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pH Modification of the Effects of Detergents on the Stability of Enteric Viruses

RICHARD L. WARD[1]* AND CAROL S. ASHLEY[2]

Division 4535, Sandia Laboratories, Albuquerque, New Mexico 87185,[1] and University of New Mexico, Albuquerque, New Mexico 87131[2]

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The effect of detergents on the stability of enteric viruses was found to be highly dependent on pH. This was demonstrated primarily with two ionic detergents, sodium dodecyl sulfate (an anionic detergent) and dodecyltrimethylammonium chloride (a cationic detergent). Both detergents were shown to be potent virucidal agents for reovirus, but the effects of sodium dodecyl sulfate were minimal near neutrality and much more pronounced at low than at high pH values. Dodecyltrimethylammonium chloride was extremely virucidal at high pH's but had little observable effect on reovirus stability at low pH values. In contrast, both detergents protected enteroviruses against heat at neutral and alkaline pH's. However, as was found with reovirus, sodium dodecyl sulfate was extremely virucidal at pH values below 5, even when the virus samples were incubated in ice. At different pH's the effects of detergents on the stabilities of coliphages T4, f2, and Qβ were qualitatively similar to those found with reovirus. Differences in viral stability in these experiments appeared to be due to the effects of pH on the ionic states of the viral capsid proteins.

Pathogens in wastewater sludge can pose a health hazard to persons involved in sludge utilization. The fate of one type of pathogen, the enteric virus, during a number of different treatment processes has already been studied, and the results have been published in a series of articles. Recently it was found that reovirus, an enteric virus commonly isolated from sewage, is inactivated by heat at much lower temperatures in sludge than in buffer at the same pH (19). The agents in sludge responsible for this effect were subsequently identified as ionic detergents (20). Many of the detergents that reduce the heat required to inactivate reovirus were found to protect poliovirus and other enteroviruses against heat (20). This led to the conclusion that detergents are one of the substances protective of poliovirus during heat treatment of liquid (22) and dried (21) raw sludge.

It was also noted during these studies that the effects of sludge on heat inactivation of reovirus are pH dependent; that is, raw sludge was much more virucidal at pH 8.5 than at pH 6 (19). To help explain this finding, an extensive study was made to determine the effects of two ionic detergents on thermal inactivation of enteric viruses. These detergents, sodium dodecyl sulfate (SDS), an anionic detergent, and dodecyltrimethylammonium chloride (DTA), a cationic detergent, have distinctly different effects on the stability of viruses, and these effects are extremely pH dependent, as is shown in this report.

MATERIALS AND METHODS

Viruses, cells, and bacteria. Four strains of enteric viruses and three strains of bacteriophages were used in these experiments. Enteroviruses (poliovirus type 1 strain CHAT, poliovirus type 2 strain 712-Ch-2ab, and coxsackievirus B1 strain Connecticut 5) were grown and assayed for biological activity in HeLa cells as previously described (16, 17). Reovirus was grown and assayed in L-cells, as described previously (18). The three bacteriophage strains used (T4, f2, and Qβ) were all grown and tested for infectivity by the plaque assay on *Escherichia coli* strain A-19. Radioactively labeled virus preparations made with either [^{3}H]uridine or ^{14}C-reconstituted protein hydrolysate (Schwarz mixture) were grown and purified as described earlier (16).

Virus inactivation studies in detergents or sludge at different pH values. To determine the effects of pH on virus inactivation, a 10-fold (enteroviruses and reovirus) or a 100-fold (bacteriophages) dilution of viral lysate was made directly into a 0.1 M solution of buffer at the appropriate pH, both in the absence and presence of 0.1% (wt/vol) detergent. The buffers used were acetate (pH 2 to 5.5), phosphate (pH 6 to 7), tris(hydroxymethyl)aminomethane (Tris) (pH 7.5 to 9), and borate (pH 9.5 to 10). The samples were then incubated for the times and at the temperatures specified, sonicated, and assayed for recoverable plaque-forming units. The pH of each treated

sample was also measured after treatment, and these are the values presented. Virus recoveries were determined relative to an untreated control sample, and the results were calculated relative to that value. It should be noted that the amount of virus inactivation was not affected by the buffer type because different buffers at the same pH yielded the same results.

Analysis of radioactively labeled poliovirions and subviral components. Sedimentation analysis of viral particles and components, phenol extraction of viral ribonucleic acid (RNA), and infectivity analyses of poliovirus RNA were carried out as previously described (16).

