml of the viral suspensions (13). After 20 to 22 h, the cultures were fixed with acetone and stained with anti-reovirus fluorescent antibody. The number of fluorescing cells on each cover slip was counted and reported as immunofluorescent cell-forming units (IFU)/ml. Results of the assays were determined by averaging the counts taken from three cover slip cultures at each dilution.

Concentration procedures. The procedure of England (2) for concentrating viruses from sewage and effluents is as follows. Before water or sewage filtration, Tween 80 was passed through all of the prefilters (Millipore Corp., no. AP20) to prevent subsequent viral adsorption to the filter disks (17). Tween 80-treated prefilters alone were used for filtration of the sewage samples and for collection of the protamine precipitate. Sample volumes of sewage ranging from 200 to 2,000 ml were filtered through a double AP20 disk by vacuum. Bovine serum albumin was then added to the filtrate to a final concentration of 0.25%. The albumin-supplemented sample was adjusted to pH 7.5 to 7.8 with 1 N HCl or 1 N NaOH. A 1% stock solution of protamine sulfate (National Biochemicals Corp.) was added to the sample to a final concentration of 0.05% for the first precipitation of the sample, and 0.025% was added for the second precipitation. The solution containing protamine and the sample was stirred for 30 min at room temperature.

The precipitate was collected by passing the sample through a Millipore AP20 disk under negative pressure. After a second precipitation and filtration, the two precipitates on the upper surfaces of the AP20 disks were dissolved and collected in a test tube in the following manner. With the vacuum off, a small volume of 1 M NaCl (usually 0.5 ml) was pipetted over the surface of the AP20 disk, allowed to soak for about 5 min to dissolve the precipitates, and then drawn into the receiving tube, using vacuum. Sterile water, six times the volume of the 1 M NaCl, was pipetted over the surface of the prefilter disk and drawn into the test tube to produce an isotonic filtrate. FBS, 10% by volume, was added to stabilize the virus. The concentrated filtrate was centrifuged at 2,500 rpm for 30 min to sediment bacteria. The supernatant fraction was inoculated onto cell cultures (2).

Modifications of the above procedure to concentrate and assay the IV and PIV were as follows. (i) FBS low in reovirus antibody was used instead of bovine serum albumin at the same (0.25%) concentration. (ii) The protamine sulfate concentration was reduced to 0.005% for the first precipitation and 0.0025% for the second. Higher protamine and FBS concentrations interfered with enzymatic enhancement of viral infectivity and with the fluorescent cell assay. (iii) Two equal volumes of 1 M NaCl, with the total volume equaling 0.5 to 1.5 ml, were used to recover virus from the protamine precipitates. (iv) After centrifugation to remove any bacteria present in the precipitated sample, the supernatant was assayed on cover slip cultures by a fluorescent-antibody procedure (13) before and after chymotrypsin treatment of the virus concentrate. All of the results represent the averages of two to four experiments involving river water samples containing 500 to 4,000 ml.

River water. Class A water (class A water is that designated for protection of cold water species of game fish, water fowl, and agricultural use. In part, the quality restrictions require a biological oxygen demand < 5 mg/liter, $NO_3 < 4$ mg/liter, and $PO_4 < 0.25$ mg/liter) was collected from the Logan River, filtered through a 0.22-μm-pore-size Millipore filter to remove suspended matter, and then inoculated with reovirus. If the water samples were not filtered, 10 to 30% of the virus adsorbed to the suspended solids and could not be recovered after precipitation.

Viral aggregation. It has been shown that reoviruses aggregate when diluted less than 100-fold into distilled water (3). This aggregation is concentration dependent and does not happen at dilutions of 100-fold or greater (3). Dilutions of stock virus were kept between 500- and 4,000-fold in filtered river water containing 0.25% FBS at a pH between 7.5 and 7.8, and comparisons between infectivity titers of stock virus and river water inoculated with reovirus (the control) were checked for unusual differences.

Sonication. Several suspensions of reovirus in river water were sonicated to disrupt viral aggregates. Reovirus that had not been enzyme treated was diluted into 500 ml of filtered river water that contained 0.25% FBS at a pH between 7.5 to 7.8 and was mixed for 5 min. A 15-ml sample of the viral suspension was withdrawn, placed in a glass tube in an ice bath, and sonicated with the small probe of the Bronwill Biosonik III. Samples were sonicated repeatedly at a maximum sonicator setting for 15 s (total of 3.5 min). After every 30 s of sonication, a 1-ml sample was removed and assayed for IV and PIV.

