Studies on the Survival and Fate of Enteroviruses in an Experimental Model of a Municipal Solid Waste Landfill and Leachate

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In laboratory scale municipal solid waste lysimeters containing simulated refuse, and seeded with either laboratory or field strains of poliovirus type 1 and echovirus type 7, viruses were not detected in the lysimeter leachate produced over a 4-month period. In addition, viruses were not detected in the lysimeter refuse contents after termination of lysimeter operation. These results appeared to be due to virus retention in the lysimeter caused by virus adsorption and virus inactivation. Evidence for virus inactivation was provided by the results of experiments on virus inactivation in composite leachate samples. Evidence for virus adsorption was supported by the rapid adsorption of viruses to various municipal solid waste components in the presence of a salt solution similar in composition to the major inorganic salts of leachates.

The disposal of municipal solid waste containing fecal material in sanitary landfills or open dumps may be a source of contamination of the surrounding environment with pathogenic microorganisms, including enteric viruses (1, 4; M. L. L. Peterson, Ph.D. thesis, Univ. of Michigan, Ann Arbor, 1972). There is particular concern for the possibility of such pathogens leaching through the refuse layer and contaminating ground and surface water. Although a number of studies on the survival and fate of enteric viruses in sanitary landfills and their leachates have recently been reported, the results of these studies are not in complete agreement and the various factors influencing the survival and fate of viruses in such systems have not been fully evaluated (3, 4; Peterson, Ph.D. thesis, 1972). Because of the leachate volumes involved in large-scale lysimeter studies, only a relatively small proportion of the total produced was examined for viruses in these previous investigations. Intermittent appearance of virus in lysimeter leachates observed by Cooper et al. (3) might not be detected if it failed to coincide with a random sampling of leachate or if the virus level was below the detection level of the virus concentration and assay methods used.

The general objective of this current study was to contribute to our understanding of the survival and fate of human enteric viruses in municipal solid waste landfills. The specific objectives were to: (i) determine the extent to which viruses applied to a municipal solid waste lysimeter would travel through the waste and appear in the leachate produced; (ii) investigate the short-term interactions of viruses with the major components of municipal solid waste; (iii) determine the survival of viruses in municipal solid waste landfill leachate; and (iv) determine whether the behavior of field and laboratory strains of viruses was detectably different.

MATERIALS AND METHODS

Cell cultures. Baboon kidney cells were trypsinized and grown in monolayer cultures as previously described (5). BSC1 cells, a continuous cell line derived from African green monkey kidney cells, were grown in Eagle minimum essential medium with 10% fetal calf serum, 0.08% NaHCO₃, 100 µg of streptomycin/ml, and 100 units of penicillin/ml, and maintained in Eagle minimal essential medium with 2% fetal calf serum, 0.12% NaHCO₃, and the same concentrations of penicillin and streptomycin as in the growth medium.

Viruses and virus assays. Two enteroviruses, poliovirus type 1 and echovirus type 7, were selected as representative of the human enteric viruses potentially present in human solid waste and used in this study. Two different strains of each virus were used: a laboratory strain which had undergone many cell culture passages and a field strain which had recently been isolated from human waste and had been passaged only once in cell cultures. The laboratory strain of type 1 poliovirus was the oral
vaccine strain (LSc). All viruses were grown in baboon kidney cells and then concentrated and purified on membrane filters following methods developed in this laboratory (10). Virus assays were done by two methods. For small sample volumes, assays were done by the plaque technique as previously described (5) by using 0.2-ml sample volumes in 1-ounce (ca. 0.30 liter) prescription bottles of monolayer cell cultures. To assay larger sample volumes for virus than was economically feasible by the plaque technique, leachate eluates and lysimeter refuse eluates were assayed by a bottle culture technique as follows. Each sample, which had been made isotonic, contained 2% fetal calf serum and had a pH of 7.5 and was filtered through a 0.22-μm porosity nitrocellulose filter which had been pretreated with Tween 80 to prevent virus adsorption (8). The purpose of this treatment was to remove bacteria and fungi, and the diameter of the filter used was dependent upon sample volume. The filtrate was diluted 1:4 in maintenance medium and then inoculated into either 1- or 16-ounce (0.473 liter) bottles of BSC1 cells. The bottles were incubated at 37°C and periodically examined over the course of 10 days for cytopathic effects. Bottles showing cytopathic effects were frozen and thawed and 2-ml volumes of the cell lysate were filtered through Tween 80-treated, 25-mm diameter, 0.22-μm porosity cellulose ester (Millipore Millex) filters to remove debris. The resulting filtrates were tested for both poliovirus type 1 and echovirus type 7 by neutralization tests as described below. Virus titer was estimated from the total sample volume inoculated and the fraction of bottles with cytopathic effects due to each virus.

