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Poliovirus Adsorption by 34 Minerals and Soils

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The adsorption of radiolabeled infectious poliovirus type 2 by 34 well-defined soils and mineral substrates was analyzed in a synthetic freshwater medium containing 1 mM $CaCl_2$ and 1.25 mM $NaHCO_3$ at pH 7. In a model system, adsorption of poliovirus by Ottawa sand was rapid and reached equilibrium within 1 h at 4°C. Near saturation, the adsorption could be described by the Langmuir equation; the apparent surface saturation was 2.5×10^6 plaque-forming units of poliovirus per mg of Ottawa sand. At low surface coverage, adsorption was described by the Freundlich equation. The soils and minerals used ranged from acidic to basic and from high in organic content to organic free. The available negative surface charge on each substrate was measured by the adsorption of a cationic polyelectrolyte, polydiallyldimethylammonium chloride. Most of the substrates adsorbed more than 95% of the virus. In general, soils, in comparison with minerals, were weak adsorbents. Among the soils, muck and Genesee silt loam were the poorest adsorbents; among the minerals, montmorillonite, glauconite, and bituminous shale were the least effective. The most effective adsorbents were magnetite sand and hematite, which are predominantly oxides of iron. Correlation coefficients for substrate properties and virus adsorption revealed that the elemental composition of the adsorbents had little effect on poliovirus uptake. Substrate surface area and pH, by themselves, were not significantly correlated with poliovirus uptake. A strong negative correlation was found between poliovirus adsorption and both the contents of organic matter and the available negative surface charge on the substrates as determined by their capacities for adsorbing the cationic polyelectrolyte, polydiallyldimethylammonium chloride.

The increasing needs for groundwater recharge and land disposal of wastewater effluents and sludges have increasingly focused interest on the fate of viruses in aquatic and soil environments. The many types of human pathogenic viruses that have been found in water and soil include polioviruses, coxsackieviruses, echoviruses, hepatitis A virus, reoviruses, rotaviruses, and adenoviruses. It is highly desirable to define optimum conditions for effluent and sludge disposal and optimum characteristics for disposal sites. Investigations of the basic mechanisms of virus-soil interactions are important in achieving this objective.

The interaction of virus particles and soils is a complex phenomenon involving adsorption and desorption and, possibly, inactivation and physical destruction. The behavior of the virus is determined by its surface chemical structure and charge, its state of aggregation, and the presence of other materials adsorbed to its surface. The behavior of minerals and soils toward virus particles depends on their composition, their surface properties, and the presence of materials which partially or completely coat them. The pH and ionic composition of the suspending medium are important additional factors which influence these interactions.

Poliovirus has been the most widely studied of all human pathogenic viruses prevalent in water and soil environments (3). However, there is little information available about poliovirus uptake by other than a few well-defined soils and minerals (4, 7, 11, 23, 27). The uptake of poliovirus by a wide variety of well-defined minerals and soils and correlation with their properties is the subject of this report.

A batch-type method used to study poliovirus uptake had the following features: (i) a nonreactive container surface to minimize virus adsorption to it, (ii) a virus preparation relatively free of other proteins and cell debris, (iii) a pH and an electrolyte content of the medium resembling

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that of freshwater, (iv) amounts of virus and substrate sufficient to permit measurement of uptake after a short time, and (v) infectious virus radiolabeled to provide more accurate information about the physical state of the virus after interaction with the substrate.

Establishment of such a batch-type method in Oak Ridge polypropylene centrifuge tubes with a synthetic freshwater enabled us to study adsorption and elution of virus and to predict the maximum adsorption under specific conditions. Here, we describe the development of the method from study of adsorption of poliovirus to a pure silica substrate, Ottawa sand, which is widely employed in water-treatment processes. This method was applied to the study of poliovirus adsorption by 34 different soils, minerals, and crushed rocks. Successive reports in this series deal with the mechanisms of virus adsorption and the effects of environmental variables, including overgrowth of soil particles by exopolymer-forming bacteria, on virus uptake.

MATERIALS AND METHODS

Virus and cell line. Poliovirus type 2 (vaccine strain P712-ch-2ab) was produced and assayed in monolayer cultures of HeLa cells grown in Dulbecco modified Eagle medium supplemented with 10% fetal bovine serum.

Virus purification. HeLa cell monolayers were washed with Dulbecco phosphate-buffered saline and infected at a multiplicity of 50 plaque-forming units (PFU) per cell. After adsorption for 1 h at 37°C fresh Dulbecco modified Eagle medium without serum was added, and 2 h later a ^{3}H-labeled mixture of amino acids was added at 0.1 mCi/ml of medium. The detached host cells and the medium were harvested 6 h after infection, and the cells were collected by centrifugation at 650 × *g* for 20 min at 4°C in a Sorvall RC-5 centrifuge with a GSA rotor.

The cell pellet was resuspended in phosphate-buffered saline and frozen and thawed three times to release intracellular virus. Cell debris was removed by centrifugation at 12,000 × *g* for 20 min at 4°C in an SS-34 rotor. The virus suspension was purified by two successive isopycnic sedimentations in cesium chloride in a Beckman L3-50 ultracentrifuge with an SW41 rotor. Virus was sedimented by centrifugation at 110,000 × *g* for 16 to 18 h at 4°C. The purified virus was dialyzed against phosphate-buffered saline at 150 to 300 times the volume with two changes of dialyzate. Stocks were stored at −70°C.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the purified virus revealed little contamination by nonviral proteins in most preparations.

Sedimentation analysis by the method of Floyd and Sharp (12) showed no significant virus aggregation.

Virus assay. Poliovirus infectivity was assayed by plaque titration with subconfluent HeLa cell monolayers in 60-mm tissue culture dishes. Cell monolayers were washed, and virus dilutions were prepared in phosphate-buffered saline. The agar overlay contained 0.95% Noble agar (Difco Laboratories, Detroit, Mich.), and Eagle minimal essential medium supplemented with 5% fetal bovine serum. After 24 h a second agar overlay containing 0.02% neutral red was added, and plaques were visualized. For measurement of radioactivity, samples were mixed with 10 ml of Aquasol (New England Nuclear Corp., Boston, Mass.) and counted in a Beckman LS 250 liquid scintillation spectrometer (Beckman Instruments, Inc., Fullerton, Calif.).

Substrates. Preliminary studies were made with Ottawa sand (VWR Scientific, Rochester, N.Y.), a quartz sand of uniform particle size (0.6 to 0.8 mm) meeting ASTM C190 specifications. All materials used as substrates for virus adsorption studies are listed in Table 1. A number of American Petroleum Institute reference clay minerals are included; further information on these clays may be obtained from American Petroleum Institute Preliminary Report 49 (1). Tofflemire and Chen (40) have described the site locations of the Riverhead and Beckett soils and a number of their physical, chemical, and mineralogical properties.

