Ultraviolet Devitalization of Eight Selected Enteric Viruses in Estuarine Water

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The effect of ultraviolet (UV) radiation on the devitalization of eight selected enteric viruses suspended in estuarine water was determined. The surviving fractions of each virus were calculated and then plotted against the UV exposure time for purposes of comparison. Analytical assessment of the survival data for each virus consisted of least squares regression analysis for determination of intercepts and slope functions. All data were examined for statistical significance. When the slope function of each virus was compared against the slope function of poliovirus type 1, the analytical findings indicated that poliovirus types 2 and 3, echovirus types 1 and 11, and coxsackievirus A-9 exhibited similar devitalization characteristics in that no statistically significant difference was found ($P > 0.05$). Conversely, the devitalization characteristics of coxsackievirus B-1 and reovirus type 1 were dissimilar from those of poliovirus type 1 in that a statistically significant difference was found between the slope functions ($P < 0.05$). This observed difference in devitalization of coxsackievirus B-1 and reovirus type 1 was attributed primarily to the frequency distribution of single and aggregate virions, the geometric configuration, the size of the aggregates, and the severity of aggregation. The devitalization curve of coxsackievirus B-1 was characteristic of a retardant die-away curve. The devitalization curve of reovirus type 1 was characteristic of a multihit-type curve. The calculated devitalization half-life values for poliovirus types 1, 2, and 3; echovirus types 1 and 11; coxsackievirus types A-9 and B-1; and reovirus type 1 were 2.8, 3.1, 2.7, 2.8, 3.2, 3.1, 4.0, 4.0 sec, respectively. These basic data should facilitate an operative extrapolation of the findings to the applied situation. It was concluded that UV can be highly effective and provide a reliable safety factor in treating estuarine water.

The fact that viruses continuously contaminate, via domestic pollution, the nation's waterways, including the saline waters of estuaries, is known not only on empirical grounds but also by experimental observation (1, 7, 9, 10, 20, 21). Of particular public health importance in this regard is the fact that artificial purification of shellfish will purportedly require large quantities of raw estuarine water. Consequently, to maximize public health safety, the raw seawater must be adequately treated so as to destroy pathogens effectively. Ultraviolet (UV) radiation has been shown to destroy coliform organisms effectively and presently is the treatment of choice for shellfish depuration systems (12, 19). Studies conducted in our laboratory to determine the inactivation rate of poliovirus type 1 (vaccine strain) in continuously flowing seawater by the Kelly-Purdy UV Seawater Treatment Unit indicated that effective virological disinfection was achieved (17). Unfortunately, because of the obvious hazards to the workers, experiments with other members of the enteric virus group could not be studied under the applied circumstances. Therefore, a basic inquiry with regard to the effect of UV on the devitalization of selected enteric viruses suspended in estuarine water was undertaken. We were aware that the kinetics of viral devitalization by chemical and physical agents, including UV, had been studied by a number of workers (2, 5, 6, 8, 13, 23). Consequently, many factors that influence the shape and slope of devitalization curves have been identified. Nevertheless, differences in experimental design, analytical treatment of the data, and test viruses used by other workers precluded the extrapolation of their data to the saline hydrosphere. The specific purpose of this longitu-

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dinal inquiry was to compare the devitalization characteristics of poliovirus type 1 (reference virus) with the devitalization characteristics of six enteroviruses and one reovirus suspended in estu-
arine water under identical conditions of UV exposure, the interpretation of the findings being based primarily on statistical inference. Subse-
quent analytical comparisons of the survival data of these seven enteric viruses with the survival data of the reference virus should then allow an academic extrapolation of the findings to the applied situation.

The experiments to be described were con-
ducted in a special UV exposure chamber de-
signed to simulate closely the geometry of the Kelly-Purdy UV Seawater Treatment Unit. In
this paper, we report the results of this longitudi-
nal study.