Virus neutralization tests. Equal volumes of sample dilution containing about 200 plaque-forming units (PFU)/0.1 ml of virus and an appropriate dilution of heat-inactivated serum were combined and incubated for 1 h at 37°C in a water bath before plating on BSC1 cells by the plaque technique. Samples were treated with four different sera: (i) control serum (no poliovirus type 1 or echovirus type 7 antisera); (ii) poliovirus type 1 antisera; (iii) echovirus type 7 antisera; and (iv) combined poliovirus type 1 and echovirus type 7 antisera. These treatments made it possible to determine if either or both viruses were present and if another, contaminating virus was present.

Physical, chemical, and bacteriological analyses of leachates from laboratory lysimeters. To monitor the behavior of the lysimeters, leachates were routinely analyzed for pH, conductivity, turbidity, ammonia nitrogen (NH₃-N), and "total" bacteria. Conductivity was measured with a HACH model 2200 conductivity meter (HACH Chemical Co., Ames, Iowa) and expressed as milligrams per liter of NaCl; turbidity was measured with a HACH model 2100 A turbidimeter and expressed as Fornazin turbidity units; and NH₃-N was determined spectrophotometrically by using Solorzano's phenolphthalein nitrite method (12). Bacteria were assayed by surface inoculation on plates of Standard Methods agar (BBL) with both aerobic and anaerobic incubation for 2 days at 25°C, and bacteria concentrations were expressed as colony-forming units per ml.

Municipal solid waste lysimeters. Two laboratory scale lysimeters were used in this study to simulate conditions in a municipal solid waste sanitary landfill. The lysimeters were constructed of plexiglass tubing (6-inch [15.24 cm] inside diameter and one-fourth-inch [0.64 cm] wall thickness). Each plastic column was 5 feet (152.4 cm) in overall length and had a one-half-inch (1.27 cm) thick plexiglass bottom plate which contained a series of 14 three-eighth-inch (ca. 0.96 cm) diameter drain holes for leachate. A 5.75-inch (ca. 1460 cm) diameter, mesh stainless-steel screen (14 by 14) was placed on top of the bottom plate to retain the gravel and refuse that would be placed in the column. A 1-inch (2.54 cm)-thick sheet of Armaflex foam plastic insulation (Armstrong Cork Co., Lancaster, Pa.) extending from the column bottom upward for a distance of 4 feet (122.92 cm) was wrapped around the outside of each column. The lysimeters were mounted on a metal frame with the column bottoms resting on a metal support plate. The metal support plate had a series of fourteen one-half-inch diameter drain holes which matched the drain holes in the Plexiglas bottom plate of each lysimeter. Six-inch diameter funnels were sealed to the underside of the metal support plate to collect the leachates produced by the lysimeters. The bottom of each funnel was connected via a 2-foot (ca. 60 cm) length of Tygon tubing to a leachate collection bottle, which was kept at 4°C in a small refrigerator mounted beneath the lysimeters.

The lysimeters were packed with a synthetic municipal solid waste mixture having the composition shown in Table 1. The synthetic refuse was used because the proportions of the components were clearly defined and their possible role as selective adsorbents of viruses might be more readily evaluated. The components, which, if necessary, had first been reduced in size to pieces of less than about 1 square inch (6.45 cm), were combined by manual

| Component                      | Total refuse by wt (%) |
|-------------------------------|------------------------|
| Corrugated paper boxes        | 27.2                   |
| Newsprint                     | 11.0                   |
| Magazine paper                | 7.8                    |
| Food wastes (garbage)         | 7.8                    |
| Glass                         | 7.3                    |
| Lawn clippings                | 6.5                    |
| Metals (tin cans)             | 6.5                    |
| Mail (bond and office paper)  | 3.1                    |
| Wood chips                    | 2.1                    |
| Brown paper                   | 1.3                    |
| Plastic (from plastic bottles)| 0.8                    |
| Rags (cotton)                 | 0.8                    |
| Vacuum cleaner catch          | 0.8                    |
| Rubber (rubber bands)         | 0.4                    |
| Leather                       | 0.4                    |
| Water (added to mixed refuse) | 16.2                   |
mixing. Seventeen pounds (7256.0 g) of the refuse mixture was prepared and immediately placed into each column, which already contained a 3-inch (7.62 cm) layer of approximately one-eighth (0.32 cm) diameter gravel. The refuse was added to the columns in small increments and manually tamped to a density of about 800 pounds/yard², giving a refuse column of 36 ± 1 inches. After the columns were packed with refuse, one-half-inch diameter holes were drilled into each column at distances of 9, 18, and 27 inches (22.86, 45.72, and 68.58 cm, respectively) from the column bottoms. Thermistor probes mounted in rubber stoppers were placed in the holes and sealed with silicone rubber glue. The probes penetrated to a distance of 3 inches into the center of the refuse column, and they were connected to a thermistor thermometer equipped with a recorder. An additional thermistor probe was placed near the columns to monitor the ambient air temperature. Temperatures were monitored from the time of initial refuse placement.