Sewage samples. Raw sewage samples were collected from Salt Lake City and Dugway, Utah. Salt Lake City sewage, collected at the north treatment facility, is greatly diluted with drainage and industrial waste water. Dugway sewage dilution is restricted to household water. Samples from both cities were collected at the point of inflow before treatment. Grab samples,

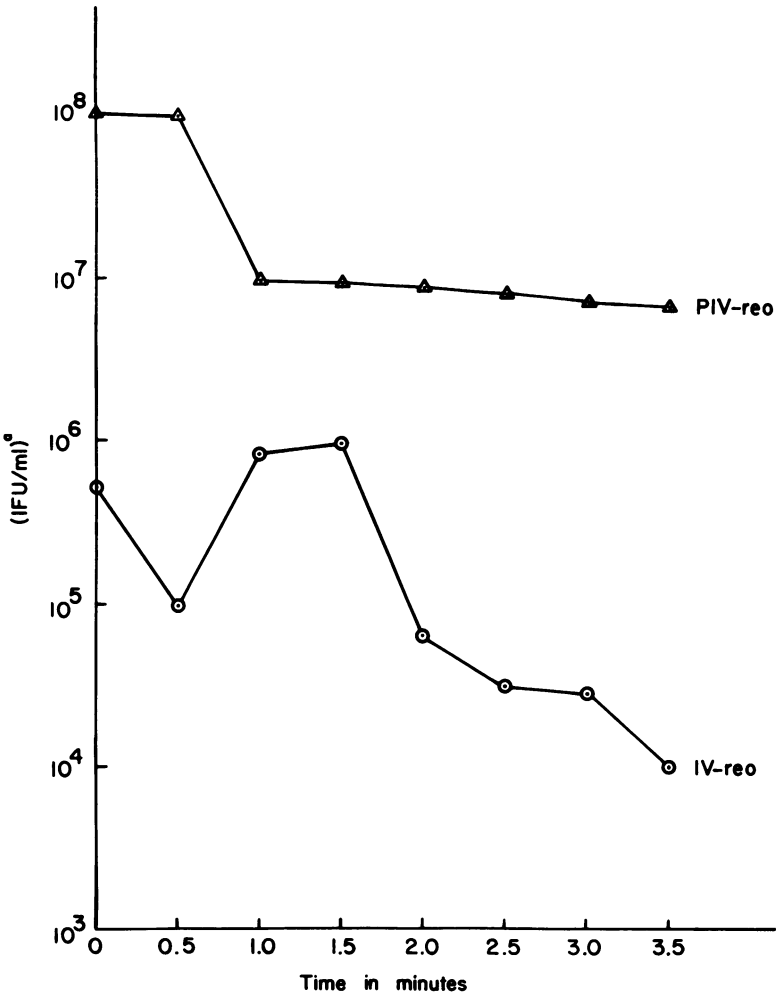

FIG. 1. Effects of sonication on reovirus. IV, Sonicated virus that was assayed before enzyme treatment; PIV, sonicated virus that was assayed after enzyme treatment. Results represent the average of two experiments. Virus controls without sonication were assayed at each time interval, and their titers remained unchanged throughout the experiments. IFU was determined by fluorescent-cell counts.

with volumes between 1,000 and 2,000 ml, were taken for testing.

RESULTS

Sonication studies of reovirus in river water. Suspensions of reovirus in river water were sonicated to disrupt viral aggregates. It appeared that, rather than breaking up aggregates, the sonication converted the PIV to IV and then inactivated the IV (Fig. 1). If viral aggregates had been dispersed, graphs of the infectivity assays of both IV and PIV would show initial increases in virus numbers. During the first half-minute of sonication, the IV was inactivated and the PIV resisted inactivation. The sonication evidently loosened the outer coat of about 90% of the PIV particles and converted them to IV particles during the next half-minute of sonication. This is shown by a loss of PIV and by an increase in IV. After PIV was converted to IV, the IV was inactivated. About 10% of the PIV resisted conversion to IV. Controls remained unchanged in titer throughout the experiments. These results indicated that our stock virus was not appreciably aggregated and would therefore permit a more than usual precision in evaluating the protamine precipitation method.

