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Field and Laboratory Investigations of Inactivation of Viruses (PRD1 and MS2) Attached to Iron Oxide-Coated Quartz Sand

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Field and laboratory experiments were conducted to investigate inactivation of viruses attached to mineral surfaces. In a natural gradient transport field experiment, bacteriophage PRD1, radiolabeled with ^{32}P, was injected into a ferric oxyhydroxide-coated sand aquifer with bromide and linear alkylbenzene sulfonates. In a zone of the aquifer contaminated by secondary sewage infiltration, small fractions of infective and ^{32}P-labeled PRD1 broke through with the bromide tracer, followed by the slow release of 84% of the ^{32}P activity and only 0.011% of the infective PRD1. In the laboratory experiments, the inactivation of PRD1, labeled with ^{35}S (protein capsid), and MS2, dual radiolabeled with ^{35}S (protein capsid) and ^{32}P (nucleic acid), was monitored in the presence of groundwater and sediment from the contaminated zone of the field site. Release of infective viruses decreased at a much faster rate than release of the radiolabels, indicating that attached viruses were undergoing surface inactivation. Disparities between ^{32}P and ^{35}S release suggest that the inactivated viruses were released in a disintegrated state. Comparison of estimated solution and surface inactivation rates indicates solution inactivation is ∼3 times as fast as surface inactivation. The actual rate of surface inactivation may be substantially underestimated owing to slow release of inactivated viruses.

Introduction

Increasing concern over microbial contamination of groundwater has spurred the U.S. Environmental Protection Agency to propose the Ground Water Rule (*1*), a set of regulations designed to reduce the public health risk associated with the consumption of waterborne pathogens in groundwater exposed to fecal contamination. Viruses were identified as the target organism of the Ground Water Rule because viruses are responsible for ∼80% of disease outbreaks for which causative agents were identified. Drinking water systems that do not disinfect groundwater drawn from "hydrogeologically sensitive" aquifers—karst, fractured bedrock, and gravel—will be subject to increased monitoring and regulations.

The current designation of hydrogeologically sensitive aquifers, which may ultimately be expanded to include sand and volcanic rock aquifers, is based solely on groundwater flow rates. Virus removal during transport by irreversible attachment and inactivation is not considered. Models of virus transport (*2*−*7*) could be used to better delineate the potential for virus transport in aquifers, but model predictions are still somewhat unreliable (*8*, *9*) owing to a lack of key parameters and, more important, a lack of complete understanding of some removal processes.

In most models, virus inactivation is portrayed simply as a first-order decrease in the number of infective viruses in solution with a rate coefficient solely dependent on temperature (*10*). It is recognized that many other factors—virus type, pH, ionic strength, ion composition, microbial enzymes, virus aggregation, and attachment to air/water and solid/water interfaces—also affect virus inactivation, but the effects of these factors are not known well enough to include in models. Recently, the role of virus attachment in inactivation has received some attention in virus transport modeling efforts (*5*, *7*). Schijven et al. (*5*) found that the rate of inactivation of attached viruses exceeded the rate of inactivation of viruses in the solution phase. Bhattacharjee et al. (*7*) concluded that inactivation of attached viruses could negate the effect of virus release from aquifer sediments.

Research investigating the effect of attachment on virus inactivation does not present a clear picture (*9*). Viruses may be protected from inactivation by attachment to some sediments, notably those with high clay and organic matter contents (*11*−*14*), whereas attachment to other surfaces accelerates inactivation (*15*−*17*). In the most detailed study of this process, Murray and Laband (*15*) incorporated radiolabels into the nucleic acid (^{3}H) and protein capsid (^{14}C) of poliovirus to examine the behavior of poliovirus components during attachment to various mineral surfaces. The extent of inactivation caused by attachment, as measured by divergent behaviors of the radiolabeled components and the intact, infective poliovirus, increased with the strength of electrostatic attraction between the poliovirus and the mineral surface.

Similar observations made during a field experiment on the transport of bacteriophage PRD1 in a ferric oxyhydroxide-coated sand aquifer (*18*) led us to adapt the dual radiolabeling method to examine surface inactivation in more detail in laboratory experiments. We performed the laboratory experiments on sediments from the field site to better understand the field data. Following Murray and Laband (*15*), we hypothesized that the strong electrostatic attraction between the ferric oxyhydroxide patches on the aquifer sediment and attached PRD1 was accelerating PRD1 inactivation.

Materials and Methods

Site Description. The field experiment, a natural gradient injection of ^{32}P-labeled bacteriophage PRD1, was conducted during the summer of 1994 at the U.S. Geological Survey's Toxic Substances Hydrology research site on Cape Cod, MA. The shallow, unconfined aquifer at the Cape Cod site is situated in Pleistocene glacial outwash composed of well-

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TABLE 1. Details of ^{32}P-Labeled Bacteriophage PRD1 Injection in the Surficial Aquifer at the Cape Cod Research Site[a]

constituent	units	uncontaminated C_0	contaminated C_0	background	precision (%)
infective PRD1	pfu mL^{-1}	1.7 (±0.3) × 10^6	3.0 (±0.6) × 10^6	nd[b]	30
^{32}P-PRD1	cpm mL^{-1}	4200 (±300)	5800 (±500)	110	10
NaBr	mM	1.63 (±0.05)	2.18 (±0.10)	0.013	5
LAS	mgL^{-1}	24 (±3)	27 (±2)	1	15

[a] The C_0 values (mean and standard deviation of triplicate analyses) are those measured in the injection multilevel sampler (MLS) immediately after injection. Also provided are background concentrations of the constituents and analytical precision (as relative standard deviations) of the measurements. [b] nd, not detected.

sorted medium and coarse sand (*19*). About 3−4% of the surfaces of the grains (primarily quartz and feldspars) are coated by ferric and aluminum oxyhydroxides and clay minerals (mainly kaolinite) (*20, 21*). Two distinct geochemical zones were created in the aquifer by the nearby infiltration of secondary sewage effluent: the uncontaminated upper zone (up to 7 mg L^{-1} dissolved oxygen, pH 5−6, specific conductance below 80 μS cm^{-1}, and <1 mg L^{-1} dissolved organic carbon) and the contaminated lower zone (<1 mg L^{-1} dissolved oxygen, pH 6−7, specific conductance up to 410 μS cm^{-1}, and 2−4 mg L^{-1} dissolved organic carbon). Details on the groundwater and sediment chemistry are available in Pieper et al. (*18*) and Ryan et al. (*21*).

Field Experiment. Injections of 100 L of groundwater amended by additions of bacteriophage PRD1, sodium bromide, and a linear alkylbenzene sulfonate (LAS) homologous mixture were made into an array of multilevel samplers (MLSs) in line with the direction of groundwater flow (Table 1). The array consisted of one injection MLS and four down-gradient monitoring MLSs spaced ~1 m apart. Groundwater was pumped from the uncontaminated zone (6.4 and 6.7 m depths) and the contaminated zone (8.7 and 9.0 m depths) into gas-impermeable, acid-cleaned, nitrogen-filled fuel bladders. Concentrated stocks of PRD1, sodium bromide, and LAS were added to the groundwater and mixed, and the amended groundwater was pumped back to depths of 6.4 and 8.7 m at a flow rate of ~1.0 L min^{-1}.

Immediately before and after the injection, the MLSs were sampled at six depths (6.2, 6.4, and 6.7 m, uncontaminated zone; 8.4, 8.7, and 9.0 m, contaminated zone) to determine the background and initial (C_0) concentration of each constituent. Samples were collected at the four down-gradient MLSs for a total of 55 days, initially at a rate of once per day (Figure 1). Samples were withdrawn at a rate of ~200 mL min^{-1} by peristaltic pumps. The average groundwater temperatures were 15.5 °C in the uncontaminated zone and 15.0 °C in the contaminated zone.

