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Induction of Cytopathogenicity in Mammalian Cell Lines Challenged with Culturable Enteric Viruses and Its Enhancement by 5-Iododeoxyuridine

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Cultures of 17 established cell lines were tested against 105 enteric virus types for capacity to support viral replication as indicated by cytopathogenic effect production. Enhancement of susceptibility by treatment of the cells with 5-iododeoxyuridine was evaluated in parallel with untreated cells. Cytopathogenic effect was produced in two or more cell lines by every virus tested except six strains of group A coxsackie virus. No cell line was found to be susceptible to these six virus types. In general, treatment with 5-iododeoxyuridine provided a more rapid onset of cytopathogenic effect in susceptible cells and in some instances resulted in refractory cells becoming permissive to viral replication. The use of 5-iododeoxyuridine allowed two human embryonic lines (HEL-299 and L-132), in combination, to be susceptible to all but the six group A coxsackie virus strains.

The presence of viruses in drinking water can be considered to represent an unacceptable risk to human health. Very few virus isolations have been reported from drinking water in the United States. However, documentation of virus isolations from drinking water in other countries is more common and in some water systems may even be a routine occurrence (15).

Methods to detect a few viable viruses in large volumes of water have only recently been developed, but these methods have already been used by numerous investigators to recover a high percentage of viruses from seeded environmental samples. However, the use of these same procedures to quantitatively detect indigenous viruses is subject to a number of uncertainties. Probably the major uncertainty is the ability of cultured cells to support growth of the viruses in question.

Not all human enteric viruses will grow in cultured cells but the majority of those known can be grown in one or more established cell line. Although a number of studies have been carried out to determine the relative recoverability of indigenous (1, 2, 7, 11, 12, 19, 20) and seeded (3, 5 16–18, 24) viruses with different cell lines, no broad-spectrum studies of this nature have been performed. This has made it difficult to predict which cell lines should be used to recover indigenous viruses from environmental samples, especially those expected to have very few viruses, such as drinking water.

A variety of materials have been used in culture media to enhance viral recoveries. One compound that has been shown to increase the susceptibility of a number of cell types to viral infection and growth is the halogenated pyrimidine 5-iododeoxyuridine (IDU) (4, 6, 9, 10, 13, 14, 21–23). Again, however, no broad-spectrum study has been performed to determine the effect of IDU on the susceptibility of a large number of cell lines to the vast array of culturable enteric viruses.

Because of the time and expense required to isolate indigenous viruses from environmental samples, it would be highly desirable to know which cell lines provide the maximum opportunity for virus detection. It is also of interest to determine the effects of IDU on virus recoveries. This report compares the susceptibility of 17 cell lines to 105 enteric virus types in the presence and absence of IDU.

MATERIALS AND METHODS

Cells. A number of viral enhancement studies have been performed utilizing adenoviruses as the challenge virus. Thus, the ability to support the replication of at least one adenovirus type, as reported in The American Type Culture Collection catalog, was used as a criterion in selection of the cell lines used. An additional criterion was to provide a variety of cell source material. The derivation of the cells used in this study is as follows: AV_3 and WISH, human amnion; BHK-21 (BHK), hamster kidney; Chang liver (CL), human liver; HEL-299 (HEL), human embryonic lung; 6000 and L-132 (132), human embryonic kidney; Intestine 407 (407), human intestine; 4000, human embryonic tonsil; LLC-MK_2 (LLC), rhesus monkey kidney; HeLa, HEp-2 (Hep), and KB, human carcinogenic

TABLE 1. Growth media for cell lines

Cell line	Medium[a]	Addition(s)[b]
AV_3	199	20% FBS
132	199	20% FBS
4000	MEM	10% FBS
6000	MEM	10% FBS
BGM[c]	L-15/MEM (1/1)	10% FBS
407	BME	15% FBS
WISH	BME	15% FBS
Vero	199	10% FBS
Hep	MEM	10% CS
HeLa	MEM	10% CS
KB	MEM	10% CS
CL	MEM	10% CS
LLC	BME	4% horse serum
RD	RPMI 1640	10% CS
BHK	BME	10% CS, 10% tryptose phosphate broth
HEL	MEM	10% FBS, 0.1% lactalbumin hydrolysate, 1 mM sodium pyruvate

[a] 199, Medium 199; MEM, Eagle minimal essential medium; BME, basal medium, Eagle.

[b] For pH control, all media contained 40 mM HEPES (*N*-2-hydroxyethyl piperazine-*N'*-2-ethane sulfonic acid) buffer. The antibiotics used were penicillin (100 U/ml), neomycin sulfate (100 μg/ml), streptomycin (100 μg/ml), and amphotericin B (1 μg/ml).