Protamine sulfate concentration of reovirus from river water. The protamine sulfate procedure of England (2) was used to precipitate IV and PIV added to river water. In these initial experiments, a heavy protamine floc was produced which rapidly plugged the AP20 filters. An additional problem was encountered when

TABLE 1. Influence of virus concentration on reovirus recovery from water by protamine precipitation

IFU/ml added to river water[a]	IFU/ml recovered in concentrate	Recovery (%)	SD	Reovirus particle form
5.1×10^4 (w/o ct)	4.4×10^4 (w/o ct)	86	±4.10	IV
1.0×10^7 (ct)	4.4×10^4 (ct)	<1		PIV
5.2×10^5 (w/o ct)	3.9×10^5 (w/o ct)	75	±6.16	IV
1.1×10^8 (ct)	3.8×10^5 (ct)	<1		PIV
5.2×10^6 (w/o ct)	3.5×10^6 (w/o ct)	67	±4.89	IV
1.0×10^9 (ct)	3.2×10^6 (ct)	<1		PIV
5.3×10^7 (w/o ct)	3.4×10^7 (w/o ct)	64	±7.54	IV
1.2×10^{10} (ct)	3.3×10^7 (ct)	<1		PIV

[a] w/o ct, Virus precipitated with protamine and assayed without chymotrypsin treatment; ct, virus precipitate assayed after treatment with 20 μg of chymotrypsin per ml for 1 h at 37°C. Four different virus concentrations were added to filtered river water and precipitated with protamine sulfate, with subsequent assay by fluorescent cell counts. After reovirus was added to filtered river water, the water was mixed for the duration of the experiment with protamine sulfate, filtered, and then assayed before and after chymotrypsin treatment. Experimental controls were treated in a similar manner, except that the protamine was not added.

cover slip cultures were inoculated with the dissolved protamine precipitate. A cloudy film formed and interfered with fluorescent-antibody staining used in the viral assay. Consequently, the concentrations of the reagents used in the protamine precipitation procedure were varied. FBS at 0.25% and protamine sulfate at 0.005% for the first precipitation and 0.0025% for the second precipitation resulted in clear cover slips for assay and precipitated between 64 and 86% of the IV. In these and other protamine experiments, virus concentration and efficiency of virus recovery were related (Table 1). The best viral recoveries were obtained when virus concentrations were low. In these experiments (Table 1), no PIV was detectable in the protamine concentrate, and less than 1% of either fraction was detected in the filtrate. Controls were run in which the virus was not precipitated by protamine, i.e., virus was added to filtered river water, mixed for the duration of the experiment, filtered, and then assayed before and after chymotrypsin treatment.

Percentages of recovery of IV (Table 1) were increased by 5 to 15% when two equal volumes of 1 M NaCl were used to elute the protamine precipitate from the AP20 disk instead of one elution with the same total volume. Each volume of 1 M NaCl elutant was left on the filters for 5 min before the elutant was dissolved. Protamine precipitate samples were collected by vacuum. The filter was then rinsed with 6 volumes of sterile water to achieve isotonic conditions in the filtrate.

Chymotrypsin treatment of reovirus before concentration with protamine. To determine why the PIV was not recovered in the protamine precipitation procedure (see Table 1), we treated the reovirus stock with 20 μg of chymotrypsin per ml before adding the stock to the filtered river water. This converted the PIV to IV. In this experiment, 79 to 95% of the total virus added was recovered (Table 2). As expected, the PIV that had been converted to IV with enzyme before being added to the river water was recovered. The control in the experiments was chymotrypsin-treated virus added to water, mixed for the duration of the experiment without protamine, filtered through an AP20 disk, and then assayed before and after a second chymotrypsin treatment of 20 μg/ml.

Increasing the chymotrypsin concentration to detect PIV. Table 3 shows the results of varying the amounts of chymotrypsin used to enhance the infectivity of the viral preparations. The best recovery was obtained by using 200 μg of chymotrypsin per ml, which is 10 times the concentration previously used. The efficiency of recovery again seems to be related to the viral concentration. The control for these experiments was the same as that used in the previous two experiments except that 200 μg of chymotrypsin per ml was used before assay of the PIV. These results suggested that the protamine or the FBS interfered with the enzymatic enhancement of the viral infectivity. Chymotrypsin was used at 200 μg/ml in all subsequent assays.

