This investigation was initiated in an attempt to delineate some factors affecting the adsorption of poliovirus to soil particles in seawater. The results could have significance in the development of assay procedures for viral contaminants in natural waters.

A sample of marine sediment, obtained from an estuary near Purdy, Washington, was characterized by X-ray diffraction and shown to consist primarily of 2:1 type mineralogical components. When poliovirus type 1 was mixed with the marine sediment, or with purified montmorillonite and kaolinite suspensions containing electrolyte, the virus was removed from the supernatant fluid of samples subjected to low-speed centrifugation.

Fetal bovine serum, in concentrations of 0.2% to 2%, prevented and reversed adsorption of the virus to the clay fraction of the marine sediment. A serum concentration of 0.02% decreased virus adsorption but 0.002% serum had no effect. Hydrogen ion
concentrations, in the range of pH 5 to 9, had no significant effect on the amount of virus adsorbed by marine sediment suspended in seawater. Adsorption of virus by the clay fractions of montmorillonite and kaolinite was more efficient than adsorption by the silt fractions, although significant removal of virus by the larger particles did occur. Adsorption of up to 60% of the virus occurred at clay concentrations as low as 1 mg/liter, while clay concentrations of 50 mg/liter resulted in almost complete removal of the virus from the supernatant fluid.

The virus did not adsorb to the clays in a deionized water medium, but when suspended in seawater diluted to salinities as low as 0.1%, or in deionized water containing $10^{-5}$ M AlCl$_3$, adsorption did occur. Clays of the 2:1 type required higher concentrations of electrolyte than did the 1:1 type kaolinite to adsorb virus. Lower concentrations of MgCl$_2$ than NaCl resulted in adsorption. Attempts to release virus adsorbed to kaolinite, by suspending in a medium of lower electrolyte concentration, were not successful.

Limited electron microscope studies did not unequivocally establish the adsorption site of virus to kaolinite crystals, but structural defects of the crystals were observed which may be important in the adsorption phenomenon. The mechanism of adsorption has not been established but a flocculation type is suggested.
Poliovirus Adsorption by Soil Particles in Seawater

by

Walter Jakubowski

A THESIS

submitted to

Oregon State University

in partial fulfillment of the requirements for the degree of

Master of Science

June 1969
APPROVED:

Redacted for Privacy

__Associate Professor of Microbiology
in charge of major

Redacted for Privacy

__Chairman of the Department of Microbiology

Redacted for Privacy

__Dean of Graduate School

Date thesis is presented ____________ October 1, 1968

Typed by Opal Grossnicklaus for _______ Walter Jakubowski _________
ACKNOWLEDGMENTS

The author wishes to express sincere appreciation and gratitude to the following people for their assistance with this project:

Dr. John L. Fryer and Dr. K. S. Pilcher for their advice and suggestions concerning this research and the preparation of the manuscript.

Dr. Paul R. Elliker and the personnel of the Microbiology Department for their cooperation and use of Departmental facilities.

Mr. C. B. Kelly and Mr. William Beck for their encouragement and assistance in obtaining approval of the training program.

Dr. John C. Hoff for stimulating my interest in the field of virology and for his many helpful suggestions concerning the conduct of this project.

Mr. L. C. Myers, Mrs. Idell Hardin, Mr. N. Anthony and the personnel of the U.S.P.H.S. Pacific Northwest Marine Health Sciences Laboratory for their assistance in collecting samples and for providing moral support.

Dr. M. E. Harward and Mr. G. A. Borchardt for supplying samples of kaolinite and montmorillonite as well as instruction in various aspects of clay mineralogy.

Mr. William Felsing, Mr. R. Eugene Hyde and other personnel of the U.S. Public Health Service National Center for Urban
and Industrial Health, Water supply and Sea Resources Program, Cincinnati, Ohio, for their helpful advice on administrative and procedural matters.

My wife, Shirley, for her patience and understanding.
# TABLE OF CONTENTS

## INTRODUCTION

## LITERATURE REVIEW

Detection of Viruses in Sewage and Water
- Viruses in Sewage
- Viruses in Water
- Virus Adsorption Phenomena

## MATERIALS AND METHODS

Soils
- Purdy Soil
- Kaolinite and Montmorillonite

Tissue Culture System
- Cell Line
- Media
- Transfer of Cell Cultures

Virus
- Source of Virus
- Propagation of Stock Virus
- High Extraneous Protein Content Virus (HPC)
- Low Extraneous Protein Content Virus (LPC)
- Virus Neutralization Tests

Seawater

Electrolyte Solutions

Virus - Soil Adsorption System

Virus Assay Method
- Dilution of Samples
- Preparation of Monolayers for Virus Assay
- Overlay Medium
- Inoculation of Monolayers
- Agar Overlay Technique

Electron Microscopy

## RESULTS

Characterization of Clays by X-Ray Diffraction
The Adsorption of Poliovirus by Purdy Clay in Seawater at Different Virus Concentrations
Effect of Serum on Poliovirus Adsorption by Purdy Clay in Seawater
TABLE OF CONTENTS (CONTINUED)

Effect of pH on Adsorption of Poliovirus by Purdy Clay in Seawater 31
Effect of Soil Particle Size on Adsorption of Poliovirus in Seawater 33
Effect of Clay Concentration on Adsorption of Poliovirus in Seawater 34
Effect of Salinity on Adsorption of Poliovirus by Clays 35
Effect of NaCl, MgCl₂ and AlCl₃ on PoliovirusAdsorption by Clays 38
NaCl 38
MgCl₂ 40
AlCl₃ 40
Effect of Decreasing Ionic Strength on Release of Poliovirus from Kaolinite 43
Electron Microscopy 44

DISCUSSION 48

Characterization of Clays 48
Virus Adsorption Experiments 52
  Effect of Virus Concentration and Serum Concentration 52
  Effect of pH 53
  Effect of Soil Particle Size 54
  Effect of Clay Concentration 55
  Effect of Electrolyte Concentration 56
  Electron Microscopy 58
Other Studies 60

SUMMARY AND CONCLUSIONS 61

BIBLIOGRAPHY 63
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>X-ray diffraction maxima produced by Purdy, kaolinite and montmorillonite clays using CuKα radiation.</td>
<td>26</td>
</tr>
<tr>
<td>2.</td>
<td>The adsorption of poliovirus (HPC) by Purdy clay (100 mg/liter) in seawater at different virus concentrations.</td>
<td>28</td>
</tr>
<tr>
<td>3.</td>
<td>The effect of 2% fetal bovine serum on adsorption of poliovirus (HPC) by Purdy clay (50 mg/liter) in seawater.</td>
<td>30</td>
</tr>
<tr>
<td>4.</td>
<td>The effect of fetal bovine serum concentration on adsorption of poliovirus (LPC) by Purdy clay (50 mg/liter) in seawater.</td>
<td>31</td>
</tr>
<tr>
<td>5.</td>
<td>The effect of pH on adsorption of poliovirus (LPC) by Purdy clay (50 mg/liter) in seawater.</td>
<td>32</td>
</tr>
<tr>
<td>6.</td>
<td>The effect of soil particle size on the adsorption of poliovirus (LPC) by Purdy, kaolinite and montmorillonite (50 mg/liter) in seawater.</td>
<td>33</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>----------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>1.</td>
<td>The effect of clay concentration on the adsorption of poliovirus (LPC) in seawater.</td>
<td>36</td>
</tr>
<tr>
<td>2.</td>
<td>The effect of salinity on the adsorption of poliovirus (LPC) by Purdy, kaolinite and montmorillonite clays.</td>
<td>37</td>
</tr>
<tr>
<td>3.</td>
<td>The effect of NaCl concentration on the adsorption of poliovirus (LPC) by Purdy, kaolinite and montmorillonite clays.</td>
<td>39</td>
</tr>
<tr>
<td>4.</td>
<td>The effect of MgCl concentration on the adsorption of poliovirus (LPC) by Purdy, kaolinite and montmorillonite clays.</td>
<td>41</td>
</tr>
<tr>
<td>5.</td>
<td>The effect of AlCl concentration on the adsorption of poliovirus (LPC) by Purdy, kaolinite and montmorillonite clays.</td>
<td>42</td>
</tr>
<tr>
<td>6.</td>
<td>LPC poliovirus specimen showing particles about 31 μm in diameter (arrows). Shadowed, reverse contrast (approximately 121,000X).</td>
<td>45</td>
</tr>
<tr>
<td>7.</td>
<td>Poliovirus-size particles (arrows) on edge of kaolinite crystal. Shadowed, reverse contrast (approximately 238,000X).</td>
<td>45</td>
</tr>
<tr>
<td>8.</td>
<td>Poliovirus-size particles (white arrows) on faces of kaolinite crystals. Note dark, crater-like areas (black arrows) on the crystal faces. Shadowed, reverse contrast (approximately 165,000X).</td>
<td>47</td>
</tr>
<tr>
<td>9.</td>
<td>Diagrammatic sketch of the structure of kaolinite (Grim, 1953).</td>
<td>49</td>
</tr>
<tr>
<td>10.</td>
<td>Diagrammatic sketch of the structure of montmorillonite (Grim, 1953).</td>
<td>50</td>
</tr>
</tbody>
</table>
INTRODUCTION

Increased demands have been placed upon our water supplies in recent years by expanding domestic, recreational and industrial needs. The result has been a greater potential hazard of transmitting disease-producing microorganisms by the water route. Routine surveillance of natural waters, and the development of adequate water treatment processes, will require reliable methods for detecting these microorganisms.