RESULTS

pH effects on reovirus inactivation in the presence of detergents. In the report that identified ionic detergents as components of wastewater sludge that reduce the heat required to inactivate reovirus (20), all experiments were carried out at pH 8.5. A constant pH was used because a previous study indicated that the rate of reovirus inactivation with heat in sludge was pH dependent (19). However, the effects of pH on the virucidal activities of ionic detergents had not been determined. Although nonionic detergents were not found to affect the rate of heat inactivation of reovirus at pH 8.5, the possibility that they may be virucidal at pH values above or below 8.5 had not been investigated. The effect of pH on the virucidal activities of standard anionic, cationic, and nonionic detergents was, therefore, tested. The ionic detergents chosen for this study were SDS and DTA, both of which remain ionized at all pH values examined (pH 2 to 10). This eliminated the possibility that a change in viral stability caused by an alteration in pH could be due to a change in the ionic state of the detergent molecules.

SDS was found to accelerate heat inactivation of reovirus at all pH values examined except, perhaps, at pH 2, where the recovery of infectious virus was below detectable limits in all samples (Fig. 1). DTA, on the other hand, was extremely virucidal at high pH values, but its effect gradually decreased with a decrease in pH. At pH 4, DTA had little effect on the heat stability of reovirus.

The dependence of reovirus stability on pH demonstrable at 45°C was also apparent when the samples were kept in an ice bath. At this temperature only DTA caused a significant reduction in reovirus infectivity at pH values between 7 and 10, but at pH's below 6, SDS was extremely virucidal (Fig. 2).

These results indicate that both anionic and cationic detergents more effectively destabilize reovirus at pH values where their charges are opposite that of the viral capsid, i.e. at acidic pH's for SDS and at alkaline pH's for DTA. It should also be noted that SDS, but not DTA, had its minimum effect on reovirus near neutrality (Fig. 1). The same observation was made

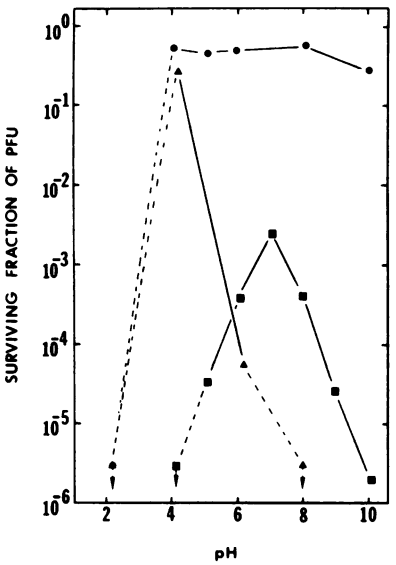

FIG. 1. *Effect of pH and ionic detergents on heat inactivation of reovirus. A preparation of reovirus was diluted 10-fold into 0.1 M buffer alone (●) or buffer containing 0.1% SDS (■) or 0.1% DTA (▲), incubated for 20 min at 45°C, and assayed for recoverable plaque-forming units (PFU).*

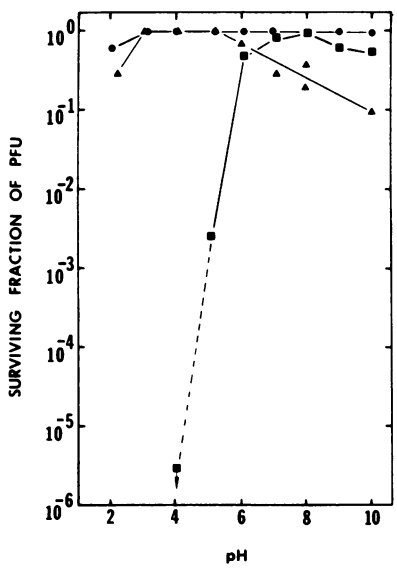

FIG. 2. *Effect of pH and ionic detergents on the infectivity of reovirus at 4°C. The symbols are the same as described in the legend to Figure 1. PFU, Plaque-forming units.*

when this experiment was repeated with another anionic detergent, sodium lauroyl sarcosine (data not shown). As shown below, maximum stability in the presence of SDS occurred near neutrality for all viruses examined in this report.