MATERIALS AND METHODS

Viruses. Eight viruses were used in the study. They were (i) poliovirus type 1 (LSc2ab), kindly supplied by James H. Nakano of the Communicable Disease Center, Atlanta, Ga.; (ii to vii) poliovirus type 2 (Lansing), poliovirus type 3 (Leon), echovirus type 1 (Farouk), echovirus type 11 (Gregory), coxsackie-
virus type A-9 (PB Bozek), coxsackievirus type B-1 (Conn.-5), obtained from the Reagents Reference Branch of the National Institutes of Health; and (viii) reovirus type 7 (176), kindly supplied by Joseph L. Melnick, Baylor University, College of Medicine, Houston, Tex. All of the viruses were propagated in HEP-2 cells with the exception of reovirus, which was propagated in mouse fibroblasts (L-929). All virus preparations were centrifuged to remove cell debris and stored at −70 C. Before storage, reovirus was pretreated for 1 hr at 35 C with 20 μg of alpha-
chymotrypsin (bovine pancreas) per ml to enhance infectivity (24). The alpha-chymotrypsin was ob-
tained from Mann Research Laboratories (New York, N.Y.).

Cell culture. HEP-2 cells or L-929 cells (for reo-
virus) were used for plaque assays. HEP-2 cells were grown in 32-oz (about 900 ml) screw-cap prescrip-
tion bottles as a monolayer in a growth medium consisting of medium 199 and 10% calf serum with penicillin (100 units/ml), streptomycin (100 μg/ml), neomycin (200 μg/ml), and amphotericin B (1.0 μg/ml). HEP-2 cell-overlay was a maintenance me-
dium consisting of Eagle basal medium, 5% chicken serum, 0.0017% neutral red, 1.1% purified agar (Difco), and the above-named antibiotics. L-929 cells were grown in suspension culture in a medium consisting of medium 199, 8% calf serum, 1% pep-
tone glucose dialyze (26), and 0.01% methyl cellulose (4,000 centipoise). Monolayers of L-929 cells were prepared in a medium consisting of medium 199, 10% agamma calf serum, and 0.5% tryptose phos-
phate broth (Difco). L-929 cell-overlay was a main-
tenance medium consisting of Eagle minimal essenti-
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UV exposure), \( a \) is the intercept and represents devitalization of the virus at the beginning of UV radiation, and \( b \) is the devitalization rate. The selection of the empirical least squares plot of the form 
\[ Y = a + bX \]
was based entirely on statistical inference, since, in all cases, statistical judgment on the intercept did not support the expectation that the line was constrained to pass through the origin. Classical single-event-type devitalization curves always pass through the origin (intercept = 0) and may be expressed as: 
\[ \frac{V_t}{V_0} = e^{-t} \]. Our data did not statistically fit the single-event-type curve. For illustrative purposes, the devitalization curve of poliovirus type 1 was constructed, and the observed surviving fractions of those viruses found not to differ significantly (statistically) in devitalization rate were plotted in Fig. 1 and 2. Devitalization rates of viruses found to differ significantly (statistically) from poliovirus type 1 were plotted on separate curves (Fig. 3, 4).

RESULTS

The calculated least-squares devitalization curve of poliovirus type 1 with the 95% confidence interval is shown in Fig. 1. The observed surviving fractions of poliovirus types 1, 2, and 3 are shown as plotted points superimposed upon the devitalization curve of poliovirus type 1. Each point represents the mean virus plaque count of five replicate titrations. Within the limits of experimental error, the observed points closely fitted the calculated least-squares devitalization curve. Statistical comparison of the slope functions by paired combinations between poliovirus type 1 and poliovirus type 2 and between poliovirus type 1 and poliovirus type 3 indicated no significant difference in devitalization rates \( (P > 0.05) \). Note also that, for the most part, all of the observed survival points of poliovirus types 2 and 3 fell within the 95% confidence interval of poliovirus type 1. This further indicated that the three viruses have similar devitalization characteristics.

The calculated least-squares devitalization curve of poliovirus type 1 with the 95% confidence interval is also shown in Fig. 2. The observed surviving fractions of echovirus types 1 and 11 and coxsackievirus A-9 are shown as plotted points. 