Originally, it was estimated that field capacity would be approximately 50% of the lysimeter placement weight or 8.5 lbs (3.86 liters) of water per lysimeter. In an attempt to reach field capacity in about 2 weeks, the lysimeters were dosed with 275 ml of distilled water daily. However, leachate was not produced by 2 weeks, and so the dosing rate of 275 ml per day was continued until leachate was produced. Lysimeters 1 and 2 began producing leachate on days 25 and 26, respectively, after refuse placement, reflecting field capacities of approximately 90% of placement weight. When leachate production began, the lysimeters were put on a distilled water dosing schedule of 204 ml twice weekly (Monday and Thursday), which corresponded to Houston's annual precipitation rate of 46 inches (ca. 116.84 cm), but allowed for no run-off.

The leachate which was constantly being produced by each lysimeter was collected three times weekly (Monday, Wednesday, and Friday) and thus represented the accumulated production of either a 2- or 3-day period. The samples were stored at 4°C and viral, bacterial, and chemical analyses were performed within 24 h of collection. A 20-ml aliquot of the collected leachate, representing about 20% of the total volume, depending on the leachate volume produced, was used for physical, chemical, and bacterial analyses. The remainder, representing about 80% of the total volume, was assayed for viruses. Of the total volume reserved for virus assay, a 2-ml portion was plated directly for virus by the plaque technique. The remaining volume was concentrated 10-fold using procedures previously described (7). Two milliliters of the concentrate was assayed for virus by the plaque technique, and, if no plaques were detected, the remainder was assayed by the bottle culture technique using 1-ounce bottles of BSC1 cells. Lysimeters 1 and 2 were operated and sampled continuously until operation was terminated on days 134 and 141, respectively, after refuse placement.

Introduction of viruses to lysimeter. Viruses were not added at the time of refuse placement to avoid the possible problem of virus inactivation due to desiccation in the dry refuse. Desiccation would have been an uncontrollable variable in the lysimeter experiments. It was originally intended to add the viruses when the lysimeters reached field capacity 2 weeks after refuse placement. However, field capacity was not achieved by the end of the second week of lysimeter operation. Both lysimeters were finally seeded with viruses on day 17 after refuse placement, which was 8 and 9 days before leachate production in lysimeters 1 and 2, respectively. By day 17, the upper portions of the lysimeter refuse columns had become saturated with liquid, and it was considered safe to add viruses without risk of desiccation. Viruses were introduced into the columns by substituting 275 ml of virus suspension in distilled water for the usual 275 ml of daily distilled water feed. Lysimeter no. 1 received 2.5 × 10⁶ PFU each of poliovirus type 1 and echovirus type 7 laboratory strains, whereas column no. 2 received 2.5 × 10⁶ PFU each of field strains of the same viruses. Immediately after adding the viruses, each lysimeter received 8 pounds (ca. 3.632 g) of topsoil to give a 6-inch cover layer. The soil cover was then saturated with 600 ml of distilled water to satisfy its water demand and to prevent desiccation of the added viruses.