The bacteriophage PRD1 is an icosahedral virus of 62 nm diameter with an isoelectric point (pH_{iep}) of <3.2 in Cape Cod groundwater (*21*). The host for PRD1 growth and plaque assay is *Salmonella typhimurium* LT2. PRD1 was radiolabeled with [^{32}P]orthophosphate by adapting the technique used Loveland et al. (*22*) for [^{35}S]methionine labeling of PRD1. Coincident fractions of infective PRD1 and ^{32}P were separated from unincorporated ^{32}P by sucrose gradient rate zonal centrifugation. Because ^{32}P was added as orthophosphate, ^{32}P was probably incorporated into both the nucleic acid and protein coat of PRD1. Infective PRD1 was measured by plaque assay in triplicate (double-layer agar technique, 1 mL samples, 1 pfu mL^{-1} detection limit). ^{32}P activity was measured in triplicate by addition of scintillation cocktail to 10 mL samples and liquid scintillation counting (Beckman LS3801; 670−1000 keV; 1 cpm mL^{-1} detection limit). Raw count rates were corrected for background radioactivity in groundwater samples taken before the injection and for radioactive decay.

Sodium bromide was added to provide a conservative tracer, bromide, which was measured with an ion-specific electrode in triplicate analyses. An LAS mixture (Vista Chemical Co.) was added to examine the effect of detergents, a significant component of the infiltrated secondary sewage, on virus transport. The LAS mixture (isomers with aliphatic chains ranging from C_{10} to C_{14}) was added at a concentration of 25 mg L^{-1}, ~5% of the critical micelle concentration of this mixture (*23*), and measured in triplicate by a methylene blue active substances (MBAS) colorimetric test kit.

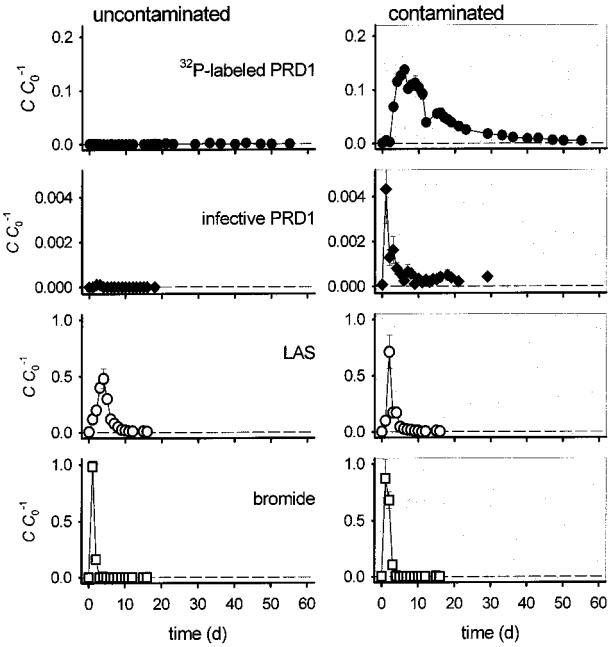

FIGURE 1. Breakthroughs of ^{32}P-labeled PRD1, infective PRD1, linear alkylbenzene sulfonates (LAS), and bromide, the conservative tracer at 1.0 m transport distance in the uncontaminated and contaminated zones of the Cape Cod aquifer. PRD1, LAS, and bromide concentrations (C) were normalized to concentrations measured immediately after injection (C_0). Relative detection limits are shown as horizontal dashed lines for each parameter. Error bars show standard deviation of triplicate analyses; most error bars are smaller than the data points.

Field Data Analysis. The relative breakthrough (RB, %) of a constituent was calculated as the ratio of the time-integrated mass of the constituent relative to that of the conservative tracer (*24*). Following the colloid filtration model of kinetically controlled irreversible attachment of colloids to collector grains (*25*), collision efficiencies (α) were calculated for the pulse inputs of PRD1 with consideration of the effect of longitudinal dispersion (*24*)

$$\alpha = \frac{d\{[1 - 2(\alpha_L/x_1)\ln RB]^2 - 1\}}{6(1-\theta)\eta_0\alpha_L} \quad (1)$$

where d is the diameter of the porous media grains, x_1 is the distance from the injection point to the sampling point, θ is the porosity, η_0 is the single collector efficiency for favorable

deposition, and α_L is the longitudinal dispersivity, which was estimated by

$$\alpha_L = \frac{x_1(\Delta t/t_{peak})^2}{16 \ln 2} \quad (2)$$

where Δt is the duration of the breakthrough for which $[Br^-] > 0.5[Br^-]_{max}$ ($[Br^-]_{max}$ is the peak bromide concentration) and t_{peak} is the time to peak bromide concentration. The single collector efficiency η_0 was calculated as only the convective diffusion contribution (*26*) owing to the small size of PRD1, using an average grain diameter of 0.6 mm, a porosity of 0.39, and fluid velocities estimated from the time of peak bromide breakthrough.

Laboratory Experiment Materials. The laboratory experiments consisted of two types of inactivation experiments: (1) solution inactivation and (2) surface inactivation. The solution inactivation experiments were conducted in raw and amended groundwater from the contaminated zone at the Cape Cod site. The surface inactivation experiments were conducted in raw and amended contaminated groundwater and contaminated sediment from the Cape Cod site. The sediment was collected within 5 m of the field experiment site using a hollow stem auger, a piston core barrel, and a liquid nitrogen circulation device that sealed the bottom of the core barrel with a plug of ice (*27*).

The laboratory inactivation experiments were conducted on bacteriophages PRD1 and MS2. MS2 is an icosahedral virus of 25 nm diameter with a pH_{iep} of 3.5 in 0.01 M NaCl solution (*28*). MS2 was grown, radiolabeled, and assayed on *Escherichia coli* ATCC 15597. Following the technique of Loveland et al. (*22*), the two viruses were dual radiolabeled by growing the host bacteria to log phase in a methionine-deficient glucose growth medium. The bacteria were infected with viruses at a multiplicity of infection (virus/host) ratio of 10−20. The radiolabels ([α-PO_4-^{32}P]DNA nucleotides, [^{35}S]-methionine) were added 5 min after infection. This method incorporates ^{32}P into the nucleic acids and ^{35}S into the protein capsid. The suspensions were incubated until lysis occurred and new viruses were released (∼3 h). Cell debris was removed from the suspensions by centrifugation (10000 rpm, 20 min, Sorvall RC-5B centrifuge, GSA rotor), and the supernatants were filtered through 0.25 μm cellulose acetate filters. These suspensions were centrifuged again (19500 rpm, 6 h, SS-23 rotor) to pellet the viruses and resuspended in phosphate-buffered saline at pH 7.5.

The radiolabeled virus stocks for the laboratory experiments were purified only by ultracentrifugation to avoid potential changes in virus surface character (*29*, *30*). A sucrose gradient rate zonal centrifugation was used to check the extent of radiolabel incorporation into the infective viruses. For MS2, 93% of the ^{35}S and 89% of the ^{32}P were found in the centrifugation fractions containing 88% of the infective MS2. For PRD1, 91% of the ^{32}S and only 16% of the ^{32}P were found in the fractions containing 90% of the infective PRD1. Because only 16% of the ^{32}P was found in the infective PRD1 fractions, we surmise that the [α-PO_4-^{32}P]DNA nucleotides were not readily incorporated into PRD1. As a result, ^{32}P results were not considered when the PRD1 surface inactivation data were interpreted.

Solution Inactivation Experiments. These experiments tested the rate of inactivation of unlabeled and dual radiolabeled viruses in raw and amended contaminated groundwater from Cape Cod at 5 °C. Groundwater was amended by addition of the LAS mixture used in the field experiment (25 mg L^{-1}) and sodium dodecylbenzene sulfonate (DBS; 25 mg L^{-1}). A 1 mL aliquot of virus suspension was added to 19 mL of groundwater in a glass vial, mixed by hand, and incubated at 5 °C for 30 days. Samples (0.1 mL) were removed every 24 h, and virus concentration was measured by plaque assay. Each of these experiments was conducted in triplicate.

