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Inactivation of Poliovirus I (Brunhilde) Single Particles by Chlorine in Water

D. G. SHARP* AND JENNY LEONG

Department of Bacteriology and Immunology, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27514

Like the Mahoney strain, the Brunhilde strain of poliovirus aggregated slowly in dilute phosphate-carbonate buffer at pH 6 but not at all at or above pH 7. Infectivity decreased at rates approximately proportional to the concentration of free chlorine present at pH 6 over the entire range of 5 to 40 μM. The addition of 0.1 M NaCl to the buffer increased the rate about twofold, but this strain was still twice as resistant as the Mahoney strain. At pH 10, inactivation was much slower than at pH 6, but when 0.1 M NaCl was added, the rate was increased 31-fold, making the OCl^- at pH 10 over three times more effective than the HOCl at pH 6.

Recent efforts to observe the effect of virion aggregation on the inactivation of some of the enteric picornaviruses by free chlorine and bromine in water (1, 5, 13) have revealed some quite unexpected facts. Not only do different viruses exhibit individual differences in habits of aggregation, but even when aggregation is clearly not involved, the kinetics of inactivation of different viruses have sometimes been so different as to suggest that entirely different mechanisms are involved (3, 9, 13). These differences become particularly conspicuous when a comparison is made of recently published works on poliovirus (1, 5) and echovirus (13, 14).

The hypothesis of Mandel, derived from his measurements of electrophoretic mobility and isoelectric points of several polioviruses, calls for the existence of two or more metastable conformational states of the proteins of the viral capsid (6). Mandel's concept has been invoked by Fujioka and Ackermann (4) to account for their observations on the inactivation of poliovirus by guanidine. This concept also seems to offer the only rational explanation of the three-phase inactivation curve that we have observed (13) with echovirus (Farouk) and possibly the somewhat similar results obtained by Kenyon and Schaub (5) with poliovirus LSc (vaccine strain). Our work with the Mahoney strain of poliovirus revealed an electrophoretic behavior different from that reported by Mandel (6) and showed no evidence of the three-phase inactivation curve that was found by Kenyon and Schaub for the vaccine strain of poliovirus or by ourselves for echovirus (Farouk) once the possibility of aggregation was eliminated.

Now, in response to the continuing need for practically useable results by those who must provide potable water as well as by others who wish to further understand the general nature of viruses, we have purposely chosen another poliovirus, presumably not very different from the vaccine strain of Kenyon and Schaub or the Mahoney strain of our own earlier work, to continue to accumulate chlorine inactivation data for polioviruses and to gain further understanding of the differences as well as the similarities in these closely related viruses.

MATERIALS AND METHODS

One of the purposes of this work was a comparison of two strains of poliovirus in their reactions with chlorine in water. The materials and methods used here with the Brunhilde strain of poliovirus were exactly the same as those used in the previous work (1) with the Mahoney strain. The culture, purification, and plaquing of the virus in HEp2 cells, the assay of virion aggregation by electron microscope and centrifuge methods, the measurement of free chlorine, electrophoretic experiments, and the inactivation of the virus in the turbulent flowing stream apparatus (10) were all the same as in the previous work.

The buffer solution used in the inactivation experiments at pH's 6 and 10 and referred to as dilute phosphate-carbonate buffer contained both KH_2PO_4 and Na_2CO_3 in the proportions given in Table 1 of a previous publication (11). In order that this buffer be chlorine demand free it was necessary to heat the dry carbonate salt for 2 h in a drying oven at 250°C before making up the buffer.

Rate-of-aggregation data were obtained by making 10-fold dilutions of the stock virus in appropriate buffer solutions at 25°C. After incubation at room temperature for 1 h, the virus suspension was centrifuged as previously described (2) for a sedimentation test. Plaque titer remaining in the supernatant fluid of the test sample divided by that of a similarly treated unaggregated (pH 7.2) control sample resulted in the ratios that were plotted as logs (see Fig. 6). At pH's 7, 8, 9, and 10 the buffer was phosphate-carbonate, at pH's 4 and 5 it was 0.05 M acetate, and at pH 3, it was 0.05 M glycine.