Recovery of virus from lysimeter refuse. It was thought that perhaps all or some of the virus introduced into the lysimeters might become strongly associated with the refuse and consequently not appear in the leachate. Therefore, after a certain period of operation, each lysimeter was opened and an attempt was made to recover virus from the refuse. Lysimeters 1 and 2 were sacrificed on days 134 and 141, respectively, after refuse placement, as follows. The plastic lysimeter housing was carefully cut open and the 3-foot (91.44 cm) refuse column was removed in 6-inch fractions beginning at the column bottom. In addition, the topsoil refuse cover was collected as a seventh fraction. Each fraction was treated by manual mixing for 10 min with 4 liters of 0.05 M glycine adjusted to pH 11.5 with NaOH. This eluent has been previously used to recover adsorbed viruses from a variety of solids and surfaces (8, 9). In addition, the eluent contained 0.01 and 0.1 M tetrasodium ethylenediaminetetracacetate (EDTA) for lysimeters 1 and 2, respectively. EDTA has been successfully used in our laboratory (7; Sobey et al., unpublished data) and by others (3) to aid in eluting adsorbed viruses from a variety of surfaces and particulates. The refuse-elucent mixture was transferred to a 100-μm porosity, viscose bag filter (GAF Corporation, Greenwich, Conn.), and the eluate fluid was recovered from the mixture by manually expressing it through the filter. The topsoil eluate was additionally filtered through a 25-μm porosity viscose bag filter. The lysimeter eluates were adjusted to pH 10.0 and those from lysimeter no. 1 were made 0.1 M with respect to EDTA. The eluates were clarified by filtering through a series of textile cartridge depth filters consisting of 10- and 1-μm porosity orlon filters and a 0.5-μm porosity, Tween 80-treated cellulose acetate filter (9). Viruses were concentrated from the clarified eluates by using methods similar to those developed for leachates (7).
Each clarified eluate was adjusted to pH 7.5 and treated with 1,000 g of Ionac A540 anion exchange resin in the chloride form. The resin-treated eluate was adjusted to pH 6.5 and MgCl₂ was added to a final 0.02 M concentration to complex residual EDTA. Each sample was adjusted to pH 3.5 and AlCl₃ was added to a final 0.005 M concentration. If possible, the sample was filtered through a virus adsorber consisting of a 142-mm diameter, 5, 1, 0.45-μm porosity Cox filter series to adsorb any viruses that may be present. The filtrate was discarded and the filters were washed with 1,000 ml of pH 3.5, 0.15 N NaCl to flush out residual Al ions. Viruses were then eluted from the filters with 100 to 200 ml of pH 11.5 glycine-NaOH eluent. The eluate was promptly neutralized with pH 1.0 glycine-hydrochloride, made isotonic with NaCl, treated with fetal calf serum to a final 2% concentration, and stored at −40°C until assayed for virus. Those pH 3.5 samples containing 0.0005 M AlCl₃ which could not be filtered directly were first centrifuged at 2,000 × g for 15 min. The resulting supernatants were decanted and concentrated on Cox filters as described above. The sediment was resuspended in about 50 ml of 0.05 M glycine, neutralized, made isotonic, treated with fetal calf serum to a final 2% concentration, and filtered through Tween 80-treated, 0.22-μm porosity cellulose membrane filters to remove bacteria and fungi. Initially, a 5-ml aliquot from each sample was screened for viruses by the plaque technique. If viruses were not detected, the entire remaining sample volume was assayed for virus by the modified bottle culture technique described above with 16-ounce prescription bottles of BSC1 cells.

Poliovirus survival in lysimeter leachates. A series of experiments was conducted to determine the survival of field strain poliovirus type 1 in the leachates of both lysimeters 1 and 2. Flow-weighted composite leachate samples were prepared from accumulated leachate samples collected from each lysimeter. These accumulated samples were the frozen, excess portions of the 20-ml leachate aliquots of each sample which had been reserved for physical, chemical, and bacteriological analyses. Freezing of the leachates caused no appreciable changes in the physicochemical properties that were routinely measured. Enough stock poliovirus was added to a 45-ml volume of each composite leachate to give an initial virus concentration of about 5 × 10⁶ PFU/ml. Each leachate was then divided into three equal volumes, and these volumes were incubated at temperatures of 4, 20, and 37°C in sealed bottles. Control samples consisting of 0.05 M glycine at the same pH as the leachate and containing the same initial virus concentration were also incubated at these three temperatures. At regular intervals small samples were taken from both the leachates and the controls for virus assay. To eliminate the possible masking effects of leachate components on virus detectability (7), the leachate and control samples taken for virus assay were treated with EDTA by diluting 10-fold in pH 7.5, 0.05 M glycine-0.01 M EDTA and holding for 20 min. The EDTA-treated samples were then further diluted serially 10-fold in pH 7.5, 0.05 M glycine-0.15 N NaCl for plaque assay.

Short-term interactions of viruses with municipal solid waste components. A series of experiments was conducted to determine if the two field strains of enteroviruses used in this study would adsorb to the major components of municipal solid waste and if such adsorbed viruses could then be eluted. These interactions were studied using relatively short contact times in either distilled water or a salt solution similar in composition to the major inorganic components of typical leachate. The salt solution had a pH of 5.5 and contained per liter: 2.5 g of NaCl, 5.5 g of CaCl₂, 5.0 g of MgCl₂, 0.75 g of NH₄Cl, and 0.7 g of H₃PO₄. The test procedure was as follows. The solid waste component was prepared as a 1% suspension in 100 ml of either distilled water or salt solution by blending for 1 min and then, if necessary, readjusted to pH 5.5. In the case of glass a 5% suspension was prepared and the blending step was omitted because the glass had already been crushed to particles of 60 to 80 mesh. Field strain poliovirus type 1 or echovirus type 7 was added to the suspension to give an initial concentration of about 5 × 10⁴ PFU/ml, and the mixture was stirred at 200 rpm on a gyrotory shaker for 60 min. After stirring, the mixture was centrifuged at 1,900 × g for 10 min, the supernatant was decanted and the sediment was resuspended in another 100-ml volume of virus-free distilled water or salt solution. This suspension was centrifuged as before, the supernatant was decanted, and the sediment was resuspended in pH 11.5 glycine-NaOH. The mixture was again centrifuged and the supernatant was decanted and promptly adjusted to pH 7.5. Both the solid waste suspension and a control solution containing no solid waste were assayed for viruses at each step of the procedure. The solid waste components which were tested by this scheme included each type of paper, glass, lawn waste, and food waste. These components plus water accounted for more than 88% of the total composition of solid waste on either a weight or volume basis.