Surface Inactivation Experiments. These experiments tested the rate of dual radiolabeled virus inactivation in the presence of sediment and groundwater collected from the contaminated zone of the Cape Cod aquifer at 5 °C. Only the contaminated zone sediment and groundwater were used in the surface inactivation experiments because PRD1 was observed only in the contaminated zone during the field experiments. A portion of 0.5−1.0 mm sieved aquifer sediment (10 g) was placed in a 20 mL glass syringe (plunger removed; polycarbonate stopcock on outlet; 105 μm polypropylene mesh to retain sand). Five pore volumes (range = 2.4−2.6 mL) of contaminated groundwater (pH 6.0−6.4) were added to the sand 1 pore volume at a time, with each pore volume remaining in the column for 15 min and draining by gravity in ∼1 min to fully saturate the sediment. With a final pore volume of water remaining in the sediment, a 1 pore volume virus suspension (either PRD1 or MS2) in raw or DBS-amended contaminated groundwater was added above the sediment. In the DBS-amended groundwater, 25 mg L^{-1} of sodium dodecylbenzene sulfonate was added to simulate the effect of the LAS mixture used in the field experiment. The virus-free contaminated groundwater was drained (and sampled), drawing the virus suspension into the sediment, where it remained for 24 h. After 24 h, another pore volume of virus-free contaminated groundwater was placed above the sediment, the virus suspension was drained and sampled, and the virus-free solution was drawn into the sediment. With virus-free contaminated groundwater, this flushing procedure was repeated 29 times, with a 24 h residence time for each pore volume. Each of these experiments was performed in triplicate.

To be sure that all of the attached viruses were released from the sediment, we used pH 11 solutions to elute the columns in single surface inactivation experiments for both PRD1 and MS2. Solutions of elevated pH effectively mobilized viruses in the field (*21*). The viruses were attached in contaminated groundwater and eluted with contaminated groundwater for 7 pore volumes, as described above. Following the seventh pore volume, the sediments were flushed with a pH 11.0 groundwater solution. Sodium hydroxide was added to the groundwater to elevate the pH. Virus inactivation in the pH 11.0 groundwater solution was very fast ($k_{i,sol} = 0.55 \pm 0.17$ day^{-1}; unpublished data), so the column samples were immediately acidified to pH 6.0 by addition of nitric acid.

Laboratory Analyses. The solution and surface inactivation samples were analyzed in triplicate by plaque assay and radioassay. Plaque assays were conducted with 0.5 mL sample aliquots (detection limit = 10 pfu mL^{-1}). For radioassay, an aliquot of each sample (1 mL) was mixed with scintillation cocktail (19 mL, Packard Ultima Gold XR) and measured for ^{32}P and ^{35}S activity by liquid scintillation counting (Beckman LS3801; ^{35}S, 400−650 keV; ^{32}P, 670−1000 keV; 1 cpm mL^{-1} detection limit). Raw count rates were corrected for background (virus-free contaminated groundwater solutions) and radioactive decay.

Laboratory Data Analyses. For the surface inactivation experiments, virus concentrations in the contaminated groundwater flushes succeeding virus attachment were reported as CC_{att}^{-1}, the concentration in the flushed pore volume, C (pfu mL^{-1} or cpm mL^{-1}), divided by the concentration of viruses attached in the first pore volume, C_{att}. The concentration of attached viruses was determined as the difference between the virus concentration in the suspension added as the first pore volume and the virus concentration in the suspension removed after 24 h.

TABLE 2. Experimental Conditions and Solution Inactivation Rate Coefficients for Non-radiolabeled and Dual Radiolabeled (^{32}P, ^{35}S) PRD1 and MS2 in 5 °C Contaminated Cape Cod Groundwater and Contaminated Groundwater (contam gw) Amended with the Linear Alkylbenzene Sulfonate (LAS) Mixture and Dodecylbenzene Sulfonate (DBS) at 25 mg L^{-1}[a]

		solution inactivation rate coefficient ($k_{i,sol}$)		
virus	C_0 (pfu mL^{-1})	contam gw (day^{-1})	LAS + contam gw (day^{-1})	DBS + contam gw (day^{-1})
PRD1	5.0 (±2.0) × 10^6	0.022 ± 0.012	0.0039 ± 0.0102	0.039 ± 0.012
^{32}P,^{35}S-PRD1	5.0 (±2.0) × 10^6	0.056 ± 0.028		
MS2	5.0 (±2.0) × 10^6	0.052 ± 0.022	0.083 ± 0.024	0.072 ± 0.020
^{32}P,^{35}S-MS2	5.0 (±2.0) × 10^6	0.093 ± 0.034		

[a] C_0 is the range of initial virus concentrations used in the experiments. Error shown for rate coefficients is the standard error of the slope of ln CC_0^{-1} versus time regressions. Differences between all pairs of rate coefficients are statistically significant (unpaired t test, $p < 0.001$).

Results

Field Experiment. Breakthroughs of the bromide, LAS, and infective and ^{32}P-labeled PRD1 were clear only at the 1.0 m transport distance in both the uncontaminated and contaminated zones (Figure 1). Owing to misalignment of the MLS with the groundwater flow direction, none of the constituents appeared at the 1.8 m distance in either zone. In the contaminated zone only, bromide and LAS breakthrough curves were measured for the 2.8 and 3.6 m distances, but infective PRD1 and ^{32}P were detected only at concentrations near the detection limits. At the end of the sampling period (55 days), ^{32}P had reached maximum CC_0^{-1} values near 0.04 and 0.03 at the 2.8 and 3.6 m distances in the contaminated zone (*31*).

In the uncontaminated zone at 1.0 m distance, bromide breakthrough occurred at 1 day and LAS breakthrough occurred at 4 days. Both infective and ^{32}P-labeled PRD1 did not arrive in detectable concentrations. In the contaminated zone at 1.0 m distance, bromide breakthrough occurred at 1 day and LAS breakthrough at 2 days. A small breakthrough of infective PRD1 (CC_0^{-1} = 0.0043) coincided with the bromide breakthrough, followed by a rapid decline to near-zero infective PRD1 concentrations. A much larger breakthrough of ^{32}P-labeled PRD1 (CC_0^{-1} = 0.14) peaked at 6 days, followed by a decline to near-background ^{32}P activity of the remaining sampling period. A small peak in the ^{32}P-labeled PRD1 breakthrough (CC_0^{-1} = 0.0058) coincided with the bromide breakthrough.

The breakthrough of infective PRD1 was characterized by a relative breakthrough (RB) of 0.00070 and a collision efficiency (α) of 0.22 in the uncontaminated zone. In the contaminated zone, infective PRD1 broke through in much larger numbers (RB = 0.0078) and exhibited a smaller collision efficiency (α = 0.12). The breakthrough of ^{32}P-PRD1 in the uncontaminated zone was characterized by RB = 0.0085 and α = 0.12. In the contaminated zone, ^{32}P-PRD1 exhibited a relative breakthrough ~2 orders of magnitude greater than the breakthrough of infective PRD1 (RB = 0.84). The collision efficiency was not calculated for the ^{32}P-PRD1 breakthrough in the contaminated zone because we surmised that this apparent breakthrough was actually the delayed release of ^{32}P-PRD1 attached as the initial pulse traveled the 1 m transport distance.

Laboratory Solution Inactivation Experiments. PRD1 appeared to be slightly more resistant to inactivation than MS2 in the contaminated Cape Cod groundwater (Figure 2; Table 2), but the differences in the inactivation rate coefficients were not statistically significant for comparisons between PRD1 and MS2 (unpaired t test, $p > 0.1$). The half-lives of PRD1 and MS2 infectivity in the contaminated groundwater were 32 ± 17 and 13 ± 6 days, respectively. The additions of LAS, DBS, and the dual radiolabels (^{32}P, ^{35}S) resulted in small, but insignificant, increases in the inactivation rates of PRD1 and MS2 (unpaired t test, $p > 0.1$).