Substrate preparation. Each sample obtained from Ward's Natural Scientific Establishment (Table 1, no. 1 through 30) was prepared as follows. When necessary, for large rocks, a 200-g amount was crushed in a jaw crusher and passed through a steel disk grinder or a tungsten-carbide ring mill; the part passing through a 0.15-mm sieve was collected. Immediately after grinding, any metal particles from the disk grinder were removed from the samples with a magnet. Each sample was stored in a polyethylene container at room temperature.

Each original sample of Riverhead or Beckett soil was stored in a closed plastic bag at 4°C; 1 kg of each was air dried at 22°C and lightly ground in a mortar and pestle, and the fraction of <2-mm particles was separated by sieving and used for these studies.

Hydrous iron oxide was prepared by hydrolysis of iron (III) nitrate solution at 100°C (2). The precipitate was washed twice at the centrifuge and then dialyzed against glass-distilled water until the ionic strength, measured by conductivity, was less than 10^{-4} M. The solid was dried at 105°C; X-ray diffraction analysis showed the material was α-Fe_2O_3 (hematite).

Substrate characterization. Nitrogen gas was used to determine surface areas by a dynamic gas adsorption procedure with a Monosorb Surface-Area Analyzer (Quantachrome Corp., Greenville, N.Y.). Samples were outgassed at 200°C for 30 min. Calibration was checked between samples. The surface area of Ottawa sand was determined by methylene blue adsorption as described by van den Hul and Lyklema (42), except that 20 g of sand and 20 ml of dye solutions were used. The sand was not further characterized.

The pH of the suspensions of the various soils and substrates in distilled water was determined by a modification of Peech's method (30). CO_2-free distilled water (0.5 ml) or the minimum volume required to produce a slurry, if 0.5 ml was insufficient, was added to 0.5 g of substrate in a 50-ml centrifuge bottle and mixed with a Vortex stirrer. After 30 min the samples were shaken, and after another 30 min they were reagitated with the Vortex stirrer. The pH of the

TABLE 1. *Soils, minerals, and other substrates used in the study*

No.	Name	Location[a]	Description
1	Dolomite	Penfield, N.Y.	$CaMg(CO_3)_2$, a crystalline sedimentary rock
2	Montmorillonite, API no. 31[b]	Cameron, Ariz.	Smectite or expansible 2:1 layer silicate clay mineral
3	Montmorillonite, API no. 26	Clay Spur, Wyo.	Smectite of marine formation
4	Quartz	Herkimer Co., N.Y.	SiO_2, crystalline rock
5	Bituminuous shale	LeRoy, N.Y.	Sedimentary rock containing hydrocarbon
6	Calcite	Rosiclare, Ill.	$CaCO_3$, crystalline rock including some silicates
7	Fossiliferous limey shale	Summit, N.Y.	Sedimentary rock of marine origin
8	Illite-bearing shale	Rochester, N.Y.	Sedimentary rock predominantly illite, a phyllosilicate
9	Argillaceous shale	Rochester, N.Y.	Sedimentary rock of consolidated clays
10	Arenaceous shale	Green Co., N.Y.	Sedimentary rock of consolidated sands
11	Calcareous shale	Lima, N.Y.	Sedimentary rock containing much $CaCO_2$
12	Kaolinite, API no. 5	Bath, S.C. (Lamar Pit)	1:1 Layer structured aluminosilicate clay mineral
13	Halloysite, API no. 13	Eureka, Utah (Dragon Iron Mine)	Hydrated kaolinite mineral
14	Dickite, API no. 16	St. George, Utah (Bull Valley)	Disordered form of kaolinite
15	Attapulgite, API no. 64	Attapulgus, Calif.	Palygorskite, fibrous 2:1 layer phyllosilicate clay mineral
16	Lake Ontario dune sand	Monroe Co., N.Y.	Predominantly silica sand, also calcareous and organic particles
17	Lake Superior dune sand	Autrain, Mich.	Predominantly silica sand, some iron oxide
18	Lake Ontario beach sand	Rochester, N.Y.	Predominantly silica sand, ca. 5% calcareous
19	Magnetite sand	Marysville, Calif.	ca. 65% magnetite (Fe_3O_4), ca. 25% hematite (α-Fe_2O_3), and ilmenite ($FeTiO_3$)
20	Colonie sandy loam	Monroe Co., N.Y.	Entisol, alfic udipsamment, mixed, mesic
21	Genesee silt loam	Monroe Co., N.Y.	Inceptisol, fluventic eutrochrepts, fine-loamy, mixed, mesic, 15 to 30 cm
22	Brown sandstone	Medina, N.Y.	Calcareous sedimentary rock of consolidated quartz sand
23	Quartz conglomerate	Near Olean, N.Y.	Calcareous conglomerate of sand and gravel-size quartz
24	Glauconite	Birmingham, N.J.	A phyllosilicate similar to illite
25	Adobe	Deming, N.M.	An unclassified heavy-textured clayey soil
26	Genesee silt	Monroe Co., N.Y.	Inceptisol, fluventic eutrochrepts, fine-loamy, mixed, mesic, same site as 21, 30 to 60 cm
27	Muck	Wayne Co., N.Y.	Histosol, terric medisoprists, euic; mesic, 6 to 12 in. (ca. 15 to 30 cm)
28	Kaolinite, API no. 4	Macon, Ga.	A 1:1 layer structured aluminosilicate clay mineral (kaolinite)
29	Kame conglomerate	Monroe Co., N.Y.	A poorly consolidated sedimentary sand and gravel, from Palmyra gravelly outwash
30	Loess	Kansas City, Kans.	Unclassified, consolidated, wind-blown soil
31	Riverhead sandy loam	Riverhead, N.Y.	Inceptisol, typic dystrochreft, coarse-loamy, mixed, mesic, acid outwash soil, 40 to 60 cm
32	Becket silt loam	Lake Pleasant, Hamilton Co., N.Y.	Spodosol, typic fragiorthods, coarse-loamy, mixed, frigid, acid till, 20 to 40 cm
33	Ottawa sand (unground)	Ottawa, Ill.	Quartz sand, 0.6 to 0.8 mm
34	Hydrous iron oxide	Synthetic	α-Fe_2O_3, hematite

[a] Sources: no. 1–3 and 5–30 were obtained from Ward's Natural Scientific Establishment, Rochester, N.Y.; no. 4 was a gift of R. M. Bryce, New York State Geological Survey; no. 31 and 32 were gifts of T. J. Tofflemire, New York State Department of Environmental Conservation; no. 33 was obtained from VWR Scientific, Rochester, N.Y.; and no. 3 was prepared by acid hydrolysis of $Fe(NO_3)_3$ at 100°C.