[c] Includes both MBGM and BBGM.

cells of the cervix, larynx, and oral cavity, respectively; RD, human rhabdomyosarcoma; Vero; BBGM and MBGM, African green monkey kidney. BBGM and MBGM are supposedly the same cell line (BGM) but obtained from different sources. However, their growth characteristics, morphology, length of viability under agar, poliovirus plaquing characteristics, and viral susceptibility were different when received and remained so after a minimum of 30 passages in this laboratory. These differences prompted the inclusion of both lines in this study.

All cells used were continuous lines with the exception of HEL, which has a finite life span of approximately 30 passages. AV_3, BHK, CL, HEL, 407, KB, 132, LLC, and WISH were purchased from the American Type Culture Collection, Rockville, Md. BBGM cells were obtained from D. Dahling, U.S. Environmental Protection Agency, Cincinnati, Ohio, and MBGM cells were from T. J. Metcalf, University of New Hampshire, Durham. HeLa, 4000, and 6000 cells were purchased from Flow Laboratories, Rockville, Md. Hep cells were obtained from the Centers for Disease Control, Atlanta, Ga. RD cells were obtained from R. Crowell, Hahnemann Medical College, Philadelphia, Pa. Vero cells were obtained from A. Fassolitis, U.S. Food and Drug Administration, Cincinnati, Ohio. The growth media for the different cell lines are shown in Table 1.

Viruses. The virus stocks used in this study were obtained from the National Institutes of Health, Bethesda, Md. Group B coxsackie viruses and adenoviruses were propagated in 407 cells. Polioviruses and reoviruses were propagated in HEL cells. Group A coxsackie virus types 2, 3, 4, 5, 9, and 10 were grown in RD cells, whereas HEL cells were used to propagate types 7, 8, 11, 13, 14, 15, 18, 20, 20a, 20b, and 21. Enterovirus types 68, 69, 71, and 275/58 were propagated in 4000 cells. Echoviruses were propagated in 407 cells. Group A coxsackie virus types 1, 6, 12, 17, 19, and 22 could not be grown in any of the cell lines. However, as shown by their abilities to kill suckling mice, all six were viable. The preparation of these viruses used in our study was as 10% suspensions (minimal essential medium) of the entire suckling mouse, diluted 1:100. Adenovirus types 9 and 11 and echovirus types 22 and 23 could not be propagated in any of the cell lines used in this study or in primary monkey kidney or human amnion cells. Because their infectivities had been determined in cell types represented in this study (adenoviruses, human embryonic kidney; echoviruses, rhesus monkey kidney), it was presumed that the seed stocks of these viruses were nonviable. Therefore, they were not further analyzed or included in the list of 105 viruses studied.

To determine the amount of each virus preparation to use in the cell susceptibility study, several dilutions of the viral preparations were made, and infectivities were determined in microtiter (MT) plates containing the cell line of propagation. The highest dilution that produced cytopathogenic effect (CPE), on the earliest day that CEP was observed at any dilution (≤4 days in all cases), was used. In most cases greater dilutions of virus permitted CPE on later days. However, to ensure a sufficient opportunity for all susceptible cell lines to show CPE within the time allotted (4 days), the more concentrated viral solutions were used. It was reasoned, for example, that if CPE were observed at a 4-log dilution of a particular virus on day 3 in the cell line used for propagation, and at a 5-log dilution on day 4 in these same cells, it might require more than 4 days for a 5-log dilution of this virus to show CPE in other susceptible cell lines. Therefore, a 4-log dilution of this virus would have been used in the study.

Preparation of cells in MT plates. Stock cultures of all 17 cell types were trypsinized and diluted to approximately 1.5×10^5 cells per ml of their respective growth medium. One-half of each cell suspension was centrifuged, and the cell pellet was resuspended in its original volume of respective growth medium containing 50 μg of IDU per ml. These cells are referred to as IDU treated.

A separate 96-well MT plate was used for each cell type. Two columns of untreated and IDU-treated cells were added alternately to the wells. Thus, wells in columns 1, 2, 5, 6, 9, and 10 received untreated cells and those in columns 3, 4, 7, 8, 11, and 12 received IDU-treated cells (Fig. 1). Each well received 150 μl of cell suspension or approximately 2×10^4 cells. MT plate covers were then added, and plates were incubated for 72 h at 37°C in a sealed plastic box containing a damp sponge to prevent drying.