The possibility that nonionic detergents affect the stability of reovirus at some pH between 2 and 10 was also examined. The two nonionic detergents tested, Igepal Co-630 and Ethosperse LA-4, were chosen because the hydrophobic portions of these molecules are identical to those of several ionic detergents previously shown to be virucidal for reovirus (20). Neither nonionic detergent significantly affected the stability of reovirus at any pH examined (Table 1). This result indicates that a detergent must be ionized in order to cause destabilization of reovirus within the pH range studied here.

pH modification of the effects of ionic detergents on poliovirus stability. In an earlier study (19), it was shown that at pH 8.5 poliovirus and other enteroviruses are protected against heat inactivation by a number of detergents, including SDS and DTA. However, Mandel has reported (9–11) that poliovirus is destabilized by SDS at pH values below about 5, and others (13) have shown that poliovirus is more rapidly inactivated at pH 10.5 than at pH 8.0 by quaternary ammonium compounds structurally related to DTA. To clarify these results, the effects of SDS and DTA on poliovirus stability were measured between pH 2 and 10.

Inactivation of poliovirus type 1 strain CHAT by heat in the absence of detergents was found to occur more rapidly at pH values above 6 than at pH's of 3 to 5 (Fig. 3A). In contrast, significant stabilization of this virus by both SDS and DTA was observed only at pH's of about 6 or greater. At pH's below 6, DTA had little effect on viral stability and, in agreement with the results of Mandel, SDS was extremely virucidal. This virucidal effect of SDS at low pH's was also quite

TABLE 1. *Effect of nonionic detergents on heat inactivation of reovirus as a function of pH*[a]

pH	% Recovery of virus with:		
	Buffer	Igepal Co-630[b]	Ethosperse LA-4[c]
2	<0.0002	<0.0002	<0.0002
4	70	89	62
6	88	100	88
8	84	107	83
10	4.6	4.9	5.4

[a] Reovirus was diluted 10-fold into buffered solutions containing no detergent (control) or 0.1% (wt/vol) detergent, heated at 45°C for 20 min, and assayed for recoverable plaque-forming units.

[b] Igepal Co-630: $CH_3(CH_2)_8C_6H_4O(CH_2CH_2O)_9H$, a product of GAF Corp.

[c] Ethosperse LA-4: $CH_3(CH_2)_{11}O(CH_2CH_2O)_5H$, a product of Glyco Chemicals, Inc.

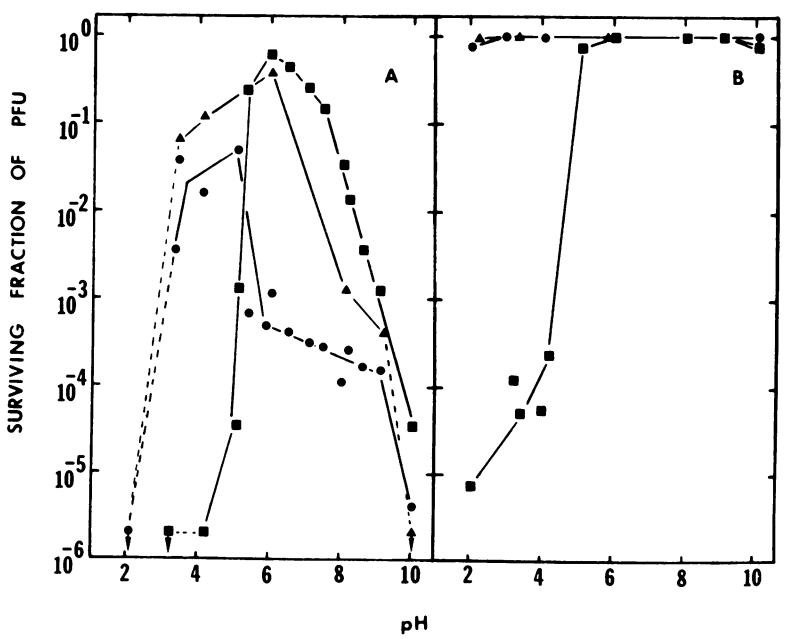

FIG. 3. *Effect of pH and ionic detergents on the infectivity of poliovirus type 1. A virus preparation was diluted 10-fold into 0.1 M buffer (●) or buffer containing 0.1% SDS (■) or DTA (▲), incubated at 47°C for 10 min (A) or at 4°C for about 30 min (B), and assayed for recoverable infectivity. PFU, Plaque-forming units.*

evident when the samples were held in ice for 30 min (Fig. 3B).