[b] American Petroleum Institute (API) reference clay mineral.

suspension was then determined by using a Radiometer PHM 4 pH meter and combination electrode.

The carbon and nitrogen contents of the various soils and substrates were determined with a Perkin-Elmer Model 240 elemental analyzer operating at 900°C (17, 29, 31). Finely divided samples were weighed on 2.4-cm glass fiber filters, rolled up on the filters, and placed in the sample boats. Analysis of such samples, carried out in duplicate, gave total carbon and nitrogen contents. To determine organic carbon, three 100-μl portions of 2 M H_3PO_4 were added to the sample wrapped in the filter, which was then dried at 85°C for 30 min. This treatment will remove up to 15 mg of calcitic carbonate or 8 mg of dolomitic carbonate. The carbon remaining after this acid treatment was assumed to be organic.

Low-temperature ashing subjects a sample to an oxygen plasma at low pressure and removes surface organic films and coatings by oxidation. Weighed duplicate samples of oven-dried materials in glass boats were subjected to the oxygen plasma of an LTA-302 low-temperature asher (LFE Corp., Waltham, Mass.), operating at about 75°C and 1 mm Hg for 4 h with occasional agitation. After cooling to room temperature in a desiccator, the weights were redetermined, and the weight loss was expressed as a percentage of the oven-dried weight.

The apparent protein content of various soils and substrates, including some which had been overgrown with bacteria, was determined in the following manner. Duplicate 0.5-g samples of each material were extracted twice with 2 ml of 0.5 M NaOH for 15 min in a boiling water bath, with occasional shaking. The two extracts were combined, and the volume was adjusted to 5.0 ml before centrifugation at 5,000 rpm for 10 min. One milliliter of the supernatant was used for protein determination by the method of Lowry et al. (24). Since the Lowry reagent is essentially an indicator of aromatic amino acids, the apparent protein content is a measure of biological activity within the soil or of the presence of incompletely degraded organic matter.

The nitric acid-extractable content of a number of elements in each substrate was determined by the method of Krishnamurty et al. (21). Duplicate 0.5-g samples were digested with 10 ml of concentrated HNO_3 in a covered flask for 2 h at ca. 100°C. After cooling, 3 ml of 30% H_2O_2 was added dropwise, and the heating and intermittent shaking were continued for 1 h. The extracting solutions were separated from the substrates by vacuum filtration through sintered-glass filters or centrifugation at 10,000 rpm for 30 min. All solutions were analyzed for phosphate by the ammonium molybdate method and for metals by atomic absorption spectroscopy according to American Public Health Association or Environmental Protection Agency standard methods or Environmental Protection Agency-approved methods.

Polydiallyldimethylammonium chloride (PDADMA) (Magnifloc 585c; American Cyanamid Co., Pearl River, N.Y.) was twice purified by precipitation from distilled water with acetone, dialyzed against distilled water, and dried at 85°C. The various substrates were taken in conical flasks and shaken with 20 ml of distilled water for about 1 h. (Substrate was used in an amount such that from 20 to 60% of the added polymer was adsorbed.) Then 20 ml of 0.01 N PDADMA solution was added, and the shaking was continued for 1 more h. The pH was determined, and the suspension was centrifuged at 3,000 rpm for 10 min (or longer if required). Three 10-ml samples were withdrawn and titrated against potassium polyvinylsulphate (Eastman-Kodak, Rochester, N.Y.) with 0.1% toluidine blue O as indicator (19). The difference between the mean titer of the sample and a suitable control (without substrate) was taken to be the amount of cationic polymer adsorbed.

Suspending solution. The composition of the synthetic freshwater used for all experiments is given in Table 2. It was prepared by dissolving KCl (8 mg/liter), $MgSO_4 \cdot 7H_2O$ (92 mg/liter), $CaCl_2 \cdot 2H_2O$ (147 mg/liter), and $NaHCO_3$ (105 mg/liter) in water filtered through a membrane filter (Millipore Corp., Bedford, Mass.). The pH was adjusted to 7.0 with 0.2 N NaOH; the solution was filtered-sterilized and stored at 4°C in borosilicate-glass or plastic bottles. The pH was readjusted to 7.0 before experimental use. The rationale for selecting this solution is outlined below.

Batch virus-substrate interactions. Adsorption experiments were performed in 50-ml Nalgene (no. 3119-0050) Oak Ridge-type polypropylene centrifuge bottles, unless otherwise stated. A 100-μl sample of the suspending medium containing 10^8 to 10^9 PFU and 10^3 to 10^4 cpm of poliovirus was added to a slurry of 500 mg of substrate in 1.9 ml of the solution. (In studies of the influence of concentration the quantities of both virus and substrate were varied.) The containers were shaken at 300 rpm on a New Brunswick G2 Gyrotory shaker for 1 h at 4°C. The substrate and suspending medium were then separated by centrifugation in a Sorvall RC-5 centrifuge with an SS-34 rotor at 12,000 × *g* for 10 min at 4°C. The pH of the suspending medium was measured, and samples were removed for measurement of virus infectivity and radioactivity. All experiments were carried out in duplicate or triplicate.

The amount of virus adsorbed was calculated as the difference between the amount recovered from suitable control samples and the amount found in the supernatant liquors of samples containing substrate. Experiments were performed in duplicate. The average variability between duplicates was typically 15 and 10% of the mean for infectivity and radioactivity, respectively. For calculations of infectivity, it was assumed that there was no change in the degree of aggregation or infectivity.

Computing. Statistical analysis of the data was performed using BMDP-77 Biomedical Computer Programs (University of California Press, Berkeley, Calif.) and a PDP 11/08 computer.