It should be pointed out that the relevance of these data to actual leachate is uncertain because leachates contain unidentified organic constituents that may compete with viruses for adsorption sites on the solid waste. However, these results do indicate the possibility of enhancement of virus adsorption to solid waste components by salts. In addition, we have previously presented evidence for the rapid association of poliovirus with leachate-suspended solids (7).

RESULTS

Lysimeter operations and leachate characteristics. The data on leachate production and its physical, chemical, and bacteriological characteristics are summarized in Table 2. Temperatures in both lysimeters were approximately the same as the ambient air temperature and did not vary much with probe location. In lysimeter 1 the leachate pH averaged 5.4 and remained in the pH 5.1 to 6.0 range throughout the entire course of the experiment. In lysime-
Table 2. Physical, chemical, and bacteriological characteristics of lysimeter leachates

| Parameter                          | Lysimeter 1 | Lysimeter 2 |
|------------------------------------|-------------|-------------|
|                                   | Mean value  | Range of values | Mean value  | Range of values |
| Daily leachate production (ml)     | 45.6        | 20–142      | 43.7        | 22.5–120      |
| Temp (°C)*                         |             |             |             |             |
| Upper probe                        | 29.0        | 25.5–33.0   | 28.3        | 25.5–31.1    |
| Middle probe                       | 28.3        | 25.5–32.0   | 28.4        | 25.5–32.4    |
| Lower probe                        | 28.5        | 25.5–32.0   | 28.5        | 25.5–32.1    |
| pH                                 | 5.4         | 5.13–5.95   | 6.1         | 5.35–7.5     |
| Conductivity (mg of NaCl per liter)| 9,080       | 3,600–22,000| 8,590       | 3,700–20,000 |
| Turbidity (FTU)*                   | 6.5         | 2.35–12     | 13          | 2.9–140      |
| Ammonia nitrogen (mg/liter as N)   | 34.4        | 8.5–68      | 37.3        | 9.9–74       |
| Nitrate nitrogen (mg/liter as N)   | ND          | ND          | ND          | ND           |
| Bacteria (CFU/ml)                  |             |             |             |             |
| Aerobic incubation                 | 5.7×10⁶     | 0–2.0×10⁶   | 5.8×10⁶     | 0–6.4×10⁷    |
| Anaerobic incubation               | 7.2×10⁶     | 0–5.0×10⁶   | 2.0×10⁴     | 1.0          |

* Ambient air temperature ranged from 25 to 32.3 °C and averaged 28.2 °C.
* FTU, Formazin turbidity units.
* ND, None detected.
* Geometric mean.

This period 2, which had an average pH of 6.1, there was a slow pH rise throughout most of the experimental period to peak values near neutral pH during the last several weeks of lysimeter operation and then finally a decline to pH values in the 5.6 to 5.9 range during the final week of lysimeter operation. Conductivity values in both leachates gradually declined throughout the course of the experiment from initial values of about 20,000 mg of NaCl per liter to final values of about 3,700 mg of NaCl per liter. Turbidity values of the leachate from lysimeter 2 were somewhat higher than those of the leachate from lysimeter 1. Ammonia nitrogen concentrations in the leachates of both lysimeters rose to maximum values within the first 2 weeks of leachate production, remained at this peak level for about 2 weeks and then steadily declined throughout the remaining period of lysimeter operation.

Appreciable differences were observed in the bacteriological characteristics of the two leachates. Initially, the leachates of both lysimeters contained bacteria concentrations in excess of 10⁴ CFU/ml on plates incubated aerobically and anaerobically. In the leachate of lysimeter 1, bacteria concentrations based on plates incubated both aerobically and anaerobically steadily declined to a level of undetectability by the end of week 4 of leachate production, and few or no bacteria were detected in the leachate during the remaining period of lysimeter operation. In the leachate of lysimeter 2 bacteria concentrations on plates incubated aerobically remained in the range of about 5 × 10⁶ to 5 × 10⁴ CFU/ml throughout the entire period of lysimeter operation. Bacteria concentrations on anaerobically incubated plates were initially about the same as those on aerobically incubated plates but became generally lower by more than an order of magnitude by week 4 of lysimeter operation and remained so throughout the rest of the period of lysimeter operation.