FIG. 1. Calculated least-squares devitalization curve with the 95% confidence interval of poliovirus type 1 in unstirred estuarine water exposed to UV radiation (116 ergs per mm² per sec). The surviving fractions of poliovirus types 1, 2, and 3 are shown as plotted points.

FIG. 2. Calculated least-squares devitalization curve with the 95% confidence interval of poliovirus type 1 in unstirred estuarine water exposed to UV radiation (116 ergs per mm² per sec). The surviving fractions of echovirus types 1 and 11 and coxsackievirus A-9 are shown as plotted points.
Table 1. Calculated slope functions, intercepts, and devitalization half-life values of selected enteroviruses exposed to UV radiation

| Virus          | Slope -b | Standard deviation of b | Intercept -a | Standard deviation of a | Half-life* (sec) |
|----------------|----------|-------------------------|--------------|-------------------------|------------------|
| Poliovirus 1   | 0.24     | 0.010                   | 0.62         | 0.009                   | 2.85             |
| Poliovirus 2   | 0.22     | 0.010                   | 0.69         | 0.010                   | 3.12             |
| Poliovirus 3   | 0.26     | 0.008                   | 0.43         | 0.008                   | 2.67             |
| Echovirus 1    | 0.25     | 0.024                   | 0.46         | 0.022                   | 2.81             |
| Echovirus 11   | 0.22     | 0.004                   | 0.71         | 0.004                   | 3.15             |
| Coxsackievirus | 0.22     | 0.014                   | 0.42         | 0.012                   | 3.09             |

* $b = \frac{\Sigma XY - (\Sigma X \Sigma Y)/n}{\Sigma X^2 - (\Sigma X)^2/n}$ of line $Y = a + bx$, where line is free to have any intercept $a$.

$\text{a)}$ $a = 2\Sigma Y - bx/n$.

* $\text{Half-life} = 0.69315/b$.

Poliovirus type 1 was considered good, as evidenced by the propinquity of the points to the straight-line curve. Statistical comparison of the slope functions by paired combinations between poliovirus type 1 and each of these three viruses by regression analysis also indicated no significant difference in devitalization rates ($P > 0.05$) between poliovirus type 1 and the respective viruses. Note that the observed survival points, with few exceptions, fell within the 95% confidence interval of poliovirus type 1. This observation paralleled the observation with poliovirus types 2 and 3. Thus, the devitalization characteristics of poliovirus types 1, 2, and 3, echovirus types 1 and 11, and coxsackievirus A-9 in un-stirred estuarine water exposed to UV were remarkably similar. Table 1 shows the calculated slope functions (devitalization rates), intercepts, and half-life values of the six viruses that exhibited similar, if not identical, survival characteristics under the conditions of the investigation. A comparison of the reaction constants substantiated the similar devitalization observed among these six viruses. In addition, considerable precision existed among the experiments, as indicated by the diminutive standard deviations observed.

The calculated least-squares devitalization curve of coxsackievirus B-1 (experiment 1) is shown in Fig. 3. The devitalization of this virus was examined by conducting three replicate experiments. The replicate experiments were done for two reasons; namely, (i) statistical analysis of the first experiment indicated that the devitalization rate was significantly different from poliovirus type 1 ($P < 0.05$) and (ii) the question of statistical confidence and reproducibility of our data was based upon statistical confidence rather than upon other considerations. Visual inspection of Fig. 3 indicates that all of the plotted virus survival points closely fit the calculated least-squares devitalization curve. Statistical comparison of the slope functions of each coxsackievirus B-1 repeat experiment by regression analysis with poliovirus type 1 resulted in identical statistical inferences and conclusions, i.e., significantly different from poliovirus type 1 ($P < 0.05$). In addition, each coxsackievirus B-1 replicate experiment was examined statistically by paired combinations. In every instance, the data indicated that the devitalization characteristics of this virus were similar in that no significant difference ($P > 0.05$) was obtained between any combination of the three experiments. The observed survival points of the three experiments, with few exceptions, fell within the 95% confidence interval of experiment no. 1. This further indicated that in all three experiments the same results were obtained. Note also that the location of the devitalization curve of poliovirus type 1 (including the 95% confidence interval) charac-