RESULTS

Effect of container composition on virus recovery. A variety of materials were tested for poliovirus adsorption to select a suitable container with minimum reactivity. The results (means of five experiments) are summarized in Table 3. Of the materials tested, polypropylene permitted the greatest recovery of radiolabeled virus. Lowest recovery was obtained in polycar-

TABLE 2. *Analysis of some New York state waters and derivation of synthetic freshwater medium*

Water	meq/liter (%) of:							
	Na^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	SO_4^{2-}	HCO_3	Total[a]
Long Island meq/liter/(meq-%)	0.339 (51.4)	0.025 (3.8)	0.088 (13.3)	0.209 (31.7)	0.197 (29.8)	0.166 (25.2)	0.295 (44.7)	0.660
Adirondack[b] meq/liter/(meq-%)	0.097 (25.7)	0.010 (2.7)	0.104 (27.5)	0.171 (45.1)	0.040 (10.4)	0.199 (52.2)	0.143 (37.4)	0.382
Central New York[c] meq/liter/(meq-%)	1.136 (3.4)	0.048 (1.2)	0.604 (15.1)	3.212 (80.3)	0.180 (4.5)	0.548 (13.7)	3.272 (81.8)	4.00
Sewage addition[d] meq/liter/(meq-%)	0.917 (20)	0.058 (1)	1.133 (25)	2.502 (54)	1.080 (23)	0.496 (11)	3.034 (66)	4.61
Synthetic freshwater meq/liter/(meq-%)	1.25 (30)	0.10 (2)	0.75 (18)	2.00 (49)	2.10 (52)	0.75 (17)	1.25 (31)	4.10

[a] As anions or cations.
[b] Orebed Reservoir, Lake George Watershed.
[c] Ocquionis Creek, Canadarago Lake Watershed.
[d] Change in concentration from source to sewage treatment plant, calculated from Lake George Village data.

TABLE 3. *Effect of container composition on recovery of radiolabeled poliovirus*[a]

Material	Virus recovery[b] (%)	
	Mean ($n = 5$)	Range
Polypropylene	83	76–88
Polyethylene	72	68–76
Polystyrene	56	46–66
Quartz glass	55	40–58
Borosilicate glass	28	20–34
Teflon	17	14–19
Polycarbonate	14	12–15

[a] The suspending medium was 0.01 M 1,4-piperazinediethanosulfonic acid sodium salt, pH 7.0.
[b] Percentage of added radioactivity.

bonate, Teflon, and borosilicate glass containers. Intermediate levels were found in containers made from polyethylene, polystyrene, and quartz glass. Based upon these data, an Oak Ridge style polypropylene centrifuge bottle (50-ml capacity) was chosen as the reaction container. The size and round configuration of the bottom of this vessel permit efficient mixing of the substrate and suspending medium on a Gyrotory shaker. This vessel permits separation of solid and liquid phases for sampling by centrifugation without requiring an additional transfer step. A large number of samples can be handled in the same fashion at one time.

Synthetic freshwater medium. The design of a formula for synthetic freshwater is by necessity a compromise. Natural waters vary widely in composition. More importantly, most natural waters are essentially solutions of calcium bicarbonate which cannot be reproduced by dissolving shelf chemicals in distilled water. Many freshwaters are not autoclavable because, at least during heating, they become strongly supersaturated with $CaCO_3$.

The average ionic content of three representative natural waters in New York State and the effect of additions of domestic waste are shown in Table 2. Our synthetic freshwater (Table 2) is representative of an average freshwater, as amended to reflect use by a residential community (i.e., supplemented with secondary sewage effluent). Calcium content was lowered to facilitate preparation.

Other properties of this water are as follows: ionic content, 4.1 meq of anions or cations per liter; ionic strength, 0.006; and point of saturation with $CaCO_3$ at 20°C, between pH 7.8 and 7.9. At 80°C, supersaturation is 0.5 mM. CO_2 pressure is three times ambient at this pH at 20°C and four times at 80°C. The pH was adjusted to 7 before adsorption studies.

Time course of virus uptake. The kinetics of poliovirus removal by adsorption to Ottawa sand was determined by monitoring the loss of virus infectivity and radioactivity from the suspending medium. Poliovirus uptake by Ottawa sand was rapid. As shown in Fig. 1, more than 70% of poliovirus infectivity was removed within 15 min, and adsorption reached a plateau after 60 min. Radioactivity measurements gave parallel results.

The effect of temperature on poliovirus adsorption was measured at 4 and 25°C. The extent of uptake at the two temperatures was indistinguishable, both by infectivity and radioactivity measurements.

Adsorption isotherms. Adsorption isotherms describing the distribution of virus at equilibrium were constructed by varying the amount of substrate or total virus while holding other factors constant. The amount of poliovirus adsorbed per unit weight of sand for various free-virus concentrations increases as free-virus concentration increases (Fig. 2). The surface concentration of poliovirus did not exceed 3×10^6 PFU/mg for Ottawa sand.

Adsorption of many materials to a surface

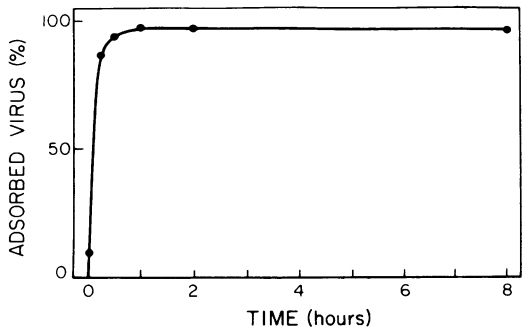

FIG. 1. *Kinetics of poliovirus adsorption to Ottawa sand suspended in the synthetic freshwater medium at pH 7 and 4°C.*

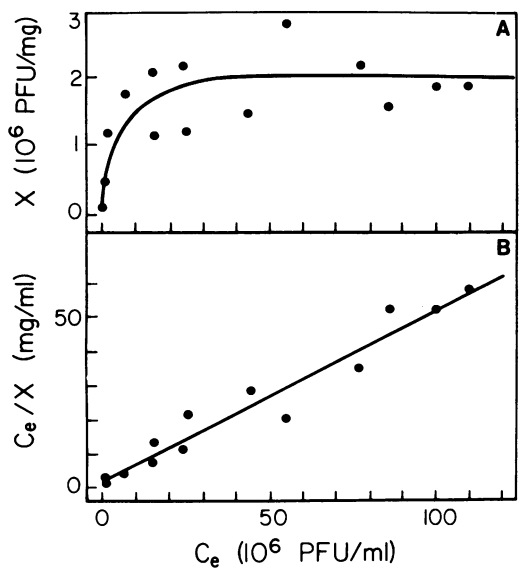

FIG. 2. *A, Influence of virus concentration on poliovirus adsorption by Ottawa sand. B, Langmuir isotherm plot. The amount of substrate varied (0 to 1,000 mg); virus concentration was constant. Abbreviations: X, amount of virus adsorbed per unit weight of adsorbent; C_e, equilibrium concentration in suspension. Adsorption was for 1 h at 4°C.*

when the apparent saturation is approached can be described by this form of the Langmuir equation:

$$C_e/X = C_e/X_{max} + 1/\alpha X_{max} \quad (1)$$

where C_e is the equilibrium concentration in solution, C is the amount of material adsorbed per unit weight of adsorbent, X_{max} is the apparent maximum surface concentration of the adsorbed species, and α is a constant.