Stability of reovirus fractions in the protamine precipitate. The stability of reovirus in protamine precipitates was investigated to determine the feasibility of precipitating viruses from water in the field and for transporting the precipitate to

TABLE 2. Chymotrypsin treatment of reovirus (conversion of PIV to IV) before precipitation with protamine sulfate

IFU/ml added to river water[a]	IFU/ml recovered in concentrate	Recovery (%)	SD	Reovirus particle form
7.7×10^5 (ct-1)	7.3×10^5 (ct-1)	95	±2.49	IV
7.6×10^5 (ct-2)	7.3×10^5 (ct-2)	93	±2.23	IV
1.1×10^7 (ct-1)	8.7×10^6 (ct-1)	79	±3.26	IV
1.0×10^7 (ct-2)	8.6×10^6 (ct-2)	84	±5.09	IV

[a] ct-1, Virus assayed after protamine precipitation and before a second chymotrypsin treatment; ct-2, virus assayed after protamine precipitation and a second chymotrypsin treatment. After chymotrypsin-treated reovirus was added to filtered river water, the water was mixed for the duration of the experiment with protamine sulfate, filtered, and then assayed before and after a second chymotrypsin treatment. Experimental controls were treated in a similar manner except that the protamine was not added.

the laboratory. The viral concentrate, with 5% FBS, was stored in glass tubes after elution from the prefilter. The stability of both IV and PIV in the protamine precipitates is shown in Table 4. The results show that the samples can be stored at −20°C or 4°C for at least 18 days with only a small loss of infectivity.

Concentration of reovirus from raw sewage. The modified protamine concentration procedure and the fluorescent-assay method were used to concentrate and assay 10 raw sewage samples from Utah sewage treatment facilities (Table 5). Five samples from Salt Lake City and five samples from Dugway, Utah, were collected. Three of the five samples collected at Salt Lake City contained detectable IV. In two of these samples, virus was also detected after enzyme treatment, at the same or lower concentration than without the enzyme treatment. The IV concentrations ranged from 2×10^3 to 5×10^3 IFU/liter. Four out of five samples collected at Dugway contained reoviruses. Three samples contained detectable IV, ranging in concentration from 0.32×10^5 to 1.2×10^5 IFU/liter. In one sample, PIV alone was detected at a concentration of 2.1×10^5 IFU/liter. These results

TABLE 3. Detection of PIV in the protamine precipitate by increasing the chymotrypsin concentration

Chymotrypsin concn (μg/ml)	IFU/ml added to river water[a]	IFU/ml recovered in concentrate	Recovery (%)	SD	Reovirus particle form
20	9.3×10^6 (w/o ct)	6.3×10^6 (w/o ct)	68	±5.31	IV
	8.5×10^8 (ct)	6.1×10^6 (ct)	<1		PIV
40	9.2×10^6 (w/o ct)	6.4×10^6 (w/o ct)	69	±5.56	IV
	8.5×10^8 (ct)	6.2×10^6 (ct)	<1		PIV
60	9.4×10^6 (w/o ct)	4.4×10^6 (w/o ct)	47	±11.04	IV
	8.4×10^8 (ct)	4.3×10^6 (ct)	<1		PIV
80	9.3×10^6 (w/o ct)	2.9×10^6 (w/o ct)	31	±11.95	IV
	8.4×10^8 (ct)	2.7×10^6 (ct)	<1		PIV
100	9.2×10^6 (w/o ct)	3.8×10^6 (w/o ct)	41	±9.39	IV
	8.7×10^8 (ct)	3.5×10^6 (ct)	<1		PIV
100[b]	4.6×10^4 (w/o ct)	3.7×10^4 (w/o ct)	80	±3.68	IV
	4.3×10^5 (ct)	3.8×10^4 (ct)	9	±5.09	PIV
150[b]	4.6×10^4 (w/o ct)	3.9×10^4 (w/o ct)	85	±4.34	IV
	4.2×10^5 (ct)	4.3×10^4 (ct)	10	±4.82	PIV
200	9.3×10^6 (w/o ct)	7.4×10^6 (w/o ct)	80	±3.24	IV
	8.5×10^8 (ct)	8.4×10^8 (ct)	99	±1.09	PIV
200[b]	4.5×10^4 (w/o ct)	4.0×10^4 (w/o ct)	89	±4.21	IV
	4.3×10^5 (ct)	4.3×10^5 (ct)	100	±0.43	PIV

[a] w/o ct, Virus precipitated with protamine and assayed without chymotrypsin treatment; ct, virus precipitated with protamine and assayed after chymotrypsin treatment.