RESULTS

The progress of inactivation of singly dispersed preparations of poliovirus (Brunhilde) by several different concentrations of HOCl at 20°C in dilute phosphate-carbonate buffer at pH 6 is shown in Fig. 1. The stock virus was diluted 60 times with phosphate-carbonate buffer, and experiments were done within 5 min of the time of dilution. The log survival ratio was essentially a linear function of reaction time. The inactivation rates did increase continuously but not in a precisely linear fashion with the HOCl concentration (Fig. 2), and there was no indication that a maximum had been reached at a 40 μM concentration of HOCl.

When 0.1 M NaCl was included in the dilute phosphate buffer, the inactivation rate was doubled (Fig. 3), but it was still not as fast as the inactivation rate of poliovirus (Mahoney) under similar conditions (1), and it is worthy of note that no transient departures from linearity were seen during the first 20 s of contact with the chlorine either with or without the salt.

At pH 10, at which the active disinfecting agent was OCl^-, the rate of reduction in plaque-forming units (PFU) in dilute buffer alone (Fig. 4) was only about one-fifth as fast as it was at pH 6, but the effect of salt was much greater. The time required to inactivate 99% of the PFU without 0.1 M NaCl was 31 times greater than when the salt was present. This means that disinfection was faster at pH 10 with salt present than it was at pH 6 whether salt was present or not.

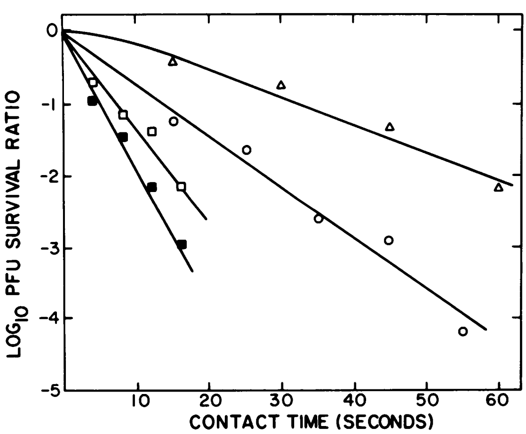

FIG. 1. *Inactivation of poliovirus (Brunhilde) in dilute phosphate-carbonate buffer at pH 6 and 20°C by several concentrations of HOCl. Symbols: △, 10 μM; ○, 20 μM; □, 30 μM; ■, 40 μM.*

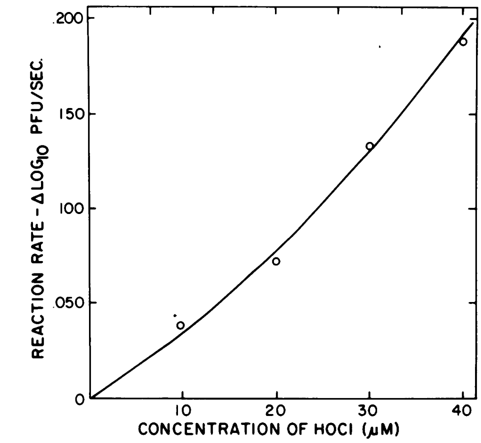

FIG. 2. *Effect of increasing HOCl concentration on the inactivation rate of poliovirus (Brunhilde) in dilute buffer at pH 6. The HOCl concentration was increased from 10 to 40 μM. There was no indication that a maximum had been reached.*