Least-squares linear analysis of the same data represented as a Langmuir adsorption isotherm produced $C_e/X = 0.38 \times 10^6 \cdot C_e + 4.8$ and a linear correlation coefficient (r) of 0.92. Thus, the maximum capacity of Ottawa sand (X_{max}) was 2.2×10^6 PFU/mg at pH 7.5. Radioactivity data, normalized by assuming a constant relationship between infectivity and particle numbers (converting counts per minute to PFU, based on specific infectivity in controls) were comparable to the infectivity data. Adsorption did not exceed 2.5×10^6 PFU/mg. The same maximum was obtained from isotherms where the concentration of virus was varied and the amount of substrate held constant (data not shown).

The Freundlich isotherm often describes adsorption on suspended colloids and surfaces at very low surface concentrations. It takes the form:

$$X = bC_e^a \quad (2)$$

where X and C_e are defined as above (equation 1), and a and b are constants. With the virus concentration varied over 4 orders of magnitude and the amount adsorbed no more than one tenth of the Langmuir maximum, a linear relationship, which conformed to the Freundlich isotherm (Fig. 3), was found between the logarithm of the virus adsorbed (infectivity) and the logarithm of the equilibrium concentration. Linear regression analysis gave $\log X = 1.2 \log C_e - 3.2$ and $r = 0.97$. Because of the small quantity of virus involved, Freundlich analysis was not applied to the radioactivity measurements.

Poliovirus adsorption by soils and minerals. The most important properties of the soils and minerals used in this study are given in Table 4. The materials range in properties from acidic to basic, from very fine to relatively coarse, and from high in organic content to organic free. The amount of cationic polyelectrolyte adsorbed by a soil is a measure of available negative surface charge, i.e., charge on external surfaces, not in very narrow pores or capillaries (D. H. Taylor, manuscript in preparation). The adsorption of PDADMA, determined at a pH close to the pH for the virus-adsorption experiments, showed that the charge of the study samples varied from low to high negative charge. Further substrate properties will be discussed by Fuhs et al. (G. W. Fuhs, R. Deibel, L. S. Sturman, M. M. Reddy, M. J. Wolin, M. Chen, T. J. Tofflemire, R. S. Moore, and D. H. Taylor, *Virus-Soil Interactions*, U. S. Environmental Protection Agency report, in press).

The results of the batch interaction studies between poliovirus and more than 30 soils, minerals, and ground rocks include both the mean and minimum amounts of adsorbed virus for all acceptable individual samples (Table 5). Each

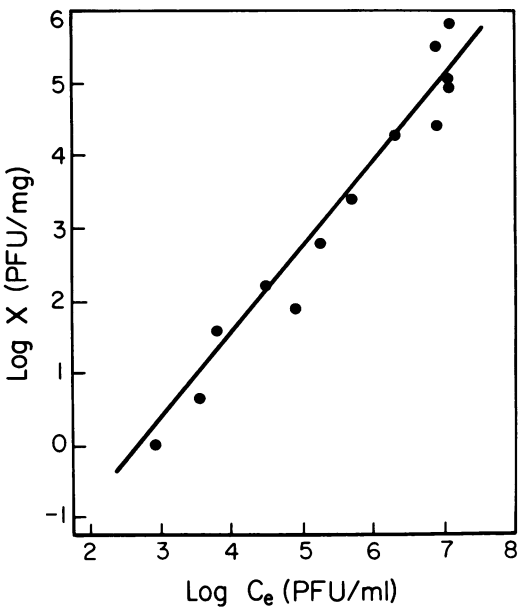

FIG. 3. *Freundlich isotherm plot. The amount of substrate remained constant (500 mg); virus concentration varied. Abbreviations: X, amount of virus adsorbed per unit weight of adsorbent; C_e, equilibrium virus concentration in suspension. Adsorption was for 1 h at 4°C.*

substrate was tested an average of six times. The minimum is the least adsorbent sample, indicating how weak and variable adsorption may be. Radioactivity data have been included only when virus purification was shown to be highly efficient. The presence of even small quantities of nonviral labeled material sometimes obscured the behavior of the virus when virus uptake was greater than 98%. In such cases, the proportion of virus adsorbed was higher than measurements of radioactivity indicated.

The removal of poliovirus from suspension in synthetic freshwater by the different soils and minerals varied widely between samples, although most substrates adsorbed more than 95% on average. Adsorption was relatively constant from one experiment to another, but a few substrates displayed erratic behavior. For instance, the muck adsorbed from between 16 and 99% of added virus, whereas the Riverhead sandy loam took up anywhere between 94 and 99.7%.

Overall, uptake of labeled and infectious matter was similar. Although excessive loss from suspension of infectious matter over radiolabeled material has no significance because of the possibility of nonviral material being present, the opposite situation (an excess of radioactivity over infectivity taken up) was observed several times. This was most notable with the loess, the muck, and the silt loam. In these cases, the virus-specific infectivity may have been enhanced over that in the controls by either an alteration of the viral particle or by dispersion of aggregates.

The soils were comparatively weak adsorbents; in fact, two soils, muck and Genesee silt loam, were the poorest adsorbents. Only one soil, Beckett silt loam, was a consistently strong adsorbent of infectious matter. The least effective virus adsorbents among the nonsoils were the two montmorillonites, the glauconite, and the hydrocarbon-bearing bituminous shale. The two most effective adsorbents were the magnetite sand and hematite. Both are predominantly oxides of iron.

Factors influencing adsorption. The coefficients of correlation between the substrate properties and the mean and minimum percent adsorption of poliovirus infectivity and radioactivity were computed. The more important results are shown in Table 6 for all substrates taken together and for the soils alone. The correlation seen with infectivity adsorption was always stronger when the worst case (minimum) was used rather than the mean. With the radioactivity results, significant correlations were found only with the cases of least uptake—none with the average. (Again, traces of nonviral radioactivity probably are responsible for this result.)

The elemental compositions of the adsorbents used in this study, as determined from X-ray fluorescence analysis or by nitric acid digestion, apparently have little effect on poliovirus uptake. Similarly, the effects of surface area and pH values in the experiments or in distilled water, were not sufficiently strong to be significantly correlated with virus uptake. The factors that were correlated significantly (although inversely) with virus uptake included the capacity of the adsorbents for the cationic polyelectrolyte (PDADMA) and the organic content of the adsorbents estimated either by organic carbon content, apparent protein content, or weight loss on oxidation (low-temperature ashing). The relationship between minimum virus adsorbed and organic content or capacity for PDADMA adsorption was significant for the soils taken as a group and also when the muck (the extreme value in both cases) was omitted from the computation. Only the capacity for PDADMA adsorption was significantly correlated with virus uptake for the remaining materials (nonsoils) taken as a group.