![Fig. 3. Calculated least-squares devitalization curve with the 95% confidence interval of coxsackievirus B-1 (experiment 1) in un-stirred estuarine water exposed to UV radiation (116 ergs per mm² per sec). The surviving fractions of coxsackievirus B-1 observed in three replicate experiments are shown as plotted points. The shaded area represents the 95% confidence interval of poliovirus type 1.](image-url)
terizes a completely different response to UV exposure during the exponential phase of devitalization when compared with coxsackievirus B-1. The remarkable agreement observed with the coxsackievirus B-1 repeat experiments unequivocally satisfied the academic question of reproducibility of our data and substantiated our confidence in the choice of the statistical treatment used. Table 2 shows the calculated slope functions (devitalization rates), intercepts, and half-life values of coxsackievirus B-1 obtained from the three replicate experiments. The reaction constants of reovirus type 1 are also shown in Table 2. Again, a comparison of the reaction constants indicated a high degree of analytical precision, as reflected in the diminutive standard deviations observed. The "negative sign" before the intercept indicates that the intercept occurred above the origin.

The calculated least-squares devitalization curve of reovirus type 1 is shown in Fig. 4. In addition, the first experiment with coxsackievirus B-1 is shown for comparative purposes. An interesting feature of the two plots was the parallelism of the devitalization curves. This indicated, of course, that the viruses behaved similarly during the exponential phase of devitalization. The major difference between the two curves was the location of the intercept with respect to the origin. For example (with one exception, Table 2), the intercepts determined for the devitalization curves of coxsackievirus B-1 occurred well below the origin. This same observation was also made for the devitalization curves of the other enteroviruses studied (Table 1). Conversely, the intercept determined for the devitalization curve of reovirus

type 1 occurred well above the origin. This latter observation supports the contention that a "shoulder" (multihit-type of curve) occurred in the reovirus curve before the exponential phase of devitalization. The position of the intercept does not indicate the initial multiplicity of virus but rather indicates, in general, the degree of clumping.

Statistical comparison of the slope functions of reovirus type 1 and poliovirus type 1 by regression analysis indicated a significant difference in devitalization rates \((P < 0.05)\). Conversely, a statistical comparison of the slope functions of reovirus type 1 and coxsackievirus B-1 indicated no significant difference in devitalization rates \((P > 0.05)\). Since a parallelism of the curves (Fig. 4) was noted previously, this analytical finding was not surprising. Again, the location of the devitalization curve of poliovirus type 1 with respect to those of reovirus type 1 and coxsackievirus B-1 (experiment 1) characterizes a completely different response to UV exposure during the exponential phase of devitalization.

![Fig. 4. Calculated least-squares devitalization curves of reovirus type 1 and coxsackievirus B-1 in unstirred estuarine water exposed to UV radiation (116 ergs per mm² per sec). The shaded area represents the 95% confidence interval of poliovirus type 1.](image)

**Table 2. Calculated slope functions\(^a\) intercepts,\(^b\) and devitalization half-life values of coxsackievirus B-1 replicates and reovirus type 1 exposed to UV radiation**

| Virus        | Slope \(-b\) | Standard deviation of \(-b\) | Intercept \(-a\) | Standard deviation of \(-a\) | Half-life \(^c\) | sec |
|--------------|--------------|-------------------------------|-----------------|-------------------------------|-----------------|-----|
| Coxsackievirus B-1 |              |                               |                 |                               |                 |     |
| Expt 1       | 0.17         | 0.013                         | 0.54            | 0.012                         | 4.04            |     |
| Expt 2       | 0.20         | 0.006                         | 0.31            | 0.006                         | 3.48            |     |
| Expt 3       | 0.19         | 0.004                         | -0.04           | 0.004                         | 3.62            |     |
| Reovirus 1   | 0.17         | 0.007                         | -0.52           | 0.006                         | 4.00            |     |

\(^a\) \(-b = \frac{(\sum XY - (\sum X \sum Y)/n)/(\sum X^2 - (\sum X)^2/n)}{\sum Y - b\sum X/n}, \) where line \(Y = a + bX\), \(a\) and \(b\) are the intercept and slope of the line.