The reasons for the rapid decrease and subsequent absence of bacteria in the leachate of lysimeter 1 are not known, and this decrease in bacteria was not accompanied by sudden, major changes in the levels of any physical or chemical characteristics of the leachate that were being measured. One possible explanation for the loss of bacteria is the presence of some toxic substance in the refuse of lysimeter 1. Although both lysimeters were prepared from the same general pool of refuse components, the refuse mixture for each lysimeter was composted separately, so it is possible that a toxic material could have been present in the refuse of one lysimeter and not the other. However, we have no direct evidence for the presence of such a toxic material in lysimeter 1. Another possibility is that lysimeter 1 became anaerobic with time with a concomitant decrease in aerobic organisms and an increase in strictly anaerobic organisms. The latter may not have been efficiently detected by our assay procedures.

Virus detection in lysimeter leachates and refuse extracts. The results for virus analysis of lysimeter leachates and lysimeter refuse extracts are summarized in Table 3. Although more than 80% of the total leachate produced by
TABLE 3. Virus recovery from lysimeter leachates and refuse extracts

| Parameter                      | Lysimeter 1 | Lysimeter 2 |
|-------------------------------|-------------|-------------|
| Total leachate produced (ml)  | 4,930       | 5,030       |
| Total number of leachate samples | 45         | 46          |
| Leachate volume assayed for viruses (ml) | 3,956 | 4,218 |
| Percent of total leachate assayed for viruses | 80 | 84 |
| Total viruses isolated from leachate (PFU) | 0 | 0 |
| Total viruses isolated from lysimeter refuse (PFU) | 0 | 0 |

Each lysimeter was subjected to virus analysis, no viruses were isolated from either leachate during the entire period of lysimeter operation. In addition, no viruses were detected in the refuse fraction extracts obtained from the lysimeters when they were sacrificed. The possibility that viruses may not have been detected in the leachates because of inadequacies in virus detection methodology was tested in a series of virus concentration experiments conducted with composite leachate samples from each lysimeter which were experimentally contaminated with poliovirus type 1 and echovirus type 7. Flow-weighted, composite leachate samples were prepared for these experiments as described in Materials and Methods. In all of these experiments viruses were concentrated 10- to 20-fold from the leachates with recovery efficiencies of greater than 50%. Apparently, the inability to detect viruses in leachates was not due to unrecognized limitations or deficiencies in virus detection methodology.

**Virus survival in lysimeter leachates.** The results of the experiments on field-strain poliovirus survival in composite leachates from lysimeters 1 and 2 are shown in Fig. 1 and 2, respectively. Virus survival in both leachates was highly temperature dependent, with maximum virus inactivation rates at 37 C and minimum rates at 4 C. In both experiments virus inactivation in control glycine solutions at the same pH levels and temperatures as leachates was lower than that in the corresponding leachate samples. In the 27-day test period virus losses in controls incubated at either 4 or 20 C were less than 1 log (90%), whereas at 37 C the rate and degree of virus inactivation was considerably greater. Virus survival at 4 C was considerably better in leachate 2 than in leachate 1, with about 70% virus inactivation in 27 days in the former and about 97% virus inactivation in 27 days in the latter. In both leachates more than 95% of the virus was inactivated in a 2-week period at 20 C, whereas more than 99% was inactivated within 6 days at 37 C. In general, poliovirus was inactivated more rapidly in leachate 1 than in leachate 2. The reasons for these differences in virus inactivation rates be-
between leachates 1 and 2 are not obvious from the available data on the physical and chemical properties of the leachates. However, it is interesting to note that bacterial survival in leachate 1 was also poor, with no detectable bacteria in the leachate after about the first month of lysimeter operation.

Short-term interactions of viruses with municipal solid waste components. The results of experiments on short-term virus interactions with paper and selected nonpaper municipal solid waste components are summarized in Tables 4 and 5, respectively. In the presence of distilled water neither poliovirus type 1 nor echovirus type 7 efficiently adsorbed to the solid waste components tested. This was indicated by the fact that most of the virus in the initial suspension remained in the supernatant after centrifugation (first supernatant). In addition, of the relatively small percentage of total initial virus sedimenting with the solids in distilled water (first resuspended sediment), much of it was recovered in the supernatant of subsequent centrifugations after resuspension in distilled water and pH 11.5 glycine-NaOH.

In general, when poliovirus type 1 and echovirus type 7 were contacted with solid waste components in the presence of salt solution, a high percentage of the total virus became associated with the solid waste components. This is indicated by the fact that in most cases little of the total initial virus was detectable in the first supernatant. Two notable exceptions are the results for lawn and food wastes. The lack of virus in the first supernatant was not due to virus inactivation by the salt solution, because control suspensions containing viruses but lacking solid waste components showed no significant decrease in virus concentration after centrifugation. Of the viruses that became solids associated, generally only a relatively small proportion was eluted when the solids were resuspended in either salt solution or pH 11.5 glycine. It is interesting to note that in many cases, some of the solids-associated viruses could be at least partially detected by direct plating of the resuspended sediment. For example, 17% of the total poliovirus could be detected in the first resuspended newsprint sediment, but only 2.2% could be detected in the second supernatant. Similar results were observed for both viruses with many of the other solid waste components.