If the adsorption data are presented on a linear scale, there is substantial deviation between values only when adsorption is weak. Thus, the results of correlation analysis reflect mostly the properties of the poor adsorbents. If

TABLE 4. *Properties of the soils, minerals, and other substrates*

No.	Name	pH in distilled water	Surface area (m^2/g)	Weight loss on LTA[a] (%)	Organic carbon content (mg/g)	Apparent protein content (mg/g)	Capacity for PDADMA (mg/m^2)	Nitric acid-extractable aluminum (mg/g)	Nitric acid-extractable iron (mg/g)
1	Dolomite	8.9	0.85	0.01	1.1	0.09	0.26	0.75	12
2	Montmorillonite, API no. 31	8.8	41	1.87	0.6	0.15	0.80	19	16
3	Montmorillonite, API no. 26	9.5	32	0.59	1.6	0.88	2.1	5.7	4
4	Quartz	8.0	0.54	0.03	0	0.15	0.48	0.7	14
5	Bituminuous shale	8.9	3.8	0.39	4.2	0.10	0.29	5.2	11
6	Calcite	8.5	4.0	0.15	5.2	0.10	0.45	11	28
7	Fossiliferous limey shale	8.5	4.5	0.77	1.4	0.09	0.22	13	30
8	Illite-bearing shale	7.1	55	2.85	13	1.7	0.19	16	30
9	Argillaceous shale	8.5	29	0.77	0.9	1.65	0.17	26	20
10	Arenaceous shale	8.8	3.5	0.34	1.1	0.07	0.36	1.3	45
11	Calcareous shale	8.2	9.4	0.59	2.4	0.25	0.27	15	26
12	Kaolinite, API no. 5	5.0	16	1.12	0.02	0.30	0.14	17	0.7
13	Halloysite, API no. 13	6.0	42	3.05	1.5	1.35	0.080	12	1.0
14	Dickite, API no. 16	8.0	2.7	0.14	0.9	0.53	0.60	4.6	56
15	Attapulgite, API no. 64	8.1	120	5.5	0.6	0.53	0.13	18	14
16	Lake Ontario dune sand	8.4	4.1	0.36	2.0	2.3	0.16	5.7	18
17	Lake Superior dune sand	8.7	1.2	0.37	0.8	0.06	0.58	2.0	28
18	Lake Ontario beach sand	8.5	0.73	0.56	2.8	1.1	1.0	2.3	14
19	Magnetite sand	8.9	5.7	0.18	0.4	0.06	0.17	4.8	15
20	Colonie sandy loam	8.0	5.3	1.12	3.6	2.3	0.58	10	17
21	Genesee silt loam	7.6	4.2	3.36	16	9.5	1.1	11	19
22	Brown sandstone	7.6	5.6	0.70	1.2	0.54	0.18	7.0	22
23	Quartz conglomerate	8.9	3.2	0.36	1.2	0.24	0.36	1.6	22
24	Glauconite	6.6	54	1.84	1.0	0.45	0.16	16	60
25	Adobe	7.9	34	1.66	5.7	1.7	0.10	19	23
26	Genesee silt	7.5	11	0.94	1.5	0.95	0.17	12	25
27	Muck	7.2	1.2	34	200	34	14	5.1	13
28	Kaolinite, API no. 4	8.0	12	0.83	0	0.25	0.23	3.5	0.5
29	Kame conglomerate	8.8	3.1	0.27	6.6	0	0.070	2.7	40
30	Loess	7.7	25	1.10	1.3	0.29	0.10	2.0	18
31	Riverhead sandy loam	4.6	6.2	1.11	2.5	0.83	0.071	8.0	6.0
32	Beckett silt loam	5.0	7.6	3.7	31.5	13	0.087	14	16
33	Ottawa sand	7.2	0.018	0	0	0	0.6	ND[b]	ND
34	Hydrous iron oxide	7.1	40	ND	0	0	0.0050	ND	ND

[a] LTA, Low-temperature ashing.
[b] ND, Not determined.

the logarithm of the amount of virus not adsorbed is used, the analysis can give much greater weight to the strong adsorbents (especially based on infectivity results, which vary by orders of magnitude). However, such analysis showed that the same factors (organic content and capacity for PDADMA) remained significantly and inversely related to virus uptake. Thus, absence of organic matter and low capacity for the cationic polyelectrolyte are properties related most strongly to poliovirus adsorption.

DISCUSSION

In a batch adsorption process the time required to reach equilibrium depends, among other factors, upon the concentration of solute, the particle size of the adsorbent, and the degree of agitation. Agitation of the suspension improves the contact of particles with liquid and decreases the mass transfer resistance at the surface (37). A contact time of only 10 to 60 min is often sufficient for equilibrium to be approached (7, 13, 38). Thus, we chose batch processing, which requires only small quantities of materials, to monitor virus adsorption. In column processes, since changes may occur over periods of 5 to 7 weeks (25), particles packed within the column must range from 0.4 to 2.5 mm in diameter to avoid excessive pressure drop and to minimize losses in handling (37). In practical fixed-bed operations equilibrium between