Bacteria are capable of reproduction in an aquatic environment and may therefore be detected with relative ease. Viral contaminants cannot reproduce outside living host cells and will be progressively diluted in natural waters. Viruses in water are likely to come in contact with natural turbidity commonly due to suspended soil particles and plankton. Viral distribution and the choice of sample assay procedure could be influenced by the results of this encounter.

These studies were initiated in an attempt to determine the effects of some physical and chemical factors on virus adsorption to soil particles in seawater. Experiments were conducted primarily with seawater in the hope of applying the results to the problem of viral accumulation by shellfish and to the detection of viruses in
an estuarine environment.

The virus preparation consisted of a partially purified suspension of Sabin type 1 poliovirus. Marine sediment, kaolinite and montmorillonite were separated into various size fractions by differential centrifugation. Virus and soil particles were suspended in the test medium, mixed and centrifuged. Deionized water, seawater and solutions of NaCl, MgCl₂ and AlCl₃ were used as suspending media. The degree of virus adsorption was determined by assaying the supernatant fluid and the sediment for virus content. Assays were performed with tissue cell cultures using an agar overlay plaque technique. In this manner, the effects of varying virus concentration, nonviral protein, pH, soil particle size, soil particle concentration and electrolyte concentration were studied.
LITERATURE REVIEW

Detection of Viruses in Sewage and Water

The viral content of a sample from a body of water, or a sewage treatment plant, will vary with distance from the source of contamination, amount of rainfall and run-off, tides, time of day and stream flow, to name a few factors. The method used in obtaining samples for virus assay will influence the reliability of the results. The volumes that must be examined usually require the sample to be concentrated prior to assay.

The type of information desired will also affect the choice of sampling procedure. Generally, it is desirable to sample water supplies under conditions where maximum virus content would be expected. The possibility of transmitting viruses may then be assumed to be lower under conditions favoring dilution and distribution of the contaminants.

Viruses in Sewage

Moore (1948) described a gauze pad method for isolating pathogenic bacteria from sewage, and in 1952, MacCallum et al. applied this method to the detection of poliovirus in sewers. A length of cotton gauze was folded into a pad and suspended in the sewage
stream for three days. Fluid was expressed from the pad by squeezing in a fruit-juice press. The fluid was treated with ether until bacteriologically sterile, and then tested for the presence of poliovirus by inoculation into monkeys. More isolations were made by this method than by examination of bulk sewage samples (also called dip or grab samples).

Melnick et al. (1954) compared grab and gauze pad sampling in greater detail for the isolation of coxsackie viruses from sewage. The pads were extracted by kneading after adjusting the pH to 8.0 with NaOH. It was presumed that there would be a little attraction between virus and cotton at this pH. Pad extracts and grab samples were concentrated by precipitation with (NH₄)₂SO₄ and ultracentrifugation. Virus isolations were more frequent from pad samples than from grab samples.

Other investigators have used the gauze pad method with minor variations in technique (Gravelle and Chin, 1961; Wiley et al., 1962; Lamb, Chin and Scarce, 1964). The principal alterations involved the length of time the pad was exposed to sewage flow, the choice of eluting medium and the method of concentrating the extract. In each case, the fluid was adjusted to an alkaline pH before extracting the pad. While the gauze pad appears to be more efficient than grab samples in detecting viruses, quantitative determination of virus content cannot be made by this method.
Viruses in Water

Efforts to detect viruses in water have been directed toward concentrating large sample volumes, containing low numbers of viruses, to amounts which may be conveniently assayed. Some procedures employed for this purpose have included membrane filtration, ultracentrifugation and absorption of water with polyethylene glycol.

Cliver (1965b) reported that polio and coxsackie viruses, in the presence of electrolytes, apparently adsorbed to 50-220 μm porosity membrane filters. Pretreating the membranes with serum or gelatin, or adding serum to the virus suspension before filtering, allowed the virus to pass through the filters.

Further studies on virus adsorption to membrane filters were made by Wallis and Melnick (1967). They used echovirus, coxsackie virus and two types of poliovirus filtered through 450 μm porosity membrane filters. When poliovirus was suspended in 0.03M NaCl only about 2% of the virus was recoverable in the filtrate. With 0.0075M MgCl₂, no poliovirus was detectable in the filtrate. Lower concentrations of both electrolytes produced increasing percentages of virus recovery in the filtrates.

Elution of the membranes with 1% sodium lauryl sulfate, 0.1% saponin or 10% bovine serum resulted in recovering 100% of the virus
adsorbed to the filters. Fifty percent of the virus could be recovered by elution with distilled water. Earle's salt solution and 0.1M ethylenediaminetetraacetic acid failed to elute any of the virus. By filtering viruses in the presence of electrolytes and eluting the membranes with bovine serum, virus suspensions were concentrated 63 to 100-fold.

Anderson (1965) described the construction of a continuous-flow ultracentrifuge rotor capable of removing more than 95% of suspended poliovirus at a flow rate to two to three liters/hour. The high initial cost of the equipment, however, would prevent the use of this centrifuge for routine assays of water samples. In addition, ultracentrifugation may not be a desirable method for processing water samples because many viruses lose infectivity after pelleting.

Cliver (1965a) concentrated virus suspensions by dialysis against polyethylene glycol. Sample volumes were reduced from 100 ml to 2 ml but only 10 to 30% of the original virus was recoverable. Cliver estimated a 50% probability of detecting virus if 100 ml of sample contained 6.64 plaque-forming units. The method, while inexpensive, would be difficult to apply to large sample volumes and is less sensitive than membrane filter techniques.
Virus Adsorption Phenomena

Considerable research has been applied to removal of viruses from water by precipitation from large sample volumes. Many studies of this type are dependent upon adsorption phenomena which allow recovery of virus with little or no loss of infectivity.

Virus adsorption procedures have found wide application in the purification of virus preparations. Sabin (1932) found that poliovirus adsorbed to alumina gel at an acid pH and could be eluted at an alkaline pH. Wenner (1945) purified poliovirus by adsorbing it from mouse-brain homogenates with cotton pledgets. The virus adsorbed best to the cotton in the pH range 4.0 to 5.5. Maximum elution of the virus occurred at pH 8.0.

Chang et al. (1958) used a flocculation procedure for removing coxsackie and bacterial viruses from water. The viruses were flocculated in the presence of Al$_2$(SO$_4$)$_3$ and FeCl$_3$. Optimum removal occurred at pH 6.7-7.2. Recovery of coxsackie virus from the Al$_2$(SO$_4$)$_3$ floc was 60% at pH 8.5 to 9.0. Virus could not be separated from the FeCl$_3$ floc.

Valentine and Allison (1959) performed a series of experiments on the attachment of vaccinia virus and polystyrene latex particles to nitrocellulose, aluminum, gold, carbon and glass. They found that the rate of collision of the latex and virus particles with the
various surfaces could be predicted almost exactly from Brownian theory. The number of particles adsorbed agreed with the collision frequency when aluminum was the adsorbing surface. The rate of virus adsorption to aluminum was not changed by the presence or absence of electrolytes. With all of the other adsorbing surfaces, the number of particles adsorbed per unit area in a given time was the same when 1% NaCl was added to the suspensions. Less adsorption occurred when virus or latex particles were suspended in distilled water and sucrose solutions.

The authors reasoned that the aluminum surface would possess a net positive charge at a pH close to neutrality. The latex spheres, virus particles and other adsorbing films would all have a net negative charge. No electrolyte was required for adsorption with the aluminum surface since an electrostatic attraction existed between latex, virus and aluminum. Electrolyte was required with carbon, glass, nitrocellulose and gold surfaces to reduce the electrostatic barrier between the spheres and the adsorbing surface to levels where the particles could approach close enough to become attached.

Valentine and Allison (1959) also found that divalent cations doubled the rate of adsorption as compared to monovalent cations. Trivalent cations had only a slight additional effect as compared to divalent cations. Mathematical treatment of electrolyte effects was based on equations involving interactions between similar surfaces.
and the differences in adsorption observed with univalent, divalent
and trivalent cations could not be explained by theoretical considera-
tions. The authors speculated that the size of the ions, rather than
the valency, may have a greater effect on adsorption rate.

The adsorption of enteroviruses by activated attapulgite, a
hydrated magnesium-aluminum silicate clay, was investigated by
Bartell, Pierzchala and Tint (1960). Suspensions of the clay were
prepared in Hanks' balanced salt solution. Centrifuging types 1 and
2 poliovirus with 100 mg/ml of attapulgite at 1,000 rpm removed
99.9% of the virus infectivity from the supernatant fluids. Virus
removal was lower with poliovirus type 3, echovirus type 9 and
coxsackie type B-3. No information was supplied on the amounts
of nonviral protein present in the stock viruses. Differences in
adsorptive levels with the several viruses may reflect differences
in nonviral protein content.

Bartell et al. found no significant difference in adsorption in
the pH range 3 to 9 or at temperatures of 4, 25 and 37 C. Only one
log of virus infectivity was removed by clay concentrations of 10 mg/
ml, increasing to three logs of infectivity at 100 mg/ml. Attapulgite
appeared to be more efficient in adsorbing virus than did kaolinite
at the higher clay concentrations. Effects of electrolyte concentra-
tion were not investigated.