Because the isoelectric points of poliovirus strain CHAT are about 4.5 and 7.5 (1), these poliovirions should be negatively charged above pH 8. However, even at pH 9 the positively charged cationic detergent DTA is not virucidal for this virus, which is in direct contrast to the results found with reovirus (Fig. 1 and 2). On the other hand, the effects of SDS at acidic pH's are similar for reovirus and poliovirus.

To determine whether these results are restricted to poliovirus type 1 strain CHAT, other enteroviruses were examined. Similar results were found with both coxsackievirus B1 and poliovirus type 2 strain 712-Ch-2ab (Fig. 4A and B, respectively). Therefore, the results found for poliovirus type 1 strain CHAT may be representative of enteroviruses.

It can be concluded from these findings that the effects of detergents on viral stability can be very different for different genera of enteric viruses.

Effects of pH and ionic detergents on the stability of several bacteriophages. To determine whether the results found with either reovirus or enteroviruses are typical of nonenveloped viruses, the effects of ionic detergents on the stability of three standard laboratory strains of coliphage were examined at different pH values. One strain, T4, is a large, double-stranded deoxyribonucleic acid phage, whereas the other two, f2 and Qβ, are serologically distinct, small, single-stranded RNA phages.

The effects of SDS and DTA on the stabilities of these strains of bacteriophage were similar to those found with reovirus (Fig. 5). SDS invariably destabilized these viruses at all pH values examined, with a minimal effect near neutrality. Furthermore, its effect was always greater at the more acidic pH when samples containing the same degree of acidity and basicity could be compared. In contrast, the effect of DTA was greater at the more alkaline pH values; at pH 4, this detergent was not demonstrably virucidal for T4, and its effect on Qβ and f2 was significantly less than it was at pH 10. The destabilizing effects of these detergents were especially evident with Qβ (Fig. 5C). For this reason, samples containing this virus were only incubated for 30 min at room temperature (21°C) before analysis.

These results support the conclusion that the quantitative effects of ionic detergents on viruses are different for each species of virus. However, the finding that enteroviruses are protected by ionic detergents at neutral and alkaline pH's may represent an exception to a rule regarding the qualitative effects of these detergents on nonenveloped viruses. The finding that rhinovirus can be stabilized by SDS against inactivation by heat or acid (8) suggests that other members of the Picornaviridae may also be exceptions to this rule.

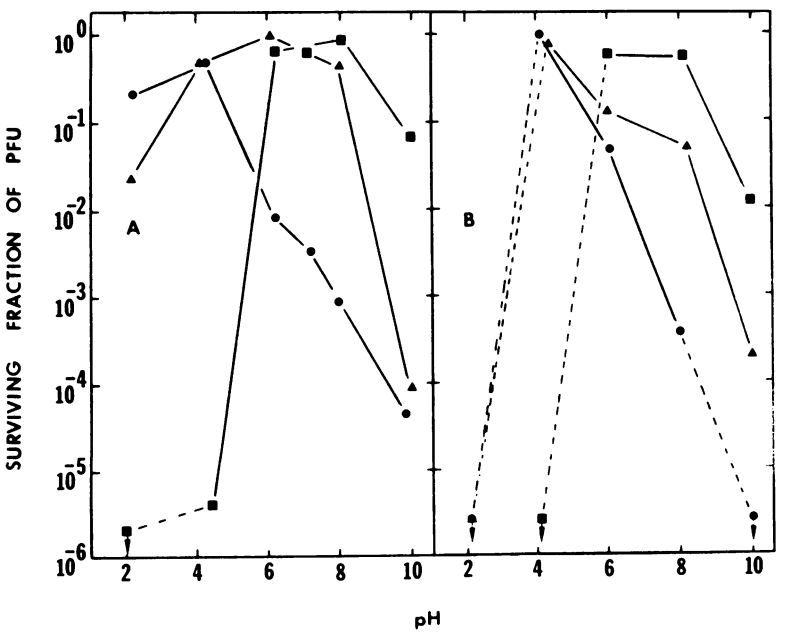

FIG. 4. *Heat inactivation (47°C, 10 min) of coxsackievirus B1 (A) and poliovirus type 2 (B) in the presence of 0.1% SDS (■), DTA (▲), or buffer (●). PFU, Plaque-forming units.*