[b] Observations taken from a second set of experiments, using a different viral stock containing fewer IV and PIV.

TABLE 4. Stability of reovirus in the dissolved protamine precipitate stored at 4°C and −20°C

Storage[a] conditions	Storage time (days)	IFU/ml remaining[b] in concentrate	Initial viral fraction remaining infectious (%)	Reovirus particle form
4°C	0	2.0×10^6 (w/o ct)	100	IV
		2.0×10^8 (ct)	100	PIV
	4	2.0×10^6 (w/o ct)	100	IV
		2.0×10^8 (ct)	100	PIV
	14	2.4×10^6 (w/o ct)	120[c]	IV
		1.6×10^8 (ct)	80	PIV
	18	2.6×10^6 (w/o ct)	130[c]	IV
		1.6×10^8 (ct)	80	PIV
20°C	0	2.0×10^6 (w/o ct)	100	IV
		2.0×10^8 (ct)	100	PIV
	4	2.0×10^6 (w/o ct)	100	IV
		2.0×10^8 (ct)	100	PIV
	14	2.1×10^6 (w/o ct)	105[c]	IV
		1.9×10^8 (ct)	95	PIV
	18	2.1×10^6 (w/o ct)	105[c]	IV
		1.8×10^8 (ct)	90	PIV

[a] Protamine-precipitated samples were eluted from the prefilters and stabilized with 5% FBS low in reovirus antibody.

[b] Numbers represent the average IFU/ml of two samples. w/o ct, Virus assayed without chymotrypsin treatment; ct, virus assayed with chymotrypsin treatment at 200 μg/ml for 1 h at 37°C.

[c] Conversion of PIV to IV during storage.

indicate that different concentrations of chymotrypsin might be added to accurately assay the PIV in sewage samples from different locations.

DISCUSSION

Since a single procedure will not efficiently recover and detect water-borne viruses of all groups, it is important that a standard method for determining water safety be developed, using viruses that best serve as indicators of fecal pollution.

We recommend that reoviruses be given serious consideration for such use because (i) they are abundant and occur consistently in sewage (2, 4, 6, 8–10). (ii) They rarely cause clinical infections, so are relatively safe to handle in the laboratory and will not be eradicated by vaccine programs. Therefore, standard tests using reoviruses are not likely to have to be changed in the future. (iii) Reoviruses have a broad host range, so fecal contamination from humans and lower animals can be detected. (iv) Detection is relatively inexpensive, since most continuous mammalian cell lines are susceptible to reoviruses. (v) Available immunofluorescent cell assay techniques can demonstrate reovirus antigen after 8 to 10 h so these assays yield quantitative data within 24 h (7, 14). (vi) The PIV particles have an intact double coat (15) that makes them exceptionally thermostable (16).

In this report, we described a modified protamine sulfate procedure for precipitating reoviruses from polluted water and a fluorescent-antibody method for assaying the precipitated virus. The procedures concentrate reoviruses more than 500-fold, recover between 80 and 100% of IV and PIV fractions, and can yield quantitative data within 24 h.

Application of these techniques to raw sewage successfully recovered both the IV and PIV. Inconsistent results were sometimes obtained when the sewage precipitates were treated with chymotrypsin. In experiments using chymotrypsin to increase the infectivity of laboratory-produced virus, the infectivity of IV was not lost. With some of the sewage isolates, however, the IV and PIV, if present, were lost upon treatment with 200 μg of chymotrypsin per ml. This could be caused by any of several factors, including heterogeneity in the components of raw sewage, the virus existing in a semi-weakened state (having altered outer or inner [or both] capsids), or the presence of chymotrypsin negative reovirus. Chymotrypsin treatment of the chymotrypsin negative reoviruses inactivates rather than enhances the virus (18).