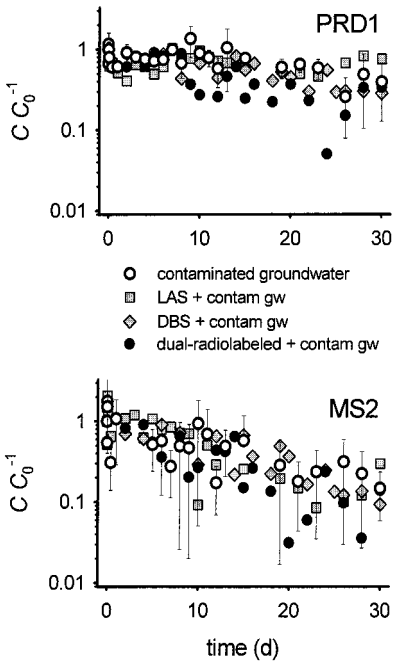

FIGURE 2. Inactivation of bacteriophages PRD1 and MS2 in contaminated groundwater from Cape Cod (contam gw), contaminated groundwater amended by 25 mg L^{-1} of the linear alkylbenzene sulfonate mixture (LAS + contam gw), contaminated groundwater amended by 25 mg L^{-1} of sodium dodecylbenzene sulfonate (DBS + contam gw), and dual radiolabeled (^{32}P, ^{35}S) PRD1 and MS2 in contaminated groundwater (dual-radiolabeled + contam gw). Data points represent the mean of triplicate analyses. Error bars (one standard deviation) are shown only for the contaminated groundwater data; similar errors occurred in other analyses.

Laboratory Surface Inactivation Experiments. The dual radiolabels (^{32}P, ^{35}S) were successfully incorporated in bacteriophage MS2, but only ^{35}S was successfully incorporated into PRD1; thus, ^{32}P data are not shown for PRD1 (Table 3). During attachment, the fraction of infective MS2 and PRD1 attached to the sediments exceeded that of the radiolabels in three of the four experiments. Some of this discrepancy can be attributed to incomplete incorporation of radiolabels in the infective viruses (11% of the ^{32}P and 7% of the ^{35}S for MS2; 9% of the ^{35}S for PRD1) or to radiolabel-containing debris of viruses that inactivated between virus purification and experiments. It is also possible that these discrepancies are attributable to measurement error given the substantial relative standard deviations associated with these measurements (Table 1).

The presence of dodecylbenzene sulfonate (DBS) resulted in a decrease in infective PRD1 attachment from 74 to 50% and a decrease in infective MS2 attachment from 92 to 78% (Table 3). The radiolabel attachment showed similar decreases in the presence of DBS.

TABLE 3. Experimental Conditions for Laboratory Surface Inactivation Experiments in the Presence of Cape Cod Contaminated Sediment with Elutions by Contaminated Groundwater at Ambient pH[a]

experiment/ constituent	units	C_0	C_{att}	C_{att}/C_0	ΣC_{rel}	$\Sigma C_{rel} C_{att}^{-1}$	$k_{i,surf} + k_{rel}$ ($^{-1}$)
surface inactivation (contam gw attachment/contam gw release)							
inf PRD1	pfu mL^{-1}	1.5 ($\pm$0.2) $\times$ 10^8	1.1 ($\pm$0.1) $\times$ 10^8	0.73 ($\pm$0.16)	3.3 ($\pm$1.1) $\times$ 10^6	0.030 ($\pm$0.012)	0.87 $\pm$ 0.19
^{32}P-PRD1	cpm mL^{-1}	57 ($\pm$50)					
^{35}S-PRD1	cpm mL^{-1}	750 ($\pm$170)	390 ($\pm$110)	0.52 ($\pm$0.26)	100 ($\pm$15)	0.26 ($\pm$0.11)	0.43 $\pm$ 0.08
inf MS2	pfu mL^{-1}	1.3 ($\pm$0.2) $\times$ 10^8	1.2 ($\pm$0.1) $\times$ 10^8	0.92 ($\pm$0.22)	1.0 ($\pm$0.3) $\times$ 10^6	0.0083 ($\pm$0.0032)	0.88 $\pm$ 0.16
^{32}P-MS2	cpm mL^{-1}	1800 ($\pm$340)	910 ($\pm$190)	0.51 ($\pm$0.20)	440 ($\pm$80)	0.48 ($\pm$0.19)	0.36 $\pm$ 0.05
^{35}S-MS2	cpm mL^{-1}	11000 ($\pm$1200)	3600 ($\pm$1100)	0.33 ($\pm$0.14)	730 ($\pm$160)	0.21 ($\pm$0.11)	0.45 $\pm$ 0.08
surface inactivation (25 mg L^{-1} DBS + contam gw attachment/contam gw release)							
inf PRD1	pfu mL^{-1}	1.5 ($\pm$0.2) $\times$ 10^8	7.4 ($\pm$1.0) $\times$ 10^7	0.49 ($\pm$0.13)	3.7 ($\pm$0.8) $\times$ 10^6	0.050 ($\pm$0.018)	1.26 $\pm$ 0.14
^{32}P-PRD1	cpm mL^{-1}	57 ($\pm$50)					
^{35}S-PRD1	cpm mL^{-1}	750 ($\pm$170)	400 ($\pm$130)	0.53 ($\pm$0.29)	100 ($\pm$10)	0.24	0.44$\pm$0.08
inf MS2	pfu mL^{-1}	1.3 ($\pm$0.2) $\times$ 10^8	1.0 ($\pm$0.2) $\times$ 10^8	0.77 ($\pm$0.27)	5.6 ($\pm$1.7) $\times$ 10^6	0.056	1.25 $\pm$ 0.10
^{32}P-MS2	cpm mL^{-1}	1800 ($\pm$340)	930 ($\pm$230)	0.52 ($\pm$0.23)	460 ($\pm$70)	0.49	0.36 $\pm$ 0.05
^{35}S-MS2	cpm mL^{-1}	11000 ($\pm$1200)	3700 ($\pm$1000)	0.34 ($\pm$0.14)	740 ($\pm$110)	0.20	0.47 $\pm$ 0.07

[a] C_0 is the virus concentration (mean and standard deviation of triplicate analyses) of the suspension added as the first pore volume. C_{att} is the virus concentration attached during the first pore volume. ΣC_{rel} is the total amount of virus release during the 29 flushing pore volumes. The first-order "rate coefficients" $k_{i,surf} + k_{rel}$ (where k_{rel} is either $k_{rel,i}$ or $k_{rel,d}$) representing the combined surface inactivation and release steps were calculated for the first five pore volumes; the units are n^{-1}, where n is the number of daily pore volumes or flushes. Virus concentrations are shown for infective (inf) and radiolabeled (^{32}P-, ^{35}S-) assays. Errors are one standard deviation for triplicate analyses (C_0) and triplicate experiments.

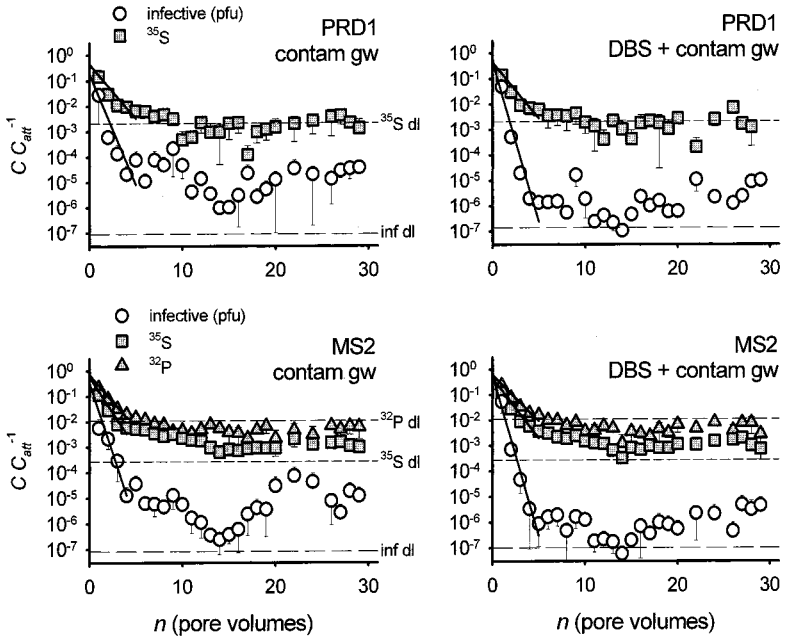

FIGURE 3. Release of bacteriophages PRD1 (upper pair) and MS2 (lower pair) as infective viruses and ^{35}S- and ^{32}P-labeled components from contaminated Cape Cod aquifer sediment. Viruses were deposited in contaminated groundwater (contam gw; left pair) or contaminated groundwater amended by 25 mg L^{-1} of sodium dodecylbenzene sulfonate (DBS + contam gw; right pair). Viruses were released by contaminated groundwater. Error bars represent one standard deviation of triplicate experiments. Relative detection limits (^{32}P, ^{35}S, inf dl) are shown as horizontal dashed lines. Divergent regression lines for infective and radiolabeled releases over the first five pore volumes indicate surface inactivation is occurring (*15*).