Virus inoculation of cells on MT plates. Growth medium was removed from the wells by inverting and shaking the MT plate once. To remove any trace of IDU, all wells were washed twice with Hanks balanced salt solution and drained. Wells of rows A, C, E, and G and columns 1, 3, 5, 7, 9, and 11 (see Fig. 1) received 100 μl of maintenance medium and 50 μl of virus suspension. Remaining wells received 150 μl of maintenance medium. This arrangement allowed each

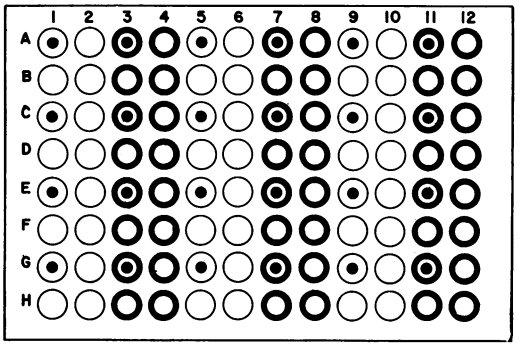

FIG. 1. Layout of MT plate. Symbols: (thin circles) untreated cells; (heavy circles) IDU-treated cells; (dotted circles) virus inoculated cells; (open circles) control cells.

inoculated well to be surrounded by negative control wells. The plates were then sealed with MT sealing tape to prevent cross-contamination, and pinholes were made in each well with a tape perforator (Microbiological Associates, Inc., Walkersville, Md.) to permit gas exchange for pH stability. The plate covers were replaced, and the plates were again incubated in the sealed box at 37°C.

Observation of MT plates for CPE. After inoculation, all plates were read daily for CPE on 4 consecutive days. No readings were recorded after day 4 due to the deterioration of some of the cell monolayers. The first day that CPE was observed was logged as the day the well became positive. However, the condition of the cells was observed and recorded each day throughout the 4-day period. Although no attempt was made to distinguish the severity of CPE, no plate was recorded as positive unless at least 10% of the cells had become rounded.

The entire experiment was carried out at two separate times. To ensure that the results of the first experiment did not bias the readings made in the second experiment, the second experiment was purposely performed in its entirety before any comparison was made with the results from the first experiment. Although this does not constitute a blind study, the methods used coupled with the large number of readings made should have removed any possible bias that may have influenced interpretation of the results.

RESULTS

The combined results of two separate determinations of the susceptibility of 17 different cell lines to 105 enteric virus types are presented in Table 2. Although the ability of any particular virus to infect any specific cell line was identical in both experiments, the day of appearance of CPE was not the same in a few instances. In those cases, the latest day of CPE appearance is presented. It should be noted that no sign of CPE was found in any control well during the 4-day observation period in either experiment.

It is evident from the results presented in Table 2 that certain cell lines are susceptible to more viruses than are others. It is also evident that IDU treatment increases this susceptibility. However, these results are more easily visualized in the composite data shown in Table 3, in which it can be seen that HEL cells are susceptible to 92 of the 105 viruses tested, the best of any cell line. The least susceptible cell line found was BHK, which showed CPE with only 32 of the viruses tested.

IDU treatment produced more rapid CPE in 256 instances (22%), slower CPE in 49 instances (4%), and no difference in time of CPE appearance in 695 instances (56%) (Table 3). In 187 instances (15%) CPE was found in IDU-treated cells when the untreated cells were negative, and, conversely, in 33 instances (3%) untreated cells produced CPE when the treated cells were negative.

DISCUSSION

From the results presented in this report it is evident that the susceptibilities of different cell lines to the different types of enteric viruses can vary greatly. In some instances the reason for this is clear. For example, BHK cells are not of primate origin, in contrast to the other cell lines tested, and therefore lack the proper receptors for poliovirus attachment (8). From the data it appears that they may also lack receptors for group B coxsackie viruses and echoviruses as well. In most instances, however, the susceptibility or lack of susceptibility of a particular cell line for a particular virus cannot be easily explained.

It is also evident from the results presented here that IDU enhances the overall susceptibility of most cell lines, but in some cases it has the opposite effect. It is possible that the concentration of IDU (50 μg/ml) and the time of exposure of cells before infection (72 h) were poor selections in certain instances. IDU concentrations reported in the literature vary from 10 (4) to 160 (25) μg/ml, and the treatment time varies from 1 (6) to 96 (22) h or until confluency is achieved (9). The concentration and time chosen here represent a compromise.