\(^b\) \(a = \frac{\sum Y - b\sum X/n}{n}\).

\(^c\) Half-life = 0.69315/b.
DISCUSSION

The effect of UV on the devitalization of eight enteric viruses suspended in estuarine water has been described.

At the outset, we decided to base our interpretation of the findings on statistical inference. Our choice for analytical treatment of the data was the standard regression analysis procedure. Obviously, this analytical treatment strongly presupposes that the expected response to UV radiation reflects linearity not incompatible with single-event phenomena. Notably, the graphic treatment in most reports concerning the effects of UV on virus survival is presented as first-order reactions. This type of data treatment constrains the line to pass through the origin, indicating the absence of a constant error (or bias) term. Our data, however, were determined statistically not to support the expectation that the line was constrained to pass through the origin. The observed virus survival fractions (points) still lie along a straight line, however, which now intercepts the Y axis at a point corresponding to the bias term. In this study, the static condition of the virus-suspending fluid coincident to our experiments may have augmented departures from single-event-type reactions. These departures could result simply by decreasing the probability of observing "single-hit effects," as evidenced by the loss of infectivity of single virions. Obviously, rotating or stirring the virus-suspending fluid would have the opposite effect by increasing the probability of observing single virion devitalization at the beginning of the UV exposure period (13). It is known that the distribution of single virions versus aggregates as well as the geometric configuration and size of virion aggregates directly affect devitalization phenomena (1, 3, 6, 13, 23). This is particularly apropos with UV, since UV devitalization of virions results from a one-dimensional collision of UV rays with the virus particles. Furthermore, there is evidence that poliovirus type 1, at least, probably exists in a more severe state of aggregation in seawater than in a viral diluent such as nutrient broth (16).

Under the conditions of our experiments, we strongly feel that the occurrence of the intercept of the devitalization curve below the origin suggests the existence of virion aggregates with a frequency distribution such that the curves actually "tail" and reflect a retardant die-away type of curve. If this hypothesis is true, poliovirus types 1, 2, and 3, echovirus types 1 and 11, and coxsackievirus A-9 occur in estuarine water in similar frequency states of single virions and aggregates. In any event, the exponential phase of devitalization among these viruses was determined to be similar. When we subjected the first 15-second and the second 15-sec UV response data of poliovirus type 1 to separate regression analyses, the results indicated that both lines were constrained to pass through the origin. These findings would, of course, indicate that the observed loss of infectivity followed first-order kinetics. Although the data were limited, this could indeed suggest that the UV response of poliovirus type 1 reflected essentially two populations of infectious units, one population consisting of single virions and small aggregates, and one consisting of virions that exist as large aggregates. This also suggests that, in reality, the devitalization curve of poliovirus type 1 is a two-component curve. We realize that we are speculating and that, to settle such a controversial issue, additional research would obviously be required. Statistical evidence would, however, support such a hypothetical contention or interpretation.