The concentration of infective viruses, ^{32}P activity, and ^{35}S activity released from the sediment decreased over the first five pore volumes of elution (Figure 3). After five pore volumes, the released concentrations reached plateaus, with the infective virus plateaus ~2−3 orders of magnitude lower than the ^{32}P and ^{35}S plateaus. In some cases (especially for ^{32}P), these plateaus coincided with the relative detection limits (calculated as the detection limit divided by the total amount of attached infective virus or radioactivity; Figure 3). Infective virus concentrations increased by 1−3 orders of magnitude after ~20 days. The concentration of infective viruses decreased much more quickly than the concentration of ^{32}P- and ^{35}S-labeled virus components (Table 3). In all of the experiments, the total release of infective viruses ($\Sigma C_{rel} C_{att}^{-1}$ = 0.8−5.6%) was substantially less than the total release of ^{32}P (48 and 49%; MS2 data only) and ^{35}S (20−26%). The difference in the amount of ^{32}P and ^{35}S recovery suggests inactivation caused disintegration of the viruses.

During the single experiments in which contaminated groundwater at pH 11.0 was used to flush the sediments after 7 pore volumes of unamended contaminated groundwater, the pH 11.0 elution resulted in an increase in the amount of infective PRD1 and MS2 released from the sediment by as much as 2 orders of magnitude relative to the amount of infective virus release in the previous elution by

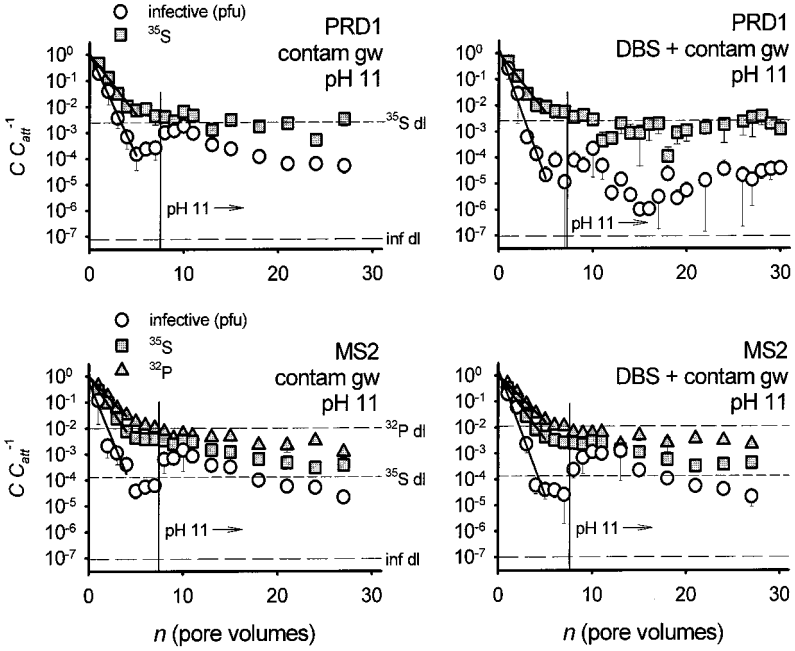

FIGURE 4. Release of bacteriophages PRD1 (upper pair) and MS2 (lower pair) as infective viruses and ^{35}S- and ^{32}P-labeled components from contaminated Cape Cod aquifer sediment. Viruses were deposited in contaminated groundwater (contam gw; left pair) or contaminated groundwater amended by 25 mg L^{-1} of sodium dodecylbenzene sulfonate (DBS + contam gw; right pair). Viruses were released by contaminated groundwater (first seven pore volumes) and pH 11 contaminated groundwater (remaining pore volumes) or deposited in and released in contaminated groundwater. Error bars represent one standard deviation of triplicate experiments. Relative detection limits (^{32}P, ^{35}S, inf dl) are shown as horizontal dashed lines.

TABLE 4. Experimental Conditions for Laboratory Surface Inactivation Experiments in the Presence of Cape Cod Contaminated Sediment with Elutions by Contaminated Groundwater at pH 11.0[a]

experiment/ constituent	units	C_0	C_{att}	ΣC_{rel} ($n = 1-7$)	$\Sigma C_{rel} C_{att}^{-1}$ ($n = 1-7$)	ΣC_{rel} ($n = 8+$)	$\Sigma C_{rel} C_{att}^{-1}$ ($n = 8+$)	$k_{i,surf} + k_{rel}$ ($^{-1}$)
surface inactivation (contam gw attachment/pH 11 contam gw release)								
inf PRD1	pfu mL^{-1}	1.5 ($\pm$0.2) $\times$ 10^8	1.2 $\times$ 10^8	2.9 $\times$ 10^7	0.24	7.0 $\times$ 10^5	5.8 $\times$ 10^{-3}	1.8 ($\pm$0.1)
^{32}P-PRD1	cpm mL^{-1}	57 ($\pm$50)						
^{35}S-PRD1	cpm mL^{-1}	750 ($\pm$170)	410	270	0.65	12	0.030	1.1 ($\pm$0.1)
inf MS2	pfu mL^{-1}	1.3 ($\pm$0.2) $\times$ 10^8	1.1 $\times$ 10^8	1.4 $\times$ 10^7	0.12	5.1 $\times$ 10^5	4.6 $\times$ 10^{-3}	2.0 ($\pm$0.2)
^{32}P-MS2	cpm mL^{-1}	1800 ($\pm$340)	1000	740	0.74	41	0.041	0.83 ($\pm$0.04)
^{35}S-MS2	cpm mL^{-1}	11000 ($\pm$1200)	7900	3400	0.43	130	0.017	1.1 ($\pm$0.1)
surface inactivation (25 mg L^{-1} DBS + contam gw attachment/pH 11 contam gw release)								
inf PRD1	pfu mL^{-1}	1.5 ($\pm$0.2) $\times$ 10^8	1.1 $\times$ 10^8	3.2 $\times$ 10^7	0.29	6.9 $\times$ 10^4	6.3 $\times$ 10^{-4}	2.3 ($\pm$0.2)
^{32}P-PRD1	cpm mL^{-1}	57 ($\pm$50)						
^{35}S-PRD1	cpm mL^{-1}	750 ($\pm$170)	390	260	0.68	15	0.037	1.1 ($\pm$0.1)
inf MS2	pfu mL^{-1}	1.3 ($\pm$0.2) $\times$ 10^8	1.0 $\times$ 10^8	2.6 $\times$ 10^7	0.26	4.7 $\times$ 10^5	4.7 $\times$ 10^{-3}	2.2 ($\pm$0.2)
^{32}P-MS2	cpm mL^{-1}	1800 ($\pm$340)	930	790	0.85	40	0.042	0.82 ($\pm$0.03)
^{35}S-MS2	cpm mL^{-1}	11000 ($\pm$1200)	7500	3700	0.49	110	0.015	1.1 ($\pm$0.1)

[a] C_0 is the virus concentration in the first pore volume. C_{att} is the virus concentration attached during the first pore volume. ΣC_{rel} is the total amount of virus release during the 29 flushing pore volumes. The first-order "rate coefficients" $k_{i,surf} + k_{rel}$ (where k_{rel} is either $k_{rel,i}$ or $k_{rel,d}$) representing the combined surface inactivation and release steps were calculated for the first five pore volumes; the units are n^{-1}, where n is the number of daily pore volumes or flushes. Virus concentrations shown for infective (inf) and radiolabeled (^{32}P-, ^{35}S-) assays. The error for C_0 is one standard deviation for triplicate analyses.

unamended contaminated groundwater (Figure 4). The amount of ^{32}P and ^{35}S radioactivity released was not increased by the pH 11.0 elutions. The amount of infective PRD1 and MS2 released by the pH 11.0 elutions never exceeded 1% of the attached infective virus concentration (Table 4).