The results of these experiments indicate

| Table 4. Short-term virus interactions with paper components of municipal solid waste |
|---------------------------------|---------------------------------|---------------------------------|
| Sample                          | Percentage of zero time control virus detectable |
|                                 | Newsprint                         | Corrugated paper                | Magazine paper                 |
|                                 | Distilled water                  | Salt solution                   | Distilled water                | Salt solution                   |
|                                 | Polio   | Echo | Polio   | Echo | Polio   | Echo | Polio   | Echo | Polio   | Echo | Polio   | Echo | Polio   | Echo |
| Initial suspension              | 94     | 86   | 90     | 110  | 84     | 74   | 84     | 88   | 99     | 80   | 83     | 86   | 95     | 82   |
| Suspension after 60 min         | 69     | 100  | 27     | 85   | 78     | 82   | 14     | 46   | 95     | 82   | 86     | 67   | 90     | 82   |
| First supernatant              | 74     | 80   | 12     | 13   | 99     | 74   | 12     | 44   | 88     | 56   | 1.7    | 9.3  | 88     | 56   |
| First sediment, resuspended    | 12     | 15   | 17     | 14   | 13     | 10   | 7.3    | 11   | 25     | 13   | 20     | 20   | 25     | 13   |
| Second supernatant             | 15     | 9.2  | 2.2    | 2.2  | 16     | 13   | 2.9    | 5.4  | 21     | 5.0  | 1.3    | 2.3  | 21     | 5.0  |
| Second sediment, resuspended   | 12     | 6.0  | 3.5    | 0.7  | 3.4    | 1.9  | 1.1    | 0.2  | 16     | 1.5  | 30     | 27   | 16     | 1.5  |
| Third supernatant              | 17     | 0    | 0      | 0    | 1.8    | 1.0  | 0.05   | 0    | 8.6    | 0    | 0.2    | 0    | 8.6    | 0    |

| Sample                          | Percentage of zero time control virus detectable |
|                                 | Bond paper                       | Envelope paper                  | Brown paper                    |
|                                 | Distilled water                  | Salt solution                   | Distilled water                | Salt solution                   |
|                                 | Polio   | Echo | Polio   | Echo | Polio   | Echo | Polio   | Echo | Polio   | Echo | Polio   | Echo |
| Initial suspension              | 102    | 104  | 98     | 98   | 90     | 114  | 71     | 98   | 90     | 86   | 87     | 82   |
| Suspension after 60 min         | 97     | 88   | 31     | 50   | 66     | 128  | 25     | 58   | 98     | 79   | 8.5    | 6    |
| First supernatant              | 55     | 84   | 10     | 44   | 68     | 92   | 2      | 24   | 94     | 90   | 3.2    | 5.2  |
| First sediment, resuspended    | 31     | 9.9  | 24     | 14   | 17     | 14   | 18     | 15   | 22     | 8.9  | 2.6    | 2.6  |
| Second supernatant             | 3.7    | 7.4  | 2.9    | 9.1  | 9.4    | 5.8  | 1.0    | 1.3  | 20     | 8.8  | 3.0    | 2.2  |
| Second sediment, resuspended   | 27     | 1.7  | 2.4    | 0.9  | 12     | 9.9  | 6.0    | 0    | 6.0    | 2.4  | 0.2    | 0.3  |
| Third supernatant              | 5.5    | 0.6  | 0.1    | 0    | 8.3    | 0.6  | 0.4    | 0.1  | 5.8    | 0.06 | 0      | 0    |
that entroviruses will adsorb to many solid waste components in the presence of high concentrations of dissolved salts but may not adsorb in the absence of dissolved salts.

**DISCUSSION**

Despite the fact that more than 80% of the total leachate volume produced by each lysimeter was assayed for viruses, no viruses were isolated from either of the leachates. The possibility of gross deficiencies in the detection methodology for viruses in leachates was ruled out by demonstrating that added viruses could be recovered with an efficiency of greater than 50% when concentrating 10- to 20-fold. One possible explanation for the absence of viruses in the lysimeter leachates was that the lysimeters may not have been operated for a sufficiently long time period for the added viruses to appear in the leachates being produced. If the lysimeters are considered to be plug flow reactors with hold-up volumes of 7 liters and daily leachate production volumes of 45 ml, then the estimated residence time for viruses added at the top of the refuse column would be about 156 days. The hold-up or void volume of 7 liters is based upon the approximate amount of water required to bring each lysimeter to field capacity. Because lysimeters 1 and 2 were operated for periods of only 117 and 124 days, respectively, after virus addition, there may have been insufficient time for the added viruses to travel the entire length of the refuse column and appear in the leachate. The assumption that the lysimeters were plug flow reactors is reasonable because the refuse size, placement procedure, and final density (800 pounds/yard$^3$) made it highly unlikely that channelling or vertical mixing to cause short-circuiting would occur.