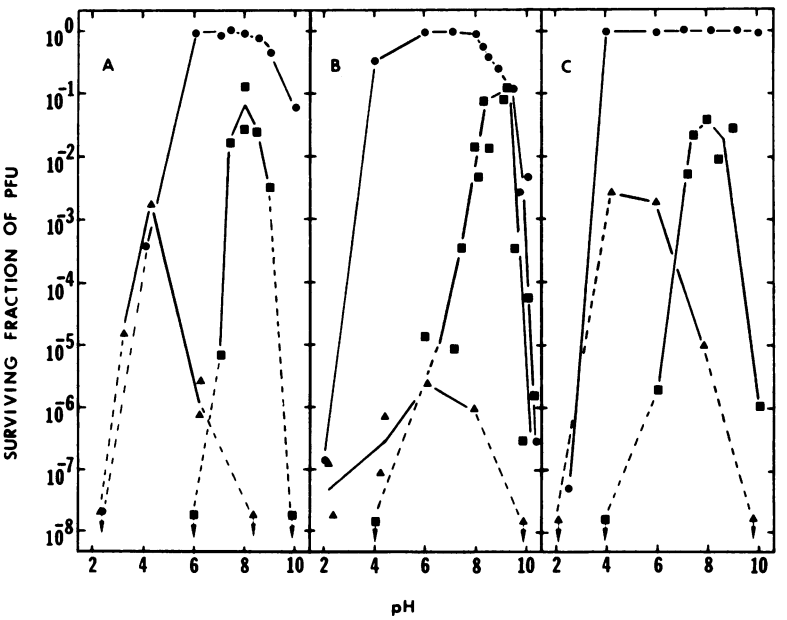

FIG. 5. *Effect of pH and ionic detergents on heat inactivation of T4, f2, and Qβ. Phage preparations were incubated in buffer alone (●) or buffer containing 0.1% SDS (■) or 0.1% DTA (▲) and assayed for recoverable plaque-forming units (PFU). (A) T4 incubated for 60 min at 50°C. (B) Bacteriophage f2 incubated for 20 min at 50°C. (C) Qβ incubated for 30 min at 21°C.*

Physical and biological effects of SDS on the components of poliovirions at different pH values. Because SDS and DTA remain ionized over the range of pH values used in these experiments, the main components of the system whose ionic states should be altered by pH are the viral particles. Furthermore, the only portion of an intact virion whose charge is expected to be altered by changes in pH is the capsid. Therefore, it is likely that the capsid is the part of the virus that determines its differential sensitivity to ionic detergents as a function of pH. The validity of this explanation was tested by an analysis of the RNA and capsid portions of viral particles after treatment.

Because the RNA genomes of poliovirus and other enteroviruses are infectious, the virus chosen for this study was poliovirus strain CHAT. To determine the effect of treatment on viral particles, purified poliovirus containing either [^{3}H]uridine or ^{14}C-labeled amino acids were analyzed by density gradient centrifugation. All samples contained untreated viruses of the opposite label to mark the position of infectious viruses. These marker viruses were mixed with the treated material immediately before centrifugation.

Incubation in buffer at 17°C for 20 min at pH 3.5 had no effect on viral infectivity, and virions treated in this manner sedimented with the marker viruses (Fig. 6A). However, the same treatment with 0.1% SDS caused viral infectivity to decrease more than 5 orders of magnitude. Virions treated in this manner were dissociated into a protein fraction that sedimented at the top of the gradient (Fig. 6B) and into an RNA fraction that sedimented at about 35S (Fig. 6C), assuming the sedimentation value of poliovirus to be 156S. Virions incubated in the same manner with 0.1% SDS at pH 7.0 remained fully infectious, and their sedimentation coefficients were unaltered (Fig. 6D). Therefore, poliovirus particles incubated at 17°C remained intact in 0.1% SDS at pH 7.0 but not at pH 3.5.

A different effect was observed after heat treatment (47°C, 10 min) of radioactively labeled poliovirions at pH 7.0. Under these conditions, the infectivity of the virus was reduced about 3 orders of magnitude in buffer but only about 70% in 0.1% SDS. In agreement with this result, virions inactivated in the absence of SDS sedimented as 80S particles which contained only a portion of the original RNA (Fig. 7A and B). The remaining RNA was released and sedimented at 35S (Fig. 7B). About one-third of the virions incubated with SDS sedimented with the marker viruses, and the remainder dissociated into a slowly sedimenting protein fraction (Fig. 7C) and 35S RNA (Fig. 7D). These results show that SDS stabilizes poliovirus particles against heat inactivation at pH 7.0.