Adsorption of virus to particulate matter in natural waters was
studied by Hill, Hamblet and Akin (1966). They examined the effects of salinity and turbidity on the recovery of poliovirus from seawater. The turbidity was supplied by "marine silt," a substance of unknown composition and undefined particle size consisting of marine bottom sediments. Different salinity levels were produced by diluting seawater with distilled water. Virus was found to adsorb to "marine silt" at turbidities of 34 to 300 Jackson Turbidity Units, but only in the presence of electrolyte. Virus did not adsorb to "marine silt" suspended in distilled water.

Carlson et al. (1968) examined virus inactivation on clay particles in natural waters. They used the term "inactivation" because they did not attempt to determine if virus could be recovered from the clays. The studies were conducted primarily with a bacteriophage but limited experiments were performed using type 1 poliovirus. Both viruses were found to adsorb to kaolinite, montmorillonite and illite clays in the presence of electrolytes. Lower concentrations of divalent cations, as compared to monovalent cations, were required to initiate adsorption. The adsorption could be prevented or reversed by the addition of egg or bovine albumin to the virus-clay suspensions. Similar results were obtained with naturally occurring Missouri River clays.
MATERIALS AND METHODS

Soils

Purdy Soil

Marine sediment (Purdy soil) was obtained from Burley Lagoon, an estuary located in Purdy, Washington. The sediment was washed several times in distilled water by sedimentation and decantation. The washed material was dispersed in 2% Na$_2$CO$_3$ solution using a mechanical vibrating mixer (Super Mixer, Lab-Line), and then centrifuged at 2,400 rpm for 30 minutes.

The sediment was resuspended in dilute Na$_2$CO$_3$ solution (1 g/9 liters) and separated into fractions of 50-20μ, 20-5μ, 5-2μ and 2-0.2μ diameter particle sizes by differential centrifugation (Jackson, Whittig and Pennington, 1950). Particle fractions greater than 5μ in diameter were stored dry. The clay fraction (2-0.2μ) and the fine silt fraction (5-2μ) were maintained as suspensions in dilute Na$_2$CO$_3$.

Light microscope examination of the Purdy soil revealed the presence of bacteria, algae, plant fragments, amorphous debris and irregularly-shaped crystalline material. Organic content of the clay fraction was 12-14% as determined by the Walkley-Black procedure (Jackson, 1958). The organic matter analyses were performed by the Soil Testing Laboratory, Oregon State University,
Corvallis, Oregon.

The Purdy soil clay component was characterized by X-ray diffraction. The criteria and methods used for this purpose were outlined by Harward (1967). The clay was divided into two samples, A and B. Sample A was prepared by oxidizing organic matter and MnO₂ with 30% H₂O₂ (Jackson, 1956). Sample B received the same treatment, and in addition, was treated for removal of iron oxides using buffered sodium citrate-dithionite (Jackson, 1956).

Portions of each sample were saturated with 1N MgCl₂ and 1N KCl solutions. Specimens were prepared for X-ray diffraction by smearing the clay paste on petrographic slides (Theisen and Harward, 1962).

Slides prepared with Mg²⁺ saturated portions of sample A received the following characterization treatments: equilibration at 54% relative humidity; solvation with glycerol and solvation with ethylene glycol. A slide containing sample A saturated with K⁺ was equilibrated at 54% relative humidity.

Slides prepared with K⁺ saturated portions of Sample B were subjected to the following treatments: equilibration at 54% relative humidity; heating at 105 C for 15 minutes and heating at 550 C for three hours. A slide containing sample B saturated with Mg²⁺ was equilibrated at 54% relative humidity.

The slides were mounted in a Philips Norelco diffractometer.
X-ray diffraction patterns were obtained using CuKα radiation, a Geiger tube detector and a potentiometric recorder. Diffraction maxima were recorded in degrees 2θ and converted to Ångstroms.

**Kaolinite and Montmorillonite**

Samples of kaolinite (No. 5, Lamar Pit, Bath, South Carolina) and montmorillonite (No. 1, Volclay, American Colloid Co.), and X-ray diffraction data for sodium citrate-dithionite treated clay fractions of these samples, were supplied by M. E. Harward, Soils Department, Oregon State University. The kaolinite and montmorillonite were separated into size fractions employing the same procedures used for the Purdy soil.

Kaolinite clay and fine silt fractions were stored as suspensions in dilute Na₂CO₃ and the remaining kaolinite fractions were stored dry. The montmorillonite clay fraction was prepared as a suspension in dilute Na₂CO₃. No fine silt fraction was available for this soil. Other montmorillonite fractions were stored dry.

All fractions of Purdy soil, kaolinite and montmorillonite were saturated with Na⁺ by washing three times with 1N NaCl using suspension, centrifugation and decantation of the supernatant fluid. Excess salts were removed by washing twice with distilled water. Only Na⁺ saturated soils were used in virus adsorption studies. All soil samples were sterilized by autoclaving at 121 C and 15 lb.
pressure for 15 minutes.

Tissue Culture System

Cell Line

A stock culture of H. Ep. -2 cells was obtained from the U.S. Public Health Service Pacific Northwest Marine Health Sciences Laboratory, Gig Harbor, Washington. The cell line was originally derived from a human carcinoma of the larynx and the cultural and morphological characteristics have been described by Moore, Sabachewsky and Toolan (1955). Cell cultures were maintained as monolayers at 37°C in 16-ounce flat, soft glass bottles. The cultures were transferred every five to seven days and the medium was changed every three to four days.

Media

H. Ep. -2 cells were maintained in Eagle's minimum essential medium (Eagle, 1959) with Hanks' balanced salt solution (HBSS). The HBSS contained phenol red indicator and was obtained in dry powder form from Grand Island Biological Co. Eagle's minimum essential medium (MEM) was prepared by aseptically adding sterile concentrates of essential amino acids, vitamins and glutamine, in amounts recommended by the manufacturer (Baltimore Biological Laboratory),
to HBSS. The pH was adjusted to 7.4-7.6 by adding sterile 7.5% 
NaHCO₃. The complete MEM was supplemented with 10% fetal 
bovine serum (Microbiological Associates, Inc.), 100 units of pen-
icillin, 100 µg of streptomycin and 2.5 µg of amphotericin/ml (Grand 
Island Biological Co.).

Transfer of Cell Cultures

Trypsin was obtained as a 2.5% solution (Grand Island Biological 
Co.) and was diluted to 0.25% in HBSS. The pH was adjusted 
to 7.4-7.6 with 7.5% NaHCO₃ solution. The medium was removed 
from a five to seven-day old cell monolayer and replaced with 10 ml 
of a 0.25% trypsin solution. After two minutes, the trypsin was re-
moved and the monolayer was left at room temperature until the 
cells detached from the glass (20 to 25 minutes). Clumps of cells 
were dispersed by pipetting with 10 ml of complete MEM. Viable 
cell counts were made by mixing 1.0 ml of cell suspension with 0.5 
ml of 0.5% trypan blue and counting the unstained cells in a Spencer 
bright line haemocytometer (American Optical Co.). The cells from 
one monolayer (about ten million cells) were dispersed in 150 ml of 
this suspension, containing about 75,000 cells/ml, into each of three 
sterile, 16-ounce flat, soft glass bottles and incubating at 37 C. 
Development of cell monolayers was usually complete within 48 
hours after transferring the cells.
Virus

Source of Virus

Attenuated Sabin type 1 poliovirus was obtained in the form of an oral vaccine (Lederle Laboratories) containing about $2 \times 10^5$ plaque-forming units (PFU)/ml. The vaccine was used to prepare a high titer poliovirus stock and was stored at -70 °C until used.

Propagation of Stock Virus

The stock virus was prepared in two passages through five-day old monolayers of H.Ep.-2 cells grown in MEM with 10% fetal bovine serum and antibiotics. The monolayers were prepared in 16-ounce glass bottles. In the first passage, the medium was removed from a monolayer and the bottle was inoculated with 6 ml of undiluted vaccine. The monolayer was incubated at 37 °C for 1.5 hours and then 25 ml of MEM with 2% fetal bovine serum were added. After 24 hours incubation at 37 °C, the bottle contents were frozen and thawed three times and the fluid was centrifuged at 2,500 rpm for 15 minutes to remove cellular debris. The supernatant fluid was stored at -70 °C and the sediment was discarded. Assay of the supernatant fluid by the plaque technique indicated that the preparation contained about $1 \times 10^7$ PFU/ml.
For the second passage, the medium was removed from five-day old monolayers in five 16-ounce bottles and replaced with 10 ml of MEM with 2% fetal bovine serum and antibiotics. Three ml of poliovirus suspension obtained from the first passage were added to each bottle. The bottles were incubated 1.5 hours at 37 C and then 22 ml of MEM with 2% fetal bovine serum and antibiotics were added to each.

After incubation at 37 C for 24 hours, the contents of the bottles were frozen and thawed three times. The fluid from all bottles was pooled and the pH was adjusted to 7.2-7.4 with 0.2N NaOH. The virus suspension was divided into three portions of about 60 ml each. One portion was stored at -70 C and the other two were subjected to further treatments.

High Extraneous Protein Content Virus (HPC)

Sixty ml of the virus preparation obtained from the second passage were centrifuged at 2,500 rpm for 15 minutes. The sediment was discarded and the supernatant fluid was stored at -70 C. Assay of this suspension by the plaque technique showed that it contained \(5 \times 10^8\) PFU/ml. The preparation contained a relatively high proportion of nonviral protein derived from the 2% fetal bovine serum in the medium and from the cell monolayer. A protein determination was not made on the HPC virus suspension itself, but 2% fetal bovine
serum was found to contain about 1,500 µg of protein/ml.