TABLE 5. *Adsorption of radiolabeled poliovirus by soils and minerals*

No.	Substrate	Viral material adsorbed (%)			
		Infectivity		Radioactivity	
		Mean	Minimum	Mean	Minimum
1	Dolomite	99.2	97.4	97.9	95.7
2	Arizona montmorillonite	91.5	78	64	55
3	Wyoming montmorillonite	94.1	74	98.3	96.3
4	Quartz	99.75	99.75	96.3	96.3
5	Bituminous shale	94.8	88	95	87
6	Calcite	98.2	95.7	95.2	93
7	Limey shale	99.40	97.1	94	91
8	Illitic shale	99.19	98.0	96.3	93
9	Argillaceous shale	99.56	98.9	95.5	89
10	Arenaceous shale	99.45	99.13	95.5	88
11	Calcareous shale	97.9	94	92	86
12	Kaolinite, API no. 5	99.53	98.2	99.0	97.9
13	Halloysite	98.6	95.2	98.4	95.7
14	Dickite	99.81	99.58	97.8	96.2
15	Attapulgite	97.9	95.2	98.2	96.0
16	Lake Ontario dune sane	99.56	99.18		
17	Lake Superior dune sane	98.0	97.0	96.1	95.6
18	Lake Ontario beach sand	99.90	99.76	92	86
19	Magnetite sand	99.994	99.99	97.0	96.7
20	Colonie sandy loam	92.5	84	94	86
21	Genesee silt loam	75	43	93	88
22	Sandstone	99.48	98.5	98.7	98
23	Quartz conglomerate	98.2	93.1	96.3	95.7
24	Glauconite	94.1	83	95.5	89
25	Deming adobe	93.9	89	91.5	84
26	Genesee silt	97.9	94.3	99.2	98
27	Muck	79	16	93.0	54
28	Kaolinite, API no. 4	98.7	95.4	99.3	97.5
29	Kame conglomerate	99.55	98.6	95.2	91
30	Kansas loess	98.3	96.1	99.6	99.5
31	Riverhead sandy loam	98.2	94.2	98.0	96
32	Beckett silt loam	99.33	99.22	97.3	96.8
34	Hydrous iron oxide	99.98	99.94	99.3	99.0

TABLE 6. *Correlation coefficients between soil properties and percent poliovirus adsorbed*

Substrate property	Virus adsorbed by all substrates				Virus adsorbed by soils			
	Infectivity		Radioactivity		Infectivity		Radioactivity	
	Mean[a]	Minimum[b]	Mean	Minimum	Mean	Minimum	Mean	Minimum
Experimental pH	0.012	0.011	−0.316	−0.163	−0.237	−0.155	−0.353	−0.428
pH in distilled water	0.015	0.003	−0.230	−0.132	−0.365	−0.301	−0.282	−0.177
Surface area	0.045	0.053	−0.055	−0.025	0.429	0.474	0.057	0.349
Extractable Al content	0.032	0.039	−0.005	0.019	0.087	0.223	−0.367	0.201
Extractable Fe content	0.132	0.143	0.040	0.1017	0.058	0.154	0.195	0.566
Weight loss in LTA[c]	−0.615[d]	−0.798[d]	−0.070	−0.635[d]	−0.578	−0.828[e]	−0.329	−0.916[f]
Organic carbon content	−0.598[d]	−0.785[d]	−0.056	−0.622[d]	−0.560	−0.812[e]	−0.317	−0.903[f]
Apparent protein content	−0.673[d]	−0.807[d]	−0.050	−0.571[d]	−0.618	−0.824[e]	−0.342	−0.839[e]
Capacity for PDADMA	−0.612[d]	−0.814[d]	−0.107	−0.658[d]	−0.601	−0.846[e]	−0.338	−0.927[f]

[a] Correlation coefficient calculated from mean value of percent virus adsorbed from all experiments.
[b] Correlation coefficient calculated from experiment in which the minimum virus adsorbed.
[c] LTA, Low-temperature ashing.
[d] Significant at 99.9% confidence level.
[e] Significant at 95% confidence level.
[f] Significant at 99% confidence level.

solid and liquid phases is rarely achieved. The distribution coefficients obtained from the equilibrium batch adsorption process can be used to estimate the upper limit of virus adsorption by columns of similar materials (13, 36).

Virus adsorption by soils and clays is usually rapid, and equilibrium may be approached within minutes (7, 22, 38). Virus adsorption is also diffusion controlled, so that uptake is more rapid to more finely divided substrates (41). The optimum time for the interaction of poliovirus with Ottawa sand was 1 h, and viral degradation was not of great significance over this period; the same interval was used for subsequent studies.

In this system adsorption was at least partially reversible. Adsorption of poliovirus from dilute NaCl by silica and other hydrous oxides has been shown to be reversible (26). Application of isotherms describing adsorption at equilibrium to uptake of the virus by Ottawa sand is, therefore, acceptable.

From the Langmuir analysis, giving an apparent surface saturation of 2.2×10^6 PFU/mg, the surface coverage of Ottawa sand by poliovirus was calculated. From the diameter of the hydrated poliovirus particle (~30 nm) and the surface area of the sand, we estimated that a monolayer of hexagonally close-packed virus represents 2.3×10^{10} particles per mg. Since 1 PFU represents approximately 50 to 100 particles (34), the maximum amount of virus adsorbed at pH 7.5 covered approximately 1% of the total surface of the sand. Thus, even coarse materials like Ottawa sand will have an enormous capacity for viruses and are unlikely to become saturated in natural systems. Of course, coating of the sand surface with competing material is more likely to cause apparent saturation. The capacity of Ottawa sand in our synthetic freshwater medium is substantially higher than that of the silicate material with higher surface areas used by Lo and Sproul (23), but their suspension medium did not contain Ca^{2+} ions.

The adsorption of virus at low concentrations followed the Freundlich isotherm, giving further confirmation that the interaction of poliovirus with Ottawa sand resembles that of viruses with other inorganic substrates (4, 5, 9, 38).

At very low concentrations ($C_e < \alpha^{-1}$) the Langmuir equation (equation 1) reduces to $X = \alpha X_{max} C_e$, a form of the Freundlich isotherm with $a = 1$ (see equation 2). Thus the slope (a) of the log-log plot (Fig. 3) should approach unity if the behavior remains described by the Langmuir equation. The actual value of a, 1.2, is not greatly different from unity. The difference may represent inhomogeneities in either the surface of the adsorbent or the virus population, or, alternatively, it may arise from some other concentration-dependent parameter.

As the slope of the Freundlich plot approaches unity, the percentage of added virus adsorbed is approximately constant even for changes of virus concentration of up to several log units. Surface saturation of Ottawa sand by poliovirus required 2.2×10^9 PFU/g; all of the other materials used for this study had surface areas at least 50 times greater than that of Ottawa sand. In no case was surface saturation by virus alone likely to have been approached for the range of virus concentrations used (10^8 to 10^9 PFU/0.5 g). Thus, it follows that for each substrate the amount of virus adsorbed as a percent of that added should be approximately constant. Deviations would be expected to reflect variations of conditions other than virus concentration. In fact, no systematic concentration dependence of the fluctuations in the amount of virus adsorbed was found; experimental variations were larger than the difference anticipated from the difference in the slope of the isotherm.

Although wide variability in the extent to which different soils bind viruses has been found by other workers (5, 12, 15, 20, 35), no previous study was as extensive as this one. To correlate observed virus adsorption with properties of the substrates, we measured over 40 different parameters for each substrate. Of these, only the eight that were significantly related to virus uptake either in this or some other study have been included in this discussion. Among the important parameters not significant in this study were pH, surface area, and, to some extent, elemental composition. Understanding why some parameters are important but others are not is aided by determining the mechanism of virus adsorption.