A range of chymotrypsin concentration from 50 to 200 μg/ml should be considered when enhancing the infectivity of reoviruses precipitated from sewage. In addition, all of the sam-

TABLE 5. Protamine sulfate precipitation of reovirus naturally occurring in raw sewage

Location	Sewage[a] sample	(IFU/liter) recovered from raw sewage	Reovirus particle form
Dugway, Utah	1	<400 (w/o ct)[b]	IV
		2.1×10^5 (ct)	PIV
	2	<400 (w/o ct)	IV
		<400 (ct)	PIV
	3	1.2×10^5 (w/o ct)	IV
		<400 (ct)	PIV
	4	1.5×10^5 (w/o ct)	IV
		<400 (ct)	PIV
	5[c]	3.2×10^4 (w/o ct)	IV
		3.0×10^4 (ct)	PIV
Salt Lake City, Utah	1	<400 (w/o ct)	IV
		<400 (ct)	PIV
	2	2.0×10^3 (w/o ct)	IV
		1.6×10^3 (ct)	PIV
	3	4.0×10^3 (w/o ct)	IV
		4.0×10^3 (ct)	PIV
	4	<400 (w/o ct)	IV
		<400 (ct)	PIV
	5	5.0×10^3 (w/o ct)	IV
		<400 (ct)	PIV

[a] Two 1,000-ml samples were used for each determination. The average is recorded in the table. Final processed sample volumes were 8 ml.

[b] 400 IFU/liter represents the detection limit of this immunofluorescent assay of 50 IFU/ml, employing an assay volume of 20 μl. w/o ct, Virus precipitated with protamine and assayed before chymotrypsin treatment; ct, virus precipitated with protamine and assayed after chymotrypsin treatment at 200 μg/ml for 1 h at 37°C.

[c] This sewage sample was stored for 3 weeks at −20°C before precipitation and assay.

ples should be assayed before and after chymotrypsin treatment. Such processing will demonstrate which viral fraction (IV or PIV) is recovered and will detect the loss of chymotrypsin negative reovirus by inactivation with chymotrypsin.

Our recovery of more than 10^5 infectious reoviruses per liter of untreated sewage (Table 5) suggests that the modified protamine precipitation and fluorescent-antibody staining procedures can be useful in determining the potential roles of the IV and PIV in water quality testing. England (2) pointed out that volumes which can be concentrated with this type of procedure fall short of what is needed to detect minimal pollution in recreational and culinary waters, but would be effective when run in parallel with or in conjunction with other large-volume concentration procedures. However, the protamine procedure could possibly be used with large volumes of water by collecting the precipitates on pleated filters or by tangential flow filtration.

Reports indicate that incoming virus loads in raw sewage range from several thousand to several hundred thousand per liter (5). Without examination of other virus populations at the locations checked in our study, reoviruses cannot be assumed to be the most abundant animal virus present. Nevertheless, their importance in these systems is evident. The results from Dugway, Utah, also indicate that the viral concentrations in undiluted raw sewage might be higher than previously expected.

During storage of the protamine concentrates, there was an increase of 5% in the IV and a 10% loss of the PIV after 18 days at −20°C. Both fractions showed more pronounced changes when stored at 4°C; i.e., the IV increased 30% and the PIV decreased 20%. These changes might be due to a conversion of the PIV to IV or a combination of events including antibody reactions and disruption of aggregates. Regardless, these results show that it would be feasible to precipitate reoviruses from water and only bring the protamine-virus precipitate to the laboratory.

Storage of a prefiltered raw sewage sample at 4°C with 0.25% FBS (Dugway sample no. 1) showed a conversion of PIV to IV within 10 days followed by a loss of infectivity of IV virus over 21 days. These observations might be explained by a number of factors: (i) reaction with antibody present in the FBS added, (ii) activation by naturally occuring proteolytic enzymes, with

subsequent inactivation, (iii) adsorption to the suspended matter and aggregation, or (iv) a combination of the above factors.

It would be premature at this time to speculate on the overall presence or absence of PIV in polluted waters. A more extensive investigation of waters and wastewaters is needed, with emphasis on the stability of IV and PIV in sewage. Currently, wastewaters are being examined for the presence of both the adsorbed and free IV and PIV fractions to further evaluate reoviruses as an indicator of viral pollution in the environment.

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