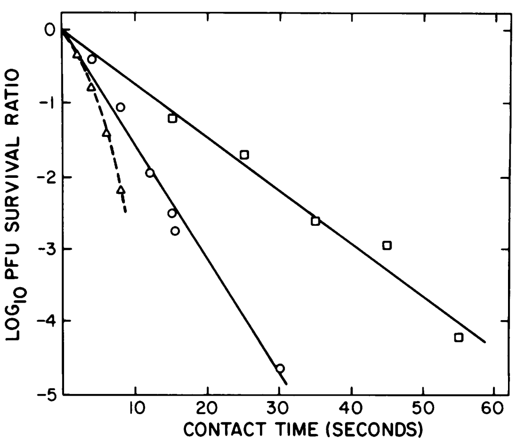

FIG. 3. *Effect of the addition of 0.1 M NaCl to the dilute phosphate-carbonate buffer at pH 6. The inactivation rate at 20 μM HOCl increased from 0.072 logs/s (□) to 0.15 logs/s (○). This was still not as rapid as the inactivation rate of the Mahoney strain (△) under the same conditions.*

The possibility that aggregation of the virions might exert an influence on these reaction rates at one or both pH values was examined in detail. Electron micrographs of the virus deposited on collodion films (2) before washing (Kinetic attachment method) were made on preparations at pH 7. There was no evidence of aggregation (Fig. 5), although the virion concentration was high (1.3×10^{12}/ml). The stock virus at pH 7 was used as the dispersed control for a series of sedimentation tests (2) that were made at other pH values (Fig. 6). At pH's 7, 8, and 9 no aggregation was detected. At pH 10 there was some loss in plaque titer, but this was found to be a

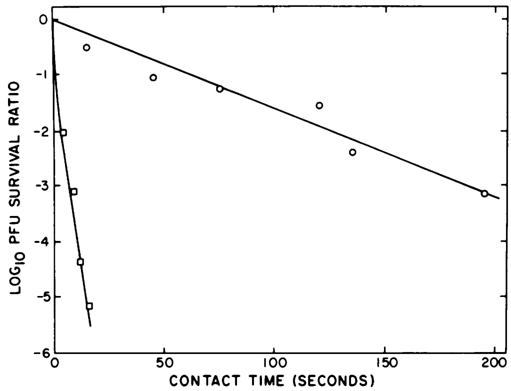

FIG. 4. *Inactivation of poliovirus (Brunhilde) in dilute phosphate-carbonate buffer at pH 10 and 20°C. The OCl^- concentration was 20 µM, and the reaction was about 31 times faster when the buffer contained 0.1 M NaCl (□) than when it did not (○).*

loss of infectivity rather than sedimentation of supernatant PFU due to virion aggregation. At pH 6 aggregation set in. It was slow when 0.1 M NaCl was present, but in the absence of salt only about 10% of the population remained as single particles after 4 h at 25°C in the dilute buffer used for inactivation experiment. All inactivation experiments at pH 6 were made within 5 min of a 60-fold dilution of the stock virus in pH 6 buffer, so very little aggregation could have occurred (2). At pH 5 aggregation was rapid and even more so at pH's 4 and 3. At pH 3 only 1 in 1,000 of the original single particles remained when 0.1 M NaCl was present, and only 1 in 100,000 remained when no salt was added to the buffer (Fig. 6). This acid aggregation profile is very similar to those of both reovirus and poliovirus (Mahoney), for which the isoelectric points have been found at pH's 3.7 and 8.2, respectively.

The isoelectric point of the Brunhilde strain of poliovirus was measured in this work by the same technique as that used in previous work (3, 13). Figure 7 shows one major peak of PFU at pH 7.3 and small, probably insignificant, amounts scattered as far as pH 9.5.