Another interesting aspect of the study was the observation that coxsackievirus B-1 (including three separate experiments) behaved differently from poliovirus type 1 with respect to devitalization rate. A precise reason for this observation remains obscure. We are assuming, of course, that errors other than random sampling error and enumerative reliability of low multiplicities of infection did not influence the observations to a greater extent with this virus than with other viruses studied. Reovirus type 1 also manifested a devitalization rate different from that of poliovirus type 1. We expected, at the outset, a different response pattern of this virus to UV exposure primarily because reovirus has physical and chemical characteristics different from those of the enteroviruses. For example, reovirus is larger (approximately 3 ×) and consists of double-stranded ribonucleic acid (RNA). Poliovirus double-stranded RNA has, moreover, been shown to be more resistant to UV than the single-stranded form (5). Our expectations were not unfounded, since we observed a significant difference in the devitalization rate of reovirus when tested against poliovirus type 1. Conversely, when reovirus was compared with coxsackievirus B-1, a similar devitalization rate was observed and confirmed statistically. There is no obvious explanation for this observation other than attributing it to virion aggregate phenomena. The devitalization curves of reovirus type 1 and coxsackievirus B-1 were parallel, but their "shapes" were considered to be different primarily on the basis of where their statistically determined intercepts fell. Much evidence exists in the literature that attributes the many possible shapes of survival curves to clumping and size of virion clumps (1, 3). We agree wholeheartedly with this position in
the absence of more detailed molecular explanations. Of course, other more speculative possibilities, such as multiplicity reactivation and nucleic acid repair, could be important under specific circumstances. Concerted effort was undertaken to compare our findings with those of other investigators. This effort was abandoned since it was evident that such an undertaking was virtually impossible because of the countless variables, gross dissimilarity of experimental designs, and the omission of details in many of the published reports. Nevertheless, our results essentially paralleled the findings of Baron et al. (Fed. Proc., 18:557, 1959), who reported that poliovirus types 1, 2, and 3, echovirus 1 and 7, and coxsackievirus A-9 all had similar inactivation curves. And, conversely, "echovirus 10" (reovirus 1) showed different characteristics. A direct comparison of our data with those of Baron and associates was precluded since their report consisted of an abstract only.

The variations in UV devitalization of the viruses herein reported may be more readily elucidated by comparing their decimal-reduction times (time of UV exposure required to effect a log reduction in PFU). A tabulation of these values as a straight-line function of slope is shown in Table 3. This table shows 1, 2, and 3 log reduction-times of the eight viruses. "Effective reduction" of a virus population is undoubtedly open to considerable scientific opinion. For example, Grossebauer (14) considers a virucidal effect can be claimed only when at least 99% of the initial infectivity has been destroyed. We consider that effective disinfection can be claimed only when the virus population is reduced by at least 99.9% (17). In keeping with this premise, it can be seen in Table 3 that, to achieve a 99.9% reduction of this group of viruses, approximately 27 to 40 sec of exposure is required when the UV dose is 116 ergs per mm² per sec. Thus, the UV dosage for a 40-sec exposure time under the conditions of our experiments is calculated to be 4,640 ergs/mm². For a 99.9% reduction, the shortest exposure time was calculated for poliovirus type 3 (26.6 sec or 3,086 ergs/mm²) and the longest exposure time was calculated for coxsackievirus B-1 (40.3 sec or 4,675 ergs/mm²). Of course, these exposure times reflect only the calculated exponential phase of devitalization of the viruses as they exist geometrically in the suspending medium (under static conditions) and should not imply a difference in resistance of individual virions per se to UV rays.

Effective virological disinfection has been achieved under field conditions with poliovirus (17). The basic data reported herein concerning effective disinfection should facilitate an operative extrapolation of the findings to the applied situation. Therefore, we contend that UV radiation can be highly effective and provide a reliable safety factor in treating estuarine water.

TABLE 3. Calculated decimal-reduction-times of selected enteric viruses exposed to UV radiation

| Virus         | 90% Reduction | 99% Reduction | 99.9% Reduction |
|---------------|---------------|---------------|-----------------|
|               | sec           | sec           | sec             |
| Poliovirus 1  | 9.47          | 18.94         | 28.42           |
| Poliovirus 2  | 10.38         | 20.75         | 31.13           |
| Poliovirus 3  | 8.87          | 17.74         | 26.60           |
| Echovirus 1   | 9.34          | 18.68         | 28.01           |
| Echovirus 11  | 10.47         | 20.94         | 31.41           |
| Coxsackievirus A-9 | 10.27      | 20.53         | 30.80           |
| Coxsackievirus B-1 | 13.44      | 26.88         | 40.32           |
| Reovirus 1    | 13.29         | 26.58         | 39.88           |

a Based on calculated least-squares devitalization rate of each virus; namely 90% reduction, 2.30259/b; 99% reduction, 4.60518/b; 99.9% reduction 6.90776/b.

b UV radiation was 116 ergs per mm² per sec in un stirred estuarine water.

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