In addition to generally increasing the susceptibility of cells for viruses, treatment with IDU had other advantages. Changes due to CPE were more easily recognized because the IDU-treated cells were larger and had more sharply delineated edges. Furthermore, cell destruction was more complete in IDU-treated cells when compared with untreated cells during the same time periods. Finally, in the cases of Hep, HeLa, 407, and AV_3, the integrity of the IDU-treated cell monolayer could be maintained for 2 to 3 days longer than that of the untreated cells. In instances of slowly replicating viruses, such as some of the adenoviruses, this prolonged mono-

TABLE 2. Day of CPE appearance by cell and virus type[a]

Virus/strain	AV_3	BBGM	MBGM	BHK	CL	HEL	HeLa	407	Hep	KB	132	LLC	RD	Vero	WISH	4000	6000
Coxsackievirus, group A																	
1	Replicated in suckling mouse only																
2	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	2/3	–/–	2/2	–/–	–/–	–/–	–/–
3	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	2/3	–/–	2/2	–/–	–/–	–/–	–/–
4	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	4/3	–/–	3/3	–/–	–/–	–/–	–/–
5	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	2/3	–/–	3/2	–/–	–/–	–/–	–/–
6	Replicated in suckling mouse only																
7	–/3	–/–	–/–	–/–	–/–	3/2	–/–	3/–	–/4	–/–	–/–	–/–	2/4	–/–	–/–	2/4	2/2
8	–/2	–/–	–/–	–/–	–/–	3/3	2/2	–/2	–/2	2/2	4/3	–/–	–/–	–/–	–/2	–/2	2/2
9	–/–	–/–	–/1	–/–	2/1	3/–	–/–	–/–	–/–	–/3	–/–	–/–	2/2	–/–	4/–	2/2	2/2
10	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/4	–/–	3/3	–/–	2/2	–/–	–/–	–/–	–/–
11	–/–	–/–	–/–	–/–	4/3	3/3	4/3	2/2	3/3	–/–	–/2	–/–	–/4	–/–	–/3	–/–	–/–
12	Replicated in suckling mouse only																
13	2/2	–/–	–/–	–/4	2/2	1/1	2/2	2/2	3/2	2/2	2/2	–/–	4/2	3/–	2/2	2/3	2/–
14	–/–	2/4	–/–	–/2	2/2	1/1	2/2	2/2	–/4	–/–	–/1	4/–	–/–	2/2	–/–	–/–	–/–
15	3/3	3/4	–/–	4/3	4/4	1/1	3/2	2/3	–/–	3/4	1/1	–/–	4/3	–/–	–/–	–/–	–/–
16	1/1	–/–	1/–	2/2	1/1	1/1	1/1	1/1	1/1	–/–	1/1	1/1	1/1	1/1	1/1	1/1	1/1
17	Replicated in suckling mouse only																
18	2/2	–/–	–/–	–/–	2/2	1/1	2/2	2/2	2/2	2/2	1/1	–/–	–/–	–/–	3/2	–/–	–/–
19	Replicated in suckling mouse only																
20	2/2	–/–	–/–	–/–	2/2	1/1	2/2	2/2	2/3	2/2	1/1	–/–	4/4	–/–	2/2	–/–	–/–
20a	2/2	–/–	–/–	–/–	2/2	–/–	–/1	2/2	2/2	2/1	2/2	–/–	–/–	–/–	2/1	2/1	–/1
20b	3/3	4/–	–/–	3/3	–/3	1/1	2/2	2/2	3/2	4/4	1/1	–/–	4/3	–/–	3/2	–/1	–/–