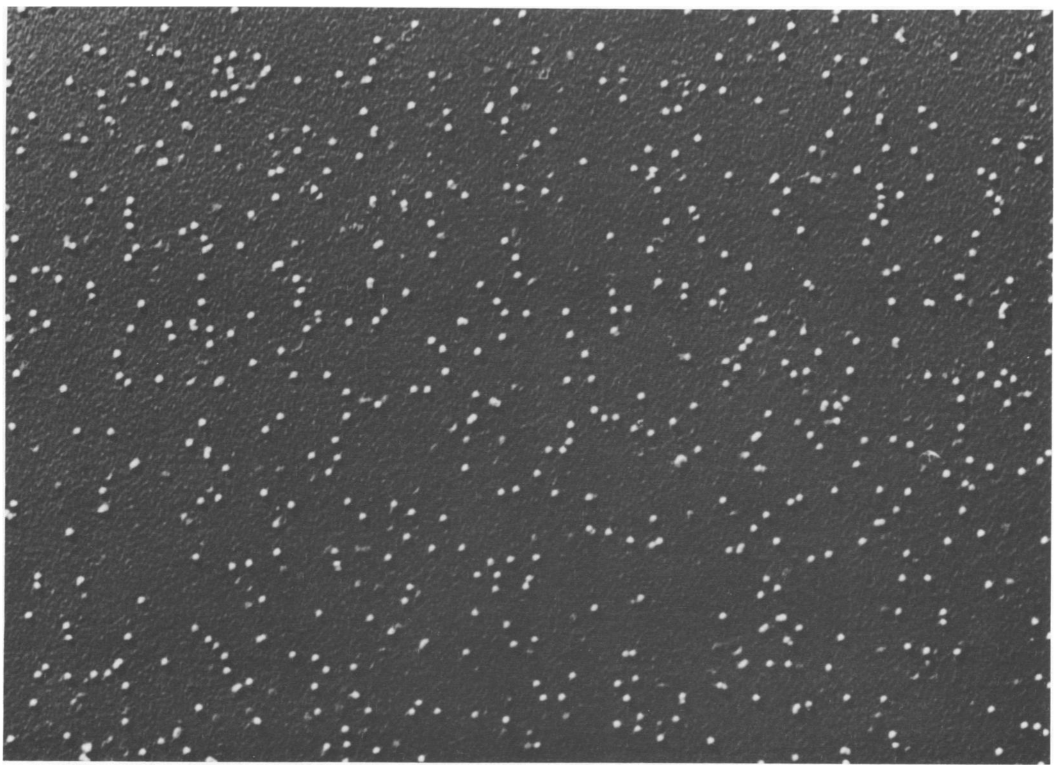

FIG. 5. *Stock poliovirus (Brunhilde) diluted 10 times with phosphate-buffered saline and deposited by Brownian activity on collodion film for electron microscopy. There were 1,679 virions in the picture of which this is a part, indicating that there were 1.3×10^{12} virions per ml of the undiluted stock virus and that there was little or no aggregation present.*

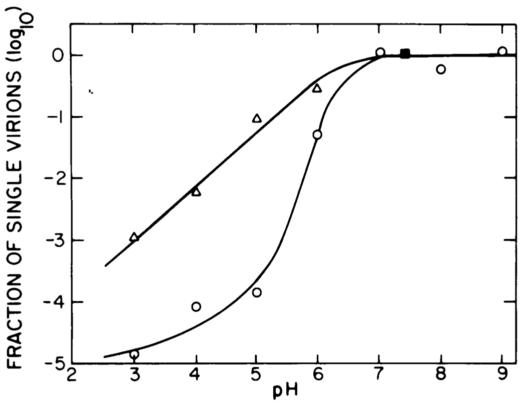

FIG. 6. *Rate of aggregation of dispersed poliovirus (Brunhilde) at 25°C as a function of pH. Ordinates are the $\log_{10}$ fraction of single particles (○) remaining after 1 h at the pH indicated. All samples tested had 1.3×10^{11} virions per ml. When 0.1 M NaCl was present (△), aggregation was slower but was not absent at any pH at which it occurred in 0.05 M buffer alone.*