Low Extraneous Protein Content Virus (LPC)

The third 60 ml portion of virus suspension from the second passage was centrifuged at 10,000 rpm and 5 C for 30 minutes and the sediment was discarded. The supernatant fluid was distributed to aluminum-capped polyethylene centrifuge tubes, pelleted at 45,000 rpm at 5 C for three hours and the supernatant fluid was decanted and discarded. Both centrifuging steps were carried out in a Spinco Model L ultracentrifuge. The pellets were washed once with 2 ml of deionized-distilled water and suspended in 20 ml of glass distilled water with a sterile applicator stick. The suspension was mixed on a mechanical vibrating mixer for 30 seconds and centrifuged at 5,000 rpm and 5 C for 30 minutes in a Sorvall RC-2 centrifuge. Fifteen ml of the supernatant fluid were removed, distributed in 1 ml aliquots to screw-capped plastic tubes (Falcon Plastics) and stored at -70 C until used. The virus titer of the LPC preparation was $4 \times 10^8$ PFU/ml and the protein content was 87 µg/ml.

Virus Neutralization Tests

The identity of the virus used in these experiments was confirmed by serum neutralization tests employing types 1, 2 and 3 poliovirus antisera (Microbiological Associates, Inc.). The three
antisera were mixed with the low extraneous protein content poliovirus diluted in HBSS to contain about 100 PFU/ml. Final dilutions of the antisera ranged from 1:20 to 1:5,120. Type 1 antiserum produced an 80% or greater reduction in plaque titer at all serum dilutions tested. Type 2 antiserum reduced the virus titer 84% in a 1:20 dilution but did not cause significant reductions in titer at higher dilutions. Type 3 antiserum did not reduce the virus titer at any dilution tested.

Seawater

Seawater was obtained from Burley Lagoon, Purdy, Washington. The salinity was 28.5 parts per thousand (‰) as determined hydrometrically (Zerbe and Taylor, 1953), and the pH was 8.0. The seawater was filtered through 450 μm porosity membrane filters (Millipore Corp.), distributed to one-gallon polyethylene containers and stored at -20°C until used.

Electrolyte Solutions

Solutions of NaCl, MgCl₂ and AlCl₃ were prepared by dissolving reagent grade chemicals in deionized water. NaCl was prepared in concentrations of 10⁻³ to 10⁻¹ M; MgCl₂ was prepared in concentrations of 10⁻⁴ to 10⁻¹ M and AlCl₃ in concentrations of 10⁻⁶ to 10⁻² M. Solutions were sterilized by autoclaving. The pH of the
$10^{-2}$ and $10^{-3}$ AlCl$_3$ solutions was 4.2 and 5.0, respectively. The pH of all other solutions was adjusted to 7.2–7.4 with 7.5% NaHCO$_3$.

**Virus - Soil Adsorption System**

Poliovirus and soils were suspended in seawater, electrolyte solution or deionized water in 50 ml polypropylene centrifuge tubes with friction-fit closures (Nalgene). Each sample contained a total volume of 30 ml. After shaking the samples, 10 ml were removed and the assay of this fraction represented the initial concentration of virus in the sample. The remaining 20 ml of suspension were shaken on a rotary mechanical shaker at 18°C for one hour. The samples were then centrifuged at 5,000 rpm and 5°C for 30 minutes in a Sorvall RC-2 centrifuge. Ten ml of the supernatant fluid were removed without disturbing the sediment. The sediment was resuspended in the remaining 10 ml of medium and virus assays were conducted on the supernatant fluid and sediment fractions. The percent removal of poliovirus by adsorption to soil was calculated by difference between the initial virus concentration in the sample and the concentration of virus in the supernatant fluid.

**Virus Assay Method**

Samples were assayed for virus content by the agar overlay plaque technique of Dulbecco and Vogt (1954) as modified by Hsiung.
and Melnick (1955).

**Dilution of Samples**

The diluent was prepared by adding 0.2% fetal bovine serum to sterile HBSS without phenol red (Microbiological Associates, Inc.), and the pH was adjusted to 7.2-7.4 with 7.5% NaHCO₃ solution. Ten-fold dilutions of the samples were made immediately after the centrifugation procedure. The diluted samples were stored at 5°C until assayed, generally, within 24 hours after preparation.

**Preparation of Monolayers for Virus Assay**

H. Ep.-2 cell monolayers were prepared in 30 ml plastic tissue culture flasks (Falcon Plastics). The cells from three 16-ounce glass bottles were trypsinized and dispersed in 350 ml of MEM with 10% fetal bovine serum and antibiotics, resulting in cell concentrations of 150,000 to 200,000 cells/ml. Cells were kept in suspension with the aid of a teflon-coated spinning bar and a magnetic mixer. Five ml of the suspension were distributed to each tissue culture flask with the aid of a Cornwall continuous pipetting syringe. After two days of incubation at 37°C, the medium was replaced with fresh MEM and the cultures were incubated for another two days.
Overlay Medium

The overlay medium was prepared by mixing 2X concentrated MEM with an equal volume of 2X concentrated agar. The MEM was prepared with Earle's balanced salt solution (EBSS) obtained from Microbiological Associates as a 10X concentrate. The EBSS did not contain phenol red. The formula for 100 ml of 2 X MEM was as follows:

| Component                          | Volume
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>EBSS, 10X concentrate</td>
<td>20 ml</td>
</tr>
<tr>
<td>Glass distilled water</td>
<td>33 ml</td>
</tr>
<tr>
<td>Neutral red, 1:1,000</td>
<td>6 ml</td>
</tr>
<tr>
<td>Essential amino acids, 50X</td>
<td>4 ml</td>
</tr>
<tr>
<td>Vitamins, 100X</td>
<td>2 ml</td>
</tr>
<tr>
<td>Glutamine, 200 mM</td>
<td>2 ml</td>
</tr>
<tr>
<td>0.2N NaOH</td>
<td>5 ml</td>
</tr>
<tr>
<td>Penicillin-streptomycin</td>
<td></td>
</tr>
<tr>
<td>10,000 units each/ml</td>
<td>2 ml</td>
</tr>
<tr>
<td>Amphotericin, 250 µg/ml</td>
<td>2 ml</td>
</tr>
<tr>
<td>NaHCO₃, 7.5%</td>
<td>4 ml</td>
</tr>
<tr>
<td>Fetal bovine serum</td>
<td>20 ml</td>
</tr>
</tbody>
</table>

The agar was prepared by mixing 1.5 g of Ionagar No. 2 (Oxoid, Consolidated Laboratories, Inc.) with 100 ml of glass distilled water, melting in a steamer and sterilizing in an autoclave.
Inoculation of Monolayers

After four days of incubation, the medium was removed from the monolayers in the 30 ml tissue culture flasks. Duplicate 0.5 ml portions of each sample were spread over the surface of two monolayers. The flasks were then incubated at 37 C for 1.5 hours to allow adsorption of virus to the cells.

Agar Overlay Technique

The 2X agar was melted and cooled to 48 C in a water bath. The 2X MEM was warmed to 48 C, aseptically combined with the agar and the mixture was kept at 48 C during the overlaying process. The agar medium, in 4.5 ml quantities, was introduced onto each inoculated monolayer with a Cornwall continuous pipetting syringe fitted with 15 to 18 gauge needles, three inches long and a different needle was used for each sample. The agar was spread over the surface of the monolayers, allowed to solidify and the flasks were inverted and incubated in the dark at 37 C. The monolayers were checked for plaques three, four and five days after overlaying. The appearance of new plaques was permanently marked each day. The final plaque count was recorded as PFU/ml and was made five days after inoculating the monolayers.
Electron Microscopy

Two different specimens were prepared: one consisted of the low extraneous protein content virus suspension alone; the other contained the virus suspension with 0.003M MgCl₂ and 25 mg/liter kaolinite. Support screens were 200 mesh copper grids coated with a film of polyvinyl formal (Formvar). Samples were applied to the grids with Pasteur pipets drawn out to fine capillary points by heating in a burner. Drops of suspension just sufficient to cover the grids were applied, allowed to remain for 30 to 45 seconds and then blotted off with Whatman No. 1 chromatography paper. Two grids of each sample were shadowed with platinum-palladium (80:20) at an angle of 15 degrees from the vertical. All specimens were examined in a Philips EM 300 electron microscope. Exposure of photographic plates and operation of the microscope was performed by Mr. Al Soldner, Electron Microscopy Service, Oregon State University.
RESULTS

Characterization of Clays by X-Ray Diffraction

Qualitative analyses of the mineralogical content of the soil samples were made by X-ray diffraction. Diffraction maxima obtained for Purdy, kaolinite and montmorillonite clays are summarized in Table 1. The kaolinite samples showed a single peak of about 7.2 to 7.3 Ångstroms. The peak disappeared entirely after heating the sample to 550 °C. These results are characteristic for kaolinite and indicate that no other mineral components were present.

The montmorillonite samples also showed a single peak ranging from 10.0 Ångstroms at 550 °C to 17.4 angstroms when saturated with Mg\(^{2+}\) and solvated with glycerol. Expansion of the mineral interlayers occurred on changing conditions from dry air to humid air, from K\(^{+}\) saturation to Mg\(^{2+}\) saturation and from water vapor to an atmosphere of ethylene glycol or glycerol. These results are characteristic for montmorillonite and further indicate that it was the only mineral present in the sample.