Poliovirus adsorption by hydrous oxides is well described by the theory of colloid stability presented by Derjaguin and Landau (8) and Verwey and Overbeek (43) as extended by Hogg et al. (18; see also reference 16) to dissimilar materials (26, 27a). Adsorption to soils and a clay mineral also follows this mechanism (39). The central premise of the theory is that there is an inherent attractive force between the particles from van der Waals interactions. Adsorption or coagulation is inhibited by coulombic repulsion if the particle charges are both similarly and sufficiently strong electrically. Van der Waals forces depend upon the compositions of mineral and the virus, as well as the distance of separation. They are essentially unaffected by the pH or the ionic strength of the suspending solution. The strength of the coulombic force

depends not only on the distance of separation but also on the surface electrical potentials at the virus and mineral surfaces as well as the ionic strength. The potentials in turn arise from the presence of surface charge, which is usually negative and often pH dependent. Elevation of the pH above neutral usually increases the negative charge on soil and virus, thereby impeding adsorption.

Since the isoelectric point (pI) of this strain of poliovirus in neutral or basic medium is 7.5 (39), the virus adsorbs readily to minerals and soils suspended in synthetic water below pH 7.6. Adsorption is weak above a narrow pH region, the position of which varies with substrate within the pH 7 to 10 range (39). At the same weakly basic pH one adsorbent may bind poliovirus strongly, whereas another does not. Even though pH is an important factor and adsorption by most neutral and acidic materials was strong, the spread of the percentage of virus bound in basic medium was such that pH did not show significant correlation with the amount of virus adsorbed.

Natural soil organic matter is a weak adsorbent for poliovirus, partly because of its low pI, which makes it anionic at the pH of most natural systems and hence unlikely to bind to other negatively charged bodies. In addition, some of this soil organic matter, particularly lower-molecular-weight fractions, is soluble and may pass into solution. It is possible that this same material may be adsorbed onto other surfaces, such as minerals and viruses, thereby giving them all low pI's. Furthermore, organic matter does not have the characteristics which induce strong van der Waals forces of attraction. This property is determined by the differences in the Hamaker constants of the interacting bodies and the medium in which they are suspended (32). The Hamaker constants of most forms of organic matter are similar to that of water (26, 44); hence the van der Waals interactions between organic colloids are weak. However, inorganic matter tends to have large Hamaker constants (44) and thus a stronger inherent attraction for virus. The variation in the Hamaker constants and the subsequent variations in the van der Waals forces agree well with differences in the observed adsorption of poliovirus by metal oxides (6, 27a). In these experiments, at pH 7, close to the virus pI, the strengths of electrokinetic interactions were calculated from electrokinetic data. When compared with the equilibrium adsorption of poliovirus to the material, there was an attractive force with a strength which increased with the Hamaker constant (and hence van der Waals attraction) of the mineral.

Virus adsorption is a surface reaction. Thus, with materials of similar surface reactivity, the proportion of added virus bound should increase as surface area increases. This effect was observed with poliovirus adsorption to Ottawa sand and to Lake Ontario beach sand (Tables 4 and 5); it was also observed in adsorption of a bacteriophage to the clay mineral allophane (38). When adsorbents of highly different reactivity are considered, however, the effect of surface area available may become secondary to the ability of each surface to bind a virus which approaches it. A measure of this is the collision efficiency, i.e., the proportion of collisions between colloid and surface which leads to binding. If the collision efficiency remains constant, increasing the surface area will increase net adsorption of the virus. However, if the collision efficiencies of the various substrates are greatly different and independent of the surface area itself, then correlation between surface area and virus uptake is not expected to be high. Thus, the parameter we measured was the amount of a cationic polyelectrolyte adsorbed per surface area unit of substrate from a moderately concentrated solution. A monolayer of the polymer used (PDADMA) is approximately 1 mg/m^2. (Higher values represent interlamellar adsorption by expandable clay minerals or dissolution and disintegration of humic matter.) Surfaces with binding capacities for PDADMA approaching monolayer have a strong affinity for cations, i.e., they are usually strongly negatively charged (38a). Substrates with much lower surface capacities have little charge or are positively charged. Such a surface has little attraction for cationic bodies—it may, however, attract matter such as virus. One difficulty with this parameter is that a multicomponent substrate may be a strong adsorbent of the polymer, as well as of the virus. Lake Ontario beach sand, for example, had discrete mineral and humus particles, the former binding virus, and the latter, polymer. However, this parameter was the most consistently reliable indicator of poliovirus behavior; it correlated well with two properties which inhibited virus uptake—the organic content and the strength of the negative charge of minerals. It is expected that anionic polymers will prove better indicators of virus adsorption by soils, as they will be bound most readily by the components which attract viruses themselves.

The variations in substrate adsorption capabilities can be interpreted on the basis of their properties. For instance, the weakest adsorbents, Monroe County muck and Genesee silt loam, contained substantial quantities of natural organic matter, confirming results of other studies

(10, 15, 35). This would appear to contradict the suggestion of Robeck et al. (33) that soils for effluent disposal sites should have significant organic content to give them adsorptive capacity. However, our soils containing the amount proposed by Robeck et al., 0.5 to 1%, were generally effective adsorbents, although most soils were poorer adsorbents than the nonbasic, organic-free minerals. A further discussion of the role of organic matter will be presented in a subsequent paper (Moore et al., in preparation).

Among the materials with little organic content which were weak adsorbents were the montmorillonites and the glauconite. By their capacity for the polyelectrolyte, each of the three minerals was shown to have a high negative surface-charge density; the montmorillonites were also basic and had low pI's (39)—properties which induce strong repulsive coulombic interactions. In contrast, the most effective adsorbents were generally free of organic matter and not basic. They also had low capacities for the cationic polyelectrolyte. Both hematite and magnetite, the two strongest adsorbents, have comparatively high pI's (28), i.e., they retain their positive charges to relatively high pH's and develop strong negative charges only in strong base. They also have relatively high Hamaker constants (44) and, therefore, experience comparatively strong attractive van der Waals interaction with viruses.

Poliovirus would be readily adsorbed from treated sewage effluents by most neutral or acidic soils which have comparatively low organic contents and moderate exposure of clean mineral surface. Saturation of a soil with virus would be difficult; saturation with other organic matter which competes with virus uptake is far more likely. Soils containing organic matter do adsorb poliovirus, but they are less effective. As a simple indicator of a soil's ability to bind virus, the amount of polyelectrolyte adsorbed per soil surface area unit should be determined.

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