Another possible explanation for the absence of viruses in the lysimeter leachates was that the viruses in the lysimeters either were inactivated or were impeded in their movement through the refuse column due to adsorption to refuse components. Indirect evidence for virus inactivation in the lysimeters is provided by the results of the experiments on virus survival in composite leachate samples. It should be recalled that at a temperature of 20°C more than 95% of the initial virus was inactivated within a 2-week period in both leachates. Considering that the lysimeters were operated for periods in excess of 100 days after virus addition at temperatures ranging from about 25 to 33°C, it is possible that an appreciable degree of virus inactivation occurred in the lysimeters. The specific factors responsible for virus inactivation in leachates were not determined in this study. Although the observed differences in virus survival in control solutions with differences in incubation temperature indicate that some of the virus inactivation in leachates may have been due to thermal effects, this mechanism does not account for all of the virus inactivation observed in the leachates. Toxic chemicals may have been partly responsible for virus inactivation in leachates, though we have no direct experimental evidence for this possibility. As previously noted, the samples were treated with EDTA before virus assay to eliminate the interfering effects of leachate components on virus detectability (7).

Engelbrecht and co-workers (4) studied the inactivation of poliovirus type 1 and a reovirus of unspecified type in centrifuged leachate supernatants from leachates of different ages at 22°C. Appreciable virus losses occurred in the older leachates, but because the leachates became cloudy and the samples were not treated with EDTA before virus assay it could not be determined if the observed virus losses were
due to inactivation or to masking resulting from virus sorption to leachate particulates. Cooper and co-workers (3) observed no appreciable poliovirus inactivation in composite leachates incubated at 20°C for up to 48 h. The leachate samples were treated with EDTA before virus assay.

Indirect evidence for the restriction of virus movement through the lysimeters due to adsorption to refuse components is provided by the results of our experiments on the short-term interactions of viruses with various municipal solid waste components. These experiments clearly demonstrated that viruses would adsorb to many refuse components in the presence of a high concentration of dissolved salts and, in many cases, they were difficult to recover from the refuse solids. The observation that viruses will adsorb to many solid waste components in the presence of high concentrations of inorganic salts is consistent with the results of a number of previous studies by us (8, 9, 11) and by others (2, 6) in which it was shown that enteric viruses will avidly adsorb to a variety of surfaces and solids in the presence of various salts. Because the solids-to-water ratio in an actual landfill will be considerably higher than that used in our experiments on short-term virus interactions with municipal solid waste components, it is possible that the extent of virus adsorption to the solid waste components in a landfill might be considerably greater than that observed in these experiments.

The absence of viruses in the lysimeter contents after termination of their operation provides further evidence for our contention that the viruses were retained in the lysimeters for a sufficiently long period to become inactivated. Differences in the behavior of field and laboratory strains of the test viruses within the lysimeters were not detected because neither was recovered, either from leachate or the refuse.

The observation that no viruses were found in the leachates from municipal solid waste lysimeters is in general agreement with the results of two recent studies in which it was found that little or no viruses could be detected in the leachates from field scale municipal solid waste lysimeters (3, 4). Engelbrecht et al. (4) found no viruses in the leachate from a large, field scale municipal solid waste lysimeter that had been experimentally contaminated with 10^6 PFU of poliovirus type 1, strain LSc. It should be mentioned, however, that only a small percentage of the total leachate produced by the lysimeter was examined for the presence of viruses. Cooper et al. (3) examined 1-gallon (3.785 liters) leachate samples periodically collected from a series of 16 pilot scale, municipal solid waste lysimeters. Some of the lysimeters were experimentally contaminated with as much as 2 × 10^4 PFU of poliovirus type 1, strain LSc, per kg of refuse, whereas other lysimeters which were not experimentally contaminated with poliovirus served as controls. Viruses were occasionally recovered from the leachates of only some of the lysimeters, including some of the control lysimeters which had not received poliovirus. The amounts of virus recovered in the leachates were small compared to the amounts originally added to the experimentally contaminated lysimeters. The virus isolates were not identified. Because the refuse in the lysimeters apparently contained appreciable amounts of naturally occurring viruses from sources such as animal (pet) feces, it was impossible to quantitatively determine the extent of virus retention in the lysimeters. In neither of these studies was the lysimeter refuse examined for its virus content after the refuse had been placed.

Although the results of this study suggest that viruses may be retained and inactivated in municipal solid waste landfills, additional studies are necessary to elucidate the actual mechanisms of virus inactivation in landfilled municipal solid waste and to determine the extent to which these processes occur in actual municipal solid waste landfills.

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