Because poliovirus RNA is released as 35S

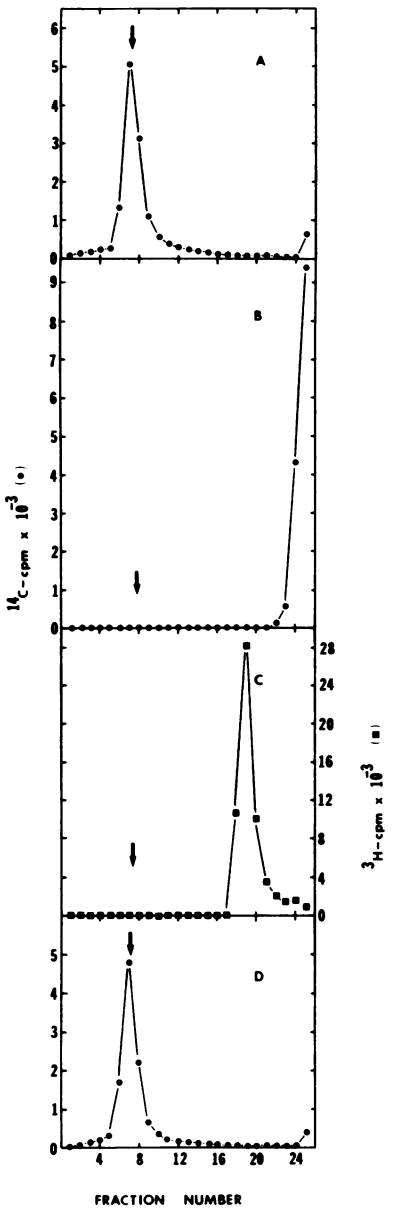

FIG. 6. *Sedimentation profiles of radioactively labeled poliovirus type 1 after treatment with SDS at pH 3.5 and 7.0. Preparations of poliovirus containing [^{3}H]uridine or ^{14}C-labeled amino acids were incubated in the absence or presence of 0.1% SDS, assayed for recoverable plaque-forming units, and analyzed by glycerol gradient centrifugation (15 to 30% glycerol in 0.1 M NaCl-0.01 M Tris [pH 7.5]-0.001 M ethylenediaminetetraacetate, SW50.1 rotor, 38,000 rpm, 1.67 h, 4°C). To adjust the pH, a one-eighth volume of 2 M Tris, pH 8, was added to each sample before centrifugation. The arrow indicates the sedimentation position of untreated infectious virus of the opposite label added immediately before centrifugation. (A) ^{14}C-labeled poliovirus incubated for 20 min at 17°C in 0.1 M acetate buffer at pH 3.5. (B) ^{14}C-labeled poliovirus incubated for 20 min at 17°C in acetate buffer containing 0.1% SDS at pH 3.5. (C) Same as (B) but with [^{3}H]uridine-labeled poliovirus. (D) ^{14}C-labeled poliovirus incubated for 20 min at 17°C in 0.1 M Tris buffer containing 0.1% SDS at pH 7.0.*

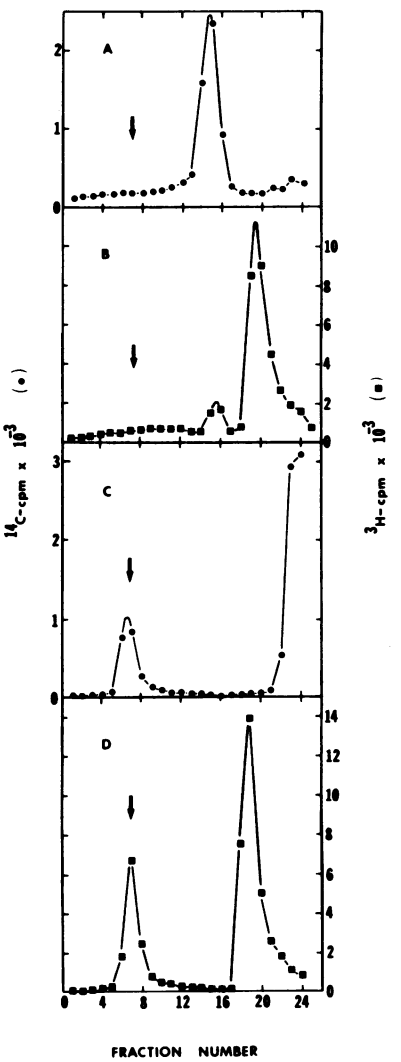

FIG. 7. *Sedimentation profiles of radioactively labeled poliovirus after heat treatment with and without SDS at pH 7.0. Preparations of labeled poliovirus were incubated (47°C, 10 min), assayed for recoverable plaque-forming units, and analyzed by gradient centrifugation as described in the legend to Fig. 6. (A) Poliovirus containing ^{14}C-labeled amino acids incubated without SDS. (B) [^{3}H]uridine-labeled poliovirus incubated without SDS. (C) ^{14}C-labeled virus with 0.1% SDS. (D) [^{3}H]uridine-labeled virus with 0.1% SDS. The arrow marks the sedimentation position of infectious virus of the opposite label added just before centrifugation.*