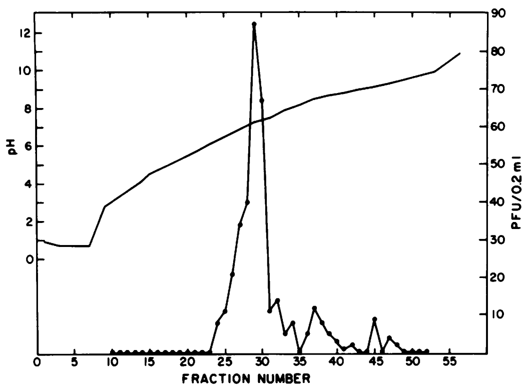

FIG. 7. *Measurement of the isoelectric point of poliovirus (Brunhilde) in a gradient of both pH and density. The electrofocused peak of PFU occurred at pH 7.3. At the start the virus was distributed equally in all parts of the gradient.*

DISCUSSION

The inactivation kinetics shown in Fig. 1 reveal no transient departure from linearity such as that so prominently displayed by echovirus (13) and poliovirus LSc (5). Three of the lines are remarkably straight down to survival levels of 10^{-2} to 10^{-4}, the only departure being the slight lag in the top line. It is unlikely that this is caused by virion aggregation. Although it is true that Fig. 6 indicates the onset of colloidal instability at pH 6, the time between the dilution of the stock virus in the pH 6 buffer and the inactivation experiment was not sufficient for aggregation of a majority of the virions to occur (2), and if a lagging curve is the result of aggregation, it can occur only if most of the virions are aggregated (8).

The steady increase in the disinfection rate with HOCl concentration (Fig. 2) distinguishes the Brunhilde strain from the Mahoney strain of poliovirus because the latter increases only very slowly with concentrations greater than 10 μM (1, 12). Like the Mahoney strain, the Brunhilde strain showed no tendency to aggregate in dilute buffers at its isoelectric point, which was revealed by electrophoretic focusing experiments to be at pH 7.3 (Fig. 7). Neither aggregated at or above pH 7, but both aggregated slowly at pH 6 and increasingly as pH was decreased to 5 and further into the acid range.

Inasmuch as virion aggregation is not likely to influence the inactivation experiments reported here, the straight line characteristics of most of the graphs might be attributed to the use of dispersed virus were it not for the fact that equally well dispersed Mahoney strain poliovirus produced predominantly curved lines in experiments of exactly the same kind (1). These results indicate that a departure from first-order kinetics of the inactivation of these viruses by chlorine is not necessarily caused by aggregation among the virions. Other influences, presumably related to the mechanism of the reaction of chlorine with individual virions, have thus been revealed.

One characteristic of the Brunhilde strain is outstanding. In the presence of the relatively weak disinfecting OCl^- form of chlorine at pH 10, the addition of 0.1 M NaCl increased the reaction rate 31-fold (Fig. 4). This was not a deaggregating effect of salt; there was no aggregation of the virions at pH 10, yet the time required to reduce the PFU survival ratio to 10^{-2} was reduced from 127 s to less than 4 s, which is less than the time required by the same 20 μM chlorine in the form of HOCl at pH 6. This effect was observed earlier with the Mahoney strain as well (11), and there the rate differential was even greater (about threefold). Although it is not at all clear why the presence of the additional Na^+ and Cl^- ions should so alter the reactivity of the OCl^-, it may be pertinent to recall the work of Salo and Cliver (7), who showed that the thermal stability of several picornaviruses was much greater in acid than in alkaline conditions. An increase in ionic strength along with high pH may loosen or otherwise weaken the protective covering capsid of the virion. Whatever the mechanism, the facts revealed are of immediate practical significance because the weak disinfection action of OCl^- previously reported by us and others (1) can apparently be augmented 30-

to 150-fold if NaCl is added. This effect must appear in experiments involving the chlorination of viruses in seawater, but we are not aware of any publication of such results. Various hard waters containing salts of several kinds are likely to show salt effects of this kind also.

ACKNOWLEDGMENTS

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