21	–/2	–/–	–/–	–/–	–/–	3/1	2/2	3/2	–/–	–/–	3/3	–/–	–/3	–/–	4/4	–/2	–/2
22	Replicated in suckling mouse only																
24	1/1	3/–	–/–	2/2	1/1	1/1	1/1	1/1	1/1	1/1	1/1	–/–	1/1	4/–	1/1	1/1	1/1
Coxsackievirus, group B																	
1	–/1	1/1	2/1	–/–	–/1	4/–	–/1	1/1	2/2	2/2	2/1	–/–	–/1	–/2	2/2	–/–	–/–
2	2/–	1/1	1/1	–/–	2/1	4/1	–/3	–/3	3/2	2/1	1/1	–/–	–/1	1/1	–/–	–/–	–/2
3	1/–	1/1	2/1	–/–	2/2	4/3	–/2	2/2	2/1	2/1	2/1	–/–	–/1	–/2	2/1	–/–	–/–
4	–/–	–/–	–/–	–/–	–/–	4/–	–/2	2/2	–/2	–/–	2/2	–/–	–/–	2/2	4/3	–/–	–/–
5	1/2	2/1	2/1	–/–	–/2	–/2	–/2	2/2	3/1	–/1	1/1	–/–	–/2	2/1	2/2	–/–	–/–
6	–/2	1/1	1/1	–/–	2/1	4/–	–/2	1/2	–/2	2/2	4/2	–/–	–/1	–/1	4/2	–/–	–/–
Echovirus																	
1	2/3	1/1	1/1	–/–	–/–	2/1	–/–	–/–	2/3	–/–	2/1	–/–	2/2	2/1	3/2	1/1	1/1
2	1/1	1/1	1/1	–/–	–/–	1/1	–/–	–/–	4/2	–/–	2/1	–/3	1/1	1/1	–/2	1/1	1/1
3	2/1	1/1	1/1	–/–	–/–	2/1	–/–	–/2	2/2	–/–	–/1	–/–	1/1	2/1	4/2	1/1	2/1
4	4/1	1/1	1/1	–/–	–/–	1/1	–/–	–/–	–/2	–/–	2/1	–/3	1/1	1/1	–/2	1/1	1/1
5	1/1	1/1	1/1	–/–	–/–	1/1	–/–	–/–	–/2	–/–	2/2	–/4	1/1	2/1	–/2	1/1	1/2
6	2/2	1/1	1/1	–/–	–/–	1/1	–/–	–/2	3/1	2/1	1/1	–/–	1/1	2/1	–/1	1/1	1/1
6′	–/3	3/2	2/2	–/–	–/–	2/1	–/–	–/–	–/–	–/–	–/1	–/–	1/1	–/–	–/3	2/1	2/1
6″	–/2	2/1	1/1	–/–	–/–	1/1	–/–	–/2	–/2	–/2	2/1	–/–	1/1	2/2	3/2	1/1	2/1
7	2/1	1/1	1/1	–/–	–/–	1/1	–/–	2/1	2/1	–/1	2/1	–/3	1/1	1/1	2/1	1/1	1/1
8	3/3	1/1	1/1	–/–	–/–	1/1	–/–	–/–	–/2	–/–	2/1	–/–	2/2	2/1	2/1	1/1	1/1
9	3/3	1/1	1/1	–/–	–/–	1/1	–/–	–/3	–/2	–/–	–/1	–/–	–/2	–/–	–/2	2/1	1/1
11	3/1	1/1	1/1	–/–	–/–	1/1	–/–	2/2	1/1	–/3	2/1	–/–	1/1	2/1	–/1	1/1	1/1
12	3/4	1/1	1/1	–/–	–/–	1/1	–/–	–/2	–/2	–/–	4/1	4/2	1/1	2/1	–/2	1/1	1/1
13	4/3	2/2	2/2	–/–	4/–	2/4	–/–	–/–	–/2	–/–	–/–	–/–	1/1	2/–	–/4	2/2	2/2
14	3/3	1/1	1/1	–/–	–/–	1/1	4/–	–/–	–/3	–/–	–/1	–/–	1/1	1/1	–/–	1/1	1/1
15	3/3	1/1	1/1	–/–	–/–	1/1	–/–	–/–	–/1	–/–	1/1	–/–	1/1	1/1	–/1	1/1	1/1
16	–/–	1/1	1/1	–/–	3/3	1/1	–/–	–/–	–/–	–/–	–/–	–/–	1/1	–/–	–/–	1/1	1/1
17	–/3	1/1	1/1	–/–	–/–	1/1	–/–	–/–	–/2	–/–	3/2	–/4	1/1	2/1	–/3	1/1	1/1
18	–/–	1/1	1/1	–/–	–/–	1/1	–/–	2/2	–/–	–/–	2/2	–/–	1/1	–/–	–/–	1/1	1/1
19	3/3	3/1	–/2	–/–	–/–	2/1	–/–	3/3	–/2	–/3	–/1	–/–	1/1	2/2	–/3	2/2	2/1
20	–/3	2/2	2/2	–/–	–/–	2/2	–/–	4/–	–/2	–/–	2/2	–/–	1/1	–/–	–/3	2/1	1/1
21	–/2	–/3	1/–	–/–	–/–	1/1	–/–	–/2	–/2	–/–	–/1	–/–	1/1	–/–	–/4	1/1	1/1
24	3/1	1/1	1/1	–/–	–/–	1/1	–/–	–/1	1/1	3/1	1/1	–/–	1/1	1/1	1/1	1/1	1/1
25	3/2	2/1	1/1	–/–	–/–	1/1	–/–	–/–	–/1	3/3	1/1	–/–	1/1	2/1	3/2	1/1	1/1