material in these experiments, and because 35S is the sedimentation value of infectious poliovirus RNA, it appears that the capsid is the portion of the virion destabilized by SDS at pH 3.5 and stabilized by this detergent at pH 7.0. To verify this conclusion, the specific infectivity of poliovirus RNA was analyzed after phenol extraction of virus samples treated in the same manner as those shown in Fig. 6 and 7. Although the specific infectivities of the virus particles were extremely different after these treatments, the specific infectivities of their RNA genomes were almost identical (Table 2). Only the RNA molecules released from virions heated at 47°C had slightly reduced specific infectivities. Therefore, the capsid and not the RNA is the portion of the poliovirion whose stability is altered by SDS at both pH 3.5 and pH 7.0.

pH effects on reovirus stability in wastewater sludge. The findings concerning the effects of pH on the stability of nonenveloped viruses in the presence of ionic detergents can have many practical applications. However, the prime motivation for conducting these experiments was to better understand the effects of pH on the inactivation of enteric viruses in wastewater sludge. If ionic detergents are one of the main components of sludge that affect the rates of enteric virus inactivation, the amount of inactivation in sludge at different pH values may be qualitatively similar to that found in ionic detergents. Because of the effects of the ammonia in sludge (15, 17), this is not true for enteroviruses. However, reovirus has not been found to be significantly affected by ammonia (17, 19). Therefore, the effect of pH on heat inactivation of reovirus was examined, and the results were compared with those found with ionic detergents.

TABLE 2. *Effect of SDS on specific infectivities of poliovirus particles and associated RNA as a function of pH and temperature*[a]

Treatment conditions	Specific infectivity (PFU/cpm)	
	Virus	RNA
pH 7.0, 4°C	2.5×10^{3}	1.0×10^{-1}
pH 3.5, 17°C, 20 min	2.5×10^{3}	9.6×10^{-2}
pH 3.5, 17°C, 20 min, 0.1% SDS	$<2.8 \times 10^{-3}$	1.0×10^{-1}
pH 7.0, 47°C, 10 min	2.8×10^{0}	4.0×10^{-2}
pH 7.0, 47°C, 10 min, 0.1% SDS	1.0×10^{3}	7.5×10^{-2}

[a] Purified poliovirus labeled with [^{3}H]uridine was incubated in buffered solutions and assayed for recoverable plaque-forming units (PFU). The remainder of the sample was extracted with phenol and monitored for recovery of counts per minute and infectious RNA.

The sludge used in this study contained a concentration of 0.06% (wt/vol) anionic detergents, determined by the methylene blue technique (5) as modified for this investigation (20). Its effect on reovirus inactivation was qualitatively similar to that found with SDS (Fig. 8; also see Fig. 1). That is, inactivation was minimal near neutrality and increasingly greater at pH values below 6 and above 8. However, in contrast to the results found with SDS, sludge had a greater effect at pH 10 than at pH 4. This result is more similar to that found with the cationic detergent DTA and suggests that significant concentrations of cationic detergents may also be present in sludge and affect the rate of reovirus inactivation with heat.

DISCUSSION

The effects of ionic detergents on reovirus found previously during studies on enteric virus inactivation in wastewater sludge (20) have now been shown to be highly sensitive to pH. SDS, an anionic detergent, was found to be virucidal at all pH values examined but had a minimal effect near pH 7. Furthermore, reovirus was much more sensitive to this detergent at acidic pH's than at alkaline pH's. In contrast, the effects of DTA, a cationic detergent, were virtually absent at pH 4 but much greater than those of SDS at alkaline pH's. Qualitatively similar results were found for the effects of SDS and DTA on bacteriophages T4, f2, and Qβ.