The Purdy samples showed three or four diffraction peaks, depending upon the characterization treatment employed. There was no significant difference between the A samples (organic matter and MnO\(_2\) removed) and the B samples (organic matter, MnO\(_2\) and iron...
Table 1. X-ray diffraction maxima produced by Purdy, kaolinite and montmorillonite clays using CuKα radiation.

<table>
<thead>
<tr>
<th>Characterization treatment</th>
<th>Diffraction Maxima (Ångstroms)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Montmorillonite</td>
</tr>
<tr>
<td>Mg²⁺ saturation;</td>
<td></td>
</tr>
<tr>
<td>54% R. H. a</td>
<td>7.3 b</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Mg²⁺ saturation; glycerol</td>
<td>ND c</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Mg²⁺ saturation;</td>
<td>7.2 b</td>
</tr>
<tr>
<td>ethylene glycol</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>K⁺ saturation;</td>
<td>7.2 b</td>
</tr>
<tr>
<td>54% R. H. a</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>K⁺ saturation;</td>
<td>ND c</td>
</tr>
<tr>
<td>105 C</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>K⁺ saturation;</td>
<td>0</td>
</tr>
<tr>
<td>550 C</td>
<td></td>
</tr>
</tbody>
</table>

a Relative humidity  
b Saturated with Ca²⁺ instead of Mg²⁺  
c Not done  
d Treated for removal of organic matter and MnO₂  
e Treated for removal of organic matter, MnO₂ and iron oxides
oxides removed). A 9.9 to 10.0 Ångstrom peak was observed regardless of the characterization treatment. This would indicate the presence of a micaceous component.

A 14.0 to 14.2 Ångstrom peak was observed, with the Purdy samples, under all treatment procedures except heating at 550 C, indicating members of the chlorite group of minerals. The \( \text{Mg}^{2+} \) saturated sample solvated with ethylene glycol produced a 16.7 Ångstrom peak not demonstrated under other conditions, and suggesting a tetrahedrally substituted smectite, probably beidellite or nontronite. The Purdy samples produced a peak with spacings varying from 6.9 to 7.1 Ångstroms under all characterization treatments except heating at 550 C. This would normally indicate kaolinite, however, in the presence of chlorite, this peak may be a second order chlorite line.

The Adsorption of Poliovirus by Purdy Clay in Seawater at Different Virus Concentrations

This experiment was conducted to determine the concentration of poliovirus to use in the adsorption studies. Suspensions containing the high extraneous protein content (HPC) poliovirus in concentrations of about \( 5 \times 10^1 \) to \( 5 \times 10^7 \) PFU/ml were prepared in seawater, with and without Purdy clay added. The clay concentration was 100 mg/liter. An aliquot of each sample, representing the initial
virus concentration, was removed before centrifuging. Virus titers of the supernatant and sediment fractions were determined after centrifuging at 10,000 rpm and 5 C for 30 minutes. In later experiments, a clay concentration of 50 mg/liter was generally used and centrifugation was conducted at 5,000 rpm. The results are shown in Table 2.

Table 2. The adsorption of poliovirus (HPC) by Purdy clay (100 mg/liter) in seawater at different virus concentrations.

<table>
<thead>
<tr>
<th>Poliovirus concentration (PFU/ml)</th>
<th>Initial</th>
<th>Supernatant fluid</th>
<th>Sediment</th>
<th>Virus removed(^a) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1 x 10(^1)</td>
<td>none detectable</td>
<td>7.7 x 10(^1)</td>
<td>&gt;99</td>
<td></td>
</tr>
<tr>
<td>3.7 x 10(^3)</td>
<td>1.8 x 10(^1)</td>
<td>8.4 x 10(^3)</td>
<td>99.5</td>
<td></td>
</tr>
<tr>
<td>5.4 x 10(^5)</td>
<td>6.5 x 10(^3)</td>
<td>1.1 x 10(^6)</td>
<td>98.8</td>
<td></td>
</tr>
<tr>
<td>4.3 x 10(^7)</td>
<td>3.9 x 10(^7)</td>
<td>5.0 x 10(^7)</td>
<td>9.3</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Decrease in virus titer of the supernatant fluid as compared to the initial virus titer.

At initial virus concentrations ranging from 4.1 x 10\(^1\) to 5.4 x 10\(^5\) PFU/ml, about 99% of the virus was removed from the supernatant fluid by adsorption to the clay. The virus was quantitatively recoverable from the clay as indicated by the titers of the sediment fractions. At an initial virus concentration of 4.3 x 10\(^7\) PFU/ml there was no significant adsorption of the virus to the clay indicating saturation of the clay by virus concentrations of 10\(^6\) PFU/ml or the presence of substances in the virus preparation which
interfered with adsorption. Control samples, containing only virus and seawater, showed no difference in the virus titers of the initial, supernatant and sediment fractions.

**Effect of Serum on Poliovirus Adsorption by Purdy Clay in Seawater**

An experiment was performed to determine the effect of serum on adsorption of virus by Purdy clay. Fetal bovine serum, in a final concentration of 2%, was added to a seawater-clay mixture 30 minutes before adding virus, and to a seawater-clay-virus mixture 30 minutes after adding the virus. The mixtures were then allowed to stand an additional 30 minutes before centrifuging. The HPC virus preparation was used and the initial virus concentration in the mixtures was about $1.5 \times 10^4$ PFU/ml. A virus concentration of $10^4$ PFU/ml was chosen in order to eliminate possible interference with the adsorption process of the serum in the $10^8$ PFU/ml HPC preparation. Dilutions of the stock HPC virus containing less than $10^4$ PFU/ml were not used in order to avoid indeterminately low titers in the supernatant fluids. The clay concentration was 50 mg/liter. Control samples containing seawater-virus and seawater-virus-clay were also prepared. All samples in this and later experiments were centrifuged at 5,000 rpm and 5 C for 30 minutes. The results are shown in Table 3.
Table 3. The effect of 2% fetal bovine serum on adsorption of poliovirus (HPC) by Purdy clay (50 mg/liter) in seawater.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Virus removed$^a$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seawater-virus</td>
<td>-6</td>
</tr>
<tr>
<td>Seawater-virus-clay</td>
<td>97</td>
</tr>
<tr>
<td>Serum added to seawater-clay</td>
<td>-12</td>
</tr>
<tr>
<td>30 minutes before adding virus</td>
<td></td>
</tr>
<tr>
<td>Serum added to seawater-virus-clay</td>
<td>-18</td>
</tr>
<tr>
<td>30 minutes after adding virus</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Decrease in titer of the supernatant fluid as compared to the initial virus titer (about 1.5 x 10^4 PFU/ml). A minus sign indicates that the titer of the supernatant fluid was higher than the initial virus titer.

When virus and seawater were centrifuged without clay the virus remained in the supernatant fluid. The addition of 50 mg/liter of Purdy clay to the mixture resulted in removal of 97% of the virus. When 2% fetal bovine serum was added to a seawater-clay suspension 30 minutes before adding virus, adsorption of virus to the clay did not occur. Addition of serum to the seawater-clay-virus mixture also resulted in the virus remaining in the supernatant fluid. These results indicate that the serum was able to prevent and reverse adsorption of the virus to the clay.

The effect of serum concentration was studied in a later experiment using low extraneous protein content virus (LPC) in a
concentration of about $4 \times 10^4$ PFU/ml. Fetal bovine serum, in final concentrations of 0.002 to 0.2%, was added to mixtures of seawater, virus and Purdy clay. The clay concentration was 50 mg/liter.

Table 4. The effect of fetal bovine serum concentration on adsorption of poliovirus (LPC) by Purdy clay (50 mg/liter) in seawater.

<table>
<thead>
<tr>
<th>Serum concentration (%)</th>
<th>Virus removed$^a$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.002</td>
<td>97</td>
</tr>
<tr>
<td>0.02</td>
<td>50</td>
</tr>
<tr>
<td>0.2</td>
<td>2</td>
</tr>
</tbody>
</table>

$^a$Decrease in titer of the supernatant fluid as compared to the initial virus titer (about $4 \times 10^4$ PFU/ml).

Adsorption of virus by the clay did not occur in the presence of 0.2% fetal bovine serum. Concentrations of 0.02% and 0.002% fetal bovine serum resulted in adsorption of 50% and 97% of the virus, respectively. Protein determinations indicated that 0.2% fetal bovine serum contained about 150µg of protein/ml.

Effect of pH on Adsorption of Poliovirus by Purdy Clay in Seawater

The LPC polio virus, in a final concentration of about $4 \times 10^4$ PFU/ml, was added to mixtures of seawater and Purdy clay which had been adjusted to pH values ranging from 2 to 9 with 0.2N HCl or...
0.2N NaOH. The clay concentration was 50 mg/liter and control samples without clay were also prepared. Results of these treatments are summarized in Table 5.

Table 5. The effect of pH on adsorption of poliovirus (LPC) by Purdy clay (50 mg/liter) in seawater.