TABLE 2—*Continued*

Virus/strain	AV_3	BBGM	MBGM	BHK	CL	HEL	HeLa	407	Hep	KB	132	LLC	RD	Vero	WISH	4000	6000
Echovirus (*Continued*)																	
26	4/3	1/1	1/1	–/–	–/–	1/1	–/–	–/–	–/3	–/–	–/–	–/–	1/1	2/1	–/2	1/1	2/1
27	3/3	1/1	1/1	–/–	3/3	1/1	3/3	–/–	2/1	3/–	1/1	4/4	1/1	1/1	–/1	1/1	1/1
28	3/3	–/2	–/–	–/–	–/–	1/1	–/3	–/–	–/–	2/–	–/–	–/–	–/3	–/–	–/–	1/1	2/2
29	3/1	1/1	1/1	–/–	–/–	1/1	3/3	–/1	–/1	3/1	1/1	–/–	1/1	1/1	–/1	1/1	1/1
30	3/3	1/1	1/1	–/–	–/–	1/1	–/3	–/2	2/1	–/2	1/1	–/–	1/1	1/1	–/1	1/1	1/1
31	3/3	1/1	1/2	–/–	–/–	1/1	–/–	–/–	–/–	2/–	–/2	–/–	1/1	–/–	–/–	1/1	1/1
32	3/1	1/1	1/1	–/–	–/–	1/1	–/–	–/–	–/1	–/–	1/1	–/–	1/1	1/1	4/1	1/1	1/1
33	3/3	–/–	–/–	–/–	–/–	1/1	–/–	–/2	–/1	–/–	2/1	–/–	1/1	–/–	–/1	1/1	1/1
Adenovirus																	
1	1/1	2/2	1/1	2/2	1/2	2/1	1/1	1/1	1/1	1/1	–/–	1/1	3/2	2/1	1/1	4/2	2/2
2	2/2	2/2	2/3	–/–	3/3	4/4	3/2	4/2	4/4	2/2	3/3	2/2	–/–	2/2	3/3	2/1	2/3
3	2/2	2/2	3/2	–/–	4/3	4/3	3/2	2/2	2/2	2/2	3/2	–/–	–/–	–/3	2/2	3/3	–/–
4	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	–/2	1/1	1/1	2/1	1/1
5	2/2	2/2	2/2	4/4	3/2	3/2	3/2	2/2	3/3	2/2	2/2	2/2	–/3	2/2	3/2	–/2	–/–
6	3/3	2/2	2/2	3/4	2/2	3/2	2/2	2/2	2/2	1/2	2/2	2/2	1/1	2/2	2/2	–/2	–/–
7	2/2	–/–	–/–	–/–	4/4	4/4	3/3	2/2	2/2	2/2	4/4	–/–	2/1	–/–	2/2	–/–	–/–
7a	2/2	–/3	3/4	–/–	3/3	3/3	3/2	2/2	4/3	2/2	3/3	–/–	2/1	–/2	2/2	–/2	–/–
8	2/2	2/2	2/3	2/2	2/2	1/1	4/3	4/3	2/2	2/2	2/2	2/2	2/2	3/3	3/3	2/2	2/2
10	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	–/–	4/2	–/2	3/2	–/1	2/1
12	2/2	3/3	2/2	1/2	2/2	1/1	2/2	–/–	2/2	1/2	1/2	1/1	–/2	2/1	2/2	2/2	2/2
13	–/–	1/2	2/2	2/2	2/1	1/1	1/1	1/1	2/1	1/1	–/–	2/2	4/1	–/3	1/1	2/2	2/1
14	2/2	–/–	–/–	–/–	2/2	1/3	3/2	2/2	2/2	1/2	2/2	–/–	–/–	–/–	2/2	–/–	–/2
15	1/1	2/1	2/2	–/4	2/1	2/1	2/1	2/1	1/1	1/1	2/1	2/2	3/1	2/2	1/1	1/1	–/1
16	1/1	2/1	2/1	–/3	3/2	2/1	2/1	1/1	1/1	1/1	1/1	1/2	3/1	–/–	1/1	2/2	–/1
17	2/2	3/2	3/4	–/4	–/3	2/2	3/2	2/2	4/3	2/2	–/2	–/–	–/4	–/–	3/2	–/4	–/2