Discussion

Experimental Controls and Artifacts. Interpretation of the results of these field and laboratory experiments depends on a level of certainty that the ^{32}P and ^{35}S radiolabels were present only in infective PRD1 and MS2. If not, the release of the radiolabels from the aquifer sediments cannot be interpreted as the product of virus surface inactivation; instead, the radiolabel release may be the result of release of radiolabels never incorporated into the viruses or release of radiolabels in the debris of viruses inactivated between virus preparation and use in the experiments. The discrepancies between the amount of infective MS2 and radiolabel attachment suggest that some fraction of the radiolabels was not associated with the infective MS2. Part of this fraction was the radiolabels not incorporated into the infective viruses (11% of the ^{32}P and 7% of the ^{35}S for MS2; 9% of the ^{35}S for PRD1), and part

TABLE 5. Comparison of Actual and Expected Radioactivity Release Attributed to Virus Surface Inactivation for the Contaminated Groundwater/pH 11 Flush

experiment/ constituent	$C_{att,virus}$ (pfu mL^{-1})	$\sum_{n=1-7} C_{rel,virus}$ (pfu mL^{-1})	$\sum_{n=8+} C_{rel,virus}$ (pfu mL^{-1})	$\sum C_{inact}$ (pfu mL^{-1})	R_{virus}^{label} (cpm pfu^{-1})	$\sum C_{rel,label}^{expected}$ (cpm mL^{-1})	$\sum C_{rel,label}^{actual}$ (cpm mL^{-1})	$\sum C_{rellabel}^{actual}/\sum C_{rel,label}^{expected}$ (%)
surface inactivation (contam gw attachment/pH 11 contam gw release)								
inf PRD1	1.2×10^8	2.9×10^7	7.0×10^5	9.0×10^7				
^{32}P-PRD1				9.6×10^7	3.4×10^{-6}	310	280	91
^{35}S-PRD1	410 (±40)	270	12		9.1×10^{-6}	870	780	90
inf MS2	$1.1 (\pm 0.1) \times 10^8$	1.4×10^7	5.1×10^5		7.2×10^{-5}	6900	3500	51
^{32}P-MS2	1000 (±370)	740	41					
^{35}S-MS2	7900 (±1900)	3400	130					
surface inactivation (25 mg L^{-1} DBS + contam gw attachment/pH 11 contam gw release)								
inf PRD1	$1.1 (\pm 0.1) \times 10^8$	3.2×10^7	6.9×10^4	7.8×10^7				
^{32}P-PRD1				7.4×10^7	3.6×10^{-6}	280	280	100
^{35}S-PRD1	390 (±50)	260	15		9.3×10^{-6}	680	830	121
inf MS2	$1.0 (\pm 0.2) \times 10^8$	2.6×10^7	4.7×10^5		7.5×10^{-5}	5500	3800	69
^{32}P-MS2	930 (±150)	790	40					
^{35}S-MS2	7500 (±1000)	3,700	110					

[a] $C_{att,virus}$ is the concentration of attached viruses, $\sum_{n=1-7} C_{rel,virus}$ and $\sum_{n=8+} C_{rel,virus}$ are the amounts of virus released over the first seven pore volumes by contaminated groundwater and over the remaining pore volumes by pH 11 contaminated groundwater, respectively, $\sum C_{inact}$ is the total concentration of viruses inactivated on the surface, R_{virus}^{label} is the specific radioactivity of the attached viruses, and $\sum C_{rel,label}^{actual}$ and $\sum C_{rel,label}^{expected}$ are the actual and expected amounts of radioactivity release attributed to virus surface inactivation.

of this fraction may have been radiolabels associated with the debris of viruses inactivated between virus purification and use in the experiments. To check the importance of the radiolabels not associated with infective viruses in interpreting the results, we performed a mass balance on the radiolabels introduced to and released from the sediments in the laboratory surface inactivation experiments.

We calculated the mass balance for the PRD1 and MS2 attachment and release with the pH 11 flushes. The specific radioactivity of the viruses (R_{virus}^{label}, cpm pfu^{-1}) was calculated as the ratio of the attached concentration of each radiolabel ($C_{att,label}$, cpm mL^{-1}) to the attached concentration of the infective virus ($C_{att,virus}$, pfu mL^{-1}):

$$R_{virus}^{label} = \frac{C_{att,label}}{C_{att,virus}} \quad (3)$$

This calculation assumes that all of the ^{32}P and ^{35}S attached to the sediments in the first pore volume was incorporated in the infective viruses attached in the first pore volume. The total concentration of viruses inactivated on the surface ($\sum C_{inact}$, pfu mL^{-1}) was calculated as the difference between the concentration of infective viruses attached (C_{att}) and the sum of the infective viruses released over the first 7 pore volumes (n) flushed by contaminated groundwater ($\sum_{n=1-7} C_{rel,virus}$, pfu mL^{-1}) and the infective viruses released by the pH 11 flushes ($\sum_{n=8+} C_{rel,virus}$, pfu mL^{-1}):

$$\sum C_{inact} = C_{att} - \left(\sum_{n=1-7} C_{rel,virus} + \sum_{n=8+} C_{rel,virus} \right) \quad (4)$$

The pH 11 flushes were used for the mass balance to ascertain that all remaining infective viruses on the surface were released. The expected amount of radioactivity release caused by virus surface inactivation ($\sum C_{rel,label}^{expected}$, cpm mL^{-1}) was calculated as

$$\sum C_{rel,label}^{expected} = \sum C_{inact} R_{virus}^{label} \quad (5)$$

The actual amount of radioactivity release is

$$\sum C_{rel,label}^{actual} = \sum_{n=1-7} C_{rel,label} + \sum_{n=8+} C_{rel,label} \quad (6)$$

The result of the mass balance shows that actual release of radioactivity ranged from 51 to 121% of the expected release. For MS2, the ^{35}S release did not balance well (51% for the contaminated groundwater release, 69% for the DBS plus contaminated groundwater release), indicating that roughly one-third to half of the radioactivity released by virus surface inactivation remained on the sediment surfaces. If significant fractions of the radiolabels were not incorporated with the infective viruses initially deposited on the sediment, we would expect to see mass balances well in excess of 100%. Only for ^{32}P-MS2 did the mass balance exceed 100%, but not by a large amount. Although some unincorporated radiolabel was present in the suspensions added to the sediment and radiolabeled debris of viruses inactivated before the experiment may have been present, the mass balances suggest that these artifacts did not affect the surface inactivation experiments significantly. These "radiolabeling artifacts" may have been removed from the system because they were not adsorbed by the sediments during the attachment pore volume.

Proposed Mechanism of Virus Surface Inactivation. The field and laboratory data indicate that attachment of these bacteriophages to Cape Cod aquifer sediments results in surface inactivation at a rate substantially in excess of inactivation in solution. To frame a detailed discussion of the data, we present a process of surface inactivation fitting the observations made in the field and laboratory experiments (Figure 5): (1) attachment to ferric oxyhydroxide patches, favored by electrostatic attraction; (2) inactivation at the surface, caused by the strong attachment force; (3) release of noninfective, disintegrated viruses to solution.

Grant et al. (*31*) introduced a similar kinetic model, the quasi-equilibrium adsorption and surface sink. In batch studies, they found that it did not describe the attachment and inactivation of bacteriophage λ in the presence of Ottawa sand treated for removal of ferric oxides. In the following discussion, we will address aspects of the proposed surface inactivation process supported by the field and laboratory data.