<table>
<thead>
<tr>
<th>pH</th>
<th>Virus removed&lt;sup&gt;a&lt;/sup&gt; (%)</th>
<th>Seawater-virus</th>
<th>Seawater-virus-clay</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>67</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>3.0</td>
<td>95</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td>4.1</td>
<td>51</td>
<td>92</td>
<td></td>
</tr>
<tr>
<td>5.0</td>
<td>-17</td>
<td>93</td>
<td></td>
</tr>
<tr>
<td>6.1</td>
<td>-18</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>7.0</td>
<td>-5</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>8.0</td>
<td>-5</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>9.1</td>
<td>13</td>
<td>95</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Decrease in titer of the supernatant fluid as compared to the initial virus titer (about 4 x 10⁴ PFU/ml). A minus sign indicates that the titer of the supernatant fluid was higher than the initial virus titer.

At pH 5.0 to 9.1 there was no significant difference in the initial virus titer and the titers of the supernatant fluids in samples containing virus but no clay. In the presence of clay, from 93 to 97% of the virus was removed at pH 5.0 to 9.1.

Below pH 5, the virus titer in the supernatant fluid decreased from 51% to 95% in the absence of clay. This was probably due to precipitation of the virus from suspension at the low pH values since the virus could be recovered from the sediment fractions of samples
containing no clay. However, when the mixture did include clay, more virus was removed from the supernatant fluids at pH 2.0 and 4.1 than when clay was not present. At pH 3.0, the decrease in virus titer was about the same either in the presence or the absence of clay.

Effect of Soil Particle Size on Adsorption of Poliovirus in Seawater

Different particle size fractions of Purdy, kaolinite and montmorillonite soils were mixed with LPC poliovirus in seawater and centrifuged. The concentration of each soil fraction was 50 mg/liter except for the < 0.2 μ fraction (25 mg/liter for kaolinite and 180 mg/liter for montmorillonite). Each sample contained poliovirus at a level of about $6 \times 10^4$ PFU/ml. Results are given in Table 6.

<table>
<thead>
<tr>
<th>Particle size (μ)</th>
<th>Purdy</th>
<th>Kaolinite</th>
<th>Montmorillonite</th>
</tr>
</thead>
<tbody>
<tr>
<td>50-20</td>
<td>96</td>
<td>21</td>
<td>61</td>
</tr>
<tr>
<td>20-5</td>
<td>95</td>
<td>75</td>
<td>95b</td>
</tr>
<tr>
<td>5-2</td>
<td>95</td>
<td>89</td>
<td></td>
</tr>
<tr>
<td>2-0.2</td>
<td>97</td>
<td>94</td>
<td>93</td>
</tr>
<tr>
<td>&lt; 0.2</td>
<td>-</td>
<td>97c</td>
<td>99, 9d</td>
</tr>
</tbody>
</table>

a - Decrease in titer of the supernatant fluid as compared to the initial virus titer (about $6 \times 10^4$ PFU/ml).
b - Particles 20-2 μ in diameter.
c - Soil concentration was 25 mg/liter.
d - Soil concentration was 180 mg/liter.
Poliovirus adsorption by the Purdy soil appeared to be independent of soil particle size, however, microscopic examination of the Purdy fractions showed that most of the particles had diameters of 2 to 3 μ or less, even in the 50-20 μ size fraction. Aggregates had apparently disintegrated resulting in clay-size particles in all fractions of the Purdy soil. No such disintegration was observed in the kaolinite and montmorillonite soil fractions.

With kaolinite, poliovirus adsorption consistently increased with decreasing particle size, from 21% for the 50-20 μ fraction to 97% for the < 0.2 μ fraction.

Significant poliovirus adsorption (61%) occurred with montmorillonite particles 50-20 μ in diameter, increasing to 95% and 93% for particles 20-2 μ and 2-0.2 μ, respectively. Montmorillonite particles < 0.2 μ in diameter almost completely removed the poliovirus from the supernatant fluid, although the concentration of soil was greater in this fraction than in the others (180 mg/liter versus 50 mg/liter).

**Effect of Clay Concentration on Adsorption of Poliovirus in Seawater**

Purdy, kaolinite and montmorillonite clays, in concentrations of 1 mg/liter to 100 mg/liter, were added to suspensions of LPC poliovirus in seawater and centrifuged. The samples were allowed to stand at room temperature for the 30 minute interval between
mixing and centrifuging. The initial virus concentration was about $5 \times 10^4$ PFU/ml. The effect of clay concentration on poliovirus adsorption is shown in Figure 1.

Only about 24% of the virus was adsorbed by 1 mg/liter, and 76% by 10 mg/liter of the Purdy clay. Adsorption reached a maximum (97%) at 50 mg/liter and was 94% at 100 mg/liter of Purdy clay.

Significant poliovirus adsorption (40%) occurred with 1 mg/liter of kaolinite clay, rising to a maximum of 99% at 25 mg/liter and declining to 96% at 100 mg/liter of clay.

With montmorillonite, 57% of the poliovirus was adsorbed by 1 mg/liter of clay, 94% at 10 mg/liter and 88 to 90% at 25 to 100 mg/liter of clay.

Effect of Salinity on Adsorption of Poliovirus by Clays

Samples with salinities ranging from 0.1 to 28.5‰ were prepared by diluting seawater with deionized water adjusted to pH 7.9 using NaHCO$_3$. The salinity of deionized water adjusted to pH 7.9 with NaHCO$_3$ was 0.03‰. The initial concentration of LPC poliovirus was about $6 \times 10^4$ PFU/ml and the clay concentration was 50 mg/liter.

Poliovirus adsorption was negligible with Purdy clay and salinities below 1‰ (Figure 2). At 1‰, 47% of the virus was adsorbed, 95% at 10‰ and 98% at 28.5‰. Kaolinite removed 75% of
Figure 1. The effect of clay concentration on the adsorption of poliovirus (LPC) in seawater.
Figure 2. The effect of salinity on the adsorption of poliovirus (LPC) by Purdy, kaolinite and montmorillonite clays.
the poliovirus from the supernatant fluids at salinities as low as 0.1%. Maximum virus removal occurred with kaolinite at a salinity of 1%. Montmorillonite adsorbed 41% of the poliovirus at 0.1% and maximum adsorption (99%) was reached with a salinity level of 10%.

**Effect of NaCl, MgCl₂ and AlCl₃ on Poliovirus Adsorption by Clays**

Solutions of NaCl, MgCl₂ and AlCl₃ were prepared in various concentrations using deionized water at pH 7 to 7.4. Purdy, kaolinite and montmorillonite clays, in concentrations of 50 mg/liter, and LPC poliovirus (about 6 x 10⁴ PFU/ml), were suspended in the different electrolyte solutions and centrifuged. Control samples, without clay, were also prepared.

**NaCl**

Purdy clay adsorbed 74% of the poliovirus at a NaCl concentration of 5 x 10⁻² M (Figure 3). Maximum adsorption (94%) occurred at 5 x 10⁻¹ M NaCl. Kaolinite did not adsorb any poliovirus below NaCl concentrations of 1 x 10⁻² M but adsorbed 95 to 96% at concentrations of 1 x 10⁻² to 1 x 10⁻¹ M. Montmorillonite adsorbed 60% of the poliovirus at 1 x 10⁻² M NaCl, rising to 97% adsorption at 1 x 10⁻¹ M. Purdy clay required the most NaCl, and kaolinite the
Figure 3. The effect of NaCl concentration on the adsorption of poliovirus (LPC) by Purdy, kaolinite and montmorillonite clays.
least, to produce maximum adsorption of the virus.

\[ \text{MgCl}_2 \]

The effect of \( \text{MgCl}_2 \) concentration is shown in Figure 4. Adsorption of poliovirus by Purdy clay was negligible at \( \text{MgCl}_2 \) concentrations below \( 10^{-2} \text{M} \), but 90 to 92% virus removal occurred in the presence of \( 10^{-2} \) to \( 10^{-1} \text{M} \) \( \text{MgCl}_2 \). Removal of poliovirus by kaolinite stabilized at 95 to 96% in \( 10^{-3} \) to \( 10^{-1} \text{M} \) \( \text{MgCl}_2 \), with insignificant adsorption occurring below \( 10^{-3} \text{M} \) electrolyte. Montmorillonite adsorbed 90 to 99.7% of the virus at \( \text{MgCl}_2 \) concentrations of \( 10^{-3} \text{M} \) to \( 10^{-1} \text{M} \). Purdy clay required about 10X greater \( \text{MgCl}_2 \) concentrations than did kaolinite or montmorillonite to adsorb the virus.

\[ \text{AlCl}_3 \]

Interpretation of results obtained with \( \text{AlCl}_3 \) concentrations greater than \( 10^{-5} \text{M} \) was complicated by virus precipitation in control samples without clay. From 73 to 88% of the poliovirus was removed from the supernatant fluids in suspensions containing \( 10^{-4} \text{M} \) \( \text{AlCl}_3 \). However, kaolinite did adsorb 97% of the poliovirus at an \( \text{AlCl}_3 \) concentration of \( 10^{-5} \text{M} \) (Figure 5). Virus was not precipitated in control samples without clay and containing \( 10^{-5} \text{M} \) \( \text{AlCl}_3 \). In samples with \( 10^{-4} \text{M} \) \( \text{AlCl}_3 \), more virus was removed from the
Figure 4. The effect of MgCl$_2$ concentration on the adsorption of poliovirus (LPC) by Purdy, kaolinite and montmorillonite clays.
Figure 5. The effect of AlCl$_3$ concentration on the adsorption of poliovirus (LPC) by Purdy, kaolinite and montmorillonite clays.
supernatant fluid when Purdy, kaolinite and montmorillonite clays were present than was removed in the control samples (94 to 97% in samples containing clay as compared to 73 to 88% in samples without clay).