18	3/–	–/–	–/–	–/–	4/–	4/2	3/3	–/–	–/–	–/–	2/3	–/–	–/–	4/1	–/–	–/4	–/–
19	1/1	1/1	1/1	2/3	2/2	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
20	1/1	1/1	1/1	3/–	3/1	2/2	1/1	2/1	3/1	2/1	1/1	2/3	3/1	1/1	1/1	2/1	2/1
21	3/1	2/2	1/1	–/–	2/1	3/2	3/1	1/1	1/2	1/1	2/1	2/2	2/1	–/1	1/1	2/1	–/2
22	1/1	2/2	1/1	–/3	2/2	2/1	1/1	1/1	1/1	1/1	1/1	2/3	–/2	1/1	1/1	1/1	2/1
23	1/1	1/1	1/1	–/–	1/1	1/1	1/1	1/1	2/1	1/1	1/1	2/1	–/1	–/2	1/1	1/1	1/1
24	2/2	3/3	2/3	3/3	3/2	3/2	2/2	2/2	2/2	2/2	3/2	–/4	–/2	2/1	3/2	1/1	1/1
25	1/1	1/2	1/1	2/2	1/1	4/1	1/1	1/1	1/1	1/1	2/2	2/2	4/1	1/2	1/1	2/1	2/1
26	1/1	1/1	1/1	3/3	2/2	1/1	1/1	1/1	1/1	1/1	1/2	1/1	–/1	2/2	1/1	1/1	–/1
27	–/–	2/1	1/1	2/2	2/1	1/1	1/1	1/1	2/1	1/1	–/–	1/2	4/1	2/2	1/1	2/1	2/1
28	–/–	1/1	1/1	–/–	2/1	2/2	1/1	1/1	1/1	1/1	1/1	3/3	1/1	–/–	1/1	–/1	2/2
29	2/2	3/3	2/2	3/3	3/2	3/2	2/2	2/2	2/2	2/2	3/2	–/4	–/2	2/1	3/2	1/1	2/2
30	–/–	2/2	–/–	3/2	4/2	2/1	2/2	2/1	2/2	2/2	–/–	–/–	–/2	–/–	1/1	–/2	–/2
31	–/–	2/2	3/3	–/–	2/2	–/2	2/2	2/2	2/2	2/2	–/–	2/–	–/2	3/2	2/2	–/2	–/–
32	–/–	3/3	4/4	2/2	4/2	2/1	2/2	2/1	2/2	1/1	–/–	1/2	2/2	3/3	2/2	2/1	2/2
33	–/–	1/2	1/1	4/2	2/1	2/1	1/1	1/1	2/1	1/1	–/–	1/2	2/1	3/2	1/1	1/1	–/1
Poliovirus																	
1	2/2	2/2	2/1	–/–	1/1	1/1	2/2	1/2	1/1	1/1	2/1	3/2	2/2	4/2	1/1	2/2	2/2
2	2/3	1/2	1/1	–/–	–/–	–/–	2/2	2/2	2/2	2/2	3/2	2/–	1/1	3/2	4/4	–/4	4/2
3	2/1	1/1	1/1	–/–	2/2	–/3	2/2	2/2	2/1	2/1	3/1	1/2	1/1	4/2	4/3	4/2	2/2
Reovirus																	
1	1/1	1/1	1/1	–/–	–/–	1/1	–/–	–/2	–/1	2/2	2/1	4/–	1/1	1/1	–/2	1/1	1/1
2	2/2	2/2	2/2	2/1	1/1	1/1	2/2	1/2	1/1	1/1	2/1	3/2	2/2	4/2	1/1	2/2	2/2
3	–/–	–/–	1/1	–/–	–/–	1/1	–/–	–/2	–/1	–/2	2/1	–/2	1/1	–/–	–/2	–/–	1/1
Enterovirus																	
68	2/2	–/–	–/–	2/–	1/1	1/1	2/1	2/1	–/–	–/–	–/3	2/2	1/3	1/3	–/–	1/1	1/1
69	–/2	–/3	–/–	–/–	–/–	1/2	–/–	–/3	2/2	–/2	–/1	–/2	1/1	–/–	3/2	1/1	1/1
71	–/–	3/–	4/–	4/–	2/3	2/1	3/3	3/3	–/–	4/3	2/3	4/4	4/4	4/3	4/4	–/3	4/3
275/58	3/1	–/3	–/1	2/–	2/2	1/1	1/1	–/3	2/1	2/1	2/1	2/–	1/1	–/3	–/2	1/1	1/1