Attachment during the Field Experiment. During the field experiment, attachment of PRD1 was extensive in both zones of the aquifer, but as we have observed in similar transport experiments (*18*, *21*), PRD1 was more mobile in the contaminated zone of the aquifer. Despite the presence

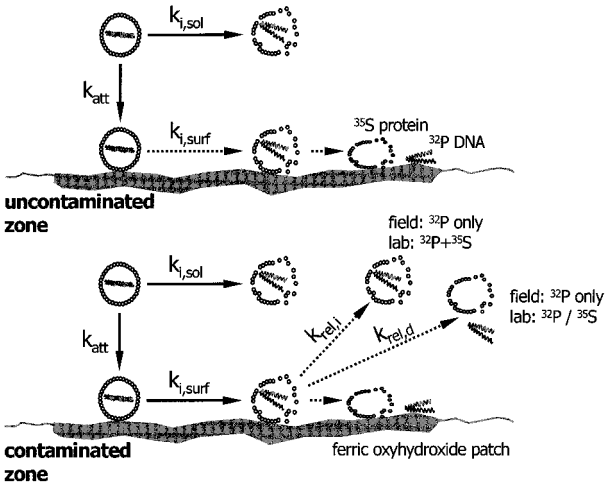

FIGURE 5. Kinetic model showing proposed surface inactivation process in uncontaminated and contaminated zones of Cape Cod aquifer. Virus attachment (k_{att}) to ferric oxyhydroxide patches is considered to be irreversible (colloid filtration). Solution inactivation ($k_{i,sol}$) occurs slowly at the groundwater temperature. Surface inactivation ($k_{i,surf}$) occurs rapidly relative to solution inactivation. Release of surface-inactivated viruses may produce intact, non-infective viruses ($k_{rel,i}$) or disintegrated viruses ($k_{rel,d}$). Only the combined rate of surface inactivation and release ($k_{rel,i} + k_{rel,d}$) can be determined from these experiments. In the laboratory, the release of intact or disintegrated viruses can be determined if the release behaviors of ^{32}P and ^{35}S are different.

of LAS, the collision efficiencies were relatively high in both zones, ~1 order of magnitude greater than collision efficiencies measured in the Cape Cod aquifer in our past work in which PRD1 was injected in unamended groundwater (*18*, *21*). We expected that co-injection of LAS with PRD1 would facilitate transport of the viruses because LAS was quite effective in promoting the release of attached PRD1 (*18*). Dodecylbenzene sulfonate, a specific linear alkylbenzene sulfonate isomer contained in the injected LAS mixture, reduced the attachment of PRD1 and MS2 in the attachment step of the surface inactivation experiments. The field results suggest that adsorbed LAS aided in the attachment of PRD1 to the sediments.

The attachment of viruses to the Cape Cod aquifer sediments can be attributed mainly to the favorable deposition of PRD1 on the ferric oxyhydroxide patches coating ~3−4% of the surfaces of quartz grains (*21*). Attachment of the negatively charged PRD1 to the patches is favored because ferric oxyhydroxides (e.g., goethite and lepidocrocite) are positively charged at the pH of the Cape Cod groundwater, ~5.5−6.0. The greater breakthrough of PRD1 in the contaminated zone can be attributed to organic matter and phosphate from the sewage infiltration—these constituents are capable of adsorbing to ferric oxyhydroxides, diminishing or even reversing the positive surface charge, and hindering virus attachment.

Attachment during the Laboratory Experiments. In the laboratory experiments, 50−92% of the infective PRD1 and MS2 attached to the contaminated zone sediments in the first pore volume. Although MS2 and PRD1 possess similar surface charges, infective MS2 attachment during the first pore volume was more extensive than infective PRD1 attachment. In contrast, attachment of ^{35}S-PRD1 was more extensive than that of ^{35}S-MS2. Most recent laboratory and field studies have found that PRD1 attachment is more extensive than MS2 attachment to a wide range of aquifer sediments (*5*, *17*, *33*, *34*), including Cape Cod sediments (*35*). We suspect these discrepancies may indicate that MS2 surface inactivation and release is more rapid than that of PRD1. Greater attachment of infective MS2 and greater release (or less attachment) of ^{35}S-MS2 activity would be consistent with more rapid surface inactivation of MS2.

As we noted above, the addition of dodecylbenzene sulfonate (DBS) to the virus suspensions reduced attachment during the first pore volume for both PRD1 and MS2. The anionic DBS is expected to adsorb readily to positively charged ferric oxyhydroxide patches, possibly to the extent that the surface charge of the patches is reversed and virus attachment is inhibited.

Surface Inactivation during the Field Experiment. The ferric oxyhydroxide patches are good PRD1 collectors owing to strong electrostatic attraction between positively charged patches and negatively charged PRD1. For the same reason, these patches should promote surface inactivation. Murray and Parks (*36*) showed that the extent of surface inactivation of poliovirus was related to the strength of attraction between the mineral or metal and the poliovirus. Aluminum metal and some transition metal oxide (CuO, α-Al_2O_3) surfaces adsorbed poliovirus more extensively and caused much greater poliovirus inactivation. Because their experiments were all conducted with negatively charged surfaces and poliovirus, which possessed a slight negative charge at the pH of their experiments, Murray and Parks (*36*) attributed the strength of attraction—and extent of surface inactivation—solely to van der Waals attraction.

A calculation of the DLVO (*37*, *38*) electrostatic interaction using constant-potential double layers (*38*), retarded van der Waals interaction (*39*), and sphere−sphere geometry for poliovirus and CuO interaction at an ionic strength of 0.305 M, a Hamaker constant of 1.0×10^{-20} J, and zeta potentials of −5.3 mV for CuO and −1.8 mV for poliovirus [values measured and estimated by Murray and Parks (*36*)] gives an attractive potential energy at a 1 nm separation distance of only 8.5 kT. The same calculation for PRD1 and a goethite surface (a model for the ferric oxyhydroxide patches) at an ionic strength of 2 mM and zeta potentials of 5 mV for goethite (for pH slightly less than the point of zero charge of goethite) and −26 mV for PRD1 (*21*) produces an attractive potential energy of 75 kT. Therefore, the ferric oxyhydroxide patches on the Cape Cod sediment should bind PRD1 strongly enough to promote surface inactivation. Finally, we note that the attachment of bacteriophage λ to Ottawa sand from which ferric oxyhydroxides had been chemically removed did not result in surface inactivation (*32*), similar to the lack of surface inactivation of poliovirus by a quartz surface observed by Murray and Laband (*15*).

Surface Inactivation during the Laboratory Experiments. The laboratory surface inactivation experiments confirmed that the Cape Cod aquifer sediments caused surface inactivation of PRD1 and MS2. The rapid divergence of the infective virus concentration and ^{35}S and ^{32}P activities as a function of the number of pore volume flushes indicates that surface inactivation occurred (*15*). PRD1 and MS2 surface inactivation and release "rates" were similar within the standard error of the first-order "rate coefficient", $k_{i,surf} + k_{rel}$, which was calculated with units of n^{-1}, where n is the number of flushing pore volumes (Table 3; Figure 3). These per-pore-volume rate coefficients were calculated over the first 5 pore volumes of release only because ^{32}P and ^{35}S radioactivities typically approached the relative detection limits for these measurements in 5 pore volumes of release. Over the first 5 pore volumes, the infective virus concentration decreased about twice as fast as the decrease in ^{35}S, which labeled the protein capsid of the viruses, for experiments in which the viruses were deposited in contaminated groundwater, and ~3 times as fast when the viruses were deposited in DBS-amended groundwater.

The presence of DBS during attachment increased the combined rate of surface inactivation and release ($k_{i,surf} +$

k_{rel}) for infective PRD1 and MS2 by ~43% (Table 3), a statistically significant difference (unpaired t test, $p < 0.05$). However, the combined rate for radiolabels was the same in groundwater with and without DBS. The presence of DBS also resulted in a greater inactivation rate for PRD1 and MS2 in solution, but the difference was not significant. Because we do not have a clear indication that DBS directly affects virus inactivation from the solution experiments, we suspect that the difference in $k_{i,surf} + k_{rel}$ for the infective viruses and radiolabels may be caused by some difference in the way that DBS affects release of intact, infective viruses and their inactivation debris (protein capsid and nucleic acid).