The effect of AlCl$_3$ concentrations greater than $10^{-4}$ M was further complicated by the relatively acid pH of these solutions (pH 5.0 for $10^{-3}$ M and 4.2 for $10^{-2}$ M AlCl$_3$). At $10^{-3}$ M AlCl$_3$, the virus removal was 32% for Purdy, 58% for kaolinite and 38% for montmorillonite clay. Virus removals of 94 to 97% were produced with all clays by $10^{-2}$ M AlCl$_3$.

**Effect of Decreasing Ionic Strength on Release of Poliovirus from Kaolinite**

Experiments were conducted to determine if adsorbed poliovirus would release from kaolinite upon going from a medium of higher electrolyte concentration to one of lower concentration. Poliovirus was adsorbed to kaolinite clay suspended in $10^{-2}$ M NaCl or $10^{-3}$ M MgCl$_2$ adjusted to pH 7.4 with NaHCO$_3$. After centrifuging the suspensions, the sediment was diluted ten-fold in deionized water at pH 7.4 to produce final electrolyte concentrations of $10^{-3}$ M for the NaCl sample and $10^{-4}$ M for the MgCl$_2$ sample. The mixtures were again centrifuged and the supernatant fluids were assayed for virus. It was found that the virus remained adsorbed to the kaolinite
after resuspending in a medium of lower electrolyte concentration. After the second centrifugation, about 96% of the virus was removed from the supernatant fluid containing \(10^{-3}\) M NaCl and 95% was removed in the sample containing \(10^{-4}\) M MgCl\(_2\).

**Electron Microscopy**

Limited electron microscope studies were conducted in an attempt to determine the adsorption site of the virus to clay particles. An electron micrograph of a shadowed specimen of LPC poliovirus is shown in Figure 6. The particles indicated by arrows appear to possess polyhedral symmetry and have diameters of about 31 m\(\mu\), indicative of poliovirus. Schaffer and Schwerdt (1959) have reported diameters of 27 to 31 m\(\mu\) for shadowed specimens of crystallized poliovirus. Portions of this same specimen, not shown in Figure 6, contained polyhedral particles about 23 m\(\mu\) in diameter which might represent ribosomal remnants derived from the H. Ep. -2 cells. However, infectious poliovirus particles with diameters of 24 m\(\mu\) were reported by Polson and Selzer (1957). Larger aggregates than the group of four pictured at the bottom-center of the field were not observed. Fibrous strands, possibly derived from serum protein, were noted in some fields of the specimen.

A specimen prepared from a mixture of kaolinite clay and LPC poliovirus is shown in Figure 7. Three particles, approximately
Figure 6. LPC poliovirus specimen showing particles about 31 mµ in diameter (arrows). Shadowed, reverse contrast (approximately 121,000X).

Figure 7. Poliovirus-size particles (arrows) on edge of kaolinite crystal. Shadowed, reverse contrast (approximately 238,000X).
31 μ in diameter and presumably poliovirus, appear to be in contact with the kaolinite crystal edges. Virus particles with an orientation to the edge of a kaolinite crystal were not observed frequently in the specimen.

A different field of the same specimen is shown in Figure 8. At least six particles with diameters of about 31 μ can be seen on the faces of various kaolinite crystals. Virus particles were more often observed on faces than on edges of the crystals. Dark crater-like areas with irregular edges are apparent on the faces of kaolinite crystals not covered by the shadowing metal. The contrast is reversed in the micrograph and these dark areas appeared light on the microscope observation screen indicating that they were less electron dense than other portions of the crystals. These craters or depressions are believed to occur in "old" kaolinite soils and may be due to weathering.
Figure 8. Poliovirus-size particles (white arrows) on faces of kaolinite crystals. Note dark, crater-like areas (black areas) on the crystal faces. Shadowed, reverse contrast (approximately 165,000X).
DISCUSSION

Characterization of Clays

X-ray diffraction of the Purdy clay demonstrated the presence of at least three mineralogical components: mica, beidellite or nontronite and chloritic intergrades. Although the characterization treatments were primarily qualitative in nature, the data indicate that Purdy clay may contain a small amount of kaolinite.

Some properties used in the classification of the clay minerals may be illustrated by using kaolinite and montmorillonite as examples. Figure 9 shows the structure of a plate in a kaolinite crystal. Kaolinite is classified as a two layer or 1:1 type clay mineral. Each plate in the crystal consists of a tetrahedral layer containing silicon and oxygen bonded to an octahedral layer composed of aluminum, oxygen and hydroxyls. The crystal is made up of a series of plates stacked one on another and the distance from an atom in one plate to a corresponding atom in the next plate is termed the 001 spacing. This distance is about seven ångströms for kaolinite, and because it does not change significantly under different conditions of hydration and cation saturation, kaolinite is considered as having a non-expanding lattice.

The structure of a montmorillonite crystal is diagrammed in Figure 10. Montmorillonite is a three layer or 2:1 type clay with
Figure 9. Diagrammatic sketch of the structure of kaolinite (Grim, 1953).
Exchangeable cations

\[ nH_2O \]

○ Oxygens  X Hydroxyls  ● Aluminum, iron, magnesium
○ and ● Silicon, occasionally aluminum

Figure 10. Diagrammatic sketch of the structure of montmorillonite (Grim, 1953).
each plate consisting of two silicon tetrahedral layers enclosing an octahedral aluminum layer. This mineral has an expanding lattice and the 001 spacing will vary from ten Ångstroms to about 18 Ångstroms with different cations and the amount of hydration.

The clay minerals have a net negative charge at pH values above about 6. The charge arises primarily through isomorphous substitution: the replacement of an Al or Si by lower valency cations. Adsorption of cations from the surrounding medium balances the charge. Very little substitution occurs within the lattice of kaolinite crystals and the particles therefore have a low net negative charge and correspondingly low cation exchange capacities. Cation adsorption that does occur must take place at the surface of the particle due to the non-expanding character of the crystal plates.

Replacement of Si by Al in the tetrahedral layer, and Al by Mg in the octahedral layer, accounts for a large part of the cation exchange capacity of montmorillonite. The cation exchange capacity of montmorillonite may be 10-20X greater than that of kaolinite. Since the crystal possesses an expanding lattice, cations may be adsorbed between the plates as well as on the surface of the particles.

Micas, or illites, are three layer non-expanding minerals. Substitution of Al for Si in the tetrahedral layer is extensive and the compensating cation is usually potassium. Because of the non-expanding lattice, cation adsorption involves only the external
surfaces of the crystals and the cation exchange capacity is lower than that of montmorillonite.

Beidellite is a three layer clay with an expanding lattice. The cation exchange capacity approximates that of montmorillonite and is accounted for by the replacement of Si by Al.

Chlorites and chloritic intergrades are mixed-layer types of minerals consisting of mica-like layers alternating with brucite-like layers. The negative charge of the mica layer is balanced by a positive charge in the brucite layer. The non-expanding character of the lattice further accounts for the relatively low cation exchange capacity (Grim, 1953; Van Olphen, 1963).

Virus Adsorption Experiments

Effect of Virus Concentration and Serum Concentration

When concentrations of the HPC poliovirus preparation, ranging from $10^1$ to $10^5$ PFU/ml, were mixed with Purdy clay, about 99% of the virus was removed from the supernatant. At a virus concentration of $10^7$ PFU/ml, only about 9% of the virus adsorbed to the clay. However, a variation of 9% in titer by the plaque technique cannot be considered significant since, in a series of assays of a single sample, mean plaque titers may be expected to vary by 25 to 30% (Cooper, 1961).
The failure of poliovirus to adsorb to the clay at the $10^7$ PFU/ml concentration might have been due to the presence of interfering serum components. The stock HPC poliovirus contained $10^8$ PFU/ml of virus and the equivalent of 0.2% fetal bovine serum. When the stock HPC poliovirus was diluted 1:10,000, the addition of 2% serum to virus-clay mixtures prevented and reversed adsorption. The experiment on the effect of serum concentration showed that 0.2% serum prevented virus adsorption completely, 0.02% resulted in 50% adsorption and 0.002% allowed 97% of the virus to adsorb to Purdy clay.

In an experiment not reported here, virus failed to adsorb to Purdy clay in the presence of 1% oyster homogenate added to seawater. The protein or phospholipid components of serum and oyster homogenate may account for the failure of virus to adsorb to clay. Adsorption experiments were not conducted with pure proteins and phospholipids, and the serum was defined only as to protein content. However, Carlson et al. (1968) demonstrated that purified egg and bovine albumin, in concentrations of 1 to 50 µg/ml, could prevent and reverse adsorption of bacteriophage to clay minerals.

**Effect of pH**

There was no significant difference in the percent removal of virus from the supernatant fluids in the presence of Purdy clay at
pH values of 2 to 9. At pH 2 to 4, removal of the virus was not due entirely to adsorption by clay, as evidenced by decreases in supernatant titers of 51 to 95% in samples containing no clay. Loss of virus from the supernatant fluids of the control samples at low pH appeared to be due to precipitation of the virus since it could be recovered from the sediment fractions.

Poliovirus and clay particles both possess isoelectric points between 5 and 6. Adjustment of the hydrogen ion concentration to a pH above 6 should produce a net negative charge on both particles resulting in mutual repulsion and a slower rate of adsorption. However, the experiment was designed to determine the effect of pH on the amount of virus adsorbed and not the effect on the rate of adsorption, although the rate apparently was not pH limited under the conditions employed.