The effects of SDS and DTA on enteroviruses at neutral and alkaline pH's were found to be quite different than those observed with reovirus and the bacteriophages examined. In the pH range of about 6 through 9.5, both SDS and

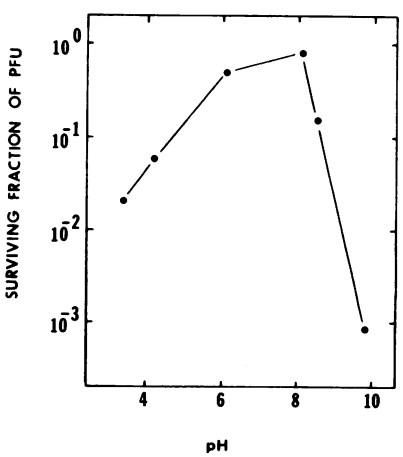

FIG. 8. *Effect of pH on heat inactivation (45°C, 20 min) of reovirus in anaerobically digested sludge. PFU, Plaque-forming units.*

DTA stabilized enteroviruses against heat. However, because of the observations of Sobsey et al. (13), it is probable that if the effects of DTA had been studied above pH 10, this compound would have been found to accelerate inactivation of enteroviruses. Below pH 5, the effects of these detergents were similar to those observed with other viruses; DTA had little observable effect on the stability of enteroviruses, and SDS was extremely virucidal. The latter observation is in agreement with the results already reported for poliovirus by Mandel (9-11).

The component of a virus particle that determines how it is affected by these detergents is probably the protein capsid. This was explicitly demonstrated with poliovirus and SDS, where it was shown that the RNA molecules obtained from poliovirions inactivated at pH 3.5 with SDS and at pH 7.0 with heat were still infectious. However, in the former case SDS destabilized the capsid, whereas in the latter case it stabilized this virion component.

Breindl (4) has reported that heat treatment of poliovirus initially causes the virions to be converted into ribonucleoprotein complexes and empty capsids (both of which sediment at about 80S) and into free RNA. This result agrees with the findings reported here (Fig. 7). Breindl also observed that both free and protein-complexed RNAs were infectious. In this report, RNA molecules extracted with phenol from heat-treated poliovirions were likewise found to be infectious (Table 2).

Because ionic detergents apparently alter the stability of nonenveloped viruses through their interactions with capsid proteins, these interactions should be modified by changes in the ionic states of the viral proteins brought about by changes in pH. Mechanisms by which ionic detergents interact with proteins have been reviewed by Putnam (12). In general, he concluded that the charged portion of a detergent molecule combines with oppositely charged residues of protein molecules, thus leading to protein denaturation. This would explain why the anionic detergent SDS usually causes more effective viral inactivation at low pH and the cationic detergent DTA is usually more effective at high pH.

Ionic detergents can also cause viral inactivation at pH's where the apparent charge on the virus surface is the same as that of the detergent. For example, several plant viruses are effectively inactivated by SDS only at neutral and alkaline pH values (1-3, 7, 14). Also, in the experiments reported here, SDS effectively reduced the heat required to inactivate reovirus at neutral and alkaline pH's as well as at acidic pH values. Because the pK for this virus has been reported to be 3.9 (6), efficient inactivation apparently occurs when the virus and detergent have the same charge. Thus, both forces of attraction and repulsion may be involved in destabilization of nonenveloped viruses by ionic detergents.

Besides causing denaturation, ionic detergents can also stabilize proteins, as was found here for enterovirus capsid proteins at neutral and alkaline pH values. A similar phenomenon has been reported for a rhinovirus (8). Explanations for these observations are not readily apparent.

The prime motivation for conducting these experiments was to attempt to understand what effects detergents in wastewater sludge might be expected to have on the stabilities of enteric viruses at different pH values. Modification of pH by lime, ferric chloride, and other chemicals is a common method of sludge stabilization. If chemically treated sludge is also subjected to heat for pathogen control, it would be important to be able to predict how the pH of the sludge might affect the rates of heat inactivation of viruses and other pathogens. Of course, sludge components other than detergents will help determine these rates. However, the finding that heat inactivation of reovirus in sludge is qualitatively similar at different pH's to that found in SDS and DTA suggests that ionic detergents may be the main components of sludge that determine reovirus inactivation rates with heat. The unpublished finding that heat inactivation of poliovirus is significantly faster at pH 3.5 than at pH 6 in sludge, a result that also occurs in the presence of SDS but not in buffer at the same pH values, supports the suggestion (20) that ionic detergents are one of the components that regulate enterovirus inactivation in sludge as well.

ACKNOWLEDGMENTS

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