[a] Numerators of each fraction represent the day of CPE appearance in untreated cells and the denominators represent that of IDU-treated cells. A dash (–) indicates no CPE found during the 4-day observation period.

TABLE 3. Relative CPE appearance in IDU-treated and untreated cells by cell type

Virus	CPE appearance[a]	No. of virus types yielding CPE per virus group																
		AV_3	BBGM	MBGM	BHK	CL	HEL	HeLa	407	Hep	KB	132	LLC	RD	Vero	WISH	4000	6000
Coxsackievirus, group A (25 types)	A	8	0	0	3	8	10	9	9	5	6	10	0	8	2	5	3	5
	B	0	0	0	1	2	2	2	1	2	1	4	0	4	0	4	2	0
	C	3	0	1	2	1	0	1	1	4	1	2	0	2	0	1	3	2
	D	0	2	0	0	0	0	0	1	1	1	1	1	1	0	0	1	0
	E	0	2	1	0	0	1	0	1	0	0	0	0	0	2	1	0	1
Coxsackievirus group B (6 types)	A	0	4	2	0	1	0	0	4	1	2	3	0	0	2	2	0	0
	B	1	1	3	0	2	2	0	0	3	2	3	0	0	1	3	0	0
	C	2	0	0	0	2	1	6	1	2	1	0	0	5	3	0	0	1
	D	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	E	2	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0
Echoviruses (34 types)	A	13	25	27	0	2	27	2	3	3	1	11	1	30	12	1	29	26
	B	10	4	0	0	0	4	0	1	5	3	10	1	0	10	7	3	6
	C	5	2	1	0	0	0	2	10	18	5	7	5	2	0	19	0	0
	D	2	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0
	E	0	0	1	0	1	0	1	1	0	3	0	0	0	1	0	0	0
Adenoviruses (34 types)	A	23	21	20	11	15	14	22	24	23	27	16	14	4	12	26	14	10
	B	1	5	2	3	15	15	10	6	7	1	5	3	13	6	5	7	6
	C	0	2	0	4	1	1	0	0	0	0	1	2	10	7	0	9	8
	D	0	1	6	3	0	2	0	0	1	3	3	4	0	1	0	0	1
	E	1	0	0	1	1	0	0	0	0	0	0	1	0	1	0	0	0

Polioviruses (3 types)	A	1	2	2	0	2	1	3	2	2	2	0	0	3	0	2	1	2
	B	1	0	1	0	0	1	0	0	1	1	3	2	0	3	1	1	1
	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
	D	1	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0
	E	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Reoviruses (3 types)	A	2	2	3	0	1	3	1	0	1	2	0	0	3	1	1	2	3
	B	0	0	0	1	0	0	0	1	2	1	3	1	0	1	0	0	0
	C	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0
	D	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0
	E	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Enteroviruses (4 types)	A	1	0	0	0	2	2	2	1	1	0	0	2	3	0	1	3	3
	B	1	0	0	0	0	1	1	1	1	2	2	0	0	2	1	0	0
	C	1	2	1	0	0	0	0	2	0	1	1	1	0	1	1	1	1
	D	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
	E	0	1	1	3	0	0	0	0	0	0	1	1	1	0	0	0	0
All viruses (105 types)	A	48	54	54	14	31	60	39	43	36	40	40	17	51	29	38	52	49
	B	14	10	6	5	19	23	13	10	21	11	30	7	17	23	21	13	13
	C	11	6	3	6	4	3	9	14	24	8	11	9	19	11	23	14	12
	D	3	4	7	3	1	5	0	5	3	4	4	6	1	1	0	1	1
	E	3	3	3	4	2	1	1	2	0	3	1	3	1	4	1	0	1

[a] A, CPE appearance on the same day in treated and untreated cells; B, CPE appearance earlier in treated cells; C, CPE appearance in treated cells only; D, CPE appearance earlier in untreated cells; E, CPE in untreated cells only.

layer integrity could have a definite advantage.

The only enteric viruses treated in these experiments that were found to be viable but produced no CPE in any of the cell lines tested were six of the group A coxsackie viruses. Three of these (A-6, A-12, and A-19) were reported to produce CPE and plaques in RD cells by other investigators (R. L. Crowell and B. Goldberg, Abstr. Annu. Meet. Am. Soc. Microbiol. 1974, V44, p. 208). Because RD cells were one of the lines used here, it is unclear why CPE was not obtained. Perhaps the strains used by the other investigators were adapted to grow in cultured cells through repeated passage or a critical assay condition was used that was not used in this study.

Adaptation of certain viruses to replicate in cultured cells may also explain another feature of the observations made here. It has been reported by Schmidt et al. (19, 20) that BGM cells are relatively insensitive to naturally occurring echoviruses. In the present study it was found that BGM cells are susceptible to all but one laboratory strain of echovirus, i.e., echovirus 33. One explanation of this apparent difference is that the laboratory viruses used in the present study had been adapted to replicate in cultured cells. Perhaps a more likely explanation, however, is that several infectious echovirus particles are required to produce CPE during the observation period used in both of these studies. It is readily apparent how these conditions could have been met in the experiments presented here but not in those reported by Schmidt et al.

The results reported here clearly showed that several cell lines were susceptible to a very high percentage of the enteric viruses tested. A combination of two of these (HEL and 132) was susceptible to all but the six group A coxsackie viruses. It is suggested, therefore, that if indigenous viruses behave like their laboratory-grown counterparts, the use of these cells, in combination with IDU, should yield optimum recoveries of enteric viruses from environmental samples.

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