Effect of Soil Particle Size

The effect of Purdy soil particle size could not be determined due to disintegration of the larger aggregates into clay-size particles (less than 2 μ in diameter). Microscopic examination of the fractions after separation by size indicated that the aggregates were sufficiently stable to maintain their size throughout the fractionation procedure. Disaggregation of the larger diameter particles apparently occurred as a result of organic matter dissolution upon suspending the soil in
This problem was not encountered with the kaolinite and montmorillonite samples. The larger diameter particles of kaolinite adsorbed less virus than did the montmorillonite particles of equivalent size, and clay-size particles adsorbed more virus than silt-size particles (50-2 μ in diameter). These results would suggest that the ability of a soil to adsorb virus might be related to the cation exchange capacity of the soil and that increasing the surface area of the particles results in adsorption of more virus.

Natural turbidity due to soil may be expected to contain a greater number of clay particles than silt particles and the clay would be subject to wider distribution in an aquatic environment than the silt. Oysters have the ability to reject certain particles as food material because of size and shape. Rejection of soil particles less than 2 μ in diameter would be less likely than rejection of silt particles. For these reasons, the adsorption studies were carried out primarily with the clay fraction of each soil.

Effect of Clay Concentration

Montmorillonite and kaolinite adsorbed significant amounts of poliovirus at clay concentrations as low as 1 mg/liter. Maximum removal of poliovirus occurred with montmorillonite at 10 mg of clay/liter, with kaolinite at 25 mg/liter, and with Purdy clay at
50 mg/liter. Higher concentrations of all three clays did not result in a higher percent removal of the virus. These samples were not shaken continuously during the 30 minute interval between mixing and centrifuging. Failure to remove more of the virus at the higher clay concentrations may have been due to a faster rate of flocculation of the clay. This could result in removal of the clay from the reaction mixture by sedimentation preventing further contact with unadsorbed virus. To provide a basis for comparison and to ensure using a system where clay was not the limiting variable, a clay concentration of 50 mg/liter was chosen for other experiments.

**Effect of Electrolyte Concentration**

In the salinity, NaCl and MgCl₂ experiments, Purdy clay required higher concentrations of electrolyte than did montmorillonite, and montmorillonite required higher concentrations than kaolinite to adsorb the virus. The virus did not adsorb to any of the clays when suspended in deionized water. Significant amounts of virus were adsorbed by montmorillonite and kaolinite at salinities as low as 0.1%, a level which may be expected to occur in coastal rivers. Brackish water has a salinity of about 5%, estuarine water 5-30% and ocean water about 35-40%.

Less MgCl₂ than NaCl was required to initiate adsorption to all three clays and less AlCl₃ than MgCl₂ was needed for virus to
adsorb to kaolinite. The AlCl$_3$ requirements for Purdy clay and montmorillonite could not be determined due to precipitation of the virus by AlCl$_3$ concentrations greater than $10^{-5}$ M.

Similar results on the adsorption of poliovirus and bacteriophage to kaolinite, montmorillonite and illite clays were obtained by Carlson et al. (1968) using NaCl and CaCl$_2$ solutions. These investigators postulated the formation of a cation bridge between the negatively charged virus and clay particles. A mechanism of this type may be involved but it does not adequately explain all aspects of the adsorption phenomenon. Clay particles suspended in deionized water would still possess an atmosphere of exchangeable cations but no "bridge" to virus occurs under these conditions.

The effect of electrolytes and serum on virus adsorption suggests that factors which influence the flocculation of clay particles alone may affect virus adsorption. Van Olphen (1963) has indicated that Van der Waals' attraction is greater than the repulsion, even though the particles have like charges. Increasing the concentration of cations in the surrounding medium causes a decrease in the zone of repulsion around clay particles. This may also explain the greater NaCl and MgCl$_2$ requirement of montmorillonite as compared to kaolinite to produce virus adsorption. A particle of kaolinite the same size as a particle of montmorillonite would possess a lower net negative charge, and a lower cation concentration would be
required by kaolinite to decrease the repelling force sufficiently for adsorption to occur. Proteins and other protective colloids have a stabilizing effect on suspensions of clays. A similar mechanism may have prevented adsorption of virus to clays in the presence of serum and oyster homogenate. Unfortunately, observations were not made on the occurrence of clay flocculation in conjunction with the virus adsorption experiments.

Decreasing the electrolyte concentration to levels which, if present initially, would not have resulted in virus adsorption to clay, failed to cause release of virus adsorbed to kaolinite. This effect was noted with both NaCl and MgCl₂ solutions and further suggests a flocculation mechanism of virus adsorption. When a clay is flocculated, much lower concentrations of electrolyte are required to maintain the flocs than are needed to initiate flocculation. However, Carlson et al. (1968) reported that virus did release from clay on changing from a medium of higher ionic strength to one of lower ionic strength.

Electron Microscopy

Aggregates were rare in the LPC poliovirus suspension, and when encountered, contained two or three particles indicating that the suspension consisted of well-dispersed poliovirus. Amorphous debris was not often observed which might suggest that the protein
content of the virus suspension (87 µg/ml) was due in large part to the presence of soluble, nonviral proteins.

In preparations containing kaolinite clay and virus, the virus was usually observed on the faces rather than the edges of the kaolinite crystals. Van Olphen (1963) suggested that the edges of kaolinite crystals may possess a positive charge while the faces carry a net negative charge. Kaolinite demonstrated a small anion adsorption capacity in a slightly acid environment but not in an alkaline environment. Van Olphen also reviewed an experiment in which a negative gold sol was mixed with kaolinite and electron micrographs showed the gold particles adsorbed only to the edges of the clay crystals.

The adsorption of poliovirus by clays might therefore be aided by an electrostatic attraction between the negative virus particle and the positive edge of the clay. More virus particles may have been observed on the kaolinite faces than on the edges due to the extreme weathering of the kaolinite. If these areas are indeed breaks in the surface of the kaolinite crystal, the craters would possess a charge distribution similar to that found on the edge of the crystals. More virus particles would be likely to come in contact with the crystal faces than the edges, and more adsorption on the face would be expected if there is no difference in electrostatic attraction of the face and edge surfaces.
Other Studies

Further information on the mechanism of adsorption of virus by clays might be obtained through more detailed electron microscope studies. Determination of the adsorption sites might be possible through the use of clay crystals without surface irregularities. Specimens of clay-virus mixtures could be examined under different conditions of electrolyte concentration and in the presence and absence of nonviral proteins.

The rate of virus adsorption to clays has not been determined. Under the conditions employed in these experiments, maximum adsorption apparently occurred within 30 minutes after mixing virus and clay. However, if a flocculation type of mechanism is operable, the rate of virus adsorption should follow the rate of clay flocculation and more virus should be adsorbed with increasing time at minimal electrolyte concentrations.

Factors which affect dissociation of virus from clay after adsorption could have significance in the distribution patterns of viral contaminants in natural waters. Comparative studies on the survival times of viruses associated with clays are also indicated.

Some natural waters remain relatively low in turbidity derived from soil particles but may be subject to plankton turbidity. The possibility that viruses may adsorb to plankton might therefore be investigated.
SUMMARY AND CONCLUSIONS

1. X-ray diffraction characterization indicated the presence of mica, beidellite or nontronite, chloritic intergrades, and possibly, some kaolinite in a sample of clay obtained from an estuarine sediment. The sample appeared to consist primarily of 2:1 type mineral components.

2. Fetal bovine serum was able to prevent and reverse adsorption of poliovirus to Purdy clay in seawater.

3. There was no significant difference in the amount of poliovirus adsorbed by Purdy clay in seawater at pH levels of 5.0 to 9.1.

4. Three different soil preparations were shown to be capable of adsorbing poliovirus from seawater and other electrolyte solutions. Montmorillonite and kaolinite silt adsorbed significant amounts of poliovirus but more was adsorbed by the clay fractions. Montmorillonite silt adsorbed more virus than kaolinite silt.

5. Montmorillonite, kaolinite and Purdy clays adsorbed maximum amounts of poliovirus at clay concentrations of 10 mg/liter, 25 mg/liter and 50 mg/liter, respectively.

6. Poliovirus did not adsorb to any of the three clays when the mixtures were prepared in deionized water. Significant virus adsorption occurred at salinity levels which may be encountered
7. Lower concentrations of MgCl₂ than NaCl were required for virus adsorption to clays. Kaolinite required less AlCl₃ than MgCl₂ to adsorb virus. The 2:1 type clays (Purdy and montmorillonite) needed higher electrolyte concentrations than did the 1:1 type kaolinite for virus adsorption to occur.

8. Decreasing the electrolyte concentration of a sample containing poliovirus adsorbed to kaolinite did not result in release of the virus from the clay.

9. Electron microscopy indicated that the LPC virus suspension contained particles with diameters and morphology consistent with reported characteristics for poliovirus. Other unidentified particulate matter was present in relatively small amounts.

10. Electron microscopy revealed surface irregularities in kaolinite crystals which may influence the adsorption site of poliovirus. Poliovirus particles were more frequently observed in association with kaolinite crystal faces than with edges.

11. The mechanism of adsorption has not been resolved but a flocculation type of mechanism is suggested.


Harward, M. E. 1967. Methods and criteria in use at Oregon State University for clay mineral identification. Corvallis, Oregon, Oregon State University, Dept. of Soils. 5 p. (Mimeographed)