The nature of the direct role of attachment in inactivation is not clear. Viruses interact with mineral surfaces through their protein capsids. It is well-known that mineral surfaces can have a denaturing effect on proteins by dehydration and disruption of interactions within peptide chains (*41*, *42*). The distortions and unfolding of protein structures may be the primary cause of virus inactivation. Such interactions could result in virus degradation and separate release of protein capsid components and nucleic acids ($k_{rel,d}$; Figure 4). For poliovirus, Murray and Parks (*36*) surmised that surface inactivation caused a conformational change resulting in a noninfective state with a decreased pH_{iep}, a feature reported for other strains of poliovirus, coxsackievirus, and echovirus, but never linked to surface inactivation. A decrease in pH_{iep} on inactivation could favor release from certain mineral surfaces; therefore, the result of surface inactivation could be the release of intact, noninfective viruses ($k_{rel,i}$; Figure 4).

Release during the Field Experiment. In the field experiment, the long, slow release of ^{32}P activity in the contaminated zone (84% of the total ^{32}P injected), which occurred without any significant release of infective PRD1, must have been the result of surface inactivation and release of inactivated PRD1. We can use the field data to determine the combined rate of surface inactivation and release ($k_{i,surf} + k_{rel}$, where k_{rel} may be either $k_{rel,i}$ or $k_{rel,d}$) but not the individual rate coefficients, because we observe the result of surface inactivation only after the ^{32}P is released. Fitting the trailing edge of the ^{32}P activity breakthrough curve (Figure 2) by first-order decay gives a combined rate coefficient of $k_{i,surf} + k_{rel} = 0.070 \pm 0.003$ day^{-1}, or a half-life of 9.9 days.

To compare this combined surface inactivation and release rate with the rate of inactivation in the solution phase, we must estimate a solution phase inactivation rate coefficient for 15 °C, the temperature of the groundwater. Bales et al. (*43*) reported a PRD1 inactivation rate coefficient of 0.10 day^{-1} (*44*) at a temperature of 11.5 °C for their Cape Cod field experiment. A two-point regression of the natural logarithm of the rate coefficient versus temperature (*9*) with 5 and 11.5 °C inactivation rate coefficients yields a 15 °C inactivation rate coefficient of 0.21 day^{-1}, or a half-life of 3.3 days. A similar estimate using data gathered by Schijven and Hassanizadeh (*9*) gives a lower inactivation rate coefficient estimate of 0.071 day^{-1}, but some of the rate coefficients used in this regression were measured in sewage effluent, which typically protects viruses from inactivation. Thus, at 15 °C, our estimated solution inactivation rate is ~3 times as fast as the combined surface inactivation/release rate. The presence of radiolabels and LAS might cause even more rapid inactivation in solution, but surface inactivation rates might be accelerated by radiolabels and LAS as well.

For two reasons, we suspect that surface inactivation is actually much more than 3 times as fast as solution inactivation. First, in the surface inactivation experiments, we observed losses of PRD1 infectivity approaching an order of magnitude per day over the first 24 h of PRD1 exposure to the sediments. In contrast, unattached PRD1 experienced less than an order of magnitude loss of infectivity over 30 days in the solution inactivation experiments. Second, we suspect that release of the inactivated PRD1 is the rate-limiting step (i.e., $k_{rel,i}$ or $k_{rel,d} \ll k_{i,surf}$) because attachment to the ferric oxyhydroxide patches is favorable. Favorable attachment conditions suggest that release would be slow or irreversible. Attachment was irreversible in the uncontaminated zone, in accord with laboratory experiments examining the reversibility of PRD1 attachment to ferric oxyhydroxide-coated quartz (*22*). Notably, Schijven et al. (*5*) obtained the best fits to field transport data for MS2 with a surface inactivation rate coefficient ~3 times greater than the solution inactivation rate coefficient. For PRD1, the best fit value of the surface inactivation rate coefficients was only about half the value of the field-measured solution inactivation rate coefficient (0.12 day^{-1}, 2−5 °C) but ~7 times greater than the solution inactivation rate coefficient measured in the same water in the laboratory (0.0094 day^{-1}, 2−8 °C). A detailed analysis of surface inactivation by Schijven and Hassanizadeh (*9*) revealed that MS2 and PRD1 inactivation is enhanced by attachment to sediment surfaces in most cases.

Because ^{32}P was nonspecifically incorporated into both the nucleic acid and protein capsid of the PRD1 for the field experiment, we cannot determine if the inactivated PRD1 was released as intact or disintegrated virus ($k_{rel,i}$ or $k_{rel,d}$). Too few viruses and too little ^{32}P activity were present in the groundwater samples to examine the molecular weight distribution of the released PRD1 material by rate zonal centrifugation. Likewise, the laboratory surface inactivation experiments for PRD1 yielded limited information on the fate of the nucleic acid and protein capsid because insufficient ^{32}P was incorporated into the PRD1.

Release during the Laboratory Surface Inactivation Experiment. The laboratory surface inactivation experiment did provide insight into the fate of MS2. Only small fractions (0.84 and 5.6%) of the infective MS2 were released during the 29 groundwater flushes, whereas one-fifth of the ^{35}S (protein capsid) and nearly half of the ^{32}P (nucleic acid) were released (Table 3). Although greater fractions of the attached ^{32}P were released, the "rate" (per pore volume) of ^{35}S release was slightly faster than the ^{32}P release rate. The difference in release rates is not statistically significant (unpaired t test, $p > 0.09$ for both cases), but the result is consistent for both surface inactivation experiments and the pH 11 flushing experiment. These differences between the amount and rate of ^{35}S and ^{32}P release suggest that the inactivated MS2 was disintegrated by surface inactivation; that is, it may have been released as separate protein capsids and nucleic acid (the release step designated $k_{rel,d}$).

The protein capsid (^{35}S) remained bound to the sediment surface to a greater extent than the nucleic acid (^{32}P), but the protein capsid was released more rapidly than the nucleic acid. DNA adsorbs readily to many mineral surfaces through interaction of phosphate functional groups with mineral surface functional groups (*45*−*49*). Proteins also readily adsorb to mineral surfaces, but the disruptions and distortions in their structure caused by attachment result in denaturing and spreading (*50*, *51*) that may strengthen or weaken attachment. For example, the "footprint" (amount of surface area occupied by a molecule) of albumin and fibrinogen molecules increases following adsorption onto hydrophobic and hydrophilic surfaces (*52*, *53*). It is not clear from the literature if this behavior promotes or inhibits protein desorption kinetics, but the adsorption of most proteins is partially reversible and the fraction of reversible adsorption is dynamic (*54*−*59*). Interestingly, an anionic surfactant, sodium dodecyl sulfate, promotes protein desorption following a change in protein conformation (*60*, *61*), suggesting another mechanism for the role of sodium dodecylbenzene sulfonate in reducing virus attachment in our experiments.

After ~5 pore volumes of flushing, the levels of infective virus, ^{35}S, and ^{32}P reached CC_{att}^{-1} plateaus near the relative detection limits for these constituents (Figure 3). After ~15–20 pore volumes, however, the infective virus release increased, most noticeably for MS2 following attachment by contaminated groundwater. Similar results have been reported for MS2 during solution inactivation studies testing the effects of iodine as a disinfectant (*30*) and temperature (*62*). In the case of iodine disinfection, this phenomenon, termed "rebound", was attributed to the consumption of iodine by beef extract. In the temperature study, the rebound was attributed to reactivation of virions that had undergone rearrangement or loss of capsid components at high temperature. We cannot speculate on the rebound observed in our experiments because we do not yet know enough about the exact mechanism of surface inactivation of viruses.

Environmental Implications. To perform the field experiment, it was necessary to use bacteriophages in lieu of viruses that could pose a health threat to humans. Nevertheless, the relevance of these results for the subsurface transport potential of pathogenic viruses such as hepatitis A, Norwalk, rotavirus, poliovirus, coxsackievirus, and other enteroviruses is expected to be high because the proposed surface inactivation mechanisms are based on electrostatic interactions between viruses and grains and virus structure.

The importance of surface inactivation as a virus removal mechanism depends on the strength of virus attachment to the mineral surface (*15*). As the strength of virus attachment increases, so does the likelihood that the attachment is "irreversible" or that the kinetics of release are extremely slow (*22*). Nevertheless, some release of infective viruses was observed in these field and laboratory experiments. We conclude that the release of infective viruses would have been much greater if inactivation of attached viruses had not been occurring.

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